A-VAX: Applying Quality by Design to Vaccines

CMC-Vaccines Working Group

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Preface

The A-VAX Case Study involved the efforts of many individuals and would not have been made possible if it were not for the countless number of hours spent by the 5 participating companies (GlaxosmithKline, MedImmune, Merck, Pfizer, and sanofi pasteur).

To that end, the Facilitation Team from Pricewaterhouse Coopers would like to thank the following participants from each company for their energy and dedication.

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1. Introduction to CMC-Vaccine Working Group (CMC-VWG) QbD Case Study

1.1. Background

Following the publication of the A-Mab case study in 2009 that applied Quality by Design (QbD) principles to the production of an example monoclonal antibody, (http://www.casss.org/associations/9165/files/Case_Study_Press_Release.pdf and http://www.ispe.org/index.php/ci_id/20555/la_id/1.htm), suggestions were made to do a vaccine case study. Considering the differences in development strategies between a monoclonal antibody and a vaccine, the rationale was clear for creating a new case study.

In early 2010, key industry and regulatory agency thought leaders were consulted to consider the feasibility of such a case study. Based on the feedback, some of these thought leaders engaged a consulting group (PRTM, now Pricewaterhouse Coopers) to further develop the feasibility package and solicit participation from the industry and regulators.

Five companies — GSK, MedImmune, Merck, Pfizer, and sanofi pasteur — responded to the solicitation and committed to participate in the Vaccine Working Group (VWG). The main objective of the VWG was to work together to see if and how QbD could be applied to vaccine development and manufacturing.

1.2. Differences in Development Strategies

Although a vaccine case study would likely emphasize some of the same QbD principles as the A-Mab case study, applying the QbD principles to a vaccine and emphasizing the differences may broaden the scope and enhance the value of the discussions.

One major difference between the A-Mab case study and a vaccine case study would be a focus on the value of QbD for non-platform products/processes typical of vaccines, rather than the platform Chinese Hamster Ovary (CHO)-based, stirred tank cell culture and column purification process typical of monoclonal antibodies). The ultimate ability to define a multivariate design space, then generate the associated process/product understanding, would be of interest for a vaccine product in light of historical challenges to develop potency assays and establish the clinical relevance of quality attributes to specifications.

Other differences for the vaccine case study arise from the fact that most vaccines are given to healthy patients prophylactically. Feedback from ongoing pharmacovigilance and the question of whether QbD can improve a manufacturer's quality management systems to lessen oversight by Health Authorities (e.g., lot releases by regulatory agencies) are also important topics for discussion. The need for consistent availability of high-quality vaccines often made from complex raw materials leads to an emphasis on the raw material attribute identification, risk

analysis, and control strategy. The final difference arises from the availability of key guidance, such as International Conference on Harmonization (ICH's) Q11 and FDA's process validation (PV) documents, that was not as fully developed at the time of the A-Mab case study.

There are some key differences between monoclonal antibodies and vaccines that influence the development and manufacturing strategy:

Monoclonal antibodies	***Vaccines***	***Implications***
Often well-characterized	*Often difficult to characterize*	*Less definitive analytical comparability pathways* *Less ability to monitor product quality in mid-process*
Clear link to mechanism of action (MoA) and/or biomarker surrogate for clinical performance	*Difficult to establish clinical potency surrogates*	*Challenging to improve process post-licensure*
Consistent process and product	*Sometimes more complex, less predictable process/product*	*Variability over product/process life cycle*
Therapeutic patient population	*Prophylactic patient population*	*"Process is product" philosophy to assure quality*
Well-understood process; good detectability for test methods	*Less understood process; difficult to measure attribute changes*	*Empirical process models for linking parameter inputs to quality outputs* *More stringent threshold for reporting manufacturing changes*

Certain differences between monoclonal antibodies and vaccines result in differences in development strategy. The aim of the case study has been to demonstrate how QbD can be applied to vaccines, emphasizing these differences.

1.3. Goals of Case Study

The goals of the case study are to present potential approaches and stimulate discussion about how to:

- Apply QbD to develop a robust vaccine manufacturing process that meets the public health need. It includes:
  - Risk-based approaches to vaccine development
  - Leveraging of science to gain process and product understanding
  - Continual improvement
  - Merging of process and analytical controls for vaccine manufacturing
- Make the rationale for development more transparent in regulatory submissions.

- Document techniques to bring safe and effective vaccines to the market more quickly.
- Strive to make reviews more efficient; decrease the number of post-approval supplements that are needed.
- Develop realistic examples to better illustrate how QbD can be applied within the development space and overall product quality system.
- Highlight and/or develop tools, frameworks, etc., to enable ICH Q8, Q9, Q10, and Q11 implementation strategies.
- Tie key benefits with the strategies illustrated in the case study.

It should be noted that this case study examines key aspects of applying QbD to vaccines. The ideas and concepts described are examples of potential strategies, but other approaches may also be appropriate. Specifically, substantial changes in manufacturing quality systems and/or regulatory approaches may be needed to fully enable application of QbD to vaccines.

1.4. Potential Benefits

The hope is that the case study may lead to a better understanding of QbD principles and their potential application to vaccine development. This may encourage promotion of QbD concepts and benefits to industry and regulatory agency management. In addition, incorporating examples of QbD applications for vaccines may challenge traditional thinking about vaccine development.

The case study will also identify the value created (e.g., business and regulatory drivers) through implementing a QbD approach to development. The value includes:

- Better understanding of the product and process, considering the different implementation tools and approaches available to attain this understanding
- Robust and consistent processes with clear understanding of the impact of future process changes
- Expedited development and regulatory review
- Cost- benefit analysis framework

The QbD approaches presented support the development of the systematic accumulation of product and process understanding that is a major pillar of the vaccine product life cycle.

1.5. Publication and Use for Educational Purposes

The case study will be published and publically publicly available through the Parenteral Drug Association (PDA) (Website: http://www.pda.org/) for use in stimulating further discussions about QbD implementation. It should be understood that that this document does not represent new regulatory policy, nor does it define a new “Gold” standard for future regulatory submissions. However, it is aligned with the available guidances available from of ICH and other sources guidances, where available. Individual companies will interpret and apply the principles differently. The extent of application applicability will vary for each development effort.

The case study is composed of thought-provoking options. The point of executing doing the case study was to push boundaries and explore scenarios, and this has been accomplished in several instances. It is critical to avoid the case study examples becoming regulatory expectations and/or standards. Vaccine development has been and continues to be an area of tremendous success and challenges. Day-to-day options differ for every project based on project its needs, timing, and markets under consideration. Although risk assessment and design of experiment (DOE) -driven development is an excellent approach, it is only one of many alternatives.

The case study is not a consensus opinion document. Working group members expressed diverse opinions regarding risk assessment tools, critical quality attribute (CQA) determination, process performance, and depth of data presented. To complete the case study, some topics were not addressed and positions were not taken even though one or more companies may have advocated for the positions.

The case study may suggest some areas where future changes to regulatory policy would benefit QbD implementation. In addition, the examples cited are meant to be illustrative of possible approaches to QbD and may not fully represent “real-life” situations. There were multiple simplifying assumptions that the case study was based on. One such simplification is that the case study does not represent the impact of collective changes across several units’ operations. There are multiple options for risk assessment, statistical analysis and establishment of a design space. It is also assumed that the manufacturer’s quality management system is augmented as needed to be able to fully support reliable QbD implementation post-licensure.

1.6. Case study focus and structure

There are many types of vaccines, including: live/attenuated/killed virus vaccines, protein conjugate vaccines, protein subunit vaccines, and DNA vaccines. Because it would be impractical to cover all vaccine types, the VWG chose to focus this case study on a fictional carbohydrate-/protein conjugate vaccine as an example of a more complex process. Also included in the case study is another example of viral vaccine production and harvest that is unrelated to the protein conjugate vaccine example but is provided to extend the concepts to more than one type of vaccine. The specific concepts and examples were selected to be complementary to those presented in the A-Mab case study, as well as illustrative of “real -world” “real-world” vaccine applications.

The case study is structured into two types of sections: general topics and process- specific. For each general topic section, the enhanced QbD approach was applied to several aspects of the selected vaccine in the case study. Within each of the process-specific sections, the enhanced QbD is approach to process development is demonstrated for process development of a single step or several steps. Example steps have been studied from upstream, downstream, and drug product functions. It is beyond the scope of this case study to demonstrate linkage of the enhanced approach across steps described in two or more of these process development sections. As such, changes proposed in one step would still be subject to downstream confirmation of no adverse impact on other steps. This document can serve as a foundational tool for further discussion leading toward that aspirational goal.

1.7. Section summaries

An executive summary of each section of the case study is included below.

1.7.1. Target Product Profile, Critical Quality Attributes, and Product Risk Assessment

A-VAX is the name of the case study vaccine. It is a pentavalent polysaccharide-VLP conjugate vaccine that has successfully completed a Phase 2 clinical trial for the prevention of cooties, an infectious disease inflicted by the organism X. horrificus in children.

The vaccine consists of five serotypes of polysaccharide that have been demonstrated to account for 80% of the disease. The exact mechanism of protection is not known. However, when conjugated to a carrier protein (VLP) and adsorbed to an adjuvant (aluminum salt), the vaccine elicits enhanced cellular and humoral responses in animals and in adult populations. These responses are similar to those observed in surviving individuals as measured after disease outbreaks. The biopharmaceutical development and manufacturing strategy for A-VAX are guided by the product's quality target product profile (QTPP).

Quality by Design (QbD) principles are applied from the onset of product definition and development and are intended to ensure the following:

- Product is designed to meet patient needs and efficacy requirements
- Critical sources of variability are identified and controlled through appropriate strategies
- Process is designed to consistently meet product critical quality attributes (CQAs)
- Process is continually monitored, evaluated, and updated to ensure that product quality is maintained throughout the product life cycle

Potential CQAs are selected on the basis of prior knowledge and current understanding of structure-function relationships, and a risk-assessment tool is developed and applied to each quality attribute. CMC-related activities focus on refining structure-function relationships and their impact on safety and efficacy through the addition of knowledge from internal studies or external publications; this information is used to iteratively update the CQA risk assessments throughout the product life cycle as it becomes available.

1.7.2. Process Development Sections (Upstream, Downstream, and Drug Product)

The process development sections are structured to illustrate different QbD elements across three categories of unit operations (Upstream, Downstream, and Drug Product). Within these categories, a number of areas are explored. These include:

- Prior knowledge and/or initial development for process definition
- Early stage process risk assessment (e.g., cause and effect (C&E) analysis)
- Identification of high-risk parameters (e.g., screening DOE, one factor at a time)
- Later stage (as well as scale-up) risk assessment (e.g., failure mode and effects analysis, or FMEA)

- DOE for understanding high-risk steps and their associated high-risk parameters (e.g., optimization DOE, design space ranging experiments, modeling simulations for defect rates)
- Scale-up confirmation
- Control strategy, process validation, and continuous improvement implications (i.e., remaining areas of high variability and high risk)

1.7.2.1. Upstream Section

The Upstream Section covers three key areas of development:

- Expression and production of both the polysaccharide and virus-like particle (VLP).
- Development of a live vaccine. (The team felt that exploring how QbD can be applied to a live vaccine could add depth to the case study.) This is included as a special section in the case study.

Polysaccharide

In the manufacturing process for polysaccharide, a well-defined upstream process is required to provide sufficient material (bulk volume) with well-defined quality attributes for the downstream processing.

The polysaccharide section describes the polysaccharide fermentation process and the effects of the complex raw materials, fermentor operating parameters, and inactivation parameters. Prior knowledge from published literature and process risk assessments are used to ascertain the factors that will be evaluated further. Ishikawa diagrams and cause-and-effect matrices facilitate the identification of process steps for further exploration via design of experiments (DOEs) or one factor at a time (OFAT) evaluations. Failure modes and effects analysis is used to assess the process risks and to develop appropriate strategies for managing critical process attributes.

VLP Example

The VLP section assesses the contribution of the upstream process in E. coli VLP production and the potential impact of the quality attributes of the upstream material to the critical attributes of the bulk VLP. The harvest step of the upstream VLP production step was selected as an example of the application of tools that provide operational confidence in selecting input parameters that may affect the quality attributes of the VLP.

Key Points from VLP Example

1. Several commonly used tools are explored throughout the document to illustrate the QbD approach for selection of critical process parameters and the design space to support the operational ranges for continuous production post validation.
2. In addition, examples of changes post validation that may or may not have any impact on the quality attributes are shown.
3. A rational approach is used to evaluate the risk of process changes associated with vaccine production with commonly used tools such as cause and effect (C&E) matrices and failure mode and effects analysis (FMEA). They assess the risk of individual process parameter changes, while taking a DOE-based approach to analyze the effects of these process parameters on the product quality attributes.

4. Scale-down models are used to reduce the number of parameters in series of fractional and full factorial designs as well as justify the design space that is defined.

Live Vaccine Example

Vaccines based on viral components represent an important segment of the vaccines available on the market including influenza, poliovirus, and hepatitis A. Because of their viral composition, these vaccines present some process requirements that must be taken into account during their development to establish robust manufacturing processes. These process constraints make it more challenging to establish a process platform than for monoclonal antibodies (mAb) processes, with a potential consequence of having less process history data and less prior knowledge in some cases.

Having these specificities in mind, the section of this case study dedicated to viral-based vaccines illustrates how Quality by Design methodology can be applied to the development of such vaccines.

Key Points from Live Vaccine Example

1. Illustrate how to consider in parallel critical quality attributes (CQAs) and key process attributes (KPAs) during the development of a viral vaccine. A specific risk assessment methodology considering CQAs as well as KPAs is proposed.
2. A methodology is proposed to ensure the definition of an efficient way to perform the scaling-up of the bioreactor size with the establishment of scale-down bioreactor model, taking into account specific aspects of micro-carrier-based cell culture (i.e., impact on mixing and shear stress).
3. The design space is built by taking into account the variability of the analytical tools used during the development of such vaccine.

1.7.2.2. Downstream Processing

The Downstream Manufacturing Process Development Section has three parts. Two parts cover the purification of the polysaccharides (PSs) and virus-like particles (VLPs) produced by the upstream processes, and the remaining part addresses the process for conjugating the PSs and VLPs. These processes are “platform-like” in that a common set of unit operations typically can be employed to purify PSs and VLPs and conjugate them. Therefore, prior knowledge is available to inform process development based on experiences with similar processes and products. However, the processes are not considered “platform” because of differences specific to the PSs and VLPs involved, which may require unique bioprocess conditions.

As with the Upstream Section, the Downstream Section uses select unit operations for the three processes to illustrate how Quality by Design principles can be applied to vaccine process development. The three parts of the Downstream Section are similarly composed for each process (PS purification, VLP purification, and PS-VLP conjugation). First, there is a description of the overall process with an explanation for the selection of the representative process step used as an example. Then, for each representative process step, there is a summary of prior process knowledge, an initial process risk assessment, and early stage process development. A late development stage process risk assessment is then presented followed by the development of a design space. This knowledge is used to demonstrate two types of post-licensure changes that can be justified, building on the design space that is defined:

- Replacement of non-recombinant enzyme (horrificase) that is purified from the bacterium *X. lyticus* with a new recombinant horrificase that is expressed in E. coli as part of a post-launch change.
- Increase in capacity in the manufacturing facility by reducing the incubation time during the conjugation step.

Key Points from Downstream Section

1. Multiple approaches for conducting risk assessments are applicable for evaluating vaccine processes
2. Definition of design space can ensure robust process operation (PS extraction)
3. Enhanced process understanding is possible regarding linkages between process parameters and both vaccine quality attributes and vaccine process performance
4. Post-licensure changes benefit from a defined design space and enhanced process knowledge achieved by using QbD development.

1.7.2.3. Drug Product

Three main processes associated with the drug product development are investigated utilizing various elements of Quality by Design. These processes are formulation development of an aluminum adjuvant vaccine development of a lyophilized formulation, and development of a sterilization process for an aluminum adjuvant diluent to ensure a homogenous product is achieved.

For formulation development efforts, understanding the optimal solution conditions that provide rapid adsorption of antigens to the aluminum adjuvant is critical since a lyophilization step is included in the process development to ensure antigen stability. Because of limited prior knowledge, a single lyophilized product containing antigens along with aluminum is not developed. Thus, it is important to clearly understand the adsorption kinetics of antigens to an aluminum adjuvant so that upon reconstitution, antigens are adsorbed quickly to the adjuvant and the administered vaccine is consistent from lot to lot.

Lyophilization cycle development is initially investigated at the laboratory scale; scalability and applicability of lyophilization are discussed in moving from laboratory to pilot to commercial scale. Prior knowledge plays a critical role in scalability aspects of lyophilization because key factors that should be investigated are very well understood to ensure a robust, fully scalable process.

The final area in the drug product section evaluates the sterilization and mixing processes associated with an aluminum adjuvant diluent. It is necessary that the aluminum adjuvant diluent is homogenous in nature and sterilized appropriately so that upon reconstitution of the drug product with diluent, proper adsorption and homogeneity are achieved in the final drug product. This ensures that, once reconstituted, an administered vaccine product is consistent from lot to lot.

Similar to the Upstream and Downstream sections, specific unit operations associated with formulation, lyophilization, and aluminum sterilization are selected to be examined using both traditional and Quality by Design approaches. An initial, early stage risk assessment (cause and effect matrix) is performed to identify process parameters where additional experiments may

have to be performed to obtain process understanding. Since the drug product processes examined are common unit operations associated with multiple vaccine drug products, the prior knowledge needed to make an informed assessment is vast.

Key Points from Drug Product Section

1. It outlines the entire drug product formulation process and indicates places where QbD can be applied.
2. It demonstrates the effective use of prior knowledge and initial risk assessment tools (multiple tools and approaches can be used) to determine where development should be focused for a robust process.
3. Development of a robust process requires multiple iterations of risk assessments, and defining the design space is critical.
4. It uses process risk assessment to link parameter risks to their respective CQAs and confirm the design space that has been defined based on the early development studies
5. The scale-up process uses a small-scale model during lyophilization development to confirm that laboratory- and pilot-scale results align with the final commercial-scale process.
6. For site to site transfer, knowledge is used to demonstrate understanding of key equipment attributes that are used to ensure proper modeling (i.e. choke flow, rate of heat transfer, freezing processes and parameters) and provide confidence that the transfer is acceptable. (It is supplemented with comparability protocols to ensure process transfer between sites is successful either before or after licensure.)

1.7.3. Control Strategy

The control strategy for A-VAX is written from a life-cycle management point of view. Early development experience, such as identification of potential critical quality attributes, and prior knowledge are built on throughout development. Nonclinical and clinical experiences are combined and are used to identify analytical attributes and process control parameters and their appropriate specifications and operating ranges.

Unique properties of some vaccines are acknowledged in development of the control strategy. Vaccine release is coupled with quality requirements to help assure acceptable vaccine properties throughout product shelf life. Key assays such as potency assays are developed to the suitable standards, employing Quality by Design principles to assure reliable measures of vaccine quality. Because of the nature of vaccine quality measurements, the case study emphasizes the roles and distinctions between specifications and control limits, as well as proper analysis of the measurements.

Critical quality attributes and their specifications are the foundation to identify and set ranges for critical process parameters. Vaccine unit operations are evaluated, both scientifically and experimentally, throughout the process to optimize it and identify the regions that yield acceptable product performance. Thus experiments are performed on a small scale to link process parameters to process performance, revealing the region where the product meets its quality specifications (the “design space”). The robustness of the control strategy is monitored, and adapted as necessary, when operated at a large scale through continuous verification. Thus the control strategy is a living plan, which is modified and improved throughout the lifetime of a vaccine.

Example scenarios are provided for assessments of quality attributes throughout development, leading to a final control strategy. Manufacturing modeling is used to inform development of nonclinical and clinical studies, which must be performed to support the control strategy. Conventional thinking is augmented by sound scientific development and documentation, which serves to communicate the control strategy and react to unexpected process and product events.

Key Points from Control Strategy Section

1. The final control strategy is the synthesis of early through late process, analytical, preclinical, and clinical experiences.
2. A sound scientific and risk-based approach to the evolution of the vaccine control strategy yields greater confidence in product quality and process control.
3. Strategic development experiments may be used to gain and communicate understanding, and to serve as a foundation for continuous process verification and improvement.

1.7.4. Regulatory Section

The environment for incorporating design space into regulatory filings for vaccines is expected to evolve in the coming years as regulators as well as vaccine companies gain more experience in application of these enhanced methodologies and they are applied earlier in the development life cycle.

With this in mind, this section of the case study explores the application of QbD concepts to the content of regulatory filings. Its purpose was to review the strategies offered in the other sections of the case study and give guidance on how best to illustrate these strategies in various types of regulatory filings. While the intent was not to “approve” a specific strategy, it did offer guidance regarding the level of data and/or justification appropriate to pursue a specific strategy. Structuring the case study in this manner generated and captured the dialog needed to better understand the challenges associated with implementing QbD within vaccine development.

The case study is a scientific document addressing the application of Quality by Design to vaccine development and product life cycle management. It is intended to serve as an example of potential ways that scientific principles and tools described under ICH documents Q8, Q9, Q10, and Q11 could be applied seamlessly during vaccine development and through post-approval life cycle management.

The examples are created as a teaching tool and as an opportunity to encourage stakeholder discussions on the application of these concepts. These examples are not presented as a mock submission, nor is there any expectation that the combination of illustrative examples would represent a realistic filing. The scientific principles are discussed and data is provided to demonstrate how the assignment of quality attributes, conduct of risk assessments, performance of experiments, and development of design space and control strategy could be utilized in regulatory filings to enhance the depth of product knowledge, increase the robustness of process control, and facilitate continuous improvement. We indicate what data could be presented to support the analysis, where summary information is appropriate, and how the data would be analyzed in each of the process sections:

- Industry will generally implement QbD for vaccines in certain process steps ("targeted QbD implementation" for vaccines), and hybrid QbD filings will be standard.
- QbD implementation for vaccines may be limited to areas that would benefit most from QbD, most likely the areas that require most of the changes post licensure (e.g., equipment changes, process changes, site changes).
- Comparability protocols, such as post-approval change management and expanded change protocols, provide a flexible mechanism to implement Quality by Design across the product life cycle (e.g., by including comparability protocols in initial marketing authorization or submitting them post approval).

Key Points from Regulatory Section

Although a few examples of vaccines developed using QbD exist, integration of key Quality by Design concepts, specifically the increased product knowledge that can be gained, will yield the following benefits:

- Provide additional strength to the data set supporting operational ranges and control strategy elements described for the product
- Justify management of change in a manner that increases the assurance of maintaining product quality. This ensures appropriate assessment across the spectrum, from full prior approval, board of health review to the firm's quality systems that oversee changes.

A summary of the type of guidance offered includes the following:

- To take advantage of the increased product and/or process knowledge that is generated it was required to capture and document the defined design space in the regulatory filings.
- Given the limited experience to date in managing change in a design space, it was recognized that to accomplish this in the EU and US filings today, a change management plan could be submitted. It would clarify the anticipated treatment of changes envisioned for the product life cycle.

The regulatory section concludes with a section on future challenges. The section introduces topics with tremendous potential value from applying the principles. However, there are also enough unanswered questions that it is important to emphasize the fluid and exploratory nature of the discussion.

One example is possible secondary or adaptive acceptance criteria in a CMP. In the development of a CMP, an acceptance criterion for CQA/CPP is required to build the control strategy. During manufacturing, a result for a CQA may be at the limit for a particular lot. This could be handled as a deviation in the usual way. Alternatively, secondary or adaptive criteria could be developed in advance and incorporated into the CMP that justify the maintained acceptability of the CQA result.

1.7.5. Implementation Section

In this section of the case study we present considerations for evaluating the business case of applying QbD to vaccine process development. The focus of this section is to present potential value drivers and evaluation tools for a step-by-step investigation of the business case development. This discussion may lead to a better understanding of the value drivers applying QbD principles in vaccine development. Also, it may encourage promotion of the concepts and benefits of QbD to industry management in situations where additional potential value is suggested. The traditional approach to vaccine process development has provided the industry with safe, effective, and reliable manufacturing processes, so the focus of evaluating the business case for QbD is to determine the specific additional value returned for the investment. The decision to apply QbD to a unit operation or step in the process is often made as a means to mitigate a risk identified in a process risk assessment. In this case study, we evaluate the potential value from the specific examples chosen in the downstream and drug product development sections.

The approach used for determining costs and benefits for these examples is a value stream measure of improved efficiency. This measure is defined in terms of the organization's "ability to predict":

- Safety and efficacy
- Product availability (robustness)
- Cost effectiveness

The business case for the QbD approach is unequivocal if this method eliminates all uncertainty and risk. However, neither the traditional nor enhanced approach is expected to produce perfectly comprehensive process and product knowledge. Thus, the key differentiator between the approaches is the value of additional process knowledge and how that information is used.

The process development risk assessment often determines where QbD will deliver the most benefit when applied. Both the traditional and QbD strategies can be applied successfully. However, in some situations the additional process knowledge gained through QbD proves useful for establishing robust control strategies and making risk-based decisions. In high-risk situations where this additional knowledge provides value to key stakeholders, the business case supports the enhanced approach. In many low-risk situations, however, the traditional approaches are very effective so there is limited value returned for the additional efforts required for QbD.

Applying this additional effort in these low-risk situations is not valuable to stakeholders and might hinder the process of delivering safe and effective drugs because of the significant increase in investment and resources required from both manufacturer and regulators. Consequently, a clear understanding of the stakeholders and value drivers for the QbD . approach improves manufacturers' and regulators' effectiveness by focusing resources where substantial value can be gained.

Key Points from Implementation Section

1. Multiple stakeholders (patients, manufacturers, and regulators) benefit from the enhanced approach to vaccine process development. (See ICH Q8 and Q11 for concepts and definitions.)
2. The enhanced approach improves the ability to predict the value stream measures of safety, efficacy, availability, and cost effectiveness.
3. A value stream approach can be used to successfully prioritize business and regulatory drivers, which supports investment in the enhanced approach.
4. ROI analysis for the enhanced approach needs to be specific to the company, regulatory agency and product because these factors drive the value stream and each situation may have unique considerations. In this case study we have provided an example framework, which can be used to develop an individualized approach.

2. Target Product Profile, Critical Quality Attributes, and Product Risk Assessment

2.1. Executive Summary

A-VAX is a pentavalent polysaccharide- virus-like particle (VLP) conjugate vaccine that has successfully completed a Phase 2 clinical trial for the prevention of cooties, a fictional infectious disease inflicted by the organism *X. horrificus* in children. The vaccine consists of five serotypes of polysaccharide that have been demonstrated to account for 80% of the disease. The exact mechanism of protection is not known; however, when conjugated to a carrier protein (VLP) and adsorbed to an adjuvant (aluminum salt), the vaccine elicits enhanced cellular and humoral responses in animals and in adult populations. These responses are similar to those observed in surviving individuals as measured after disease outbreaks.

The biopharmaceutical development and manufacturing strategies for A-VAX were guided by the product's quality target product profile (QTPP). Quality by Design (QbD) principles were applied from the onset of product definition and development and were intended to ensure that:

i. The product would be designed to meet patient needs and efficacy requirements
ii. Critical sources of variability were identified and controlled through appropriate control strategies
iii. The process was designed to consistently meet product critical quality attributes (CQAs)
iv. The process would be continually monitored, evaluated, and updated to maintain product quality throughout its life cycle

Potential CQAs were selected on the basis of prior knowledge and current understanding of structure-function relationships for conjugate vaccines, and a risk-assessment tool was developed and applied to each quality attribute. Chemistry, Manufacturing and Controls (CMC)-related activities focused on refining structure-function relationships and their impact on safety and efficacy. As new information becomes available throughout the product life cycle, it is used to iteratively update the quality attribute risk assessments, CQA classifications, and acceptance criteria.

2.2. Scientific Rationale and Disease Area Overview

In child lore, "cooties" is a fictional, widespread infectious disease. Infection with the fictional bacteria *X. horrificus* causes the rapid onset of a short-lived illness (usually lasting for a week or less) called cooties, which generally occurs in children. Cooties is typically a mild, self-limited illness manifest by fever and rash In some cases, however, cooties may be complicated with a bloodstream infection, pneumonia, or meningitis, thus requiring treatment with systemic antibiotics.

Once an episode of cooties has resolved, recidivism is rare (the bacteria is essentially eliminated from the body by the immune response), and re-infection also is rare (protection via an adaptive immune response to the natural infection). Cooties most commonly occurs in children aged 4 to 10 years as they enter school settings; however, it is also occasionally confirmed in those older than 10.

A-VAX's target product profile (TPP), a prospective summary of the vaccine development program described using labeling concepts, is located in Table 2-1: TPP for A-VAX.

Table 2-1: TPP for A-VAX

Mechanism of Action	• A-VAX (drug product) is a pentavalent vaccine containing the capsular Ps of *X. horrificus* serotypes 1-5, individually linked to a recombinant, non-infectious virus-like particle (VLP) and adjuvanted with an aluminum salt. • A-VAX is expected to provide an enhanced cellular (Th1) and humoral (Th2), antigen-specific, protective immune response when compared with a natural *X. horrificus* infection.
Indication	A-VAX is indicated for the active immunization of 2-month-old to 60-month-old infants for prevention of cooties-related illnesses caused by *X. horrificus*.
Primary Endpoints	• 70% reduction of *X. horrificus*-confirmed cooties disease within one year after dosing (below 60% is a no go) in the target population • Safe and tolerable as defined by solicited symptoms, adverse events, and serious adverse events (no evidence of enhanced *X. horrificus* disease)
Key Claims	• Has a favorable risk-benefit profile • Can be dosed with other pediatric vaccines • Universal recommendation except for premature infants (<36 weeks), immunocompromised infants, or infants with previous adverse reactions to A-VAX • Achieves World Health Organization (WHO) stability requirements
Secondary Endpoints	• Analysis supportive of primary endpoint in target population • Reduction in *X. horrificus*-specific hospitalizations and emergency-room visits • Reduction in *X. horrificus*-specific rates of bacteria-confirmed cooties disease • Reduction in antibiotic use for *X. horrificus*-confirmed cooties disease • Duration of protection >1 year (with/without booster)

Formulation/Dosing	• Antigen and adjuvant in pre-filled syringe or single-dose vial • Antigen and adjuvant containers are pre-mixed prior to injection • 3 doses administered 2 months apart (preferably 0-, 2-, and 4-month pediatric vaccine schedule)
Approvals and Recommendations	• Expecting Advisory Committee on Immunization Practices and other universal recommendations (i.e., United States, European Union, Canada, Japan, and WHO)

2.3. Biological Target and Its Role in the Disease Area

The exact mechanism by which *X. horrificus* bacteria causes cooties disease is not known, but anticapsular polysaccharide (Ps) antibody levels (humoral response) and an enhanced cellular response correlate with a significantly reduced incidence of invasive *X. horrificus* infection. These humoral and cellular responses are similar to those observed in surviving individuals who fully recovered from the disease.

Five *X. horrificus* strains, each composed of a unique polysaccharide serotype (1, 2, 3, 4, or 5), account for about 80% of the total disease. A-VAX is indicated for the active immunization of 2-month-old to 60-month-old babies for prevention of cooties-related illnesses caused by *X. horrificus*, and the vaccine is designed to elicit antibodies to *X. horrificus* capsular Ps.

A-VAX is a pentavalent vaccine that has finished Phase 2 clinical trials and contains the capsular Ps of *X. horrificus* serotypes 1-5, individually linked to a recombinant, non-infectious VLP and adjuvanted with an aluminum salt. The mechanism by which A-VAX stimulates the cellular and humoral immune response is not fully understood; however, prior knowledge supports the assumption that only the Ps-VLP conjugate can initiate a protective immune response to Ps in this age group. Ps 1-4 are more immunogenic than Ps 5 (no neutralizing monoclonal antibody [Mab] is available for Ps 5). A murine challenge-protection model is available for each of the serotypes. However, no *in vitro* model exists that can be correlated with human protection for serotype 5.

The total pAb titer (Th2) and cytokine panel (Th1) show a dose response to each adjuvanted Ps-VLP (either separately or in combination). No synergistic immune response is observed – the immune response to each serotype is independent. Unconjugated Ps does not illicit an immune response; for this reason, the level of free Ps and VLP, in addition to their extent of conjugation, must be controlled. The immune response to the conjugate promotes phagocytosis and microbial killing; the opsonophagocytic killing assay (OPA) is widely accepted as the reference method for measuring the protective capacity of *X. horrificus* antibodies, and it is employed for serotypes 1-4. An OPA level of 90% of subjects with 1:8 OPA titers is considered effective.

2.4. Status of Clinical Development

The concept of clinical design space, the link between the clinic and CQAs, and approaches to quantify the clinical experience with a biotech product candidate have been reviewed (A.S. Rathore and H. Winkle, *Nature Biotechnology* 27, 26-34 [2009]).

The clinical development program for A-VAX has completed a Phase 2 study, with an 87% response rate for each serotype. Key assumptions in the clinical development program included:

i. The "null hypothesis" was that at least one serogroup has a seroresponse rate with a lower bound of the 95% confidence interval (CI) being less than 70%.
ii. The 70% bound was selected on the basis of a sample-size estimation involving 90 participants in the study group providing 80% power to reject the null hypothesis
iii. Enrollment was, therefore, 100 subjects with an assumed 10% drop-out rate to have 90 subjects available for the assumed immunogenicity analysis (Table 2-2: Assumed Seroresponse Rates*) and reactogenicity profile (Table 2-3: Assumed Reactogenicity, Infant Stage*).

Table 2-2: Assumed Seroresponse Rates*

Serotype	Seroresponse Rate % (95% CI)
1	92 (84, 97)
2	96 (89, 99)
3	97 (91, 99)
4	94 (86, 98)
5	92 (84, 97)

* Adapted from: Immunogenicity of a Tetravalent Meningococcal Glycoconjugate Vaccine in Infants, A Randomized Controlled Trial. Matthew D. Snape, JAMA, January 9/16, 2008—Vol 299, No. 2, corrected on February 15, 2011

Table 2-3: Assumed Reactogenicity, Infant Stage*

Adverse Event	UK234 (n = 90)
Local Reactions	
Erythema	
Any	69 (77)
Grade 3	1 (1)
Pain	
Any	40 (44)
Grade 3	6 (7)
Induration	
Any	21 (23)

Adverse Event	UK234 (n = 90)
Grade 3	0
Systemic Reaction	
Irritability	63 (70)
Sleepiness	56 (62)
Diarrhea	29 (32)
Reduced Feeding	28 (32)
Vomiting	28 (31)
Persistent Crying	19 (21)
Axillary Temperature	
≥38 °C	7 (8)
≥40 °C	0
Analgesic/Antipyretic Use	43 (48)

* Adapted from: Immunogenicity of a Tetravalent Meningococcal Glycoconjugate Vaccine in Infants, A Randomized Controlled Trial. Matthew D. Snape, JAMA, January 9/16, 2008—Vol 299, No. 2, corrected on February 15, 2011

2.5. Key Molecular Characteristics of A-VAX

Table 2-4: QTPP for A-VAX lists the vaccine's quality target product profile. The QTPP is a prospective summary of the desired quality characteristics of the drug product that will ideally be achieved, taking into account the safety and efficacy of A-VAX (ICH Q8):

Table 2-4: QTPP for A-VAX

Key Claims	• Easy to administer, 0.5-mL subcutaneous delivery in a healthcare (outpatient) setting using a 1-mL syringe (27G × ½ inch needle) • Stability: 2 years at room-temperature storage or 4 years at 2–8 °C, and 24 hours' physical and chemical stability following reconstitution at 2–8 °C or 8 hours at room temperature (achieves WHO stability requirements) • No animal- or human-derived products are used in the manufacture of A-VAX
Formulation/ Dosing	• Sterile product: the drug substance (Ps-VLP) can be sterile filtered • 3 doses (containing 5 mcg each of Ps 1-4 and 50 mcg Ps 5; adsorbed to 300 mcg aluminum adjuvant as Ps-VLPs) administered 2 months apart (preferably 2, 4, and 6 months pediatric vaccine schedule) • Lyophilized and reconstituted with standard diluents containing adjuvant: rapid reconstitution profile with viscosity of 1-3 cP • Composition: sugar, surfactant, buffer (isotonic pH), and Ps-VLP conjugate • Label volume 0.5 mL filled (actual fill volume will be greater than the

	label volume to account for losses) • Single-dose vial (ISO2R vial, clear, Type I glass), latex-free stopper (13-mm coated stopper) and seal (13-mm aluminum seal with flip-off plastic button) • Secondary packaging and shipping: allowed shipping-excursion temperature 2-40 °C for 3 days in a carton (10 vials/carton)

A-VAX consists of polysaccharides purified from fermentation of *X. horrificus* on a large scale, conjugated to VLPs, and then adsorbed to an aluminum salt adjuvant. Each *X. horrificus* serotype is fermented, and the individual Ps are purified by a series of chemical and physical methods. The Ps are sized (average of 15 repeat units, each representing the critical epitope), chemically activated to aldehydes, and directly conjugated to the VLP carrier protein through reductive amination to form the Ps-VLP conjugate.

VLPs are composed of individual polypeptides of a recombinant protein. The VLP is produced in *E. coli* and is purified by a series of chemical and physical methods. VLPs first assemble through non-covalent forces (hydrogen bonding and hydrophobic interactions), followed by the formation of inter-chain disulfide bonds. The fully assembled VLP ranges in diameter from 20 to 50 nm.

Individual Ps are conjugated to the VLP through the accessible amino groups on the exterior of the VLP. The individual Ps-VLP conjugates (drug substance) are then formulated to create a polyvalent drug product containing the five different Ps-VLPs, followed by vial filling and lyophilization.

Candidate selection experiments established that A-VAX provides an enhanced cellular (Th1) and humoral (Th2), antigen-specific, protective immune response, which is observed only for the Ps-VLP conjugate. Non-conjugated Ps are unable to illicit an immune response in the target population. Experience with other conjugated vaccines using the same VLP carrier identified T-cell epitopes critical for obtaining a robust response and long-term immunity.

For the analytical development strategy, the initial focus was to support an Investigational New Drug-application, enabling activities for the Phase 1 study. Particular focus was on lot-release assays and characterization of key neutralizing epitopes during manufacture and storage. The main emphasis was on developing and implementing analytics for monitoring clinically relevant epitopes. This involved establishing antigenicity-immunogenicity correlates with the critical structural attributes of the antigen:adjuvant complex.

To support later stages of development, the analytical strategy included assays for monitoring potency, identity, purity, product- and process-related impurities, stability, and drug titer of the soluble-protein antigen in the presence and absence of the adjuvant.

A key development tool for A-VAX was the availability of a murine-potency assay (with both serology and neutralization readouts); it was used for establishing the important link between immunogenicity (and its mechanistic relevance) in an animal model and antigenicity in ligand-binding assays [in this case study, we assume enzyme-linked immunosorbent assays (ELISAs)] for serotypes 1-4. Selection of neutralizing mAbs for use in the ligand-based assays for these serotypes was confirmed using the murine-potency assay. Clinical results (human serology) support the conclusions that:

i. The ELISA is predictive of human immunogenicity
ii. Antigenicity, as defined by the specific epitope, can be used as a surrogate for immunogenicity
iii. The ELISA is suitable as the sole potency assay for serotypes 1-4 since a correlation with animal model and human immunogenicity has been demonstrated for serotypes 1-4, but not serotype 5

Serotype 5 potency was evaluated using the *in vivo* animal model only, though an antigen-binding assay (rate nephelometry) was also performed in hopes of establishing a correlation and replacing the animal model in the future.

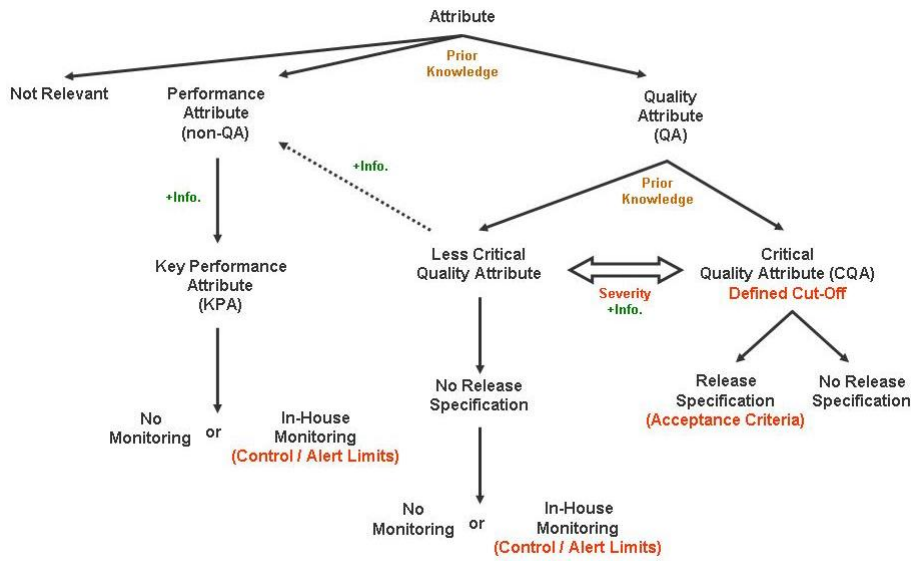
2.6. Product Risk-Assessment Tool and Potential Critical Quality Attributes

CQAs are the molecular and biological characteristics found to be critical in ensuring the safety and efficacy of a drug product. Because of the complexity of vaccine products, defining their CQAs often is difficult. Therefore, many attributes are explored during development.

For A-VAX, an initial list of quality attributes to be assessed included all product attributes that could be characterized using existing technology and analytical methods. A risk-assessment tool was developed and applied for each A-VAX quality attribute. Potential CQAs were identified on the basis of prior knowledge and current understanding of structure-function relationships. Then initial acceptance criteria were established for each CQA on the basis of prior knowledge, as well as manufacturing experience, clinical or pre-clinical data, and stability. It is important to note that knowledge gained from other conjugate products, in addition to polysaccharide products, and relevant published literature articles were evaluated in the assessment of CQAs and acceptance criteria.

Activities then focused on refining structure-function relationships and assessing the impact of their ranges on safety and efficacy of the product. As new information is discovered during the product's life cycle, it is used to iteratively update the CQA risk assessments (outlined in Figure 2-1: CQA and Control Strategy Information 'Decision Tree'*).

Figure 2-1: CQA and Control Strategy Information 'Decision Tree'*



* The approach of using a criticality continuum (⇔) is a key aspect of the control strategy in the case study. The exercise of classifying each attribute into quality attribute (QA) or performance attribute (non-QA) should have been done prior to Phase 2. A less critical QA is a quality attribute that has a relatively lower risk of impacting safety and efficacy of the product. Using the risk-assessment tool, the criticality continuum allows adjustments within the QA "envelope" as new information is obtained. A performance attribute is designated as a key performance attribute (KPA) if it affects process performance (e.g., yield or duration), but not product quality.

A questionnaire-based severity analysis was performed to identify potential CQAs. Each quality attribute was assessed for:

i. level of impact on clinical performance (safety and efficacy, see Table 2-5: Impact Scores)
ii. level of uncertainty associated with this prediction of the impact (see Table 2-6: Uncertainty Scores)

In this case study, we define (very high) uncertainty as a situation where the current state of knowledge about an attribute is such that the consequences, extent, or magnitude of a change event is unpredictable, and credible probabilities cannot be assigned to possible outcomes.

The quality attributes that have "severity" scores ≥25 are initially categorized as "critical" (Equation 2-1).

Equation 2-1: Severity

Severity = Impact × Uncertainty

Quality attributes slightly below the cutoff value are further evaluated and discussed to confirm their level of criticality. The ≥25 cutoff limit is justified even if all the uncertainty is removed from the evaluation, because any parameter with a potential high impact will still remain a potential CQA. Furthermore, the quality attributes with only moderate impact can be considered critical if there is high uncertainty.

This case study illustrates how different risk-assessment approaches and types of knowledge (prior or platform knowledge, laboratory data, non-clinical data, and clinical data) may be used to assess quality attributes. The case study used the risk-assessment tools to evaluate the impact of quality attributes on safety and efficacy. It did not consider process or manufacturing capabilities or ability to detect an important process attribute in the evaluation. Prior knowledge gained from the protein carrier generated for other conjugate products, in addition to polysaccharide products, was considered relevant.

The risk-assessment process is composed of several steps, including product definition (see Table 2-1: TPP for A-VAX and Table 2-4: QTPP for A-VAX), the identification of relevant stakeholders and subject matter experts for the exercise, and the evaluation of new and previous knowledge. Rather than describing the assessment of all quality attributes for the case study, a subset of quality attributes was selected. Each attribute has a different level of criticality, varies in the impact on efficacy and safety, and varies in the amount and types of information available to assess criticality:

i. As part of the preparation work for the risk assessment exercise, all relevant quality attributes should be identified (starting with the DP), taking into consideration the target product profile (refer to Table 2-1: TPP for A-VAX and Table 2-4: QTPP for A-VAX).
ii. Impact scores (Table 2-5: Impact Scores) were created that take into consideration the most important attributes of a vaccine: safety and efficacy (refer to Table 2-2: Assumed Seroresponse Rates* and Table 2-3: Assumed Reactogenicity, Infant Stage*).

In contrast to other biologics, issues such as unwanted immunogenicity and pharmacokinetics do not normally apply to vaccines. Because the ultimate goal is to link product attributes either directly or indirectly to clinical performance, the impact score is restricted to characteristics that have the potential to impact clinical performance, as assessed by clinical, animal, or *in vitro* studies. The impact score is also simplified compared with other biologics because *in vivo* data tend to be highly variable. Studies conducted with similar products, including published journal articles, also provide information to help assign the impact scoring for a product.

Table 2-5: Impact Scores

Impact Score	Efficacy	Safety and Tolerability (Adverse Events, AEs)
Very High 25	Significant Change	**Severe** AE prevents normal, everyday activities (e.g., prevent attendance at school/kindergarten/day-care center, requiring medical attention or advice). Significant increase in severity and/or frequency.
Moderate 8	Moderate Change	**Moderate** Sufficiently discomforting to interfere with normal everyday activities. Moderate but detectable increase severity and/or frequency over placebo.
Minimal 2	Minor to No Change	**Mild** Easily tolerated, causing minimal discomfort and not interfering with everyday activities. Similar to placebo.

Uncertainty scores (Table 2-6: Uncertainty Scores) were based on the availability of relevant information about the quality attribute under evaluation. The level of uncertainty ranges from a minimal value of 1 (little or no uncertainty) to a high of 5 (total lack of information). Supportive data from small clinical studies provides some level of assurance, but may not be statistically powered to detect minor changes. Pre-clinical data and data from similar vaccines require a more extensive discussion with relevant experts to determine their applicability to A-VAX assessments. Literature searches about related vaccines, although useful, may not fully represent A-VAX characteristics (e.g. conjugation process, formulation).

One important feature of the scoring system is that if there is data confirming a high impact or high risk for the attribute (e.g., impact score = 25), it will result in assigning a high severity score (e.g., severity score will be ≥25). Such attributes should be automatically considered as critical (CQA defined as any product attribute with severity score ≥25), no matter the level of uncertainty. Thus, any product attribute with high impact is automatically considered a CQA. The uncertainty score is based on availability of information that supports an acceptable change to the attribute.

Table 2-6: Uncertainty Scores

Score	Uncertainty
Very High 5	No information available
High 4	*External* information available from literature on related vaccine(s)
Moderate 3	Data from *internal* laboratory or nonclinical studies with this antigen:adjuvant complex, or *internal* data extrapolated from related vaccine(s)
Low 2	Supportive data from *clinical studies* with this antigen:adjuvant complex
Minimal 1	Published limits widely accepted by regulatory and scientific community

Severity scores are summarized in **Table 2-7: Severity Scores***. Using equation 1 with the scores for impact (Table 2-5: Impact Scores) and uncertainty (Table 2-6: Uncertainty Scores) assigned as part of the risk assessment, a potential critical quality attribute was assumed to have a severity score ≥25 and a less critical quality attribute was assumed to have a severity score ≤24. To score using the definitions in **Table 2-7: Severity Scores***, the risk-assessment team evaluated the potential impact of an attribute being outside its acceptable range. As a first pass, the team may consider the potential effect of the attribute as if it cannot be controlled.

It is important to note that an “iterative triage” was applied to all attributes, with particular attention paid to scores near the cut-off (indicated as yellow), which involved reassessment of impact and uncertainty scores as updated information became available. Time points for conducting iterative triage are not defined, but rather the triage is done when new information on clinical, non-clinical, or manufacturing data becomes available. This iterative triage allowed severity scores to be adjusted on the basis of new impact and uncertainty information.

It is particularly important that a rationale is provided for any adjustment and a record of how severity scores evolve is available for product life-cycle management and justification of control strategies.

Table 2-7: Severity Scores*

Uncertainty Score

Impact Score	1	2	3	4	5
2	2	4	6	8	10
8	8	16	24	32	40
25	25	50	75	100	125

Severity Score

* Severity scores are categorized as critical (≥25, red), borderline (10-24, yellow), and less critical (≤10, green). As stated previously, those scores considered borderline (10-24, yellow) require further evaluation and discussion among the relevant technical experts. Note that scoring granularity and severity scoring are provided as an example in this case study. Manufacturers should score and granulate as they consider appropriate. For example, more granularity in the ranking system could be considered for either uncertainty or impact.

Upon completion of the CQA-scoring process (summarized in Table 2-8: Initial CQAs and Risk Assessment for Reconstituted A-VAX **(adjuvant + Ps-conjugate)**) and Table 2-9: Triage Round 1 CQAs and Risk Assessment for Reconstituted A-VAX (adjuvant + Ps-conjugate)), the full list of attributes should be reviewed to ensure that the output of the scoring system is realistic.

In particular, attributes that score as less critical (not listed in Table 2-8 and Table 2-9) should be reviewed carefully with consideration of whether they may be important markers of process consistency or have been shown to be essential for the efficacy/safety of other vaccine products.

For example, product-specific data may suggest that completeness of adsorption is not linked to clinical performance. However, if the literature for a previously licensed vaccine suggests a link between completeness of adsorption and safety or clinical performance, then it may be necessary to adjust the interpretation of the scoring for this parameter to address the knowledge gained from the other vaccine.

826 **Table 2-8: Initial CQAs and Risk Assessment for Reconstituted A-VAX (adjuvant + Ps-conjugate)**

Quality/Product Attribute	Method	I*	U*	S*
Potency				
Serotypes 1-4 (correlation)	mAb-based Competitive ELISA (adsorbed)	25	2	50
Serotype 5 (no correlation)	Rate Nephelometry (desorbed)	8	2	16
Animal Model (confirms correlation)	Murine Serology (adsorbed)	25	2	50
Th1/Th2 Profile	Cytokine-panel ELISAs (adsorbed)	25	2	50
Purity (desorbed Ps-VLP)				
Peptidoglycan Level	Calculated	8	3	24
Monomer	Reducing CGE	25	2	50
Complexes/Aggregates	Non-reducing CGE	25	2	50
Product-derived Impurity (desorbed Ps-VLP)				
Fragments	Reducing CGE	8	3	24
Complexes/Aggregates	Non-reducing CGE	25	3	75
Process-derived Impurity				
Activation and Conjugation Reactants	Calculated	8	5	40
Structure/Function (Charac.) (adsorbed Ps-VLP unless indicated)				
VLP Structure	Cryo-TEM	8	5	40
Ps/VLP/Adjuvant Ratio	Calculated	8	5	40
VLP Linear and Conformational Epitopes	mAb-based ELISA (desorbed)	8	5	40
Ps Size Distribution	HPSEC-MALLS-RI	25	5	125
Size of Aggregates	DLS (desorbed)	25	5	125
Extent of Conjugation (as Ps-VLP, free Ps, and free VLP)	Reducing CGE	25	3	75
Other				
Quantity (as Protein Content)	Calculated	25	2	50
Quantity (as Ps Content)	Calculated	25	2	50
Fill Volume in Container	Compendial	25	1	25
Endotoxin	Compendial	25	1	25
Completeness-of-Adsorption (Adsorption to Al)	mAb-based ELISA (adsorbed)	25	5	125
Aluminum Content	ICP or AA	25	1	25

827 * Impact = I, Uncertainty = U, and Severity = S (see Equation 2-1 and Table 2-7).
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Table 2-9: Triage Round 1 CQAs and Risk Assessment for Reconstituted A-VAX (adjuvant + Ps-conjugate)

Quality/Product Attribute	Method	I*	U*	S*
Potency				
Serotypes 1-4 (correlation)	mAb-based Competitive ELISA (adsorbed)	25	2	50
Serotype 5 (no correlation)	Rate Nephelometry (desorbed)	8	2	16
Animal Model (confirms correlation)	Murine Serology (adsorbed)	25	2	50
Purity (desorbed Ps-VLP)				
Peptidoglycan Level	Calculated	8	3	24
Monomer	Reducing CGE	25	2	50
Complexes/Aggregates	Non-reducing CGE	25	2	50
Product-derived Impurity (desorbed Ps-VLP)				
Fragments	Reducing CGE	8	3	24
Complexes/Aggregates	Non-reducing CGE	25	3	75
Process-derived Impurity				
Activation and Conjugation Reactants	Calculated	8	5	40
Structure/Function (Charac.) (adsorbed Ps-VLP unless indicated)				
VLP Structure	Cryo-TEM	8	5	40
Ps/VLP/Adjuvant Ratio	Calculated	8	5	40
VLP Linear and Conformational Epitopes	mAb-based ELISA (desorbed)	8	5	40
Ps Size Distribution	HPSEC-MALLS-RI	25	5	125
Size of Aggregates	DLS (desorbed)	25	5	125
Extent of Conjugation (as Ps-VLP, free Ps & free VLP)	Reducing CGE	25	3	75
Other				
Quantity (as Protein Content)	Calculated	25	2	50
Quantity (as Ps Content)	Calculated	25	2	50
Fill Volume in Container	Compendial	25	1	25
Endotoxin	Compendial	25	1	25
Completeness-of-Adsorption (Adsorption to Al)	mAb-based ELISA (adsorbed)	25	5	125
Aluminum Content	ICP or AA	25	1	25

* Impact = I, Uncertainty = U, and Severity = S (see Equation 2-1 and Table 2-7).

The quality attributes for the A-VAX final drug product, including severity scores from the risk assessment, are summarized in Table 2-10: Triage Round 2 CQAs and Risk Assessment for Reconstituted A-VAX (adjuvant + Ps-conjugate). Although only the reconstituted drug product CQAs are presented and less critical QAs are not included, this assessment was done for each drug substance and drug product and their intermediates. More detailed information on the evolving potential CQAs, risk assessments, and specifications is provided in the Appendix

(hyperlink). This information was then used to update the risk assessments in an iterative manner.

Table 2-10: Triage Round 2 CQAs and Risk Assessment for Reconstituted A-VAX (adjuvant + Ps-conjugate)

Quality/Product Attribute	Method	I*	U*	S*
Potency				
Serotypes 1-4 (correlation)	mAb-based Competitive ELISA (adsorbed)	25	2	50
Animal Model for Type 5	Murine Serology (adsorbed)	25	2	50
Purity (desorbed Ps-VLP)				
Peptidoglycan Level	Calculated	8	3	24
Monomer	Reducing CGE	25	2	50
Complexes/Aggregates	Non-reducing CGE	25	2	50
Product-derived Impurity (desorbed Ps-VLP)				
Complexes/Aggregates	Non-reducing CGE	25	3	75
Process-derived Impurity				
Activation and Conjugation Reactants	Calculated	8	5	40
Structure/Function (Charac.) (adsorbed Ps-VLP unless indicated)				
VLP Structure	Cryo-TEM	8	5	40
Ps/VLP/Adjuvant Ratio	Calculated	8	5	40
VLP Linear and Conformational Epitopes	mAb-based ELISA (desorbed)	8	5	40
Ps Size Distribution	HPSEC-MALLS-RI	25	5	125
Size of Aggregates	DLS (desorbed)	25	5	125
Extent of Conjugation (as Ps-VLP, free Ps, and free VLP)	Reducing CGE	25	3	75
Other				
Quantity (as Protein Content)	Calculated	25	2	50
Quantity (as Ps Content)	Calculated	25	2	50
Fill Volume in Container	Compendial	25	1	25
Endotoxin	Compendial	25	1	25
Completeness of Adsorption (Adsorption to Al)	mAb-based ELISA (adsorbed)	25	5	125
Aluminum Content	ICP or AA	25	1	25

* Impact = I, Uncertainty = U, and Severity = S (see Equation 2-1 and Table 2-7).

It is recognized that use of the risk-ranking tool and the assessment of criticality can be considered a subjective process. To effectively utilize the tool, manufacturers should do their best to consider many types of information and rely on relevant experts in a variety of relevant fields. Thus, the risk assessment is considered a tool to help prioritize efforts during development and highlight risks that should be communicated both internally and to regulatory agencies.

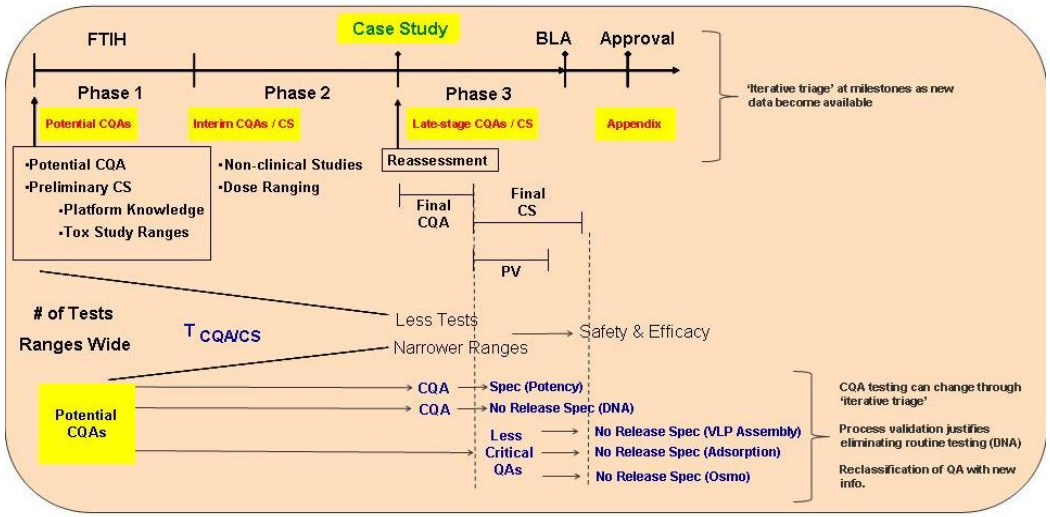
It is not anticipated that the risk assessments provide a final decision on the justification of criticality for a product, but rather that the assessments assist in the justification of CQAs selected by a manufacturer. In the end, the manufacturer and regulatory agency will need to agree upon the determined CQAs for a product, so discussions with the agency are recommended to begin early in development.

Acceptable ranges for a subset of these CQAs were established based on a combination of clinical experience, non-clinical studies, laboratory studies, and prior knowledge. The acceptable ranges were used to establish the boundaries for the design spaces in the Upstream, Downstream, and Drug Product sections of this case study.

It is important to note that testing for an attribute considered critical for the vaccine drug product may be moved upstream in the process when acceptable business or testing reasons exist to routinely control and monitor the CQA. As an example, the size of the polysaccharides was identified as a CQA since it is important in eliciting an appropriate immune response. However, for analytical reasons, testing for Ps size cannot be performed on the final drug product. Thus, size testing was moved upstream to the first potential chance to test, which is on the activated polysaccharide following size reduction. In addition, residual host-cell protein (HCP) or DNA levels would be evaluated on the drug substance, rather than the drug product, for business-efficiency reasons.

The overall CQA/risk-assessment workstream and control strategy (as outlined above) that was conducted for A-VAX is summarized in Figure 2-2: CQA/Risk-Assessment Workstream for A-VAX. It includes connections to the phase of clinical development and the "iterative triage" of the CQAs and specifications as new information becomes available. Note that it is expected that a manufacturer will begin with a relatively large number of tests (with broad acceptance ranges) and narrow the number of tests, acceptance ranges, and criticality on the basis of knowledge gained during development.

Figure 2-2: CQA/Risk-Assessment Workstream for A-VAX*



* CQA acceptance criteria generated from existing data – clinical data, non-clinical data, literature, and experience with similar products. The abbreviation CS means control strategy, and $T_{CQA/CS}$ means triage of CQAs via the control strategy.

CQAs, risk assessments, and specifications evolve with control strategy input as new information is obtained with increasing biopharmaceutical development, manufacturing, and clinical experience. Abbreviations and details are provided in the Appendix (hyperlink).

It is essential to document progression of quality attributes through the product's life cycle. Quality attributes that are considered potential CQAs early in development may be further defined as true CQAs later in development.

However, not all of these CQAs will be release specifications. For example, potency may be part of the release specifications, but residual DNA may not be if the process routinely demonstrates adequate clearance of the impurity, as demonstrated through process validation. Furthermore, a quality attribute (VLP assembly in the example above) may be downgraded from a CQA to a less critical QA during development. In addition, some QAs may be removed from the release specification as they are confirmed to be non-essential for efficacy or safety (adsorption in the example above).

2.7. Caveats and Limitations

"State-of-the-art" analytical methodology currently in practice is not advanced enough to allow the classification of most vaccine candidates, including the conjugates described here. With further advances in analytical methodology for vaccine candidates, QbD principles may be more readily applied to provide for more meaningful specifications and improved understanding of product design space.

3. Control Strategy Section

3.1. Introduction

An integrated approach to a control strategy for a vaccine product includes elements which impact both the process and the product. In addition to process and product controls at the point of manufacture, the control strategy should include appropriate consideration of bulk and final product stability, as well as strategies for addressing changes in manufacturing and analytical methods.

A risk based approach should be taken in developing a vaccine control strategy. This commences from the bottom up, in determining product quality attributes which are related to the safety or efficacy of the vaccine. Also included are attributes which combine to affect those attributes which impact safety or efficacy over the shelf life of the product. Thus while moisture of a lyophilized product has no direct impact on safety and efficacy, it may impact the preservation of potency throughout shelf life.

In conjunction with process development, preclinical and clinical development may be engaged to explore vaccine quality attributes which may be related to clinical safety and efficacy, and develop experimental plans which facilitate setting of specifications.

An iterative triage of potential critical quality attributes (CQAs) is undertaken during vaccine development. Depending upon factors such as direct evidence of clinical impact, the ability to manage the level of the CQA through the process, and others, the manufacturer will decide how to incorporate the CQA into the vaccine control strategy. Thus while some CQAs will have release and/or stability specifications (acceptance criteria) others will be managed as part of the routine quality system. Testing of others may be eliminated after successful demonstration of process control during validation.

Following the identification of attributes which are critical to quality, raw material, equipment, and process factors may be explored to determine control points in the manufacturing process. Prior knowledge combined with strategically designed experiments help identify those parameters which will become a part of the vaccine control strategy, and the control levels which must be maintained to ensure quality.

Stability studies are performed during development which helps reveal degradation pathways of a vaccine product, which define optimal formulation, packaging, handling and shipping conditions, and support vaccine shelf life. The information collected from development stability studies is also valuable to support post licensure stability monitoring and comparability.

Given the importance of some vaccine assays, such as potency assays, a strategic approach to analytical method development and maintenance may be undertaken and quality by design principles can be employed during assay development to optimize assay performance. An assay control strategy should utilize similar elements as a process control strategy, such as method

quality control, method change protocols and method change control which help ensure continued quality of vaccine measurements.

The elements of a vaccine control strategy evolve over the course of development. Thus a lifecycle approach should be taken in the development of a vaccine control strategy. This section describes the evolution of the vaccine control strategy from early development when vaccine quality attributes are identified for evaluation, through development studies to establish specifications and process controls, to the final commercial control strategy which will be used help ensure robust supply of safe and effective vaccines are administered to the target population.

3.1.1. Terminology

Wherever possible terminology has been used which is in accordance with regulatory guidance and industry technical reports but new terminology has also been used in this case study to introduce the concept of evolution of attributes throughout the product's life cycle and the continuum of criticality of the attributes. The terminology also introduces the notion of process performance attribute. As stated in the introduction to this case study, this approach is illustrative of one possible approach to definition of terms and companies may or may not adhere to this terminology. Companies should nevertheless consider including concepts related to this terminology in the development practices and in their vaccine control strategy. The terminology used throughout this section and other section of the case study follows.

Table 3-1: Control Strategy Terminology

Terminology	Definition
Quality attribute (QA)	A physical, chemical, biological, or microbiological property or characteristic of the product whose variability might have a potential impact on the safety and efficacy of the product. At early stages of development some of these quality attributes are likely to be equivalent to "potential CQA"
Critical quality attribute (CQA)	A physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality - ICH Q8(R2)
Less critical quality attribute (less critical QA)	A quality attribute determined through risk analysis to be less critical to assurance of desired product quality, efficacy and safety.
Acceptance criteria	Numerical limits, ranges, or other suitable measures for acceptance which the drug substance or drug product or materials at other stages of their manufacture should meet to conform with the specification of the results of analytical procedures - ICH Q8(R1)
Performance attribute (PA)	A physical, chemical, biological, or microbiological property or characteristic whose variability might have a potential impact on process performance

Terminology	Definition
Key performance attribute (KPA)	A parameter than when controlled ensures optimal process performance
Critical process parameter (CPP)	A process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality – ICH Q8(R1)
Key process parameter (KPP)	An adjustable process parameter (variable) of the process that, when maintained within a narrow range, ensures optimum process performance. A key process parameter does not meaningfully affect critical product quality attributes. Ranges for KPPs are established during process development, and changes to operating ranges will be managed within the Quality System – aMab
Design space	The multidimensional combination and interaction of input variables (eg, material attributes) and process parameters that have been demonstrated to provide assurance of quality – ICH Q8(R1)
Formal experimental design	A structured, organized method for determining the relationship between factors affecting a process and the output of that process. Also known as "Design of Experiments" – ICH Q8(R1)

3.1.2. Lifecycle approach to identifying and controlling critical quality attributes

1. Identification of critical quality attributes

ICH Q1 (R2) defines a critical quality attribute (CQA) as "A physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality." Quality is defined as "The suitability of either a drug substance or a drug product for its intended use. This term includes such attributes as the identity, strength, and purity." Thus vaccine critical quality attributes are properties which are either directly or indirectly related to clinical safety or efficacy of the vaccine.

A risk analysis is performed early in product development to identify quality attributes (QAs) which may be related to the clinical safety and efficacy of a vaccine and considered as CQAs. The factors which should be considered in earmarking a quality attribute as potentially critical are:

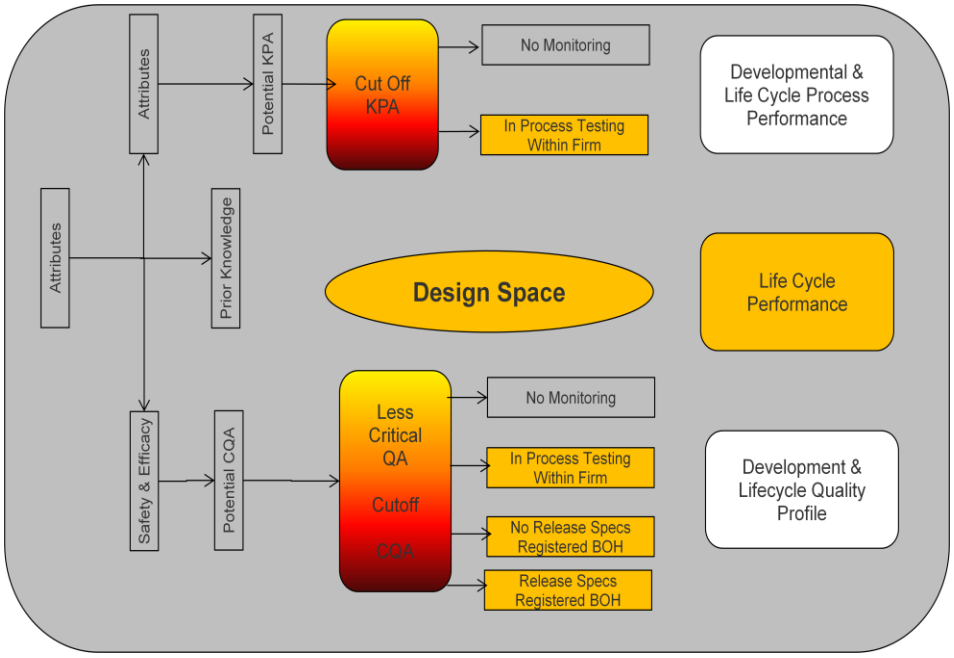
1. Local and worldwide compendial requirements;
2. Pre-clinical data;
3. Clinical experience;
4. Requirements of a downstream process step;
5. Assurance of stability; and
6. Process capability (if known).

Prior knowledge as well as scientific understanding of the mechanism of action of the vaccine are used to rank attributes according to impact on clinical safety or efficacy, as well as uncertainty based on the strength of the evidence for a link to safety or efficacy. A threshold is determined to provide guidance as to which CQAs should be further evaluated, to confirm their impact on vaccine quality and as an aid in establishing acceptance criteria wherever relevant.

In addition to QAs, performance attributes (PAs) may be identified which are potentially related to acceptable manufacturing throughput. A risk analysis is performed on the PAs to identify those which should be within an acceptable limit, range or distribution to ensure effective process performance and adequate product supply. These attributes are defined as KPAs (e.g. the viscosity or pH of an upstream material with impact on subsequent purification step, yield). The manufacturers may decide to include these KPAs in their control strategy.

The following scheme (Figure 3-1) depicts the classification of attributes into KPAs and CQAS.

Figure 3-1: Classification of attributes into KPAs and CQAs



2. Framework for identifying critical quality attributes

The vaccine manufacturer has multiple potential tools for further assessment of the "criticality" of quality attributes. In some instances, this may include *in vivo* studies in a suitable animal model. Routine safety assessment is performed on products throughout development, while vaccine efficacy can sometimes be forecast with the combination of an animal species which is sensitive to the target immunogen, and a readout which is linked to the vaccine effect. Thus, for example a murine model might be used in combination with immunogenicity readout to evaluate the impact of changes in level of a quality attribute. Likewise, *in vitro* systems may provide valuable information regarding impact on vaccine quality attributes. Infectivity in cell culture is a classical mechanism for determining changes in potency of formulations which may differ in their levels of a potentially significant quality attribute.

An additional consideration in the selection and use of an *in vivo* or *in vitro* model to assess "criticality" of a quality attribute is the variability of the model. The criticality of a quality attribute might be determined on the basis of changes in pre- clinical (*in vivo* response) or in clinical with changes in levels of the attribute. The useful model would be capable of detecting (or excluding) meaningful changes in response against the backdrop of uncertainty associated with model variability. Thus, experiments should be designed to address uncertainty, and control the risks associated with decisions made using these models.

The commercial control strategy for the vaccine will include acceptance criteria on critical quality attributes which help ensure that product is fit "for its intended use." Normal variability may have negligible impact on safety and efficacy of a vaccine in most quality attributes; however, excess variability in a critical quality attribute may lead to product, that when released is unsafe or ineffective. Experiments (*in vivo* or *in vitro*) which attempt to establish "criticality" should be performed in a range which is indicative of potential quality attribute variability. Manufacturing modeling can be utilized using mechanistic understanding, planned experiments, early development experience, and experience with platform technologies to determine the range of a quality attribute which must be supported in experiments to assess "criticality" of a quality attribute.

In instances where robust *in vivo* or *in vitro* models are not possible, evidence of immune responses and process consistency of CQA may be the primary factors considered in development of an appropriate control strategy.

Thus, some combination of these elements form the framework for a strategy to assess the "criticality" of quality attributes which have been identified through risk analysis:

- A sensitive model of product quality, performed *in vivo* or *in vitro*, and using a readout which forecasts safety or efficacy of the vaccine.
- A forecast of the range of quality attribute variability based on manufacturing modeling.
- Adequate model design, to assess "criticality" against the backdrop of model variability.

An experiment showing no impact on *in vivo* or *in vitro* response over a range spanning potential process capability could lead to either setting acceptance criteria on the basis of manufacturing variability or declaring the quality attribute as less critical (less critical QA). A quality attribute showing significant response across the range is a CQA. Acceptance criteria might then be set on some combination of the basis of "scalability" of laboratory limits or process capability to the clinical experience and prior knowledge.

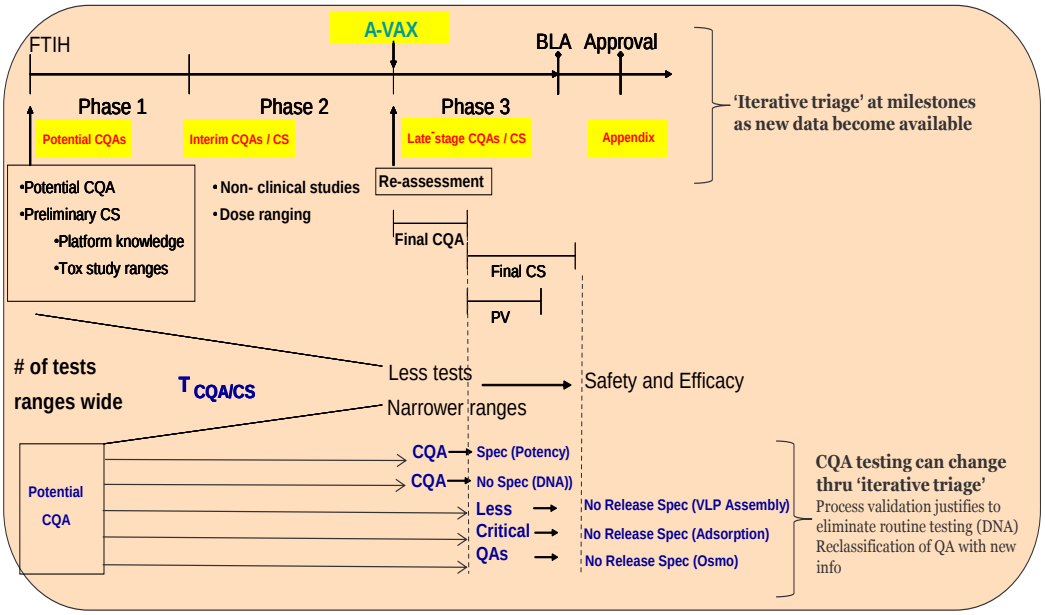
3. Lifecycle of critical quality attributes for A-VAX

A preliminary control strategy is established prior to first time in humans (FTIH). Potential CQAs are identified by risk analysis, and preliminary acceptance criteria are established and challenged in toxicology studies. The resulting list of CQAs, together with their associated tests, will be continuously evaluated throughout early development. In some cases a test might be eliminated or a criterion may be refined to reflect the evidence obtained in nonclinical studies, as well as strategic clinical studies. The total experience throughout Phase 1 and Phase 2 is utilized to reassess the list of potential CQAs. The list of final CQAs with associated acceptance

criteria is determined prior to process validation and incorporated into the final control strategy. These limits are re-evaluated and re-defined, if necessary, prior to submission of the Biological License Application (BLA). On occasion, once new data become available, the CQAs and criteria will be re-evaluated yet again, as further understanding of the product and process become available.

A life cycle approach is considered in the framework of the overall clinical and nonclinical development program. This is depicted in Figure 3-2.

Figure 3-2: Life cycle approach to management of critical quality attributes



The early risk analysis supporting A-VAX yielded a list of potential CQAs for the drug substances (PS and VLP), intermediate conjugated bulks (PS+VLP), and final drug product (PS+VLP+Alum).

A subset of potential CQAs and "less critical" QAs from the A-VAX early risk analysis are used to illustrate the lifecycle approach (Table 3-2).

Table 3-2: Subset of critical quality attributes and less critical quality attributes from the early risk assessment

Risk Analysis Category	Quality Attribute	Early Score	Process step	Preliminary Specification
Potential CQA	Potency	50	Ps+VLP and DP	0.5 – 2.00 (rel to ref std)
Potential CQA	Host Cell DNA	32	VLP	<100 ng/dose
Less Critical QA	Fragments	24	Ps+VLP and DP	<10%
Less Critical QA	Osmolality	8	DP	280-350 mOsm/kg

A combination of prior knowledge, and nonclinical and clinical studies were utilized to control substances throughout development, and to develop a final control strategy for commercial product.

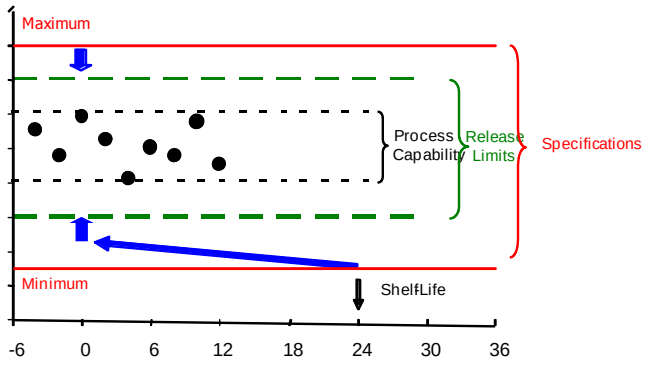
Potency

During early development, immunogenicity is measured in the conjugated bulk drug substance, and in the adjuvanted drug product. Potency of early development materials is measured both in a murine immunogenicity assay, and in a mAb-based competitive ELISA for 4 of the 5 serotypes. An appropriate monoclonal antibody could not be identified for the 5th type which was tested instead by rate nephelometry with polyclonal antiserum. A standard was introduced into each assay, to calibrate potencies across time as well as across assays.

Experiments were performed throughout early development to establish a concordance between the clinically validated *in vivo* murine immunogenicity assay and the *in vitro* assays. Potency was modified in a series of samples by temperature inactivation, and the modified and unmodified samples were tested in both assays. Excellent concordance (equivalence of relative potency across modified levels) was observed between the *in vivo* murine assay and the mAb-based competitive ELISAs for serotypes 1-4. Concordance could not be established, however, between the murine assay and the rate nephelometry assay for serotype 5. Testing in both the *in vivo* and *in vitro* assays was carried forward throughout development.

Manufacturing modeling was used to establish a range of potencies which is forecast to support commercial product capability. The predicted range drives development to support commercial release and expiry acceptance criteria. Manufacturing modeling was performed to support the potency ranges required for A-VAX. The target potency range between maximum and minimum potencies in an ideal situation is depicted in Figure 3-3.

Figure 3-3: Minimum and maximum potencies, release potencies, and process capability of A-VAX vaccine



It is recognized that complexity of manufacturing of many vaccines, and the balance required in setting limits for quality attributes that may be influenced in opposing ways by a specific change in process parameters, may result in relatively few situations where this ideal situation of release limits significantly wider than processs capability and comfortably nested within legal specifications. Routine manufacturing data for a licensed product which is manufactured and controlled similarly to A-VAX was obtained to forecast process capability of A-VAX. Accelerated stability studies show that A-VAX has similar stability as the licensed vaccine. The range in

maximum to minimum potencies was determined through a process capability analysis (See Formula in Annex 1).

The final commercial lot control strategy for potency was based on the compiled experience throughout development. Based upon the excellent concordance observed between the *in vivo* murine immunogenicity assay and the *in vitro* mAb-based competitive ELISAs for the 4 serotypes, and due to the ethical implications of using experimental laboratory animals in routine batch release, *in vitro* potency testing will be performed for commercial materials for these types. The *in vivo* assays will only be used as characterization assays to support major process and facility changes. Potency testing for the 5th type will be carried using the rate nephelometry testing out on every lot as part of the in-house management system. Due to the use of state-of-the-art production processes and intensive in-process monitoring of both process parameters and quality attributes through the use of state-of-the-art analytical tools and of strict quality systems such as GMP and QA, once confidence in the consistency of the production process has been demonstrated through validation of every step of the manufacturing process, the murine *in vivo* test will be omitted and replaced by the rate nephelometry test for routine commercial release . The final control strategy for potency of the vaccine is summarized in Table 3-3.

Table 3-3: Final Control Strategy for potency of A-VAX

Stage	Risk Analysis Category	Process Component	Serotype	Test	Specification
Early	Potential CQA	Ps+VLP and DP	All	All	0.50 – 2.00
Final CS	CQA	Ps+VLP	A-VAX$_1$-A-VAX$_4$	Release	0.77 – 1.30
			A-VAX$_1$-A-VAX$_4$	Expiry	0.50
			A-VAX$_5$	Release	0.50 – 2.00
			A-VAX$_5$	Expiry	0.35

Host cell DNA

Host cell DNA is an impurity that originates from fermentation of X. horrificus (polysaccharides) and E. coli (VLP). Each polysaccharide serotype is purified by a series of chemical and physical methods, while the VLP is purified by a series of physical methods only. Host cell DNA was identified as a potential CQA in an early risk analysis due to a combination of a moderate impact score, and high uncertainty of the impact.

Based on this, downstream process development was undertaken to remove host cell DNA. Process development was successful in that spiking experiments were performed at small scale demonstrate the removal of host cell DNA to levels below the limit of detection of the assay. Continued testing of small and large scale batches, including process validation batches manufactured at commercial scale, showed successful clearance of even high levels of the residual.

Based on the implementation of a purification process which was demonstrated to successfully eliminate host cell DNA from purified batches of VLP and polysaccharides, the specification on

host cell DNA was eliminated. In the control strategy VLP will to be tested for host cell DNA in process validation batches to verify clearance at manufacturing scale. However, in the final control strategy, the test will be eliminated as a routine test following demonstration of clearance during process validation. Host cell DNA testing will be used to characterize major process and facility changes thereafter.

Table 3-4: Final control strategy for host cell DNA

Stage	Risk Analysis Category	Process Component	Serotype	Test	Specification
Early	Potential CQA	VLP	All	Release	<100 ng/dose
Final CS	CQA	VLP	All	Not required	Non detectable*

* Release testing eliminated after confirmation of clearance during process validation and small scale spiking experiments

Fragments

VLP fragments were identified as a less critical quality attribute due to uncertainty in the impact of a high level of unassembled fragments. Percent of unassembled fragments was judged a potential efficacy concern, and was not believed to be a potential safety concern.

Phase 1 clinical studies were performed with materials with high amounts of unassembled fragments. Further development of the VLP process resulted in considerable improvement in the assembly process, resulting in an insignificant residual of unassembled fragments. Clinical studies performed with VLP materials with fully assembled particles yielded similar responses as early development experience with high levels of unassembled fragments.

On the basis of the lack of impact of unassembled fragments on clinical response, and a robust final reassembly process, the final control strategy does not include a specification for fragments. However, data will continue to be reported and maintained in the quality system as a means to evaluate excursions in the level of fragments during commercial manufacturing.

Table 3-5: Final control strategy for fragments

Stage	Risk Analysis Category	Process Component	Serotype	Test	Specification
Early	Less Critical QA	VLP & Ps	All	Release	<10%
Final CS	Less critical QA	VLP	All	Report	NA

Free polysaccharide

The level of free polysaccharide after conjugation was identified as a potential CQA. Drug product development was able to achieve >80% conjugation in early small scale formulations of the vaccine. Similar high levels of conjugation were sustained throughout development, and into process validation lots (>90% conjugation in full scale PV lots).

While published literature shows a negligible impact due to lower conjugation in animal studies for a similar vaccine utilizing materials which were artificially formulated to span 20% to 95% conjugation, prior knowledge with other similar vaccines indicates an impact at higher % free polysaccharide levels. Animal studies were therefore performed in a similar manner as described in the literature, on artificially formulated batches of the A-VAX polysaccharide conjugates with levels of 5-40% free polysaccharide and only a modest effect over this range was observed with immunogenicity endpoints met in each instance.

On the basis of the prior knowledge and confirmation of a modest effect over the expected range defined by the conjugation properties of A-VAX extent of conjugation by reducing CGE was retained as a release test in the commercial lot control strategy. The final control strategy does include a specification for % free polysaccharide. However, it is based upon the broadest ranges demonstrated to generate an adequate immune response. Additionally, data will continue to be reviewed in the quality system against tighter internal limits as a means to evaluate excursions during commercial manufacturing.

Table 3-6: Final control strategy for free polysaccharide

Stage	Risk Analysis Category	Process Component	Serotype	Test	Specification
Early	Potential CQA	DP	All	Release	<=20%
Final CS	CQA	DP	All	Release	<=40%

Osmolality

The osmolality of the final adjuvanted vaccine was identified as a less critical QA due to publications identified early in development that show no impact on local tolerance or pain at the vaccine injection site, in addition to the small volume of A-VAX administration (0.5mL) versus other products administered by IV infusion.

The final adjuvanted drug product vaccine was tested for osmolality during development and results were consistently within the range of 280-350 mOsm/kg water, which is similar to the osmolality of serum.

On the basis this information, osmolality was classified as a less critical QA in early development and later eliminated from the specification and testing strategy for commercial manufacturing.

Table 3-7: Final control strategy for osmolality

Stage	Risk Analysis Category	Process Component	Test	Specification
Early	Less Critical QA	Adjuvanted DP	Report	NA
Final CS	Less Critical QA	Adjuvanted DP	Not Required	NA

3.1.3. Specifications versus control limits on quality attributes

Specifications (acceptance criteria) should be contrasted with control limits, which are typically based on process performance and used to monitor a manufacturing process for potential shifts and trends in a quality attribute, as described above for %FS, where both types of limits are utilized. While the manufacturer may set acceptance criteria based on process performance, there are several advantages for considering alternatives.

Key among the advantages is the opportunity to develop a more flexible control strategy, which is responsive to both manufacturing drift as well as quality excursions. Using control limits as specifications may hinder a manufacturer's ability to monitor product and to make process improvements. This was highlighted in a PhRMA paper on *A Rational Approach for Setting and Maintaining Specifications for Biological and Biotechnology-Derived Products*. Separating specifications from control limits provides protection to the patient from receiving a product which is not fit for use, and protection for the manufacturer of potentially discarding acceptable product.

Furthermore, manufacturing flexibility and even improvement is difficult to achieve when specifications are based primarily on normal manufacturing variability. The experimental paradigm for defining the "design space" for a manufacturing process is the intersection of responses across a range of process parameters, with the product acceptance criteria. A design space which has been constrained by the normal performance of the process is the normal operating ranges of the process. Thus there is no opportunity to move outside the normal operating range, and thus limited opportunity to change or improve the process without significant effort.

When acceptance criteria are based upon normal manufacturing variability, special consideration should be given the risks associated with the proposed limits. Inherent in the approach are the following considerations:

1. The only risk which can be controlled using limits based on manufacturing variability is the manufacturer's risk of an out of specification (OOS) result.
2. The risk of a product batch failure is the compound risk of not meeting one or more of the batch acceptance criteria.
3. The manufacturer's risk can be controlled through consideration of the number of batches utilized to calculate the process limits, and the maturity of the process including normal process events such as variation in raw material inputs as well as other operational parameters.

Based upon these considerations, the manufacturer must develop a strategy for setting acceptance criteria which provides an adequate system of control, while assuring satisfactory product supply.

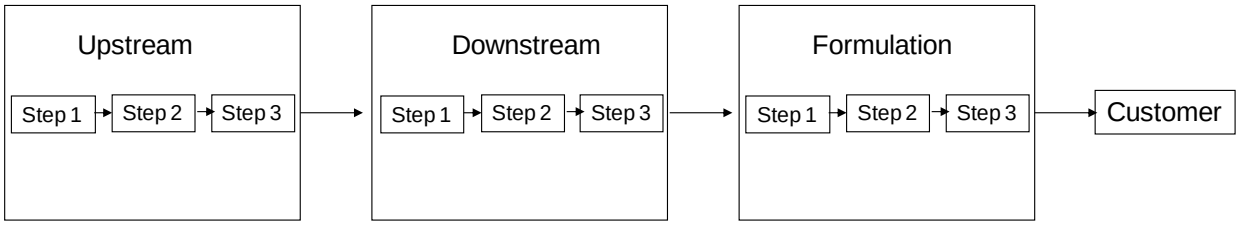
3.2. Framework for identifying critical process parameters, and definition of design space

A key element of the vaccine control strategy is management of critical process parameters. ICH Q8(R2) defines a critical process parameter (CPP) as "A process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality." Additionally, key process parameters (KPPs) which do not meaningfully affect critical quality attributes but ensure optimum process performance are identified during development. CPPs and KPPs are identified through a process of risk analysis, followed by univariate or multivariate experiments. Subsequent experiments may be performed on confirmed CPPs and KPPs to define the "design space" for the process step.

As noted in the ICH definition, key to the identification of critical process parameters is their association with critical quality attributes and their acceptance criteria. In fact acceptance criteria are the basis for development of a control strategy across process steps.

The vaccine process can be conceptualized as a series of contiguous unit operations. The major operations are: (1) upstream synthesis of the API; (2) downstream purification; and (3) drug product formulation. Each of these may have multiple steps or sub-processes. Thus purification may be a series of steps, each expected to purify away one or several components of the input material. A schematic of the overall process might be depicted in Figure 3-4.

Figure 3-4: Schematic of overall A-VAX process

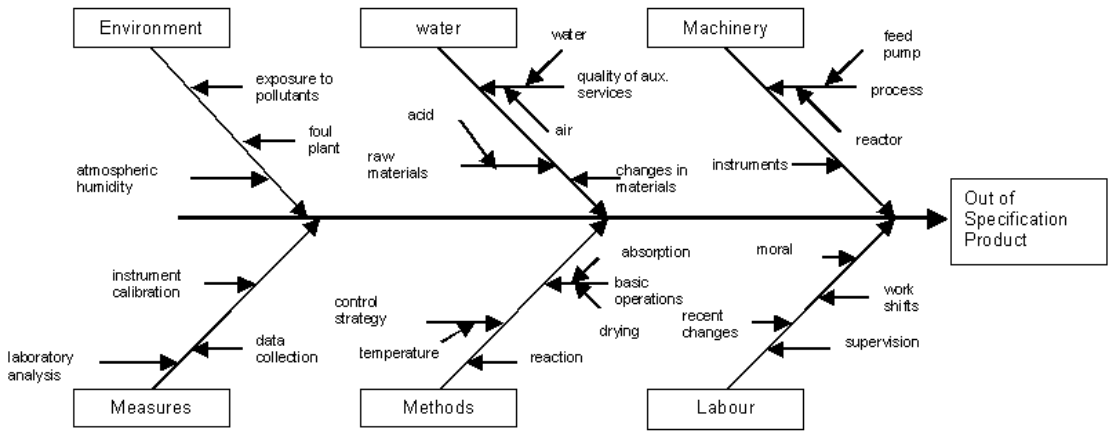


In this scheme the arrows represent the quality attributes which are known to impact a subsequent step in the process. These may affect the next immediate step, or a step further downstream in the process. For simplicity these are shown as impacting the next immediate step. Limits on a quality attribute which ensure satisfactory performance in a step are an acceptance criterion that must be met by the previous step. Thus step k must output product with a quality attribute which meets specifications on the attribute defined by step k+1.

With such linkages between process steps and unit operations, it's possible to establish the design space for each process step. The design space is the "established range of process parameters that has been demonstrated to provide assurance of quality." Said otherwise, the design space for a process step is the ranges on critical process parameters which have been demonstrated to deliver output with quality attributes which meet the acceptance criteria defined by subsequent steps of the process.

The course of demonstrating satisfactory performance begins with a risk analysis of the process factors. That risk analysis can be carried out in a number of ways, and may use various sources of process information. It should begin, however, with a thorough understanding of the factors that could impact the process. A process map might be developed utilizing a "fishbone" or cause-and-effect diagram (Figure 3-5).

Figure 3-5: Example of a process map (fishbone or Ishikawa diagram)



Scientific understanding and historical information can be utilized to eliminate or select process parameters which may impact the quality attributes that have been identified to be important to a subsequent process step. One tool that is useful for documenting factor risks is Cause and Effects analysis, which scores process parameters and quality attributes in a matrix fashion. A rigorous scoring system utilizes mechanistic or empirical understanding of the parameter or the attribute, prior knowledge from other vaccine programs which follow a similar process, or early development experience with the process. A thorough analysis of the matrix scores, including a scientifically justifiable threshold will earmark factors which should be studied in subsequent development.

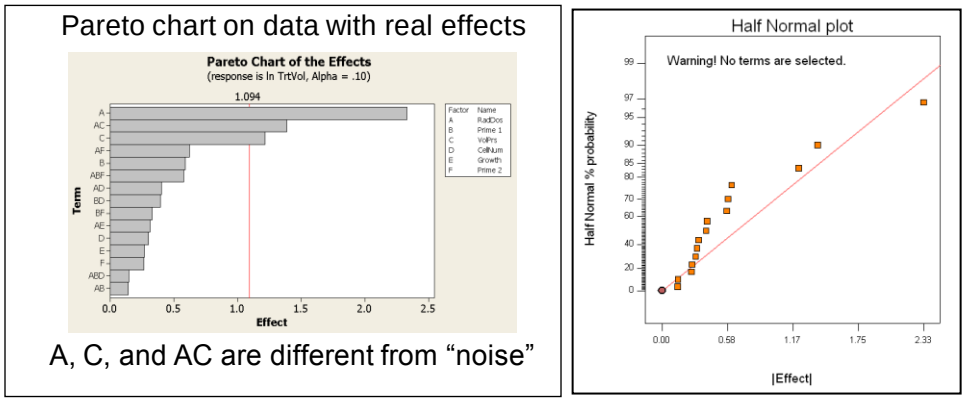
Process factors which have been identified by risk analysis to have a potential impact on subsequent process steps may be studied using multifactor design of experiments (DOE). The purpose of early studies are to "screen" out factors which have limited or no impact on a process step, and identify potential critical process parameters (CPPs) for further evaluation. DOE has the advantages over traditional "one-factor-at-a-time" (OFAT) experiments of being more efficient as well as more effective than OFAT strategies. DOE is more efficient in (1) requiring fewer numbers of experimental runs, and (2) in covering a broader "knowledge space" than OFAT experimentation. It is more effective in (1) addressing potential interactions among process factors, (2) in addressing artifacts such as experimental clustering and run order through randomization, and (3) in making use of "hidden replication," and thus in having better sensitivity for detecting important effects due to process factors or interactions.

For screening purposes, highly fractionated designs can be used to screen large numbers of factors simultaneously. Care must be taken to use sound scientific justification for the selection of a design, as highly fractionated designs lose their resolution to identify interactions among process factors. Thus scientific judgment and prior knowledge should be utilized to select a

design which preserves the ability to discover significant factors and potential interactions. The levels which are set for the factors should also be varied according to sound scientific and statistical principles. These should vary far enough outside the expected normal operating range of the factor to establish an impact, if present, and thus help guide the future control strategy as necessary.

An additional consideration in design of a screening study is the approach which will be taken to identify "significant" effects (factors and interactions). Some approaches use statistical graphics, such as Pareto plots or normal plots (Figure 3-6), to highlight "unusual" effects.

Figure 3-6: Pareto plot and half-normal plot for experimental effects



A more rigorous statistical approach involves determining the P-value for effects which are estimated from the statistical model (ANOVA approach), or estimating the effects and declaring the effect non-significant if the estimate or a confidence interval on the effect falls within some margin which is determined to be an important variation in a quality attribute.

Both approaches require some consideration of the number of experimental runs which will need to be performed to mitigate study risks. There are two types of risks associated with factor screening: (1) the risk of missing a potentially important factor; and (2) the risk of detecting a practically insignificant factor. Screening should err on the side of minimizing the risk of missing an important factor which should be controlled to ensure acceptable process performance. Statistical support of these considerations should be sought to properly balance the risks against the number of runs which will be performed in the study.

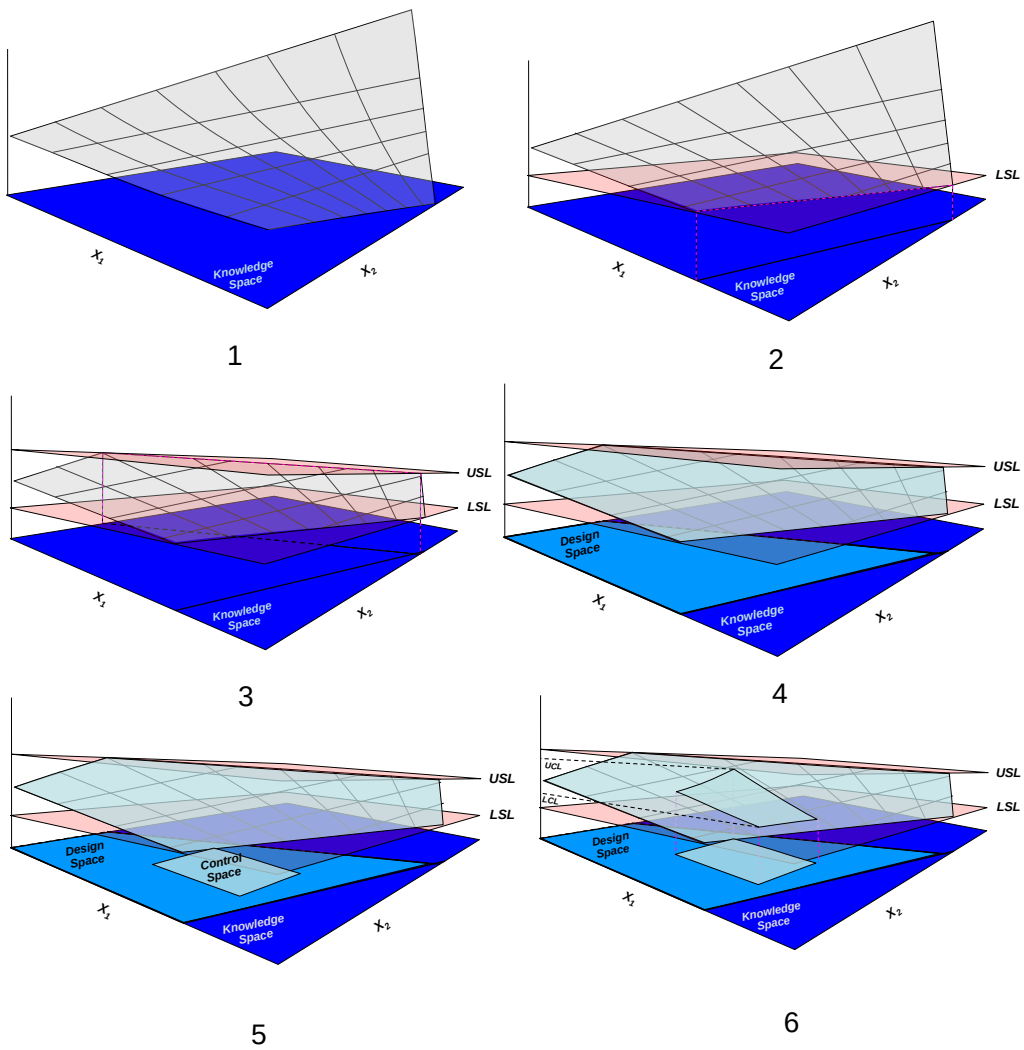
Continuous process verification is another resource for identifying critical process parameters. While all parameters may not be evaluated in development studies, some of these may assert influence during routine manufacture. For example if the process monitoring shows that a quality attribute is OOT yet all of the identified CPPs are within their control ranges, then there most likely is a parameter not identified as critical that has a significant impact on the process. An investigation may reveal additional process parameters which must be controlled to ensure product quality and optimal process performance.

Those process parameters which have been identified in screening experiments to have impact on one or more quality attributes may be further studied using enhanced experimental designs, such as response surface designs. Response surface designs are carried out to derive a

mathematical model of the responses in a quality attribute with changes in the process parameters. These are approximations to the true mathematic relationships. Mechanistic modeling can also be utilized when the relationship is known.

The mathematical model which is derived from DOE can be used together with acceptance criteria on the measured attributes to define the design space for the process step. This is depicted in Figure 3-7. Two process parameters (X1 and X2) are studied across the knowledge space defined by the multifactor DOE and yield a response surface in a critical quality attribute (Panel 1). The response surface intersects the lower (Panel 2) and upper (Panel 3) specification limits (USL and LSL) for a subsequent process step to yield its design space (Panel 4). The control space represents the normal operating ranges for the factors, falling well within the design space (Panel 5). Operating within this control space will yields quality attribute measurements falling within the upper and lower control limits (UCL and LCL in Panel 6). Since LCL and UCL fall well within LSL and USL, the process step is predicted to be highly capable of delivering product which meets the requirements of subsequent steps in the process.

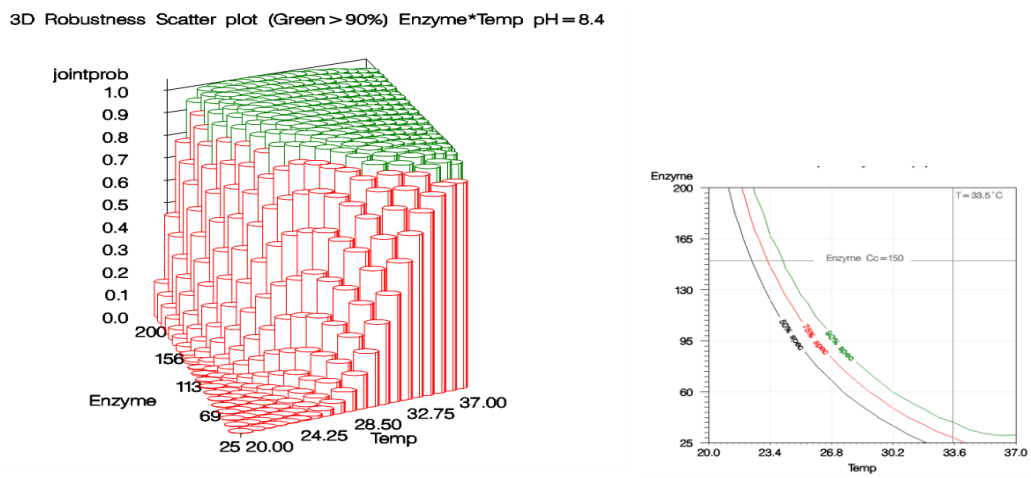
Figure 3-7: Schematic illustrating determination of design space



Excursions outside the control space are predicted to deliver product with quality attributes which fall within the specification limits for the next step of the process, as long as the operating parameters are held to limits defined by the design space.

A risk based approach may be taken in the definition of design space. Mathematical modeling can be used together with simulations, to forecast the probability of out-of-specification (OOS) results within the experimental region. An example of a design space defined through the probability of OOS is illustrated in Figure 3-8.

Figure 3-8: 3-D and contour plots of experimental results for enzyme kinetics

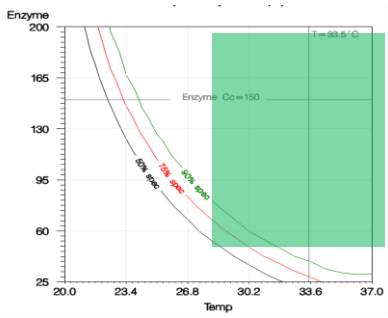


Here the region where the joint probability of an OOS among multiple quality attributes is depicted in green in the tower plot, and shown together with regions of 75% and 50% probability in the associated contour plot. The contour plots are useful to assess the “steepness” of the region associated with acceptable capability.

One consideration in applying this approach to definition of design space is the following. The design space defined by placing a limit on the probability of an OOS result provides protection to the manufacturer (or an upstream process step) of failing to meet the acceptance criterion for a quality attribute. Adequate protection should be built into the acceptance criterion to protect the customer (or the downstream step) of receiving material which has unacceptable quality.

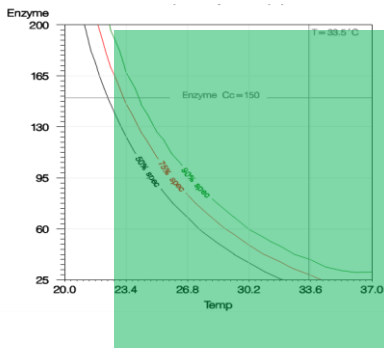
The design space for a process has traditionally been reported as a set of ranges on the relevant CPPs. Issues related to using ranges are the following:

1. ICH Q8(R2) has depicted the ranges based on an inscribed rectangle within the design space.



This has the advantage of ensuring product quality within the design space (here >90% process capability). However, it is conservative because it doesn't capture the entire design space. In addition, here is no unique solution as an infinite number of rectangles can be inscribed in the non-rectangular region.

2. Manufacturers might set the limits of design space to the extremes of the CPP ranges.



This generates a larger design space, but the probability of OOS ranges from >90% to <50% across the ranges.

Based on these limitations, design space should not be defined using ranges. Design space might be reported as a multivariate function of CPPs, or more reasonably as an algorithm which is maintained as part of the control strategy for the product.

The design space for a manufacturing step need not be defined as limits on process parameters which ensure satisfactory performance (i.e., ensure specifications are met). This might be called the "edge of failure" approach. Alternatively experiments may be performed at ranges of process parameters that the manufacturer is comfortable can be maintained, to demonstrate "robustness"of the process step across these ranges.

3.3. Manufacturing Control Strategy

Once the Critical Quality Attributes and Critical Process Parameters have been identified a control strategy must be put in place to ensure the process meets each of the elements of control. That strategy will be comprised of:

- Input Materials Controls
  - Input materials can have significant effects on a manufacturing process. Challenges such as undefined media components to subtle vendor changes must be managed via risk assessment and mitigation.

- Process Controls which include
  - Procedural controls

    A comprehensive set of facility, equipment and quality system controls which result in robust and reproducible operations supporting the production of product of the appropriate quality. These controls are supported by a quality risk management system.
  - Process parameter controls

    Critical process parameters that are linked to Critical Quality Attributes (CQAs) that when controlled within the limits of the design space ensure product quality. Key process parameters that are linked to Key performance Attributes (KPAs) that when controlled within the limits of the design space ensure product consistency. The control strategy during A-VAX manufacture will include the identification of CPPs and KPPs. The parameters will require process controls to ensure they remain in the limits identified to ensure the overall process meets its CQA and KPAs. The identification of the process controls is an evolutionary process developed using risk assessment and DOE.
  - Process development: During process development a preliminary list of CQAs has been developed to meet the requirements of the TPP. From these CQAs a process would be developed to produce a product that meets requirements. This process will be developed with little process variability in mind. Process parameters will be identified through the use of prior knowledge, literature searches and pilot lots. These same methods will be used to identify set points that each of the parameters will be run at during the development process. At this point, we are looking to develop a process that will produce a product that meets the TPP and the preliminary CQAs but not concerned with understanding the inherent variability of the process.
  - Process Characterization:Once a process has been identified and proven to meet the product CQAs a second risk assessment will be performed to identify those parameters that truly have an effect on the CQAs. Here the first attempt to define the ranges for the CPPs will be performed. If this step is performed with prior knowledge techniques only, the CPPs and their ranges will be identified using prior experience with similar products, previously published experimentation and scientific knowledge. The use of Design of Experiment techniques will identify CPPs that influence the CQAs as main affects and if the proper techniques are used interactions can be identified. If no interactions are identified the ranges used during the DOE exercises will be used as the ranges for the process. If interactions are identified then Response Surface Modeling DOE techniques should be used to identify the extent of the interactions and also set the ranges for the

CPPs. Those parameters that are not identified as CPPs might not be included in the control strategy.

- Test Controls

  As part of a comprehensive approach to the control and verification that the process can produce product that meets the assigned CQAs a testing strategy is employed to verify that the process and procedural controls performed as expected. The control strategy includes:
  - In-process testing
    - Measurements typically conducted using analytical test methods or functionality tests to ensure that selected manufacturing operations are performing satisfactorily to achieve the intended product quality.
  - Specifications (release testing)
    - Tests with associated acceptance criteria conducted at final lot release on a set of quality attributes to confirm quality of drug substance for forward processing and drug product for distribution.
  - Characterization or comparability testing
    - Testing of certain attributes outside of lot release testing for the purposes of demonstration of comparability. A specific testing plan would be developed based on risk to product quality.
  - Process monitoring
    - Testing or evaluation of selected attributes and/or parameters to trend product quality or process performance within the design space and/or to enhance confidence in an attribute's normal distribution. The frequency of monitoring is periodically reviewed and adjusted based on trends. The process monitoring program may include limits for evaluating data trends.

- Continuous Process Verification (Process Monitoring)
  - The control strategy approach to this point has been focused on developing a process that will produce product that meets the predetermined CQAs and KPAs utilizing parameters identified as critical. This identification is based on risk assessments, univariate and multivariate experimentation and validation performed in process development. Using multivariate and univariate statistical process control, data generated during the manufacturing process will be evaluated to verify that the most influential parameters were chosen to control the process and to also identify manufacturing trends. The set of parameters that constitutes the quality product profile is routinely monitored to ensure consistency of the manufacturing process.

3.3.1. A-VAX Process Controls

Process control and control of material inputs are both elements of a robust control strategy for the manufacture of A-VAX. In characterizing the process through a combination of risk assessments and the resulting multivariate and univariate experimental designs, the CPPs that control the CQAs and KPAs are identified. For the limited set of CQAs discussed in this case study, the correlation between the CQAs and KPAS and the CPPs and KPPs is given below.

Control of material inputs (either directly through knowledge of first principles or deduced from observed correlations) can be assessed in a similar manner to process parameters.

Three attributes were studied in the fermentation of the polysaccharide: number of unitrepeats, polysaccharide length and percent lysis, which influences the polysaccharide length. A risk assessment identified four variables that have potentially significant effects on these CQAs: the concentrations of raw material 1 (RM #1) and characteristics of raw material 2 (RM#2) as a material input, as well as time to inactivation and incubation temperature. The number of unit repeats was influenced by RM #2. The percent lysis was influenced by time to inactivation and incubation temperature.

Five attributes, purity (as measured by DNA, protein and lipids), SDS-PAGE profile and percent monomer were studied for the manufacture of VLP. A preliminary risk assessment determined that the quality attributes chosen for study were predominantly affected in the primary recovery of the VLP. A second risk assessment identified nine parameters in primary recovery as potentially critical. After an initial screening DOE, four parameters were identified for further study: homogenization pressure, pass number, temperature and time of solubilization. The data for the upstream operations is given in 9.

Table 3-8: CQA/CPP Correlation for Upstream Operations

	PS **Fermentation**	**VLP** **Primary Recovery**
CQAs to Control	1. Number of unit repeats 2. Percent Lysis	1. Purity (DNA, protein, lipid) 2. SDS-PAGE profile 3. % Monomer
CPPs Identified	1. RM #2[1] 2. RM #2, Time to inactivation and incubation temperature	Data for CPP vs CQAs to be collected post licensure and control strategy updated

Downstream operations were also studied using risk assessments in conjunction with multivariate and univariate experimental designs. Three downstream steps of the manufacture were studied: PS extraction, PS activation and PS/VLP conjugation.

The risk assessment process identified temperature, pH and horrificase concentration as potential CPPs for the extractions step. The QAs and CQAs measured were PS size, O-acetyl content and residual peptidoglycan content. Both residual peptidoglycan content and PS size were significantly affected by the temperature and pH, but none of the three operating parameters affected the O-acetyl content.

[1] RM 2 as a material input is treated in a manner equivalent to a CPP, though strictly speaking it is not a process parameter, though individual attributes of the material act to influence the process much as a process parameter does.

Time, pH and PS concentration were similarly identified as potential CPPs for the activation step. Quality attributes measured were reducing activity, PS size and O-acetyl content. These attributes are not necessarily CQAs but are required to ensure successful conjugation to the VLP. A screening DOE revealed that temperature, over the range studied, had no effect on the quality attributes. However, time, pH and PS concentration were observed to have effects on the three quality attributes. The PS size was also measured at line by HPSEC HPLC to ensure the size was less than 15,000 kD. This is a true in-process test.

DAPS and VLP concentrations, temperature, agitation during VLP addition, $NaCNBH_4$ concentration and time were identified as potential CPPs for conjugation. The CQAs measured were free PS, VS/VLP ratio and PS/VLP size. Only the DAPS and VLP concentrations had a significant impact, over the ranges studied, on the measured CQAs. The results for each of these three downstream operations are summarized in Table 3-10.

Table 3-9: CQA/CPP Correlation for Downstream Operations

	PS Extraction	**PS Activation**	**PS/VLP Conjugation**
CQAs to Control	1. Residual peptidoglycan content 2. PS size 3. O-Acetyl content	1. Reducing activity 2. PS size 3. O-Acetyl content	1. Free PS 2. PS/VLP ratio 3. PS/VLP size
CPPs Identified	1. Temperature, pH 2. Temperature, pH 3. No effect of Temperature, pH or enzyme concentration	1. pH, Time, PS concentration 2. pH 3. Time, PS concentration	1. DAPS concentration 2. No effect of parameters studied 3. DAPS concentration, VLP concentration

Two process steps, drug product formulation and lyophilization, were addressed in this case study. Again, extensive use was made of risk assessments to aid in the design of multivariate experiments.

In the first set of experiments, the excipients, sucrose and NaCl, along with pH and $AlPO_4$ were varied to determine the effects in binding of the PS/VLP to the aluminum adjuvant. Sucrose, pH and NaCl concentrations had significant impact on the binding of the five PS/VLP serotypes to the adjuvant. In the second set of experiments, the concentrations of excipients sucrose, histidine and polysorbate 80 were varied and the formulated PS/VLP containing all five serotypes was lyophilized under standard conditions. No significant effects of the excipients were observed on the VS/VLP binding, moisture content or reconstitution time.

Next, the lyophilization conditions were studied with the standard formulation. The parameters varied were sucrose concentration, chamber pressure, primary drying shelf temperature, shelf temperature ramp rate, secondary drying shelf temperature and secondary drying duration.

Moisture of the cake, reconstitution time and potency were measured. The moisture level was impacted by the sucrose concentration, shelf temperature ramp rate and the secondary drying temperature and time, and the reconstitution time was impacted by the secondary drying temperature and time. None of the parameters had impact on potency or cake appearance. The results for each of these three downstream operations are summarized in Table 3-11.

Table 3-10: CQA/CPP for Drug Product Operations

	Formulation	Lyophilization
CQAs to Control	1. PS-VLP Binding 2. Moisture 3. Reconstitution time	1. Moisture 2. Reconstitution time 3. Potency 4. Cake appearance
CPPs Identified	1. pH, sucrose and NaCl 2. No effect of excipients 3. No effect of excipients	1. Sucrose, Shelf temperature ramp rate, SD temperature, SD time 2. SD temperature and SD time 3. No significant effects of the parameters studied 4. No significant effects of the parameters studied

Test Control

1. The control strategy during A-VAX manufacture includes raw material testing, in-process testing, intermediate polysaccharides (Ps) and virus-like particle (VLP) acceptance testing as well as drug substance and drug product release testing. Raw material testing is discussed in Section X.X.X. In-process tests have been developed for fermentation operations as well as for the downstream and conjugation processes.
2. The testing component of the integrated approach to the control strategy is given in Table 3-12 through Table 3-14. Table 3-12 lists the release and stability CQAs and associated assays registered for the initial filing for both release and in-process testing. It is comprehensive and includes the CQAs assayed at not only the drug product stage, but also for the process intermediates. In addition, Table 3-2 lists several CQAs that are assayed but not registered at the initial filing and are used for additional process monitoring. Finally, Table 3-3 lists those CQAs for which additional clearance studies will become available or are assayed earlier in the process and may be redundant. If, after suitable validation and continuous process monitoring, these CQAs are under control they would be eliminated from the control strategy.

Testing Controls

Table 3-11: Initial DRAFT of Control Strategy: Registered Release Tests [a, b]

Specification Tests

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Physical Properties							
pH		5.5-6.5	✓	5.5-6.5	5.5-6.5	5.5-6.5	Compendial
Appearance	White to off white powder	Clear, colorless & essentially free from visible particles	✓	White to off-white cake	Homogeneous white suspension	Homogeneous white suspension	Compendial
Residual Moisture*	≤ 5%			3-9%			Compendial
Quantity	95% monosaccharides	0.9-1.1 mg/mL				✓	PS: High-pH HPAEX-PAD VLP: BCA
Size*	Type 1: 6.6-9.2kD	20-50 nm diameter ≤ 0.07 polysispersity index	50 nm				PS: HPSEC-MALS-RI VLP: DLS
	Type 2: 8.8-12.3kD						
	Type 3: 6.6-9.2kD						
	Type 4: 11.0-15.3kD						
	Type 3: 13.2-18.4kD						

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Conjugation Sites*	> 0.5 site/repeating unit						1H-NMR
Ps/VLP Ratio*			✓	0.2-0.4Ps/ VLP monomer			Calculated from Extent-of-Conjugation Data
Quantity (as PS Content)			5 mcg each of Ps 1-4 50 mcg Ps 5	5 mcg each of Ps 1-4 50 mcg Ps 5		5 mcg each of Ps 1-4 50 mcg Ps 5	DS:HPLC
Quantity (as Protein Content)			TBD g/mL				BCA
Reconstitution Time						≤ 180 sec	Visual
Particle Size*					5-40 µm		Particle sizer
Zeta Potential*					-10 mV		Zeta potentiometer
Fill Volume in Container					≥ 0.5 mL	≥ 0.5 mL	Compendial
Aluminum Content					0.3±0.05 mg/mL as $AlPO_4$	0.3±0.05 mg/mL as $AlPO_4$	Compendial

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Identity							
Identity	Western blot – positive for each subtype	ELISA – positive	Western blot – positive for each subtype	Western blot – positive for each subtype	Homogeneous white suspension. Positive for aluminum	Western blot – positive for each subtype	Specific to drug intermediate, substance, adjuvant or drug product.

a. CQAs marked with an "*" are stability indicating.
b. CQA in grayed cells are marked for potential removal

1635 **Table 3-12: Registered Release Tests (continued)[a,b]**

1636 **Specification Tests**

1637

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Potency							
Serotypes 1-4 (correlation) *			70-130%	70-130%		70-130%	mAb-based Competitive ELISA
Serotype 5 (no correlation)*			70-130%	70-130%		70-130%	Rate Nephelometry
Purity							
%Purity*	≥ 95%						1H-NMR
Integrity and Degradation Products*	≤ 5%						1H-NMR
Monomer*	≤ 5%	80-90%	≥ 95%	≥ 95%		≥ 95%	VLP: Asymmetrical Flow FFF DS and DPLyo: Reducing CGE
Complexes* (dimer + trimer)		≤ 10%	≤ 10%	≤ 10%		≤ 10%	VLP: Asymmetrical Flow FFF DS and DPLyo: Non-reducing CGE
Aggregates* (>trimer)	≤ 5%	≤ 1%	✓				PS: HPSEC-MALS-RI VLP: Asymmetrical Flow FFF DS: DLS
Fragments*		≤ 1%	≤ 7%	≤ 7%		≤ 7%	VLP: Asymmetrical Flow FFF

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
							DS and DPLyo: Reducing CGE
Post-Translational Modifications		Comparable to reference standard					Peptide map
Free Amino Groups*		Comparable to reference standard					Peptide map
Host Cell Proteins	< 10 ng/mg	< 10 ng/mg					Anti-HCP ELISA
Host Cell DNA	≤ 10 ng/100 mcg	≤ 10 ng/100 mcg					qPCR
Free Ps*			≤ 10%	≤ 10%			High-pH HPAEX-PAD
Free VLP			✓				Reducing CGE
Conjugation Reactants			✓				RP-HPLC
Free Phosphate					✓		Compendial

a. CQAs marked with an "*" are stability indicating.
b. CQA in grayed cells are marked for potential removal for final control strategy

Table 3-13: Control Strategy: Registered Release Tests [a,b]

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Safety							
Endotoxin	< 5EU/kg of body mass	< 5EU/kg of body mass	< 5EU/kg of body mass	< 5EU/kg of body mass	< 5EU/kg of body mass	< 5EU/kg of body mass	Compendial
Sterility					Meets compendial requirements	r	Compendial
General Safety				Meets compendial requirements	Meets compendial requirements	Meets compendial requirements	Compendial

In-process tests

Attribute	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Bioburden	< 10 cfu/mL	Meets compendial requirements	Meets compendial requirements	Meets compendial requirements			Compendial
Reducing Activity (PAT)			Activation:				HPSEC
Polysaccharide size			Activation				PS: HPSEC

a. CQAs marked with an "*" are stability indicating.
b. CQA in grayed cells are marked for potential removal for final control strategy

1647 **Table 3-14: Additional Release Tests for characterization, Not Registered [a]**

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Critical Epitope(s)*			Report results	Report results			mAb-based Competitive ELISA (1-4) or Rate Nephelometry (5)
Linear & Conformational Epitopes		Report results					mAb-based ELISA (desorbed) or Peptide Map
Mass-to-charge ratio		Report results	Report results				CZE
Quantity (as protein content)				Report results			BCA

1648 a. CQAs marked with an "*" are stability indicating.

1649

1650 **Table 3-15: Tests Targeted for Removal**

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Comments
Host Cell Proteins	✓	✓					Process validation demonstrates easily removed.
Host Cell DNA	✓	✓					Process validation demonstrates easily removed.
Ps/VLP Ratio*				✓			Measured on drug substance
Fill Volume in Container				✓			More relevant with adjuvanted diluents.
Quantity (as PS content)						✓	Applies to DPLyo, no change upon

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Comments
							dilution
Aluminum Content						✓	Applies to adjuvant only, no change upon dilution of DPLyo
Ps/VLP/Adjuvant Ratio*						✓	Validated to use stability
Serotypes 1-4 (correlation)*						✓	Applies to DPLyo , no change upon dilution
Serotype 5 (no correlation)*						✓	Applies to DPLyo , no change upon dilution
Monomer*						✓	Applies to DPLyo no change upon dilution
Complexes*						✓	Applies to DPLyo , no change upon dilution
Sterility						✓	Applies to DPLyo and Adjuvent only. Reconstitution not performed under aseptic conditions.
Endotoxin*						✓	Applies to DPLyo and Adjuvent only. Reconstitution not performed under aseptic conditions.

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Comments
Rabbit Pyrogenicity				Meets compendial requirements	Meets compendial requirements	Meets compendial requirements	Compendial; Test replaced by endotoxin test

Input Materials Control

- Input materials required for the manufacture of A-VAX are determined by process development and are controlled by procedures within the quality control and quality assurance organizations. Quality control is responsible for executing the appropriate tests to ensure that the materials meet pre-determined specifications. Quality assurance is responsible for procedures to ensure the operations fall within cGMP guidelines including receipt, testing, and storage, order of use and disposal of out-dated materials. Compendial and well-characterized input materials are tested by analytical methods appropriate for each chemical.
- Input materials that are not well characterized are assayed for ability to promote the expected response in an appropriate biological system. The lack of ability to assay these materials by more precise methods requires additional procedures to ensure that they meet use specifications on a regular and continuing basis. Such additional procedures include regular audits of the supplier(s) ensure that the input material manufacturing processes remain consistent and that any changes are communicated to the A-VAX manufacturer to ensure that such changes do not affect A-VAX production in an adverse manner.
- A robust development program is in place to identify the critical and active components of the not well-characterized input material mixture. As information is developed it will be communicated to the input material manufacturer to determine if there are opportunities to upgrade the manufacturing process to gain a more consistent and robust control of the incoming raw material. Also in place is a procedure of process monitoring (refer to Continuous Process Verification section) to identify shifts and changes in the process. This process can identify important aspects of an input material. For example, process monitoring for complex raw material #2 for the polysaccharide fermentation indicated a reduction in variability occurred after a vendor change (refer to Upstream section) The subsequent investigation revealed that the new vendor had better control of nitrogen levels which ultimately affected OD levels in the fermentation. With this information the specification for the material was changed to include a requirement for nitrogen levels. In the event of any potential change to the raw material manufacturing process, multiple lots will be evaluated for performance in the A-VAX manufacturing process. Such evaluations would include, but are not limited to, process performance and consistency as well as process validation including characterization of the intermediate materials, drug substance and drug product, in a comparability study.

Continuous Process Verification (or Process Monitoring)

- At the completion of developing a control strategy for the processes involved in the manufacture of A-VAX, continuous process verification should be implemented to ensure that the control strategy is appropriate. Multivariate Statistical Process Control (MSPC) will be used for the process parameters implemented in the upstream and downstream processes. Univariate SPC will be used for attributes. Routine monitoring of data will further increase the understanding of the sources of variation in the process and ensure the most influential parameters were selected to control the process.
- The data for MSPC will be collected from the various processes via online and at-line collection points. The advantage of MSPC vs. Univariate SPC is that it can detect shifts in the mean or the relationship (covariance) between several related parameters. After the

collection of a minimum of 30 lots of data, control limits should be put in place. Control limits will be reevaluated after process changes are implemented.

- The data for Univariate SPC on the attributes will be collected from release testing. After the collection of data from a minimum of 30 lots control limits should be put in place. Run rules, eg. Western Electric Run rules can also be utilized to further enhance the process and can detect more subtle shifts in processes. Control limits should be reevaluated after process changes are implemented.
- The level of monitoring should be statistically sound and appropriate based on the criticality and impact of the parameters and should be reevaluated on a routine basis.
- Learnings from the verification process should be evaluated on a regular basis to determine if changes are required for the control strategy.

Annex 1

The following formula was used in the analysis:

Equation 3-1: Process Capability Analysis Formula

$$\mathrm{Cp} = \frac{(\mathrm{Maximum} - \mathrm{Minimum}) - \mathrm{Release\ Ranges}}{6 \cdot s_{\mathrm{Process}}},$$

$$\mathrm{Maximum} - \mathrm{Minimum} = 6 \cdot \mathrm{Cp} \cdot s_{\mathrm{Process}} + \mathrm{Release\ Ranges},$$

where s_{Process} is the variability estimated from manufacturing data or obtained from manufacturing modeling.

This is related to a capability index, Cpm, which is commonly used to assess the impacts of process variability on process capability.

$$\begin{aligned}\mathrm{Cp} &= \frac{(\mathrm{Maximum} - \mathrm{Minimum}) - \mathrm{Release\ Ranges}}{6 \cdot s_{\mathrm{Process}}}, \\ &= \frac{\mathrm{Maximum} - \mathrm{Minimum}}{6 \cdot s_{\mathrm{Process}}} - \frac{\mathrm{Release\ Ranges}}{6 \cdot s_{\mathrm{Process}}} \\ &= \mathrm{Cpm} - \frac{\mathrm{Release\ Ranges}}{6 \cdot s_{\mathrm{Process}}}.\end{aligned}$$

Cpm is related to the proportion of lots which are predicted to fall outside of release limits. Thus for example Cpm=1.0, which corresponds to 3 standard deviations on either side of the process mean, is associated with a failure rate equal to 0.0027, or 3 in 1000 failures. Cpm=0.67 is associated with a rate of 1 in 20 failures.

Release ranges are calculated for the upper release limit and the lower release limit as follows:

Equation 3-2: Release Range Formula

$$\text{Upper Release Range} = t_{\alpha,df} \cdot s_{Assay},$$

$$\text{Lower Release Range} = t \cdot b + t_{\alpha,df} \cdot \sqrt{(t \cdot s_b)^2 + s_{Assay}^2},$$

where $t_{\alpha,df}$ = value from t-distribution with error degress of freedom (df),
s_{Assay} = release assay variability estimated from stability evaluation,
b = estimated loss rate at labelled storage temperature,
s_b = standard error of the estimated loss, and
t = product shelf-life at lablelled storage temperature (24 months).

Summary measures from analyses of manufacturing and stability data for a licensed product similar in process and in the potency assay to A-VAX, along with the calculated upper and lower release ranges are presented in **Error! Reference source not found.**. The results are expressed in log (natural log) units due to the distributional characteristics of the potency measurements of the licensed product.

Table 3-16: Summary measures from analyses of manufacturing and stability data for a similar licensed vaccine

Component	Process Variability (sProcess)	Loss Rate (b)	Standard Error (sb)	Assay Variability (sAssay)	Upper Release Range	Lower Release Range
A-VAX$_1$ - A-VAX$_4$	0.0608	0.0100	0.0062	0.0461	0.0800	0.5101
A-VAX$_5$	0.1596	0.0100	0.0062	0.1210	0.2098	0.5726

The loss rate, standard error of the loss rate, and assay variability (for A-VAX$_1$ - A-VAX$_4$) were obtained from an analysis of stability data for 3 lots of the similar vaccine. The t-value associated with the estimate of assay variability is equal to $t_{0.10,18}$ = 1.734. This gives upper and lower release ranges as follows:

Equation 3-3: Release Range Calculation

$$\text{Upper Release Range} = t_{\alpha,df} \cdot s_{Assay} = 1.734 \cdot 0.0461 = 0.0800,$$

$$\begin{aligned}\text{Lower Release Range} &= t \cdot b + t_{\alpha,df} \cdot \sqrt{(t \cdot s_b)^2 + s_{Assay}^2} \\ &= 24 \cdot 0.0100 + 1.734 \cdot \sqrt{(24 \cdot 0.0062)^2 + 0.0461^2} = 0.5101.\end{aligned}$$

The release ranges for A-VAX$_5$ were calculated from the stability results obtained from the in vitro assay, but using the assay variability for the *in vivo* assay (s = 0.1210 from long term control data for the *in vivo* assay). Process variability was likewise scaled up in proportion to the difference in variability of the *in vivo* and *in vitro* assays.

The minimum to maximum potency ranges and values supporting several levels of process capability are given in Table 3-5.

Table 3-17: Potency ranges and minimum and maximum potencies for values two levels of process capability (probability of OOS)

Component	Cpk	Prob(OOS)	Range (loge)	Minimum at Expiry	Minimum at Release	Maximum at Release
A-VAX$_1$ - A-VAX$_4$	0.67	0.05	0.8340	0.53	0.80	1.22
	1	0.003	0.9555	0.50	0.77	1.30
A-VAX$_5$	0.67	0.05	1.4208	0.40	0.60	1.70
	1	0.003	1.7400	0.35	0.50	2.00

The minimum and maximum potencies are derived from the target potencies for the 5 components of A-VAX (1.00). Potencies were determined to support good process capability (Cpm=1.0). It should be noted that the probability of OOS for one or more of the serotypes is equal to $1 - (1\text{-}0.003)^5 = 0.015$ (i.e., 1.5%). Target potencies, together with minimum and maximum potencies are given in **Error! Reference source not found.**.

Table 3-18: Target potencies, and minimum and maximum potencies

Component	Target	Minimum	Maximum
A-VAX$_1$ - A-VAX$_4$	1.00	0.50	1.30
A-VAX$_5$	1.00	0.35	2.00

It should be noted that minimum and maximum potencies are not (geometrically) symmetric about the target (1.00). This is caused by including stability in the determination of minimum expiry potency.

The forecast minimum and maximum potencies were utilized to guide manufacture of clinical lots to be performed in Phase III clinical studies. The clinical lots were manufactured from common conjugated bulks in order to preserve the planned differences (minimum to maximum) in potencies. The source conjugate bulks were tested in an enhanced potency assay format in order to better target clinical lot potencies.

Annex 2:

This begins with determining an appropriate level of risk of batch failure due to one or more false positive (false OOS) results. The overall failure rate is a function of the number of tests and the risk of failure in each individual test. The overall risk associated with either 95% or 99% limits for various numbers of tests is given in Table 3-8.

Table 3-19: Overall risk for various numbers of tests

No. Tests	95% Limits	99% Limits
1	5%	1%
2	10%	2%
3	14%	3%
6	26%	6%

Significant overall risk results from using 95% limits. The overall risk using 99% limits results in a more realistic false failure rate for a moderate number of tests. The number of tests can be tests on multiple components of a vaccine (e.g., multiple polysaccharides) or multiple quality attributes.

Excess risk also results from redundant or correlated tests. Tests which measure the same or related properties of a vaccine will be correlated. Thus for example, potency measured by both an *in vivo* assay and an *in vitro* assay will likely be highly correlated, resulting in higher than expected product failure. Effort should be made to select a single measure of a quality attribute, or to utilize an alternative strategy for controlling the vaccine such as multivariate quality control.

Acceptance criteria which have been established from process data are estimates of the true limits and subject to uncertainty. Like all statistical estimates, their reliability may be a function of the number of data points (batches) used to calculate the limits. The risks associated with estimating acceptance criteria using simple 2- or 3-sigma limits are high for small numbers of batches. Tolerance limits are utilized to control risk of false failure for small and large numbers of batches alike. This comes at a cost, however, of excessively wide limits with small numbers of batches. A lifecycle approach to establishing acceptance criteria using tolerance limits should be utilized. Early limits should be updated when a sufficient number of batches (and adequate long term experience with the process) has been acquired.

4. Upstream (Polysaccharide) Section

4.1. Executive Summary

In the manufacturing process for polysaccharide,, a well-defined upstream process is required to provide sufficient material (bulk volume) with well-defined quality attributes for the downstream processing.

This document describes the polysaccharide fermentation process and the effects of the complex raw materials, fermentor operating parameters, and inactivation parameters. Prior knowledge from published literature and process risk assessments are used to ascertain the factors that will be evaluated further. Ishikawa diagrams and cause-and-effect matrices facilitate the identification of process steps for further exploration via design of experiments (DOEs) or one factor at a time (OFAT) evaluations. Failure modes and effects analysis is used to assess the process risks and to develop appropriate strategies for managing critical process attributes.

4.2. Brief Description of Each Process Step

The following is a step-wise description of each process step at Phase 2 starting with the *H. horrificus* background. Post-Phase 2 changes are discussed at the appropriate section of the document.

H. horrificus is a lactic acid-producing, gram-negative anaerobic bacteria. It is aero-tolerant; however, it is sensitive to vigorous mixing and prolonged exposure to elevated levels of oxygen. It typically grows as single cells. There are 11 serotypes, of which eight are pathogenic in otherwise healthy individuals. Five serotypes are responsible for >95% of clinically reported cases in both the developed and developing worlds, although the distribution among the five varies by region. The serotype-specific capsular polysaccharide (Ps) is constitutively expressed through the growth cycle. Therefore, Ps yield correlates with biomass. Under stressed conditions, such as nutrient limitation, *H. horrificus* expresses the enzyme polysaccharidase, which will digest the capsular Ps to monomer units.

4.2.1. Cell Banks

Master and stock cell bank vials are prepared in the logarithmic growth phase according to standard procedures to generate a sufficient inoculum per vial to initiate a viable culture of the organism. The choice of a glycerol-based cryo-preservative was made based on characteristics of the organism. Maximum viability of freshly thawed vials will ensure a robust process.

4.2.2. Media

There is significant prior knowledge for the media. It is a proprietary media with two complex non-animal-derived components (raw materials designated RM 1 and RM 2). Glycerol is the carbon source (5 g/L for shake flasks and 10 g/L for seed and production fermentors) and is the limiting nutrient. Experimental results indicate that the media can support fourfold biomass achieved in fermentor, given a concomitant increased in glycerol. Remaining media components are amino acids, salts, and one growth factor/vitamin. The only other difference in shake flask media contains 1M PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid)). Fermentor pH is controlled with the automated addition of a 1N sodium hydroxide solution.

4.2.3. Shake Flask: Stage 1

The stage 1 shake flask purpose is to robustly culture the organism after cryo-preservation and increase the biomass for the shake flask stage 2 inoculation. Two (1.5ml each) WCB vials are thawed for 20 minutes at room temperature. The vials inoculate 72ml shake flask media (4% v/v) in 250ml disposable shake flasks. The flasks are incubated at 25 ±5 RPM and 37 ±2 °C. Transfer to stage 2 is triggered at an optical density (OD) target of 2 Absorbance Unit (AU) (range 1.5 to 3).

4.2.4. Shake Flask: Stage 2

The stage 2 shake flask purpose is to robustly culture and increase the biomass for the seed fermentation inoculation. Inoculate 2 x 768ml media in 2L disposable shake flasks with 32ml (4% v/v) each from the stage 1 culture. The flasks are incubated at 30 ± 5 RPM and 37 ± 2 °C. Transfer to stage 2 at an OD target of 2 AU (range 1.5 to 3). In Table 4-1, the shake flask data is summarized from prior knowledge.

Table 4-1: Shake Flask Data from Prior Knowledge

Process Step	Doubling Time (h)	Lag (h)	Total Time to Transfer (h)
Shake Flask Stage 1	0.83 ± 0.06	2.3 ± 0.23	7.8 ± 0.6
Shake Flask Stage 2	0.80 ± 0.05	N.D	4.7 ± 0.5
Seed Fermentor	0.74 ± 0.05	1.08 ± 0.15	5.8 ± 0.6

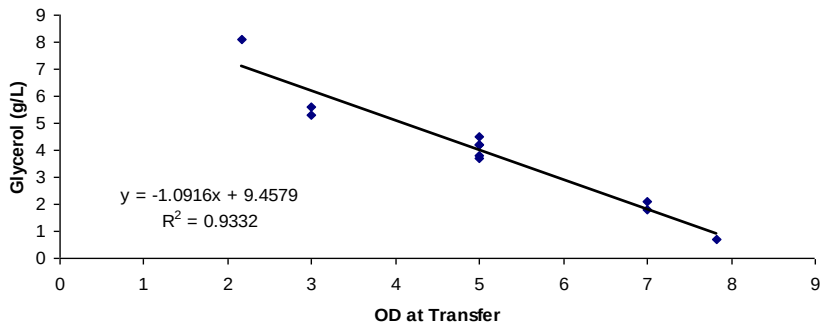
4.2.5. 50L Seed Fermentation

The seed fermentor purpose is to increase biomass for the production fermentor inoculation, and it is performed as a batch fermentation. Inoculate 38.4L fermentor media with 1.6L (4% v/v) stage 2 culture. Transfer to the production fermentor is triggered at an OD target of 3 AU (range 2.5–5). The fermentor operation parameters are summarized in Table 4-2.

Table 4-2: Seed Fermentor Parameters

Parameter	Set-point and Range
Back Pressure	2 ± 1 psig
Air Overlay	4 ± 2 LPM
Temperature	37 ± 2 °C
pH	7 ± 0.5 pH units
Agitation	40 ± 10 RPM

Figure 4-1: Seed Fermentation Transfer Criterion Data from Prior Knowledge



4.2.6. 1,000L Production Fermentation

The purpose of the production fermentation is to provide sufficient biomass for a consistent culture substrate for the down stream inactivation step. It is a batch fermentation in which 760L of fermentor media is inoculated with 40L (2% v/v) of seed fermentor culture. The fermentor operation parameters are summarized in Table 4-3.

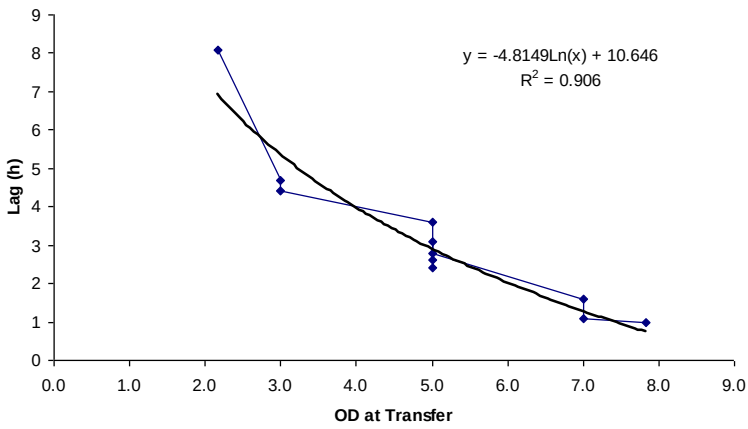
Because the process involves cultivation of an aero-tolerant anaerobe, mixing and aeration conditions were not deemed critical to quality and conditions from a previous production platform were implemented. Temperature and pH ranges were established at typical ranges for this production platform based on a series of early stage experiments, which are not included herein. Phenol is added 60 minutes post glycerol exhaustion.

Table 4-3: Production Fermentor Parameters

Parameter	Set-point and Range
Back Pressure	2 ± 1 Psig
Air Overlay	10 ± 2 LPM
Temperature	37 ± 2 °C
pH	7 ± 0.5 pH units

Agitation	30 ± 10 RPM

Figure 4-2: Effect of Seed Fermentor Transfer on Lag from Prior Knowledge

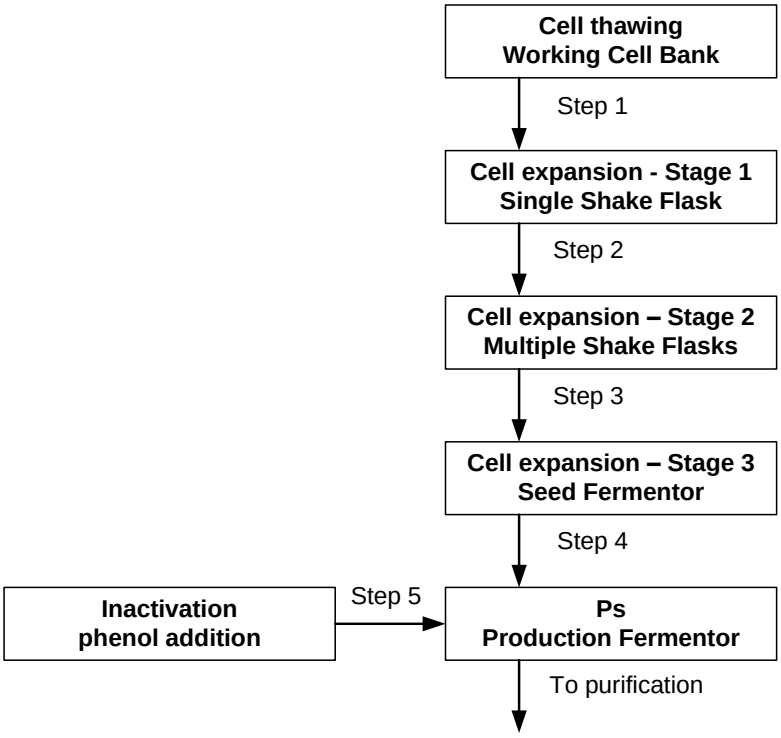


4.2.7. Inactivation

Phenol is added to a final concentration of 1% (w/w). Studies to determine inactivation kinetics were performed prior to initiating development work. The results were mostly independent of serotype. A 7-log reduction in viable cells is achieved in 27 ± 3 minutes at the stated inactivation conditions. A threefold safety factor was used to determine the 90-minute time for inactivation. After 90 minutes, a sample is submitted to confirm culture inactivation. After inactivation, *X. Horrificus* culture OD (600nm) is adjusted at 5 with Water for Injection (WFI) to normalize the biomass. Assuming a constant peptidoglycan content in the cell wall, this dilution is expected to normalize the enzyme substrate concentration. The diluted inactivated broth is then sent to purification.

4.2.8. Process Diagram

Figure 4-3: Process Diagram



4.2.9. Source of Prior Knowledge

Numerous articles exist giving general cultivation parameters such as pH and temperature. Literature also exists for media and nutritional requirements but is less numerous. The process risk assessment was executed by subject matter experts. Similar data is available from other Ps processes (one licensed, one in development) derived from other species of lactic acid-producing bacteria. Also, the final manufacturing facility is planned to be the same facility as the licensed Ps product.

4.3. Process Risk Assessment

The following section summarizes the process of defining and executing the risk assessment.

4.3.1. Process Analysis (Ishikawa Diagram)

This Ishikawa diagram illustrates a comprehensive analysis of how all aspects of the development and manufacturing process potentially impact drug substance quality. The process-specific parameters are only a subset of the parameters to control the overall process. Nonetheless, these parameters are the most direct routes to ensure consistent product quality.

Figure 4-4: Ishikawa diagram built around parameters that include process, materials, people, and facilities.

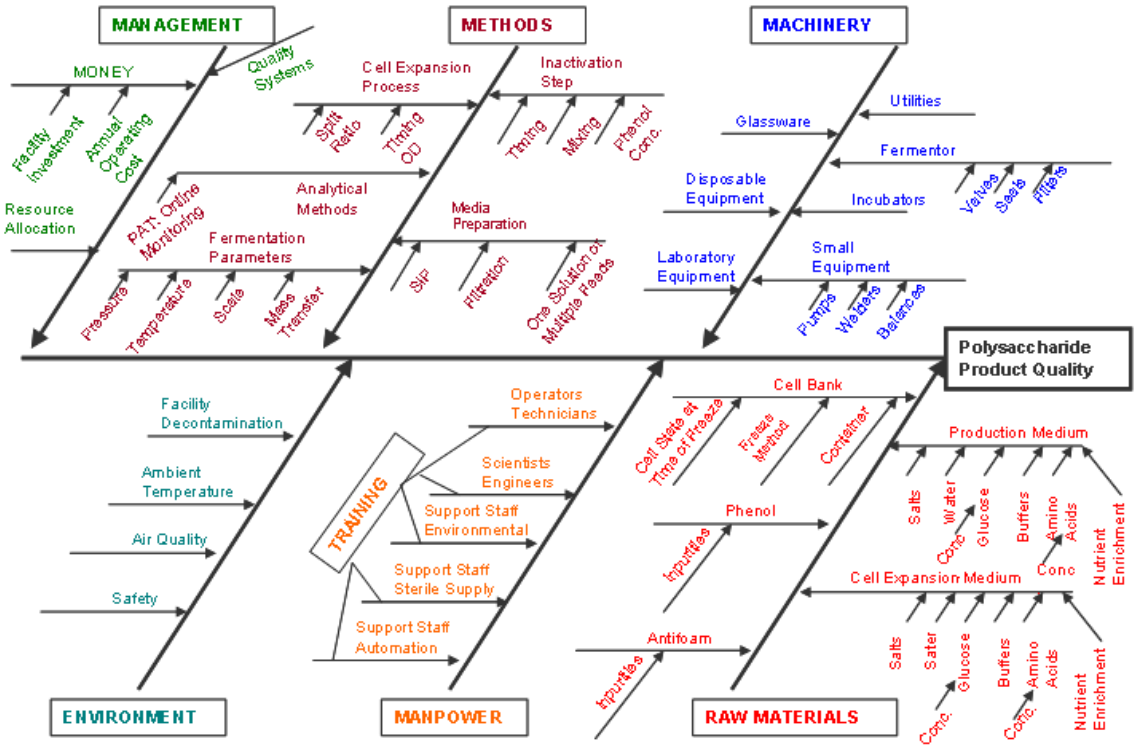
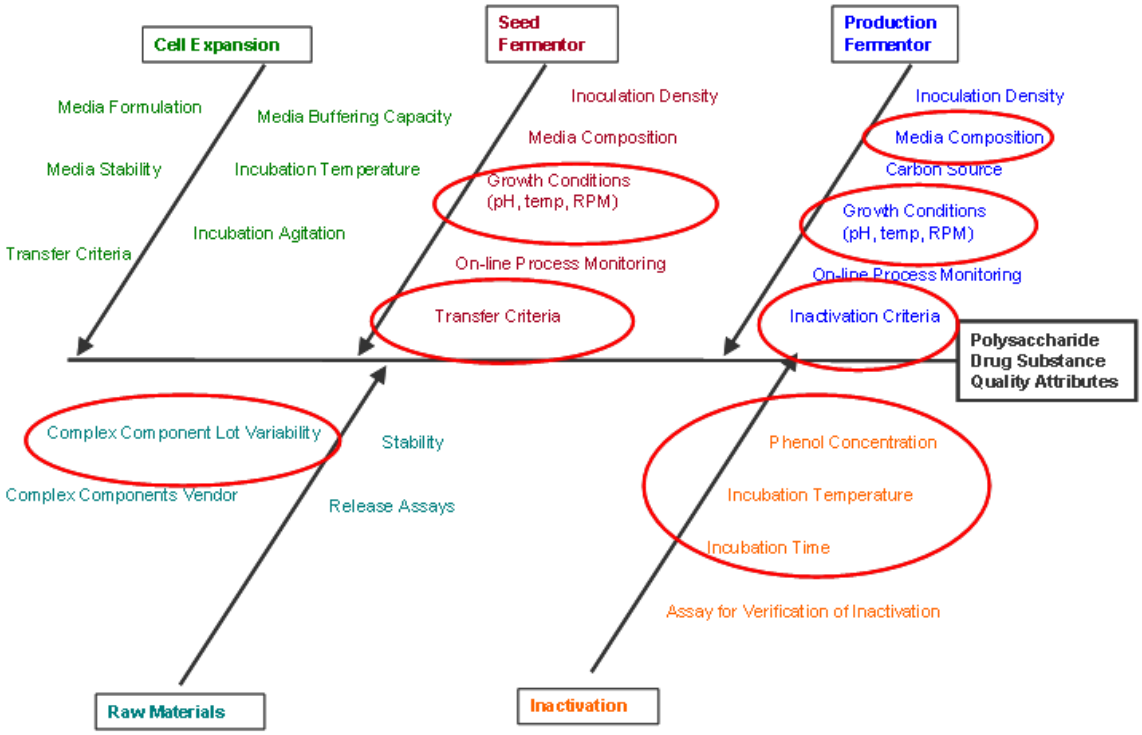


Figure 4-5 is an expanded Ishikawa diagram built around the process-related factors. The expansion was performed to identify the key parameters at each process step. This information will be used for analysis once the process step for Quality by Design (QbD) analysis is identified.

Figure 4-5: Process Ishikawa Diagram



This Ishikawa diagram isolates process-specific parameters for their potential impact on drug substance quality attributes. The outcome of this analysis is a list of parameters that can be taken forward for further analysis or experimentation to begin identifying key and critical process parameters. Font colors have been assigned to each process step to better visualize the specific parameters involved in it.

Elements of prior knowledge were used to identify process steps (cell Expansion, seed fermentor) that were NOT taken forward with additional QBD approaches. Various parameters of raw materials, production fermentor, and inactivation steps (see circles) were analyzed with further QbD approaches.

4.3.2. Rationale for Selecting the Production Fermentation/Inactivation as a Unit of Operation for QbD Analysis

The results of the Ishikawa analyses and cause-and-effect matrix identified process steps and parameters that required further experimentation to define critical and key parameters. Most of the “no relationship” scores were based on prior knowledge. The “relationship known” or “relationship expected” scores were determined based on scientific first principles. The quantitative ranking structure was based on a typical scoring matrix.

Table 4-4 defines the weight given to each ranking value. A total score of 66 was estimated to represent “greater than moderate impact” (i.e., score of 5.5) across all 12 quality attributes. Process steps with scores or 66 or higher were taken forward for further exploration via DOEs and OFAT experiments to determine critical parameters and ranges. The scores are shown in

Table 4-5 and illustrated in the Pareto chart in

	PS Yield	Host-cell Protein	Host-cell DNA	Size	Integrity & Degradation Products	Aggregation	Conjugation Sites	Polysaccharide Length	Quantity (as Monosaccharide Content)	Endotoxin	Bioburden	Appearance	TOTAL
Cell Bank Vial Thaw	4	1	1	4	1	1	4	1	1	1	1	4	**24**
Cell Expansion	4	1	1	4	4	1	4	1	4	1	1	4	**30**
Seed Fermenter	7	4	4	7	4	4	4	4	4	4	7	4	**57**
Production Fermenter	10	7	7	4	4	7	4	7	7	4	7	7	**75**
Inactivation	7	10	10	7	7	7	4	7	4	10	7	7	**87**
Harvest Process	4	4	4	4	7	7	4	4	4	10	4	7	**63**
Raw Materials	10	4	4	7	4	4	4	7	7	4	7	4	**66**

Figure

Figure 4-6. Those steps with borderline scores (seed fermentor and harvest process) were not considered for further experimentation in this case study, although prior knowledge was used to mitigate risk around these steps.

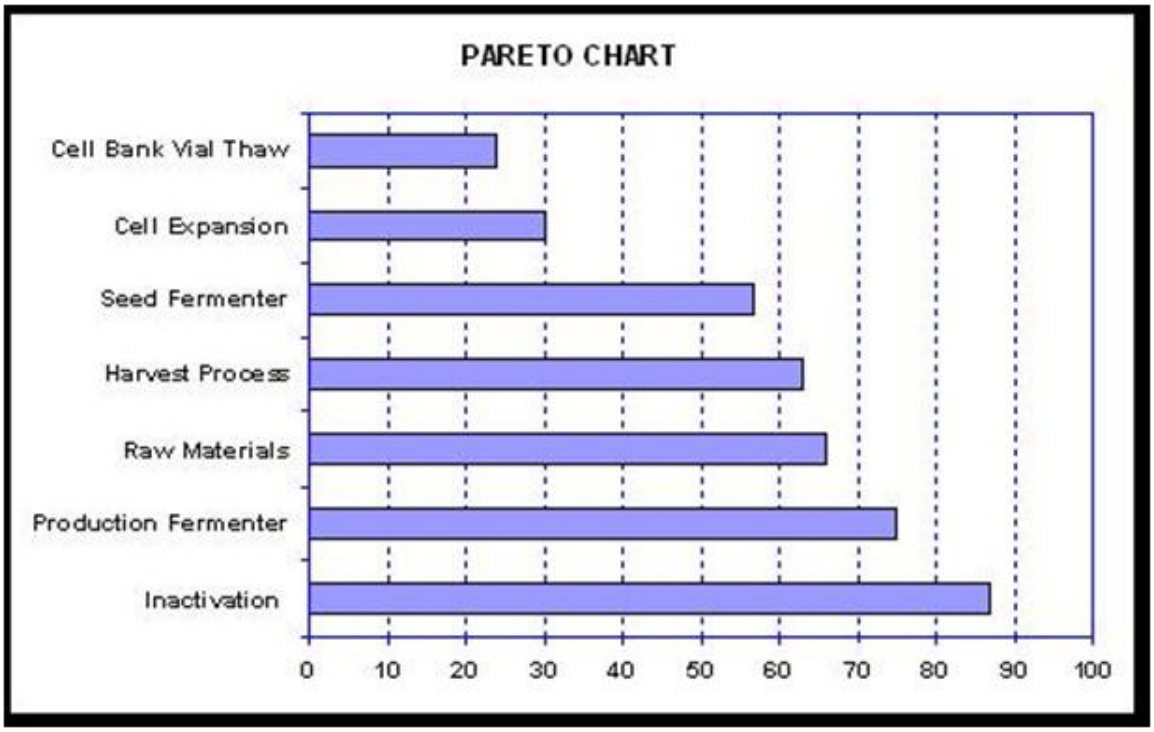
Table 4-4: Cause-and-Effect Ranking Definition

Rank	Input Process Steps to Critical Quality Attributes (CQA) and Key Process Attributes (KPA)
10	Relationship Known
7	Relationship Suspected or Unknown
4	Slight Relationship
1	No Relationship

Table 4-5: Cause-and-Effect Process Step Ranking

	PS Yield	Host-cell Protein	Host-cell DNA	Size	Integrity & Degradation Products	Aggregation	Conjugation Sites	Polysaccharide Length	Quantity (as Monosaccharide Content)	Endotoxin	Bioburden	Appearance	TOTAL
Cell Bank Vial Thaw	4	1	1	4	1	1	4	1	1	1	1	4	24
Cell Expansion	4	1	1	4	4	1	4	1	4	1	1	4	30
Seed Fermenter	7	4	4	7	4	4	4	4	4	4	7	4	57
Production Fermenter	10	7	7	4	4	7	4	7	7	4	7	7	75
Inactivation	7	10	10	7	7	7	4	7	4	10	7	7	87
Harvest Process	4	4	4	4	7	7	4	4	4	10	4	7	63
Raw Materials	10	4	4	7	4	4	4	7	7	4	7	4	66

Figure 4-6: Pareto Chart



4.4. Design of Experiment

Based on a combination of historical knowledge and process risk assessment (cause-and-effect analysis (Table 4-5), the raw materials, fermentor operating parameters, and inactivation parameters (see Pareto Chart, Figure 4-6) were analyzed through a multivariable central composite design of experiments.

Note that at this stage the central composite design was selected in place of a more routine screening design for a number of reasons. First, it was known from early process development (and prior knowledge from similar programs) that polysaccharide production yield and quality are directly tied to biomass production. Therefore, conditions that promoted optimal biomass productivity would generate optimal Ps yields. As the production process involves cultivation of an aero-tolerant anaerobe, screening of mixing and aeration parameters was not prioritized. Instead, greater emphasis was applied to identify potential interacting parameters using an experimental design that was best suited for this. The following factors were explored:

- Concentration of complex RM #1 (18–22 g/L)
- Concentration of complex RM #2 (8–12 g/L))
- Time to inactivation (time post glycerol depletion) (-30 – 150 minutes)
- Incubation temperature (35–39˚ C)

Appropriate analytical tools were developed through the early stages of process development to determine the cell lysis during the fermentation process. In addition, analytical methods were developed to determine the polysaccharide repeat units and quantify the yields at the laboratory scale.

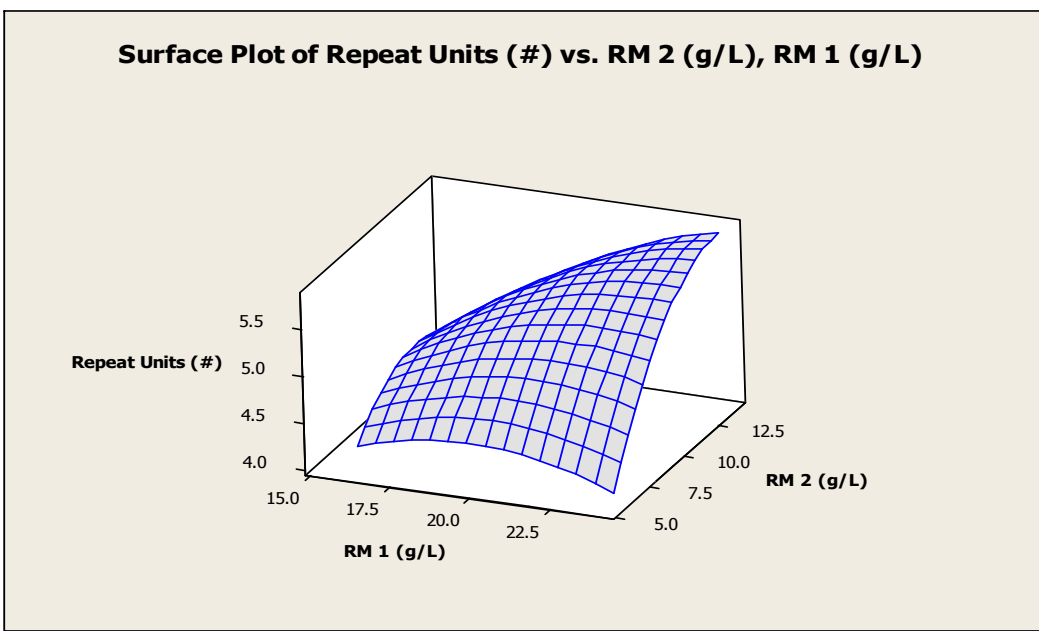
4.4.1. Response Surface and Analysis of Variance for Repeat Units

Response variables were identified primarily by leveraging prior knowledge from early and late stage upstream process development of a polysaccharide production platform. Early development indicated that polysaccharide length and the number of polysaccharide repeat units were variable with incorporation of upstream process changes. Polysaccharide yield is a major process economics consideration. Most critically, the link between polysaccharide length and percent lysis was well established early on in the upstream process development. Extended time post lysis resulted in degradation of mean polysaccharide lengths and therefore negatively impacted product quality. The following response variables were explored:

- Number of polysaccharide repeat units, identity, and integrity are measured by 1H-NMR. This parameter impacts potency CQA, measured in Ps-VLP through ELISA.
- Polysaccharide size is measured by HPSEC-MALLS-RI on the purified Ps, following fermentation. Furthermore, each type is sized to a particular molecular weight in the downstream purification process (summarized in Table 7-12 in the Control Strategy section). The final size of the Ps impacts potency CQA and is measured in Ps-VLP through ELISA.
- Ps yield (key process attributes, referred to as quantity in the cause-effect matrix) is measured through the hydrolysis of the purified polysaccharide using high-pH HPAEX-PAD
- Percentage lysis, which is tied to Ps length and subsequently the potency critical quality attribute (CQA)

The outcome of the DOE is to understand interactions and identify potential Critical Process Parameters (CPPs), without defining clear parameter limits or ranges. The CPP candidates identified from the DOE underwent further analysis via FMEA and OFAT experiments to conclusively define their overall criticality and establish ranges.

Figure 4-7: Response Surface for Impact of RM 1 and RM 2 on the Polysaccharide Repeat Units



The results of the DOE (see surface response plot Figure 4-8) indicate that the concentration of RM 2 has a direct impact on the number of polysaccharide repeat units. Considering the direct impact of RM 2 concentration on this critical quality attribute, this parameter was defined as a CPP.

The following table lists the analysis of variance (ANOVA) regression for the number of repeat units versus block, RM 1, and RM2. The analysis was performed using coded units.

Table: 4-6 Response Surface Regression: Number of Glucose Repeats versus Block, RM 1 (g/L), RM 2 (g/L). Estimated Regression Coefficients for number of glucose repeat Term.

	Coef	SE Coe	T	P
Constant	5.30000	0.18982	27.921	0.000
Block 1	-0.01000	0.12005	0.083	0.935
Block 2	0.04500	0.12005	0.375	0.714
RM 1 (g/L	0.17083	0.09491	1.800	0.095
RM 2 (g/L)	0.23333	0.09491	2.458	0.029
Inactivation Time (min)	0.02083	0.09491	-0.220	0.830
Temperature	0.14583	0.09491	1.537	0.148
RM 1 (g/L)*RM 1 (g/L	-0.06563	0.08878	-0.739	0.473
RM 2 (g/L)*RM 2 (g/L	0.11563	0.08878	-1.302	0.215
Inactivation Time (min)*Inactivation Time (min)	0.00312	0.08878	-0.035	0.972
Temperature*Temperature	0.01562	0.08878	-0.176	0.863
RM 1 (g/L)*RM 2 (g/L)	0.10000	0.11624	0.860	0.405
RM 1 (g/L)*Inactivation Time (min)	0.01875	0.11624	0.161	0.874
RM 1 (g/L)*Temperature	0.09375	0.11624	0.807	0.434
RM 2 (g/L)*Inactivation Time (min)	0.08750	0.11624	0.753	0.465
RM 2 (g/L)*Temperature	0.15000	0.11624	1.290	0.219
Inactivation Time (min)*Temperature	-0.03125	0.11624	-0.269	0.792
S = 0.464961 PRESS = 17.2224				
R-Sq = 57.56% R-Sq(pred) = 0.00% R-Sq(adj) = 5.32%				

RM 2 was the only significant ($p<0.05$) term for this response, while RM 1 had borderline-significant response ($p<0.10$). Because inactivation time and temperature were shown not to impact the number of repeat units in this experiment, these variables were excluded from the DOE analysis to repeat the statistical analysis with increased degrees of freedom. When the DOE was re-analyzed with number of repeats as the response variable and only RM 1 and RM 2 as

the model effects (Table: 4-7), RM 2 again was the only significant factor ($p<0.05$), with RM 1 showing borderline significance ($p< 0.10$).

The following table lists the ANOVA regression for the number of repeat units versus RM 1 and RM 2. The analysis was performed using coded units.

Table: 4-7, Response Surface Regression: Number of Repeat Units versus Block, RM 1 (g/L), RM 2 (g/L) Estimated Regression Coefficients for number of repeat units:

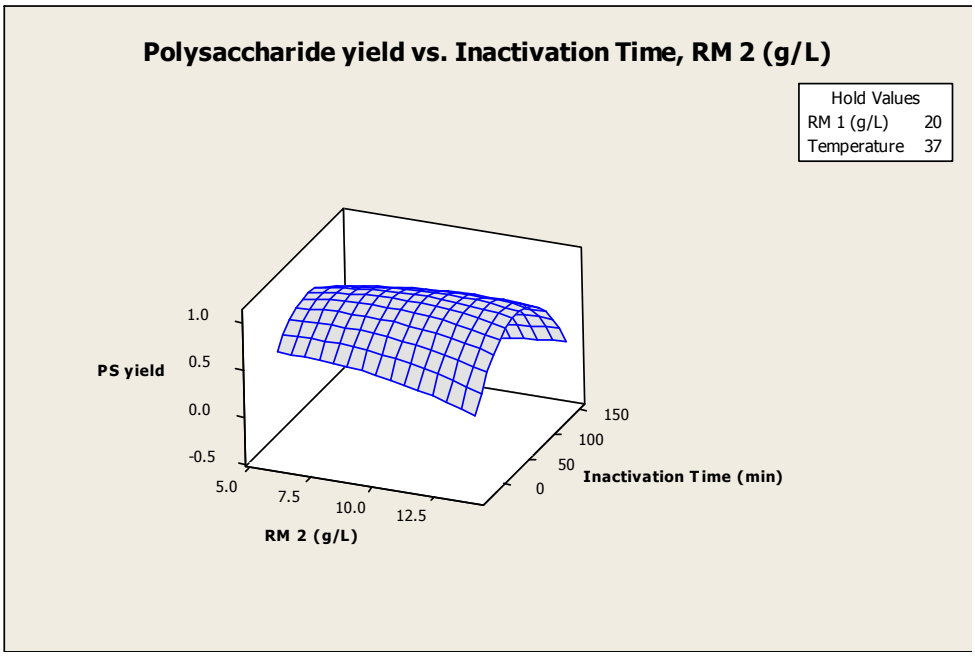
Term	Coef	SE Coef	T	P
Constant	5.28125	0.12282	43.000	0.000
Block 1	-0.01000	0.10985	-0.091	0.928
Block 2	0.04500	0.10985	0.410	0.686
RM 1 (g/L)	0.17083	0.08685	1.967	0.062
RM 2 (g/L)	0.23333	0.08685	2.687	0.013
RM 1 (g/L)*RM 1 (g/L)	-0.06328	0.07977	-0.793	0.436
RM 2 (g/L)*RM 2 (g/L)	-0.11328	0.07977	-1.420	0.170
RM 1 (g/L)*RM 2 (g/L)	0.10000	0.10637	0.940	0.357
S = 0.425462 PRESS = 8.10926				
R-Sq = 39.86% R-Sq(pred) = 0.00% R-Sq(adj) = 20.73%				

The range for RM 2 was subsequently determined by OFAT experiments. Since no interaction effects were shown in the DOE, an OFAT experiment was chosen to better define the response to a range of RM 2 values.

4.4.2. Response Plots for Polysaccharide Yield

Polysaccharide yield is sensitive to inactivation time (not RM 2)
Concentration of phenol required for inactivation of the bacterial strain was obtained from prior knowledge. Considering the historical data, it was deemed not to be a critical parameter, as long as it was well controlled above a threshold. Inactivation time was critical to maintaining high polysaccharide yield. Ps yield was insensitive to changes in concentration of RM 2. Maximum polysaccharide yield was obtained when inactivation was initiated 50–100 minutes following glycerol depletion.

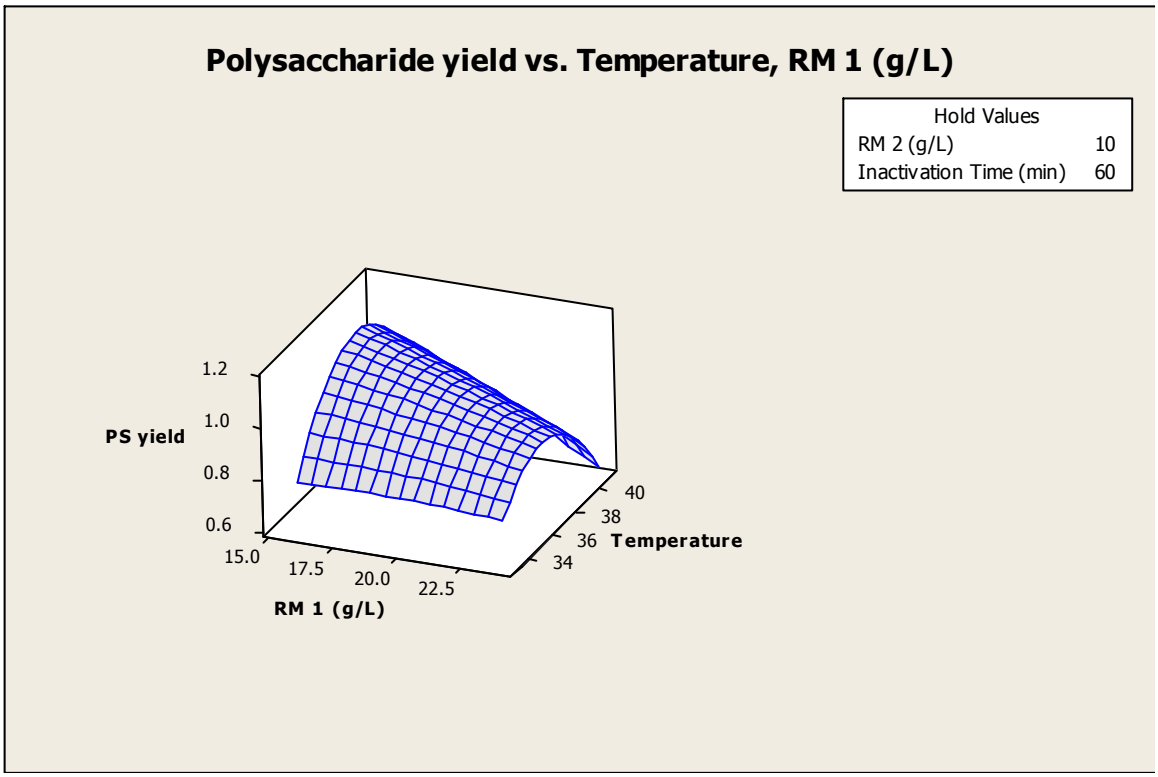
Figure 4-8: Impact of Polysaccharide yield on Inactivation Time



Polysaccharide yield (potency) is sensitive to temperature (not raw material)

Ps yield was sensitive to fermentation temperature but not to the concentration of RM 1. Incubation temperatures of 36–38˚C delivered the highest polysaccharide yield relative to the lowest and highest temperatures explored.

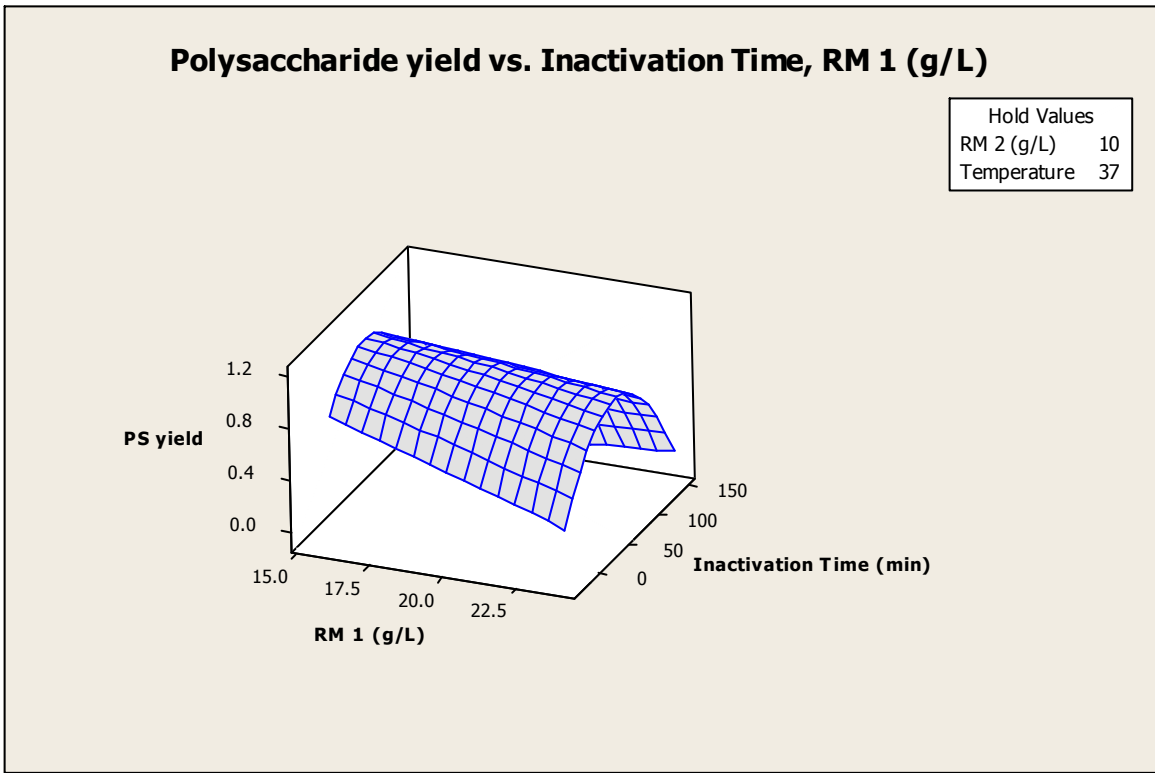
Figure 4-9: Impact of Polysaccharide yield on Temperature



Polysaccharide yield is sensitive to Inactivation Time (not RM 1)

Inactivation time was critical to maintaining high polysaccharide yield. While it is known that the enzyme polysaccharidase is expressed under these conditions, therefore reducing the Ps overall MW, it is balanced with the rate of Ps release yield. Ps yield was less sensitive to changes in concentration of RM 1. Maximum polysaccharide yield was obtained when inactivation was initiated 50–100 minutes following glycerol depletion.

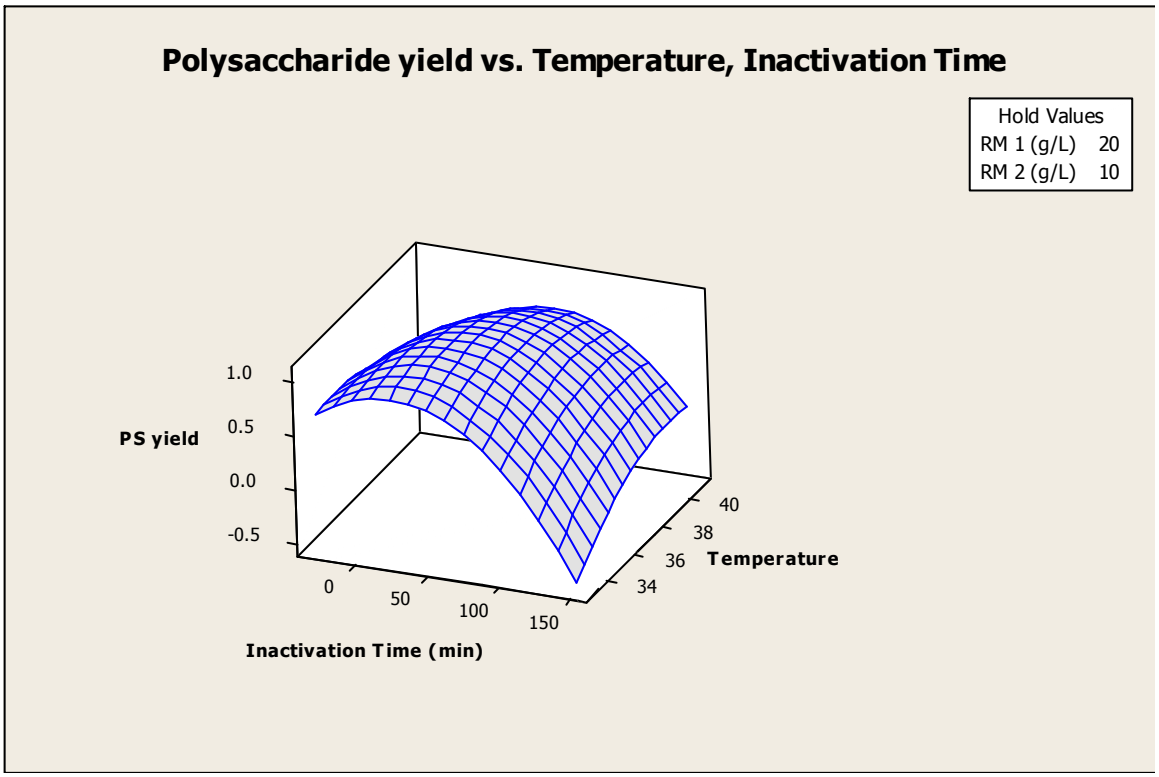
Figure 4-10: Impact of Polysaccharide yield on Inactivation time



Polysaccharide yield is sensitive to both inactivation time and temperature

Polysaccharide yield was most sensitive to changes in inactivation time and temperature, as described in previous slides. Considering the direct impact of these process parameters to polysaccharide critical quality attributes, these two parameters were defined as CPPs.

Figure 4-11: Impact of Polysaccharide yield dependence on Inactivation time and Temperature



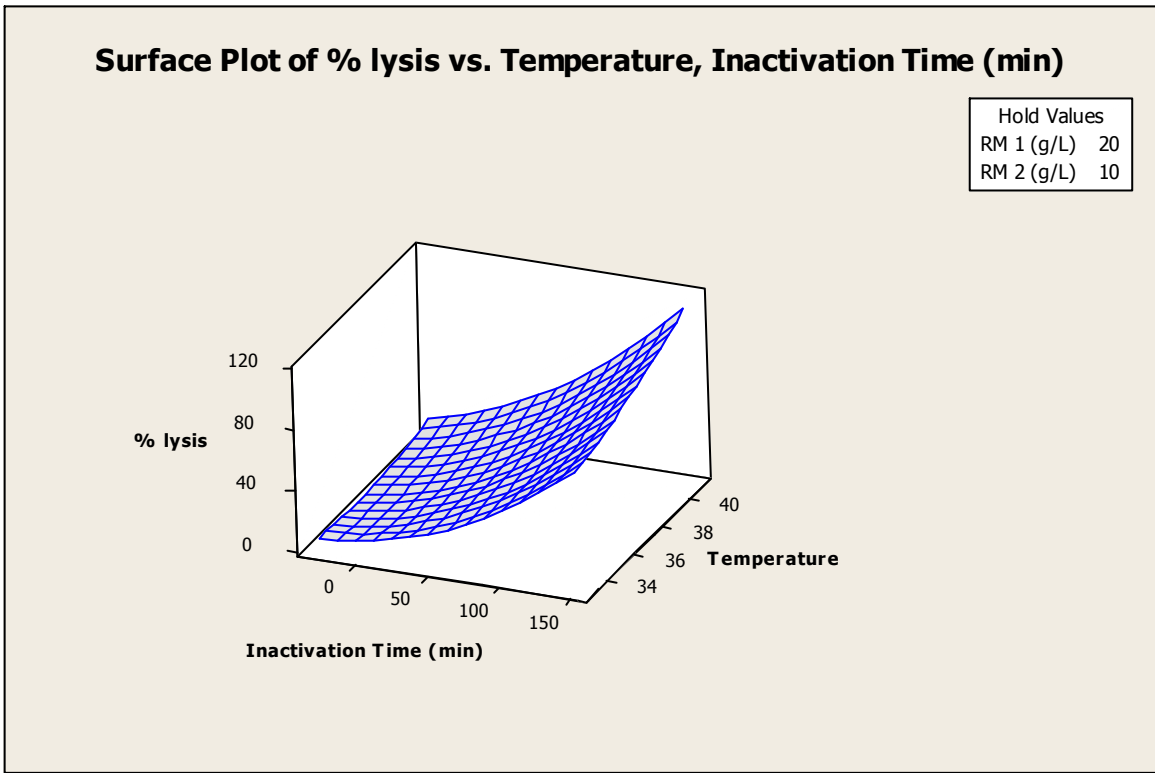
4.4.3. Response Surface Plots for Cell Lysis

Figures below illustrate the impact of DOE parameters on % lysis (a key process attribute and measure of overall process performance). Cell lysis is a negative attribute that is coupled with cellular degeneration and endotoxin release. Factors explored included temperature, inactivation time, and raw materials 1 and 2 concentration.

Cell lysis is sensitive to inactivation time and temperature

Minimal cell lysis was observed when inactivation was initiated by 50 minutes post glycerol depletion. This is also within the window of maximum polysaccharide yield as described in previous figures. Longer time prior to inactivation is coupled with increased cell lysis and higher risk of exceeding endotoxin limits, which is a CQA.

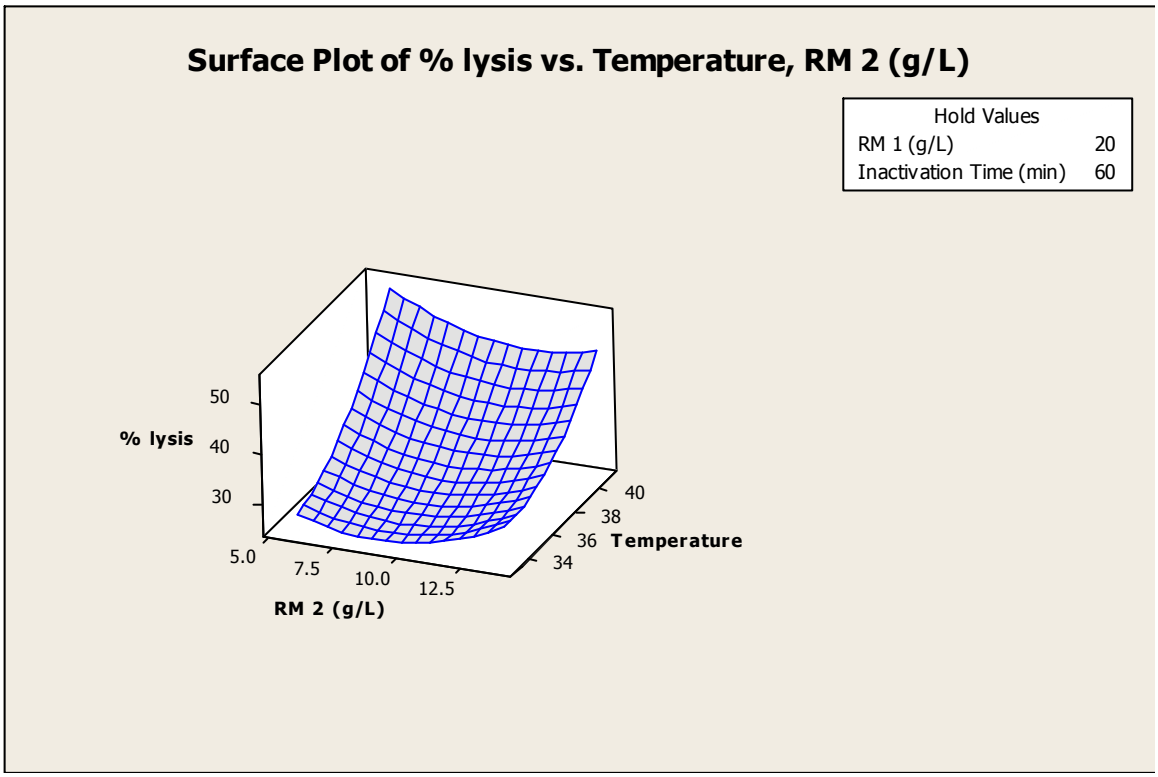
Figure 4-12: Impact of Inactivation time and Temperature on cell lysis



Cell Lysis is sensitive to temperature (not RM 2)

Higher levels of cell lysis occurred when fermentation was incubated above 37°C. This correlates with higher endotoxin levels and therefore is undesirable.

Figure 4-13 Impact of Temperature and RM2 on cell lysis



% lysis is sensitive to inactivation time (not RM 1)

Minimal cell lysis was observed when inactivation was initiated by 50 minutes post glycerol depletion. This is also within the window of maximum polysaccharide yield as described in previous figures. Longer time prior to inactivation is coupled with increased cell lysis and higher risk of exceeding endotoxin limits, which is a CQA. Concentration of RM 1 and/or 2 did not impact the degree of cell lysis.

Figure 4-14: Impact of Temperature and RM2 on cell lysis

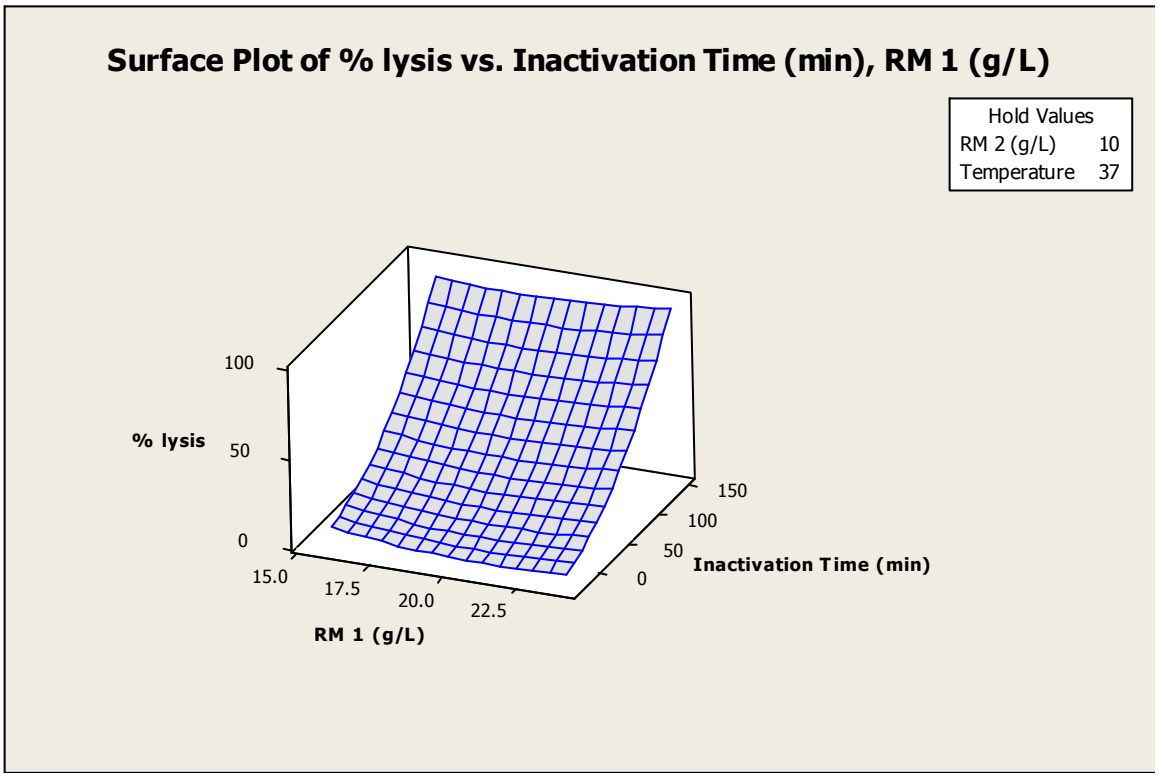
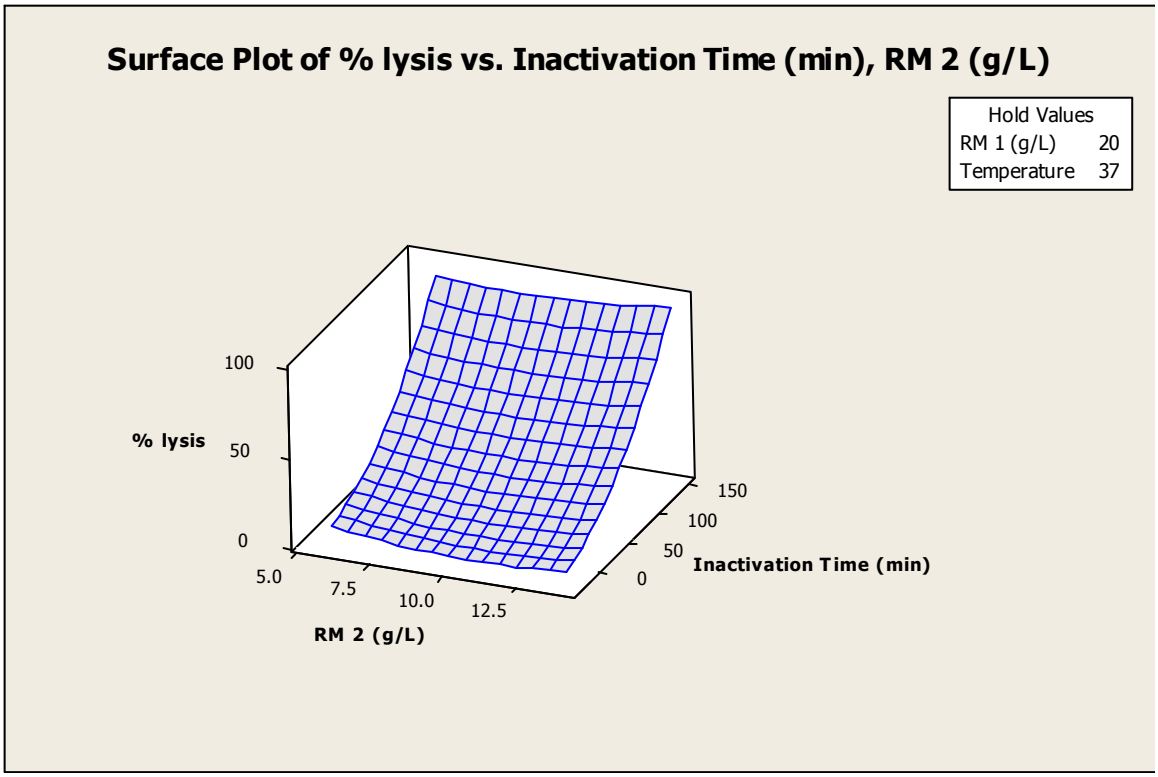


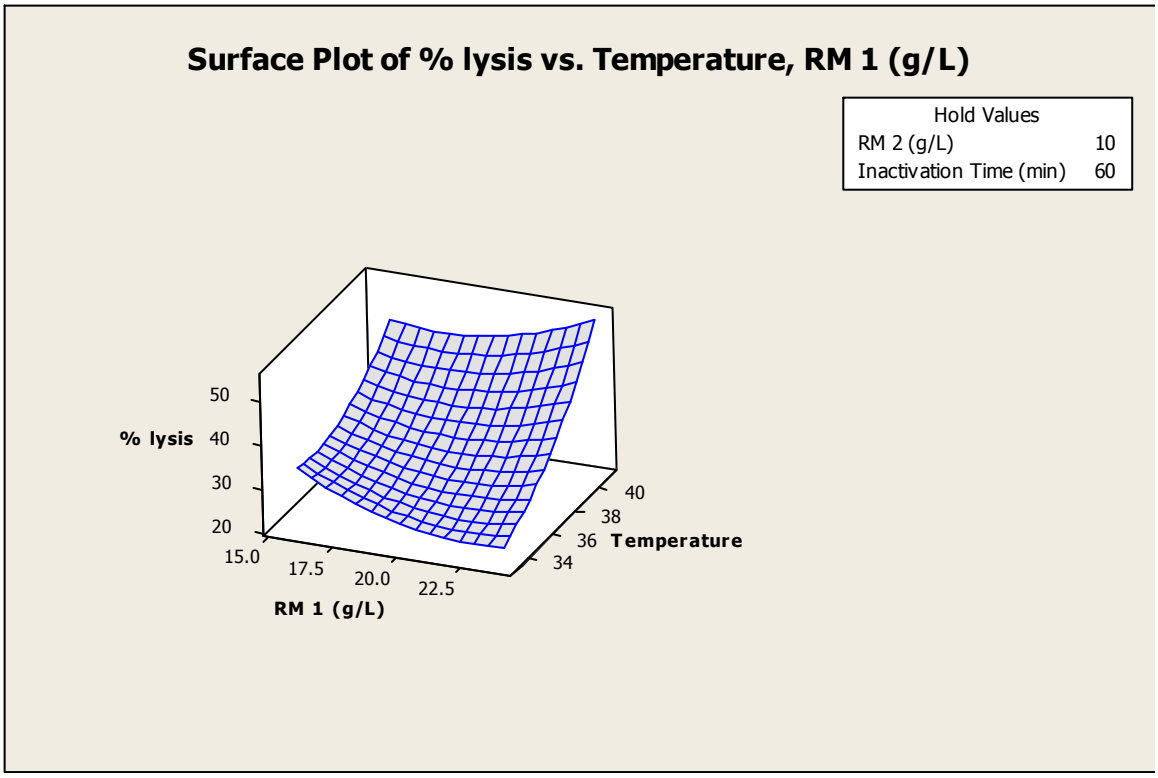
Figure 4-15: Impact of Inactivation Time and RM2 on cell lysis



% lysis is sensitive to temperature (not RM 1)

High incubation temperature promoted a higher degree of cell lysis. Target temperature (35–38 C) supported lower levels of cell lysis.

Figure 4-16: Impact of Temperature and RM1 on cell lysis



4.5. Selection of Critical Process Parameters (CPPs)

Parameters, that influence the number of polysaccharide repeat units, polysaccharide yields and lysis of the cells, were identified using the design of experiments (DOE) and one factor at a time (OFAT). The factors are summarized in Table: 4-8.

Table: 4-8, Summary of Production Bioreactor Parameters' Impact on Polysaccharide CQAs Parameter ranges were defined based on DOE and OFAT experiments (provided in next section).

Process Parameter	IMPACT: Number of PS Repeat Units	IMPACT: Polysaccharide Yield (potency)	IMPACT: % Lysis	OVERALL Parameter Assessment
Concentration of Complex RM #1 (18- 22 g/L)	NO	NO	NO	NOT a CPP
Concentration of Complex RM #2 (8– 12 g/L)	YES	NO	NO	Key Operating Parameter
Time to Inactivation (time post glycerol Depletion): 30– 150 min)	NO	YES	YES	CPP
Incubation Temperature (35 – 39?C)	NO	YES	YES	Well Controlled CPP

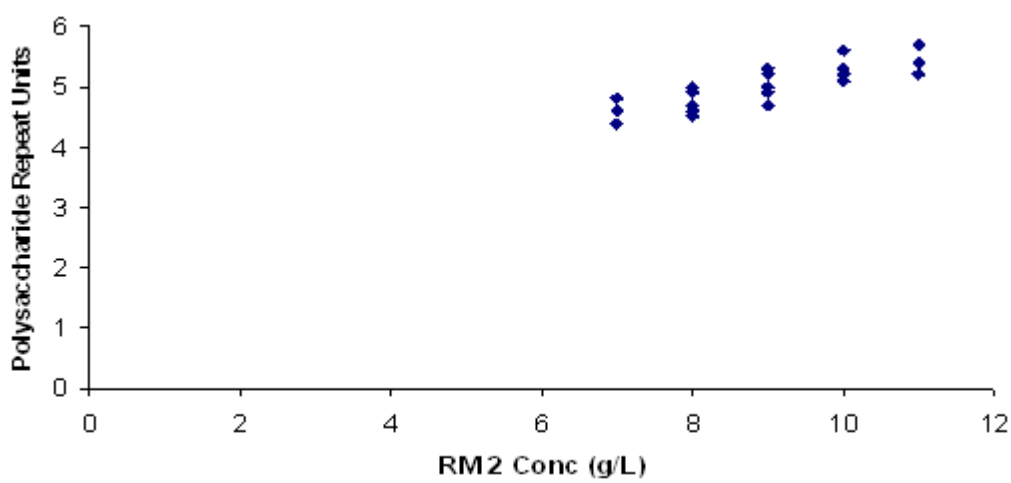
Note that while the Ps is sized to a particular molecular weight (MW) in downstream steps, it is possible that the fermentation could produce a Ps of a MW less than the minimum size needed. This may also happen if the number of repeat units differs significantly. A well-controlled CPP has been defined in this case when redundant automation system in the overall manufacturing process is able to control the operating parameter in a very narrow range, as compared with the design space.

4.6. One Factor at a Time Experiments to Establish Critical Process Parameters (CPPs) Range

After the DOE and CPP selection, the critical ranges were determined for each parameter by OFAT. Again OFAT was chosen to define the range since there were no significant interactions among the parameters as determined by the DOE. Both the RM 2 concentration and time to inactivation) were further defined around their respective set points using experimentation. Incubation temperature was not further explored by experimentation despite being a CPP since it was determined to be a well-controlled parameter and a sufficient range was tested in the initial DOE.

For RM 2, the concentration was explored in the range of 7 to 11 g/L. The experimental range was skewed to the lower concentration since the effect on the response in the DOE was much more pronounced. The experimental results are shown in Figure 4-17.

Figure 4-17: Polysaccharide Repeat Response to RM 2

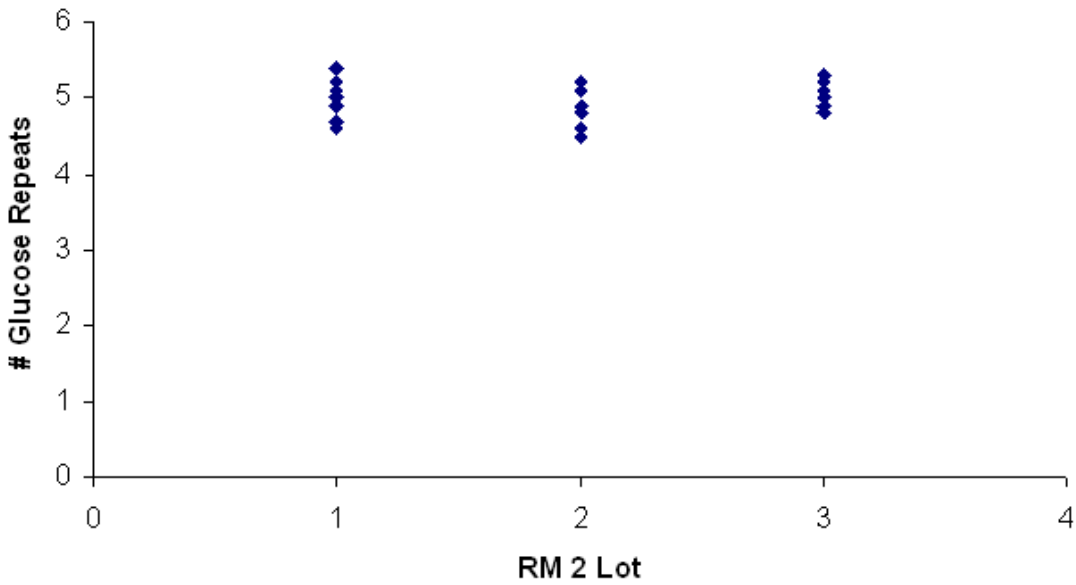


This figure shows a threshold concentration of RM 2 is needed to yield a consistent number of polysaccharide repeat units. This RM 2 value is 10 ± 2 g/L.

4.7. Exploration of RM 2 Lot-to-Lot Variability

Because of the fact that RM 2 concentration is a CPP and the material is derived from an undefined plant, an initial screen was performed to assess the lot-to-lot variability. This was accomplished via an OFAT experiment with three independent lots of RM 2. The results are illustrated in Figure 4-18. Note that results of post-implementation early manufacturing data with more than 100 lots in consideration (section 1.9) subsequently revealed that lot-to-lot variability in RM 2 led to variability in product yield, which was not evident through this initial series of OFAT experiments.

Figure 4-18: Polysaccharide Repeat Response to RM 2 Lots



The results were compared by a T-test analysis, and there is not a significant difference among the lots ($p < 0.05$).

4.8. Failure Modes and Effects Analysis

4.8.1. FMEA Methodology

The failure modes and effects analysis is a risk assessment tool used to proactively identify and mitigate potential failure scenarios. The initial step in the analysis is to generate a list of process parameters to assess in the FMEA. Next, a risk prioritization number (RPN) is generated for each parameter based on assessment of the severity (S), occurrence (O), and the ability to detect (D) failures (see FMEA for full list). The product of these scores is used to determine the RPN (Equation 4-1), which enables a semi-quantitative ranking of process parameters.

Equation 4-1: FMEA Risk Prioritization Number (RPN)

S x O x D = RPN

Severity was defined based on the potential impact to the process and/or product as evaluated by the effect on in-process CQAs and final release tests (which include final product CQAs). Occurrence was defined as the likelihood that the failure mode would take place. The detection score was defined as the ability to recognize the potential failure (i.e. excursion of measured parameter from a pre-defined range) of a process parameter before the consequences are observed either in additional processing or after product release. A summary of the parameters is given in Table 4-9: FMEA Scoring System. The levels were chosen with weighting of 1, 3, or 9 to clearly delineate the results.

Table 4-9: FMEA Scoring System

SEVERITY (S)	1 =	No impact to customer	
	3 =	Probable generation of impact on CQA	
	9 =	Known product quality impact or likely to fail release testing	
OCCURRENCE (O)			Frequency
	1 =	Likelihood of occurrence is remote	1 in 100
	3 =	Moderate failure rate without supporting documentation	1 in 20
	9 =	Assured of failure based on warranty data or significant testing	1 in 2
DETECTION (D)	1 =	Certain that failure will be found (including calibration errors)	
	3 =	Moderate chance that failure will be undetected (or detected after additional processing, but before release)	
	9 =	Certain that failure will be undetected (or detected after release)	

In addition to the RPN, the FMEA was also used to evaluate operating ranges and process control. All parameters and potential failure modes were discussed and agreed upon jointly by a cross-functional team. Table 4-10: RPN Results Classification summarizes the classification of RPN results and the classification of the parameters as a CPP, non CPP, or potential CPP. The Failure Modes Effects Analysis is summarized in Table 4-11

Table 4-10: RPN Results Classification

RPN RESULT	CLASSIFICATION
1–8	Not a CPP
9–26	Potential CPP
27–729	CPP test experimentally for process range

Table 4-11: Failure Modes Effects Analysis

Failure Modes Effects Analysis

Process Step or Variable or Key Input	Operating Parameter	Typical Operating Range	Sensitive to Scale of Operation	Potential Failure Effects	SEV	Potential Causes or Route of Failure	OCC	Current Process Controls	DET	RPN	Actions Recommended
What is the process step?	What is the operating parameter?	What is the targeted operating range?	Is the processing step sensitive to scale? (Y/N)	What is the impact on the Key Output Variables?	How Severe is effect to the customer?	What causes the Key Input to go wrong? (How could the failure mode occur?)	How frequent is cause likely to Occur?	What are controls that prevent the failure mode from occurring or detect it should it occur?	How probable is Detection of cause?	Risk Priority # to rank order concerns	What are the actions for reducing the Occurrence of the cause, or improving Detection? Should have actions on high RPN's or Severity of 9 or 10.
1000L Fermentation	Innoculate from Seed Fermentor	40 +/- 8L	N	Growth Failure	3	Failure in due to extreme varaition in growth paramter (pH, temperature)	1	On-line monitoring with automated alarms	1	3	N/A
	Media Addition	800 +/- 40L	N	Slight variability in volume of inoculum	3	load cell miscalibration	1	depends on load cell or not? Scale check?	1	3	N/A
	Complex RM 1 addition	16000 +/- 1600 g	N	possibility growth inhibition at high concentration; at low slight inpact to growth	3	Incorrect wieghment	1	Documenation	1	3	N.A
	Complex RM 2 addition	8000 +/- 800 g	N	High: impact to PS structure variability (glucose repeats); low = minimal impact to biomass; lot to lot variability may lead to non-robust productivity or PS structure variability	9	Incorrect wieghment	1	Documenation	3	27	Procedural Controls, scale calibration
	Glycerol Addition	8000 +/- 800 g	N	Change in final biomasl	3	Incorrect weighment	1	Documentation, scale calibration	1	3	NA
	Agitation	100 +/- 20 RPM	Y	Loss of agaition	3	Mechanical Failure	1	Preventative Maintenance, on-line monitoring with alarms	1	3	NA
	Pressure	Target 2 PSIG	N	Contamination	3	Loss of back pressure valve control	1	Preventative Maintenance, redundant control, on-line montoring with alarms	1	3	NA
	Air Overlay	10 +/- 1 LPM	N	Contamination	1	Loss of clean air, used for pressure control	1	Preventative Maintenance, on-line monitoring with alarms	1	1	NA
	pH	7 +/- 0.5 units	N	transient: minimal impact to biomass; sustained excursion: growth inhibition	3	Value failure, probe failure	1	On-line monitoring with automated alarms	1	3	NA
	Temperature	37 C +/- 2 C	N	transient: minimal impact to biomass; sustained excursion: growth inhibition	9	Value failure, probe failure, steam and or glycol loss	1	On-line monitoring with automated alarms	1	9	N/A
	Inactivation Criterion (glycerol concentration)	inactivation 30 minutes (+/- 10 minutes) post glycerol depletion (<0.1 g/L)	N	Cell lysis	9	Incorrect glycerol measurement, insturmentation failre	3	on-line monitoring manual hourly recorded off-line final sample	3	81	Maintian back up insturment
Phenol Inactivation	Phenol Concentration	0.5 +/- 0.1%	N	Incomplete inactivation / safety	9	Incorrect weighment	1	Documentation, scale calibration	3	27	In process phenol assay
	Incubation Time	1h +/- 15min	N	Incomplete Ps release, yeild loss	3	Human error	1	Documentation	1	3	N/A
	Incubation Temp	37 +/- 2 C	N	Incomplete inactivation / safety	9	Probe failure, temp control loss	1	On-line monitoring with automated alarms	1	9	N/A
	Agitation Rate	60 +/- 20 RPM	N	Incomplete inactivation / safety	3	Mechanical Failure	1	Preventative Maintenance, on-line monitoring with alarms	1	3	N/A

The results from the FMEA are as follows. The inactivation criterion had the highest RPN score of 81 and is a CPP. RM 2 had a score of 27 and is a CPP as a result of the significance of the concentration on the PS.

Note that two parameters resulted in borderline RPN scores of 9, although upon further analysis only one of these parameters was carried forward as a CPP because of its differential in potential impact on product quality. The phenol concentration had a score of 9 due to the safety aspect for completing inactivation, but because it does not have direct quality impact on the product it was not determined to be a CPP. Incubation temperature also had a score of 9, although this was determined to be a CPP because of its impact on the quality attribute Ps size. However, as the redundant automation systems in the process are able to control the processing parameter in a very narrow range, as compared with the design space, incubation temperature is classified as a well-controlled CPP. The previously mentioned parameters would all require special attention during the scale-up to final manufacturing.

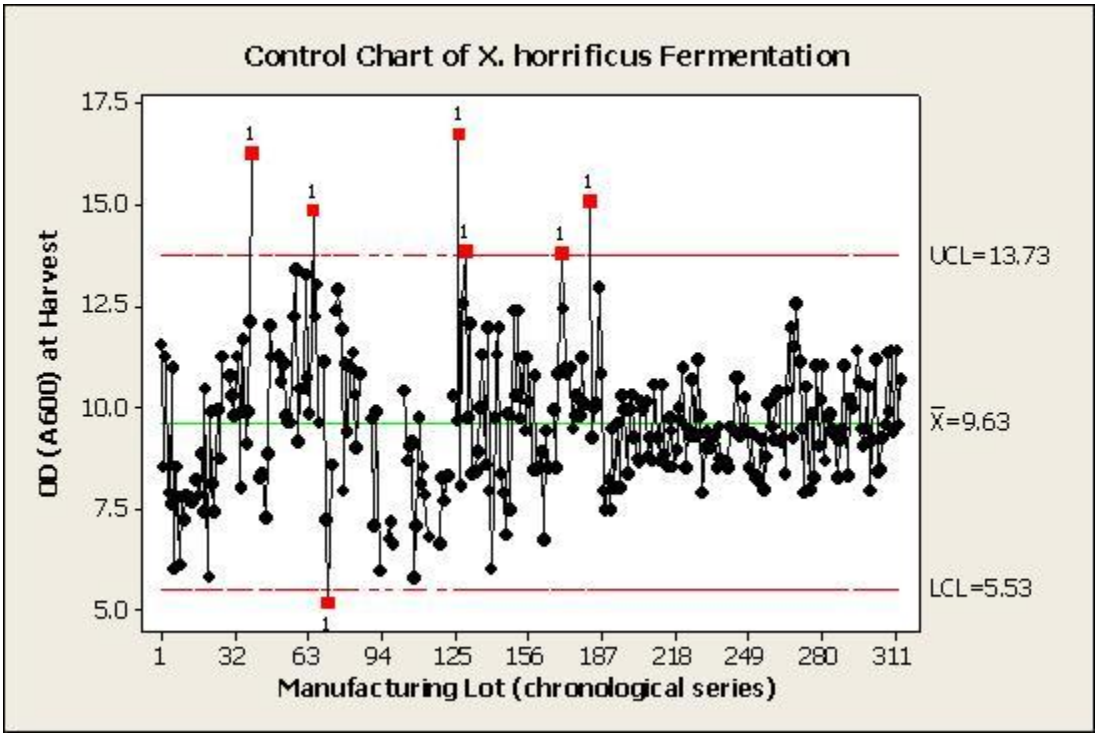
In addition, as part of the scale-up to final manufacturing, the ability of the downstream process to consistently clear residual host cell impurities, including proteins and host cellular DNA, is verified through process validation.

4.9. Continuous Improvement Based on Process Understanding

Proactive monitoring of the fermentation process was implemented to leverage new technology to build scientific understanding. During the manufacturing, multivariate tools (random forest analysis) were used as a proactive process monitoring initiative to identify correlations between variability among input parameters to variability in process attributes such as OD at harvest. The random forest analysis has the ability to evaluate hundreds of process input parameters with respect to their impact on a given process attribute.

The multivariate analysis identified that variable nitrogen content contained in various lots of complex RM 2 was related to variability in cell mass at harvest. By controlling nitrogen content through setting acceptability criteria and implementing a release test and/or by procuring large volumes of a single lot of raw materials within these specifications, the variability in cell yield at the production stage was reduced.

Figure 4-19. Control Chart of Fermentation Output (Optical Density)



5. Upstream (VLP) Section

5.1. Executive Summary

In the manufacturing process for recombinant VLP in gram negative organisms, the criticality of the final attributes is largely determined by the efficiency of the downstream processing. However, there should be a well-defined upstream process to provide a sufficient yield of upstream material with well-defined quality attributes for the downstream processing.

This document assesses the contribution of the upstream process in E. coli VLP production. Also, it looks at the potential impact of the quality attributes of the upstream material on the critical attributes of the bulk VLP. The harvest step of the upstream VLP production step was selected as an example of the applications of tools that would provide operational confidence in selecting input parameters that potentially can affect the quality attributes of the VLP.

Several commonly used tools have been explored throughout the document to illustrate the approach for selection of critical parameters and the design space, which support the operational ranges for continuous production post validation. Examples of post-validation changes that may or may not affect the quality attribute have also been shown. A rational approach to evaluate the risk of process changes associated with vaccine production has been taken. Common tools such as cause-and-effect (C&E) matrices and failure modes and effects analysis (FMEA) have been used to assess the risk of individual process parameter changes. Also, a DOE-based approach has analyzed the effects of these process parameters on the product quality attributes.

For the case study, the responses measured upstream do not directly impact the critical attributes of the bulk VLP after downstream processing. However, the downstream process involves a series of purification steps to achieve the final vaccine's desired critical attributes, such as size distribution, tertiary structure, purity etc.. So the overall efficiency of sizing depends on modeling a downstream process based on expected specific protein activity of the inclusion bodies upstream while assessing the initial purity of the material to ensure consistency of material delivered for downstream purification. The critical quality attributes of the bulk VLP will be defined downstream of the VLP harvest step.

For the E. coli VLP primary recovery steps, the following response parameters were assessed: protein content, pellet mass for each wash, purity (DNA, protein, lipid), SDS-PAGE profile, and percentage of monomer measurement. The scale-down models were used to reduce the number of parameters in series of fractional and full factorial designs. For the screening experiments (DOE #1), all these tests were performed for 16 runs in a fractional factorial design with all eight parameters.

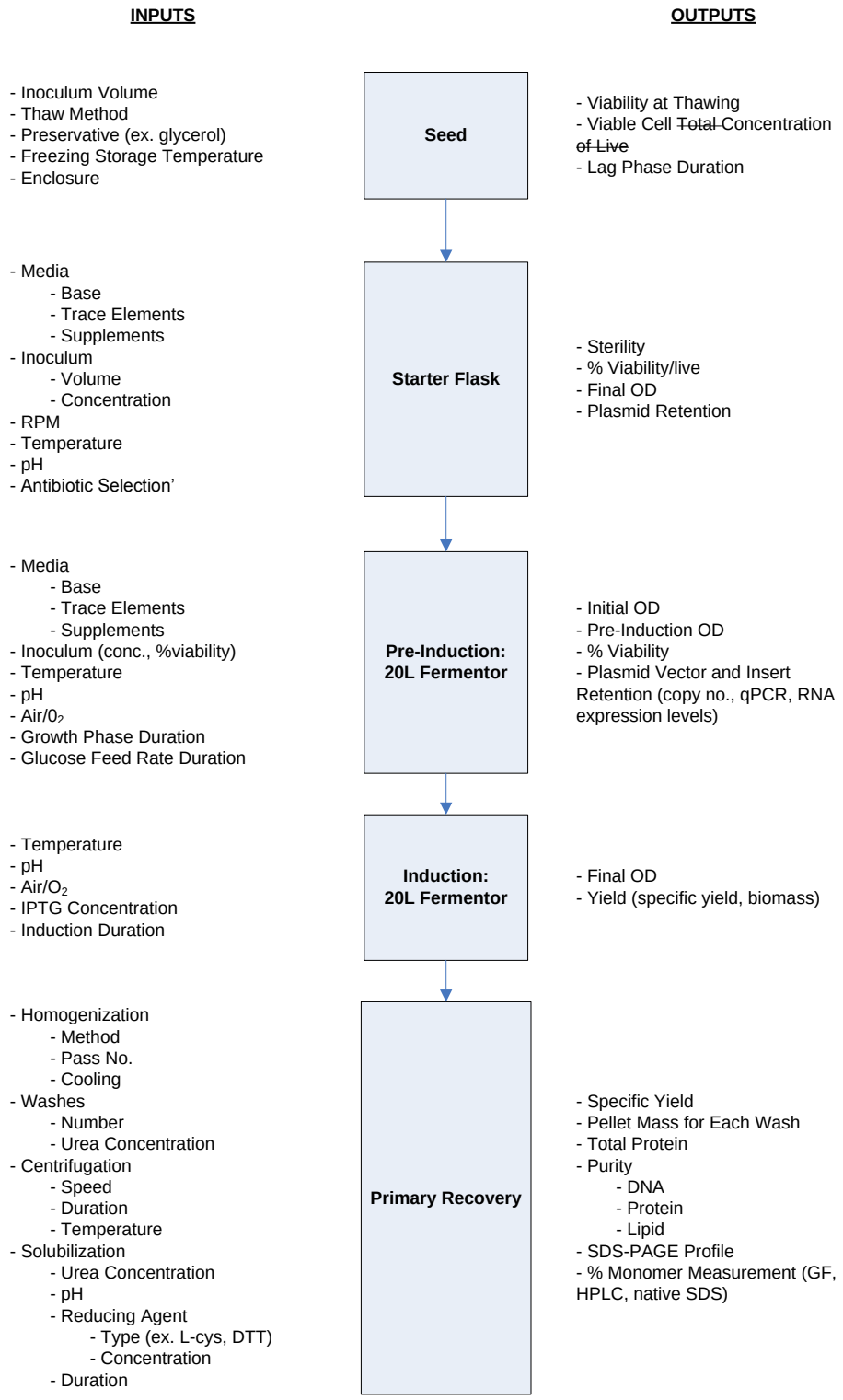
In DOE #2, the design space was also defined using scale-down models from four factors that were selected from DOE #1. For the optimization studies to define the design space, a central composite rotatable design with 29 runs was used, and the design space was defined from analysis simulations using MATLAB software to generate response surface models. The control space was verified at scale with 16 repeat runs at the same conditions. This provided enough confidence to establish the protein content expected downstream for the VLP process. In all, the eight parameters were eventually reduced to four by relative importance for the harvest step.

For the purposes of illustration, only responses for protein content are used throughout the document. Primarily, the reason is that the quality and quantity of the protein upstream impact the downstream processing, during which the critical quality attributes of the bulk VLP are assessed for the vaccine. These responses will then be monitored on a continuous basis. Downstream processing tests will include tests for purity and percentage of monomers.

Combining with the downstream purification and drug product analysis, this document can contribute to development of a more systematic way to validate the manufacturing processes at late stages of vaccine development and production.

5.2. Process Descriptions

Figure 5-1: General Process Flow Diagram (Upstream)



5.2.1. Brief Description of Each Process Step

The following is a brief description of the process steps outlined in the proposed VLP primary recovery process. Variables and key considerations are presented where applicable.

5.2.1.1. Seed

Seed vials are prepared in a logarithmic growth phase according to standard procedures to generate sufficient inoculum per vial to initiate a viable culture of the desired recombinant organism. Antibiotic selection on the culture prior to cryopreservation is optional but likely in order to ensure a high percentage of recombinant organisms at the time of thawing. If present, nonrecombinants may overwhelm a culture, resulting in reduced protein content per biomass.

The choice of preservative is made based on characteristics of the host organism and for bacterial hosts is likely to be a glycerol-based cryopreservative. Maximum viability of freshly thawed vials will ensure a prompt initiation of the culture in the starter flask, reducing process time and maximizing expression levels. Plasmid copy number is to be assessed at the end of the starter culture.

5.2.1.2. Starter Flask

Generally richer than cultures in subsequent steps, the starter culture ensures maximal recovery of an organism post cryopreservation. Organisms are usually in logarithmic growth at the end of culturing, creating a consistently high concentration of cells prior to inoculation into the pre-induction fermentor. Vial-to-vial variations in total number of organisms, concentration, volume, viability, etc., are usually minimized during starter flask culturing such that the inoculum for the 20L fermentor is consistent from batch to batch.

5.2.1.3. Pre-induction Culture: 20L Fermentor

The pre-induction culture is inoculated with sufficient starter culture to initiate a logarithmic growth of the organism in the absence of an inducer. Log phase cells are maximally viable such that once they are induced, a maximum amount of VLP monomer is expressed. Final pre-induction optical density should be maximized while ensuring that the culture remains at log phase prior to induction. Protein contents depend on culture condition at the time of induction.

5.2.1.4. Induction Culture: 20L Fermentor

Induction is performed by addition of an appropriate inducer and as defined by the host vector expression system. Duration, temperature, and concentration at induction all affect the final protein content. The desired conditions at this stage are those that maintain the metabolism of the cell for the longest time to maximize continued expression of the desired VLP monomer. The expressed VLP monomers are accumulated as an inclusion body (IB) in the recombinant organisms.

5.2.1.5. Primary Recovery

Recovery of the product from inclusion bodies requires disruption of the cell wall/membrane such that IBs are released. Passage through a homogenizer or microfluidizer can result in heat transfer and cause enzymatic and/or thermal degradation of the product. To minimize this potential negative effect, cooling is often employed during IB release. In addition, ineffective

homogenization may cause incomplete release of IBs from the cell and thus their loss in subsequent centrifugation steps. Passage number, channel width, and other factors including pressure determine the efficiency of cell disruption.

Furthermore, denaturation/solubilization is a critical step in primary harvest. It separates the aggregated IB mass and generates individual proteins, which can then be recovered by standard chromatographic techniques. Inefficient denaturation/solubilization results in aggregated material and poor recovery of VLP monomer, especially during subsequent centrifugation steps. Duration of denaturation and denaturant concentration both affect the degree of solubilization and overall protein content.

5.2.2. Prior Knowledge

The primary objective of the upstream process is to have a maximal amount of product for downstream processing while taking into consideration any conditions that will impact the purity percentage of the IBs going downstream. Impurity at the IB stage is generally less than 5% and dependent on inclusion body washing efficiency. Based on this prior knowledge, purity assessment as a potential CQA for the upstream process has been excluded. The overall efficiency of sizing the downstream process to achieve the desired CQAs is dependent on modeling a process based on expected protein contents of the IBs upstream. The CQAs of the VLP will be defined downstream of the VLP harvest step.

The purification of IBs from over-expressing host cells generally involves the process of cell lysis and subsequent centrifugation. The IBs are a high-density, intracellular body resistant to the effects of cell lysis. Once lysis is complete, the IBs are released and easily separated from all other solubilized cell debris by low-speed differential centrifugation. The pellet resulting from such centrifugation is highly enriched in over-expressed protein. However, resolubilization of the pellet without further washing fails to remove contaminating proteins, which are readily identified by SDS-PAGE. IB washes result in a much cleaner product, but the washes are often accompanied by some product loss.

From prior knowledge, the presence of the contaminating material results mainly from nonspecific adsorption on the surface of the inclusion bodies following cell lysis and contaminating proteins/nucleic acids, etc., that are not likely integrated into the IB. Furthermore, the IB can be considered a highly pure aggregate of the over-expressed protein of interest, which if purified appropriately should yield protein purity levels >95%.

5.2.2.1. Quality at Upstream/Primary Recovery

Unlike most other cell-derived recombinant products, proteins over-expressed in hosts such as E. coli are segregated into inclusion bodies that do not preserve the secondary and tertiary structure of the protein of interest. As such, the product is recovered during primary recovery as a nonfunctional protein, which is refolded during intermediate processing steps into a functional product with the desired structure. Subsequent purification steps are employed to remove residual impurities as well as product that lacks the desired functional structure.

Since the quality of the product is determined only during the intermediate refolding steps, the harvest and primary recovery steps that precede this refolding play no role in the final product

quality beyond the yield of the intact (full-length) protein within the structure of the inclusion body. Inclusion bodies effectively remove the product from the general metabolism of the cell, notably from the action of proteases that would otherwise degrade the product. As such, the recovered product from inclusion bodies tends to be full-length intact protein, abrogating the need for additional design requirements to ensure product quality. This leaves the overall product yields as a priority in a well-designed upstream process.

5.2.2.2. Optimizing Yields Vs. Optimizing Purity at Primary Recovery

Although it is pointed out that yields are potentially higher if modified conditions are applied during primary recovery, this increase in yields comes at the cost of decreased purity of product. Although the downstream process can be modified to accommodate a larger impurity capacity, this generally becomes cost prohibitive relative to the gains achieved in product yield.

The proposed criteria for the primary recovery are expected to generate estimated impurity levels that are well within the capacity of the downstream process to remove them. The loss of product is therefore offset by the reduced costs downstream. It is a common occurrence that a compromise between product yield and purity is made throughout a mature purification process. It is also possible that the desired compromise can be adjusted depending on protein expression levels, product value, downstream processing costs, etc. These can be finalized once the process is better defined.

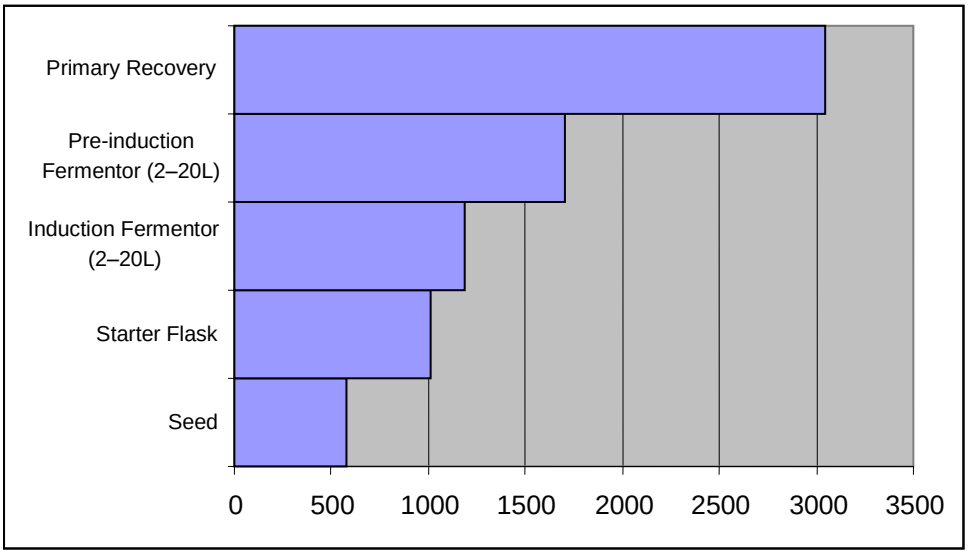
5.2.3. Rationale for Selecting Primary Recovery as a Unit of Operation for Quality by Design Analysis

Primary recovery is the last step in VLP production prior to purification. It is complex and is known to be affected by more than a dozen process parameters. This is twice as many as some other single steps during the upstream manufacturing process, considering the number of factors that affect product quality and quantity.

The primary recovery step is also impacted by other changes accumulated through the upstream process optimization and manufacturing. Thus, it can be a direct measurement of the effect of these process modifications. In addition, what is generated through this step is used in the next stage of the VLP production. The step has a significant impact on all subsequent manufacturing processes, especially purification, which takes place following completion of the primary recovery step. Finally, risk assessment using cause-and-effect (C&E) matrices suggests the primary recovery impacts the quality of VLP to a considerable extent during VLP production.

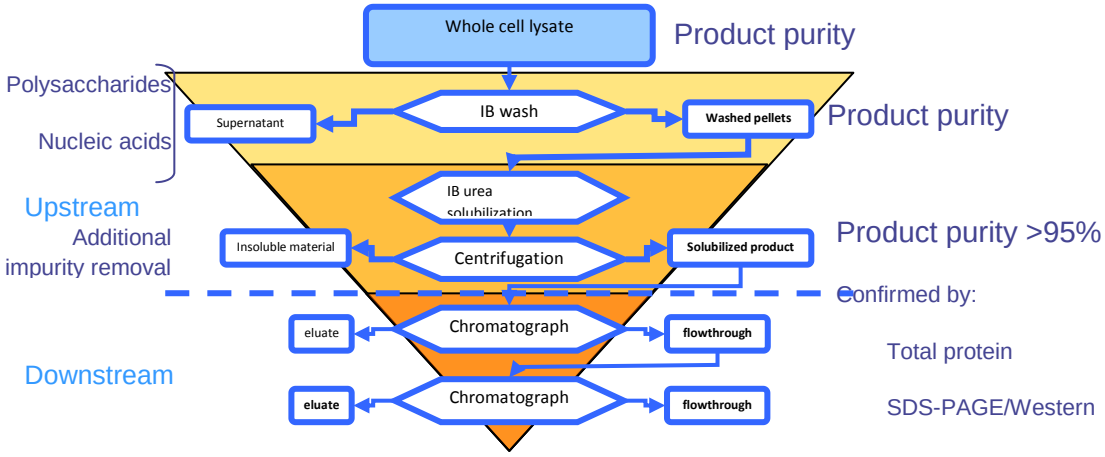
The complexity of the primary recovery step and its bridging function in determining the protein content and initial quality characteristics of the VLP for downstream processing demonstrate its importance to be chosen as a unit of operation for the VLP Quality by Design case study.

Figure 5-2: Pareto Graph (by Process Step)



5.2.4. Summary Process Flow Diagram of VLP Primary Recovery Step

Figure 5-3: Summary Process Flow Diagram of VLP Primary Recovery Step



5.3. Process Risk Assessment

5.3.1. Risk Assessment Using Cause-and-Effect Matrices

Table 5-1: Scoring of Process Parameters and Quality Attributes

Process Parameters		**Attributes**[1]	
Impact Score	Ranking Criteria	Weight Score	Ranking Criteria
10	Strong relationship is known based on available data and experience.	10	Established or expected direct impact on safety and/or efficacy of product.[2]
7	Strong relationship is expected.	7	Moderate or indirect impact on safety and/or efficacy. Direct impact on efficiency.
5	Not-so-strong relationship is expected or unknown.	5	Low or unlikely impact to product safety and/or efficacy. Moderate or indirect impact efficiency.
1	Known to not have a relationship.	1	No impact to product safety and/or efficacy. Low or unlikely to impact efficiency.

[1] Process performance attributes may have no direct impact on product quality, safety, or efficacy but are assessed where they are important indicators of focus area function or performance consistency. Examples include step recoveries and overall protein content.
[2] May include efficiency attributes, but most efficiency attributes are not a 10 unless they significantly impact product viability.

Total Score = ∑ (impact score * weight score)

2447 **Table 5-2: Cause-and-Effect Matrix**

		Protein Content (Specific Activity by ELISA)	Pellet Mass for Each Wash	Total Protein	Purity (DNA, Protein, Lipid)	SDS-PAGE Profile	% Monomer Measurement (GF, HPLC, Native SDS)	Total Score
Quality Attributes Score		5	5	5	10	10	7	
Process Step	Parameter							
Seed	Inoculum Volume	5	5	7	1	1	1	112
	Thaw Method	5	5	7	1	1	1	112
	Preservative (ex. glycerol, DMSO)	5	5	7	1	1	1	112
	Freezing Storage Temp.	5	5	7	1	1	1	112
	Enclosure	5	5	10	1	1	1	127
Starter Flask	Base Media + Trace Elements/Supplements	5	10	10	1	1	1	152
	Inoculum Volume	5	7	10	1	1	1	137
	Inoculum Conc.	5	7	10	1	1	1	137
	RPM	5	7	10	1	1	1	137
	Temp.	5	7	10	1	1	1	137
	pH	5	7	10	1	1	1	137
	Antibiotic Selection	10	10	10	1	1	1	177
Pre-induction Fermentor (2–20L)	Base Media + Trace Elements/Supplements	10	10	10	1	5	1	217

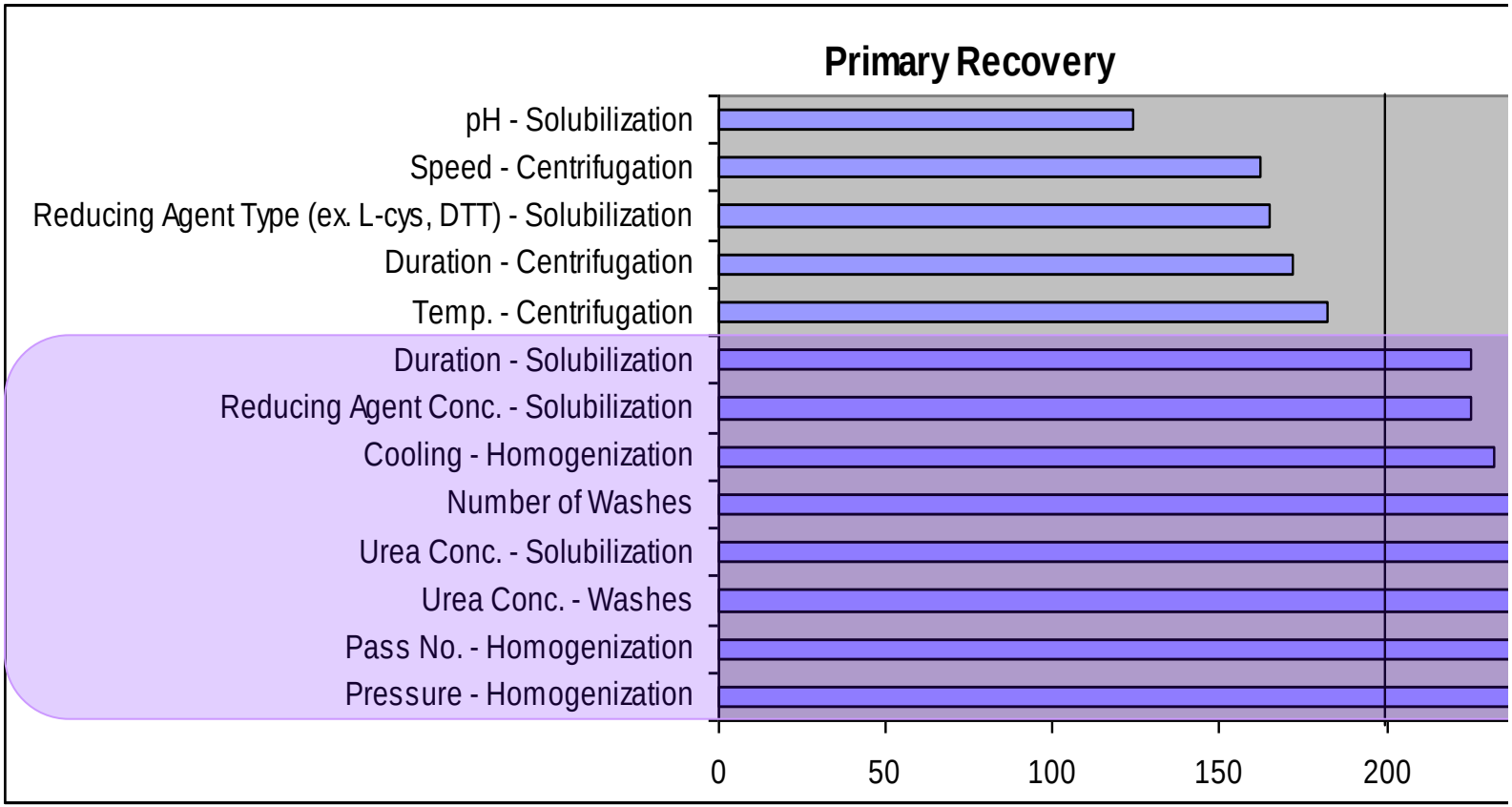
		Protein Content (Specific Activity by ELISA)	Pellet Mass for Each Wash	Total Protein	Purity (DNA, Protein, Lipid)	SDS-PAGE Profile	% Monomer Measurement (GF, HPLC, Native SDS)	Total Score
	Inoculum (conc., % viability)	10	10	10	1	5	1	217
	Temp.	10	10	10	1	5	1	217
	pH	7	10	10	1	5	1	202
	Air/O2	7	10	10	1	5	1	202
	Growth Phase Duration	10	10	10	1	5	1	217
	Glucose Feed Rate	10	10	10	1	5	1	217
	Glucose Feed Rate Duration	10	10	10	1	5	1	217
Induction Fermentor (2–20L)	Temp.	10	10	10	1	10	1	267
	pH	7	10	10	1	5	1	202
	Air/O2	10	10	10	1	10	1	267
	IPTG Conc.	10	5	5	1	10	1	217
	Induction Duration	10	7	7	1	10	1	237
Primary Recovery	Pressure - Homogenization	1	10	10	10	10	1	312
	Pass No. - Homogenization	1	10	10	10	10	1	312
	Cooling - Homogenization	1	5	5	7	10	1	232
	Number of Washes	1	10	10	7	10	1	282
	Urea Conc. - Washes	1	10	10	10	10	1	312
	Speed - Centrifugation	1	7	7	7	1	1	162

		Protein Content (Specific Activity by ELISA)	Pellet Mass for Each Wash	Total Protein	Purity (DNA, Protein, Lipid)	SDS-PAGE Profile	% Monomer Measurement (GF, HPLC, Native SDS)	Total Score
	Duration - Centrifugation	1	5	5	10	1	1	172
	Temp. - Centrifugation	1	5	5	5	7	1	182
	Urea Conc. - Solubilization	1	10	10	1	10	10	285
	pH - Solubilization	1	5	5	1	1	7	124
	Reducing Agent Type (ex. L-cys, DTT) - Solubilization	1	1	1	1	7	10	165
	Reducing Agent Conc. - Solubilization	1	7	7	1	7	10	225
	Duration - Solubilization	1	7	7	1	7	10	225

5.3.1.1. Parameters with the Highest Potential Impact on Quality Attributes

From the Pareto, the parameters with the highest potential to impact any of the response attributes have been highlighted. These attributes include protein content measured as specific activity by ELISA as the critical quality attribute of the primary recovery step.

2451 **Figure 5-4: Pareto Graph (Primary Recovery Step)**



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5.4. Addressing High-Risk Process Parameters/Material Attributes

5.4.1. Selection of Parameters (from Primary Recovery Step) for DOE

5.4.1.1. Parameters' Selection Scoring Guidelines

- **Technical impact:** Using technical literature and/or theory as a guide, how important is this process variable?
  - 1 = Not important
  - 3 = Relatively important
  - 9 = Extremely important
- **Ability to adjust:** When working with the manufacturing process, how easy is it to make changes to this process variable?
  - 1 = Difficult
  - 3 = Moderate difficulty
  - 9 = Very easy to change
- **Support by process data:** When assessing the process control and performance, how much does the process data support the relative importance of this variable?
  - 1 = No importance observed
  - 3 = Moderate importance observed
  - 9 = High level of importance

5.4.1.2. Parameters' Selection Scores

2470 **Figure 5-5: Parameters' Selection Scores**

Process Input or Factor	Purpose	Investigational Range		Units	Type	Technical Impact	Ability to Adjust	Supported by Process Data	Importance Index
		Low	*High*			*3*	*1*	*9*	
Pressure – Homogenization	release of the product from intracellular compartment	10000	20000	psi	Continuous	9	1	9	109
Pass No. – Homogenization	no. of repeats with which to achieve maximum product release	1	3	N/Ap	Continuous	9	9	9	117
Cooling – Homogenization	prevention of product degradation due to excessive heat buildup	5	15	min	Continuous	3	9	9	99
Number of Washes	removal of impurities	1	4	N/Ap	Continuous	3	9	9	99
Urea Conc. – Washes	efficiency of impurity removal	1	5	M	Continuous	3	9	9	99
Speed – Centrifugation	pelleting of product inclusion bodies	10000	20000	g	Continuous	1	9	3	39
Duration – Centrifugation	pelleting of product inclusion bodies	10	60	mins	Continuous	1	9	3	39
Temp. – Centrifugation	minimizing of product enzyme degradation	4	24	°C	Continuous	3	9	3	45
Urea Conc. – Solubilization	solubilization of product	5	10	M	Continuous	9	9	9	117
pH – Solubilization	solubilization of product	5	10	N/Ap	Continuous	3	3	3	39

Process Input or Factor	Purpose	Investigational Range		Units	Type	Technical Impact	Ability to Adjust	Supported by Process Data	Importance Index
		Low	*High*			*3*	*1*	*9*	
Reducing Agent Type (ex. L-cys, DTT) – Solubilization	solubilization of product – reduction of disulfide cross-linking	DTT	L-cys	N/Ap	Discrete	3	3	**3**	**39**
Reducing Agent Conc. – Solubilization	solubilization of product – reduction of disulfide cross-linking	0.5	50	mM	Continuous	**9**	**9**	**9**	**117**
Duration – Solubilization	solubilization of product – reduction of disulfide cross-linking	3	15	hrs	Continuous	**9**	**9**	**9**	**117**

Top 80% of parameters ranked by importance index chosen as candidate factors for DOE

- Minimum: 39
- Maximum: 117
- Selection Boundary: 94 (= 0.8*117)

5.5. DOE #1: Fractional Factorial Design (Scale-Down Model – 2L Fermentor)

DOE#1 consisted of 16 runs using eight factors - Resolution 4, designed to assess some two-factor interactions. From the C&E and selection of parameter analysis, eight factors are potentially critical to all the performance attributes at the VLP harvest step. Since there are eight factors, a fractional factorial design at a small scale is used as the first screening step to assess interaction and confounding effects and to select the parameters that have the highest impact for the next series of experiments.

5.5.1. Analysis of the Fractional Factorial Design

Table 5-3: Analysis of the Fractional Factorial Design (DOE #1)

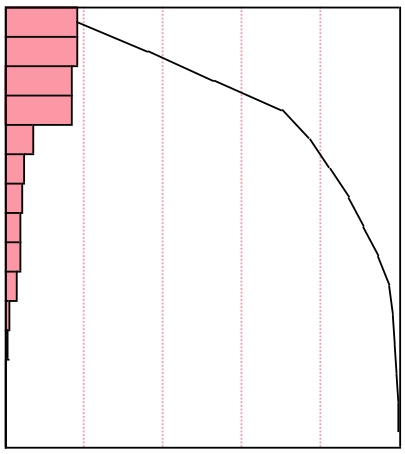
Run #	Pattern	Pressure – Homogenization (psi)	Pass No. – Homogenization	Cooling – Homogenization (min)	Number of Washes	Urea Conc. – Washes	Urea Conc. – Solubilization(M)	Reducing Agent Conc. – Solubilization (mM)	Duration – Solubilization (hrs)	Specific Activity Protein Content
1	−−−−−−−−	10000	1	5	1	1	5	0.5	3	0.92
2	−−−++++−	10000	1	5	4	5	10	50	3	1.03
3	−−+−++−+	10000	1	15	1	5	10	0.5	15	1.34
4	−−++−−++	10000	1	15	4	1	5	50	15	1.43
5	−+−−+−++	10000	3	5	1	5	5	50	15	1.19
6	−+−+−+−+	10000	3	5	4	1	10	0.5	15	1.24
7	−++−−++−	10000	3	15	1	1	10	50	3	1.23
8	−++++−−−	10000	3	15	4	5	5	0.5	3	0.97
9	+−−−−+++	20000	1	5	1	1	10	50	15	1.40
10	+−−++−−+	20000	1	5	4	5	5	0.5	15	1.29
11	+−+−+−+−	20000	1	15	1	5	5	50	3	1.34

Run #	Pattern	Pressure – Homogenization (psi)	Pass No. – Homogenization	Cooling – Homogenization (min)	Number of Washes	Urea Conc. – Washes	Urea Conc. – Solubilization(M)	Reducing Agent Conc. – Solubilization (mM)	Duration – Solubilization (hrs)	Specific Activity Protein Content
12	+−++−+−−	20000	1	15	4	1	10	0.5	3	1.50
13	++−−++−−	20000	3	5	1	5	10	0.5	3	0.51
14	++−+−−+−	20000	3	5	4	1	5	50	3	0.50
15	+++−−−−+	20000	3	15	1	1	5	0.5	15	1.17
16	++++++++	20000	3	15	4	5	10	50	15	1.18

5.5.1.1. Pareto Plot of Estimates

Figure 5-6: Pareto Plot of Estimates (DOE #1)

Term	Estimate
Pass No. - Homogenization(1,3)	-0.1412500
Duration - Solubilization(3,15)	0.1400000
Cooling - Homogenization(5,15)	0.1300000
Pressure - Homogenization*Pass No. - Homogenization	-0.1300000
Pressure - Homogenization*Cooling - Homogenization	0.0562500
Urea Conc. - Solubilization(5,10)	0.0387500
Urea Conc. - Washes(1,5)	-0.0337500
Pressure - Homogenization(10000,20000)	-0.0287500
Pressure - Homogenization*Reducing Agent Conc. - Solubilization	-0.0287500
Reducing Agent Conc. - Solubilization(0.5,50)	0.0225000
Pressure - Homogenization*Duration - Solubilization	0.0087500
Pressure - Homogenization*Number of Washes	0.0037500
Pressure - Homogenization*Urea Conc. - Solubilization	-0.0025000
Number of Washes(1,4)	0.0025000
Pressure - Homogenization*Urea Conc. - Washes	0.0025000



5.5.1.2. Conclusions from DOE #1

Main factors Pass No. – Homogenization, Duration – Solubilization, Cooling – Homogenization, and interaction Pressure – Homogenization*Pass No. – Homogenization show relatively higher estimates compared with the other factors based on the Pareto Plot (Figure 5-6). Thus, these four factors will be used for the next experimental design runs.

5.6. DOE #2: Central Composite Design for Control/Manufacturing Space (Scale-Down Model – 2L Fermentor)

Based on the knowledge learned from the first run, four factors (Pressure – Homogenization, Pass No. – Homogenization, Cooling – Homogenization, and Duration – Solubilization) were used for a central composite design run in DOE #2. DOE #2 consisted of 29 runs using the four factors, designed to assess the design space and optimum responses.

5.6.1. Analysis of the Central Composite Design

Table 5-4: Analysis of the Central Composite Design (DOE #2)

Run #	Pattern	Pressure-H (x1)	Pass #-H (x2)	Cooling-H (x3)	Duration of Solubilization (x4)	Specific Activity by ELISA - Protein Content (y)
1	−+−−	1000	3	5	3	*0.68*
2	+−++	2000	1	15	15	*0.91*
3	−−++	1000	1	15	15	*0.65*
4	00a0	1500	2	0	9	*0.45*
5	0	1500	2	10	9	*0.95*
6	+−+−	2000	1	15	3	*0.89*
7	++−−	2000	3	5	3	*0.44*
8	+++−	2000	3	15	3	*0.7*

Run #	Pattern	Pressure-H (x1)	Pass #-H (x2)	Cooling-H (x3)	Duration of Solubilization (x4)	Specific Activity by ELISA - Protein Content (y)
9	−−−+	1000	1	5	15	*0.49*
10	+−−+	2000	1	5	15	*0.52*
11	++++	2000	3	15	15	*0.69*
12	−−−−	1000	1	5	3	*0.59*
13	000A	1500	2	10	21	*0.58*
14	00A0	1500	2	20	9	*0.73*
15	0	1500	2	10	9	*0.77*
16	0a00	1500	0	10	9	*0.5*
17	0	1500	2	10	9	*1.05*
18	−+++	1000	3	15	15	*0.65*
19	−++−	1000	3	15	3	*0.53*
20	0	1500	2	10	9	*1.23*
21	++−+	2000	3	5	15	*0.78*
22	−−+−	1000	1	15	3	*0.71*
23	000a	1500	2	10	-3	*0.49*
24	+−−−	2000	1	5	3	*0.53*
25	A000	2500	2	10	9	*0.41*
26	−+−+	1000	3	5	15	*0.64*
27	a000	500	2	10	9	*0.43*
28	0A00	1500	4	10	9	*0.98*
29	0	1500	2	10	9	*1*

5.6.1.1. Conclusions from DOE #2

The central composite design data in Table 5-4 is used to develop a quadratic Response Surface Model RSM model (second-degree polynomial) that can capture the curvature in the data.

The RSM model :

y = 0.65+4.18E-06*x1+0.03*x2+0.01*x3+0.004*x4-4.71E-09*(x1-15000).^2-3.75E-06*(x1-15000).*(x2-2)-0.038*(x2-2).^2+1.95E-5*(x1-15000).*(x3-10)-0.013*(x2-2).*(x3-10)-0.003*(x3-10).^2+8.75E-07*(x1-15000).*(x4-9.2069)+0.006*(x2-2).*(x4-9.2069)-0.0003*(x3-10).*(x4-9.2069)-0.003*(x4-9.2069).^2;

where y = Specific activity by ELISA protein content , x1 = Pressure – H, x2 = Pass # - H, x3 = Cooling – H, and x4 = Duration of solubilization

5.7. Constraints for Maximum Protein Content

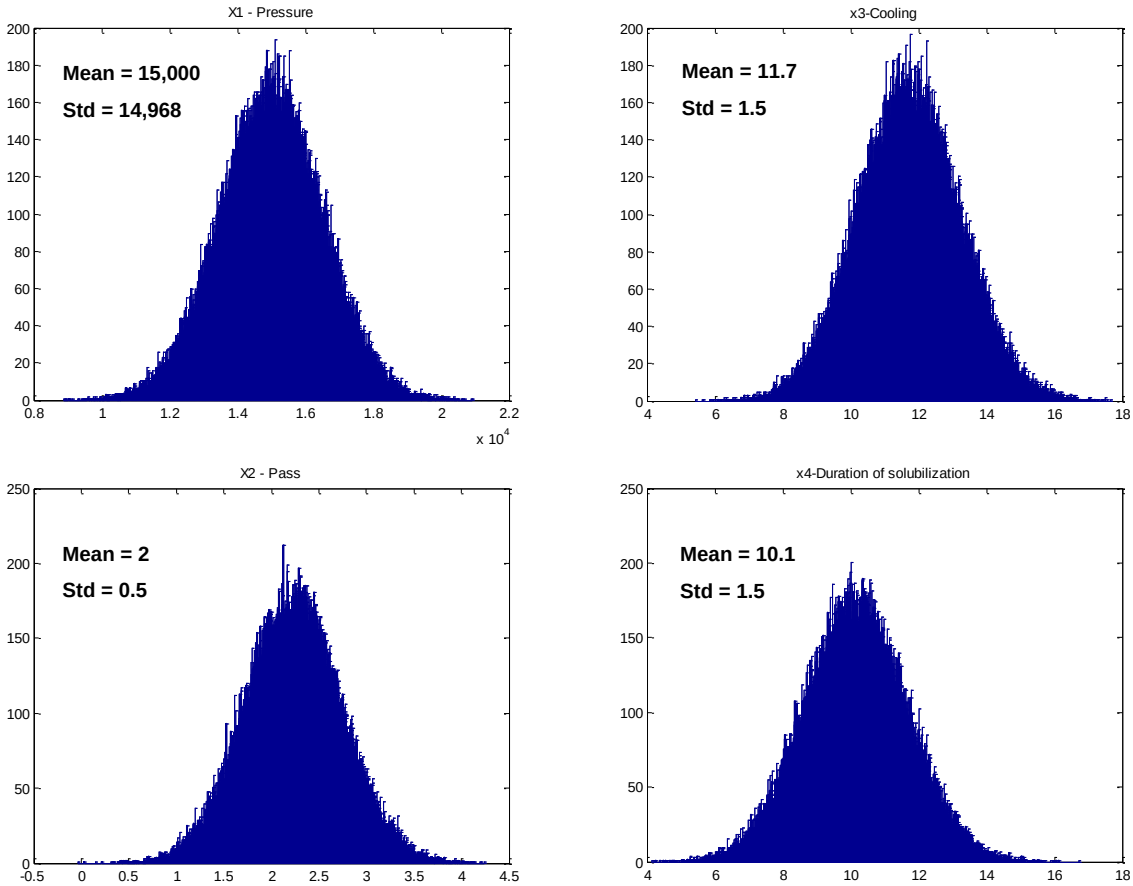
Monte Carlo simulation was performed to obtain the optimal constraints for maximum protein content. In the simulation, 100,000 realizations were sampled from normally distributed populations to evaluate the RSM model for protein content. The mean values used were the optimum point based on the model, and the standard deviations were tuned to reduce the chances for the protein content to fall below a value of 0.77. The optimum constraints based on ± 3σ are given below (the values are rounded):

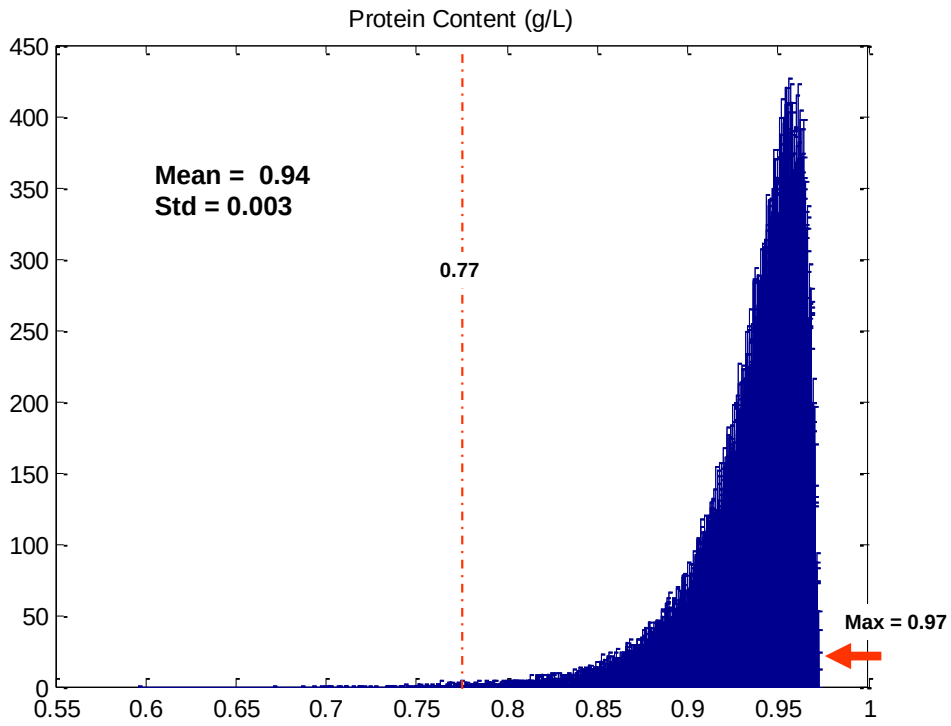
	X1 Pressure(psi)	**X2 Pass #**	**X3 Cooling(min)**	**X4 Duration(min)**
min	10,000	1	7	5
max	19,000	3	16	14

- The statistics of the resulting protein content distribution are given as follows:

Mean = 0.94, Std = 0.003

The histograms of the inputs as well as the protein content are shown below.





The shape of the resulting distribution is skewed toward the maximum value of 0.97, as can be seen in the protein content histogram.

5.8. Design Space for the VLP Primary Recovery Step

- A Central Composite Rotatable Design (CCRD) (Schmidt and Launsby, 1992) is chosen to optimize the VLP recovery step. This design is more useful in practice than other designs; it requires fewer experimental points to determine polynomial coefficients and also measures the lack of fit of the resulting equation.
- A CCRD was used to study how variations in Pressure – Homogenization, Pass No. – Homogenization, Cooling – Homogenization, and Duration – Solubilization affect the purity and quantity of protein content responses of VLP from the primary recovery step.
- Responses, namely protein content, pellet mass for each wash, total protein, purity (DNA, protein, lipid), SDS-PAGE profile, and percentage of monomer measurement (GF, HPLC, native SDS-PAGE), were studied.
- Optimization of the protein content is provided as surface plots to illustrate the process capability within the design space.

5.9. Design Space Identification

Simulations were performed in MATLAB using the RSM model from the factors in DOE #2. The worst-case protein content was set to 0.77, and the sweet spot plot was then used to visualize the resulting design space based on the model. The area is encapsulated with the relaxed boxlike space, which is given by the following vertices:

	X1 Pressure (Psi)	X2 Pass #	X3 Cooling (min)	X4 Duration (min)
min	9,000	0	1	3

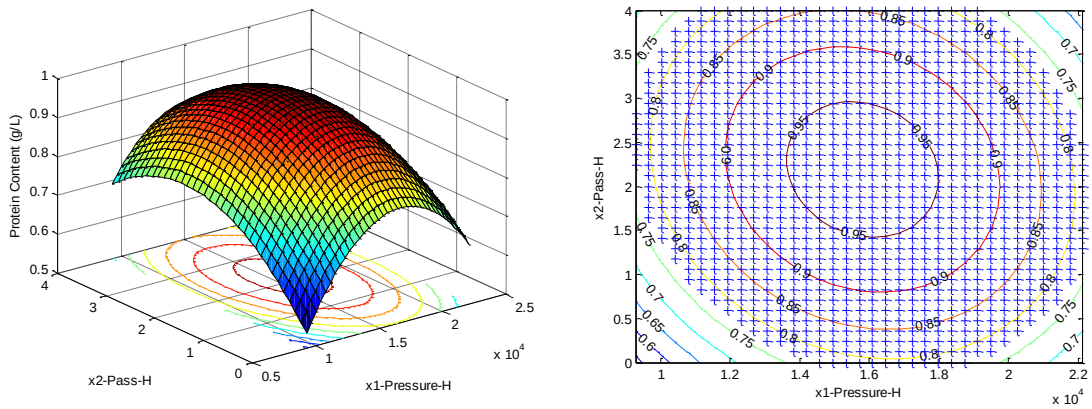
max	22,000	4	20	18

It should be pointed out that with the relaxed space, some combinations when selected will result in protein content lower than the worst-case value of 0.77. Also, to be close to the optimum operations, the control space should be strictly inside the space represented by the sweet spot plot because the boundary itself is associated with uncertainty resulting from model errors (i.e., close to the 0.77 boundary).

The surface response profiles and sweet spot plots are shown in the following figures for all binary combinations. In these plots, perturbations were made around the optimal point obtained from maximizing the protein content based on the RSM model. The maximum protein content obtained is 0.97 (according to the model) with the following optimum conditions: X1 = 15000, X2 = 2, X3 = 12, and X4 = 10.

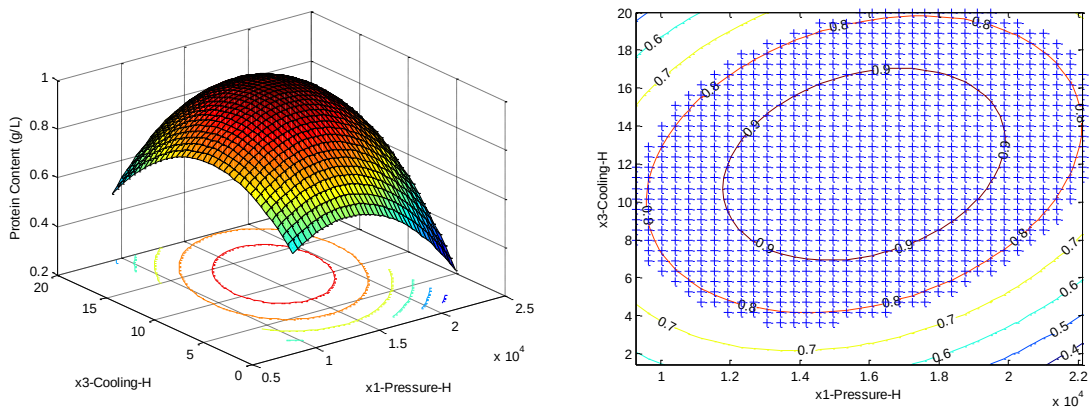
DOE #3 will be designed to confirm the model and assess the noise in the control space. Based on analysis of the contour plot, DOE #3 will be repeat runs with Pressure – Homogenization (15,000), Pass No. – Homogenization (2), Cooling – Homogenization (12), and Duration – Solubilization (10).

Figure 5-7: Surface Response Profile and Sweet Spot Plot (Pressure and Pass Number)



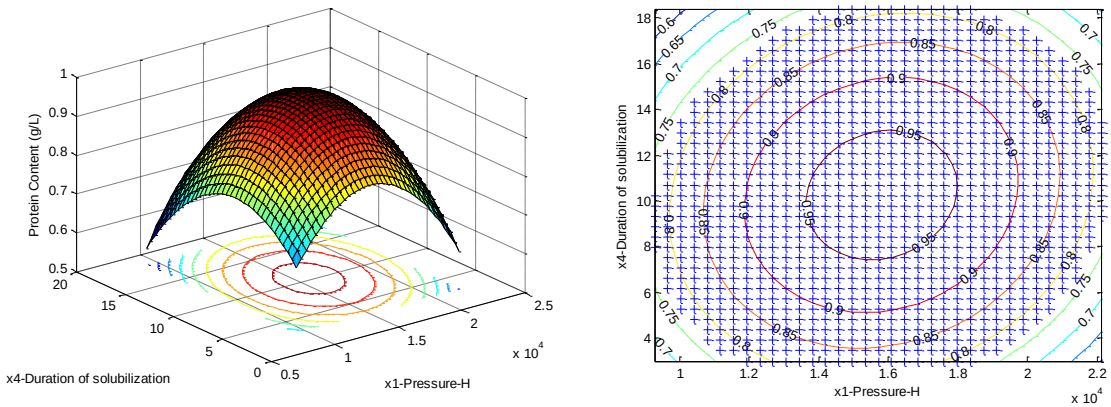
Surface response profiles and the contour plot with sweet spot area (+) for the binary interaction between the **pressure and pass number.** The optimum is inside the operating range.

Figure 5-8: Surface Response Profile and Sweet Spot Plot (Pressure and Cooling)



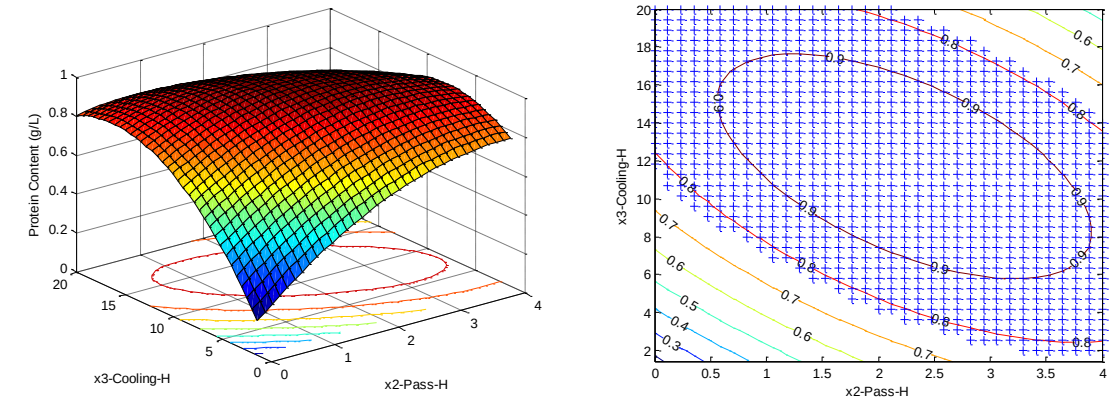
Surface response profiles and the contour plot with sweet spot area (+) for the binary interaction between the **pressure and cooling**. The optimum is inside the operating range.

Figure 5-9: Surface Response Profile and Sweet Spot Plot (Pressure and Solubilization Duration)



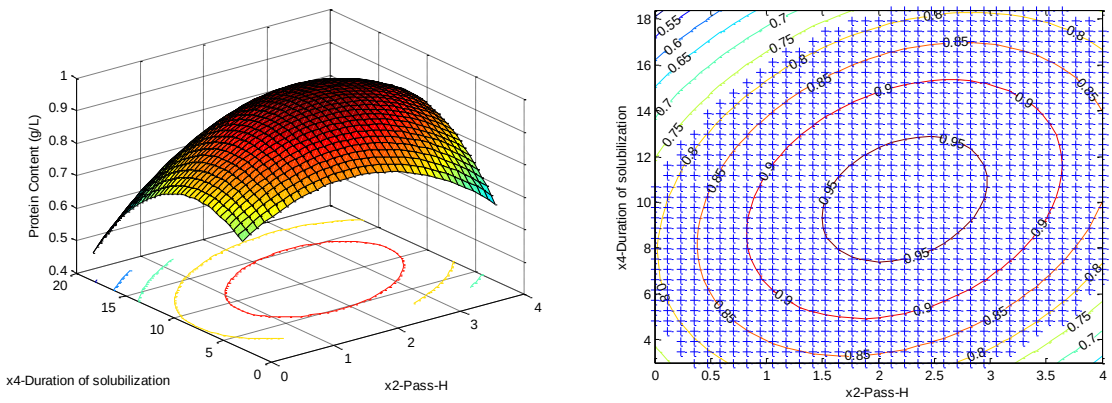
Surface response profiles and the contour plot with sweet spot area (+) for the binary interaction between the **pressure and solubilization duration**. The optimum is inside the operating range.

Figure 5-10: Surface Response Profile and Sweet Spot Plot (Pass Number and Cooling Time)



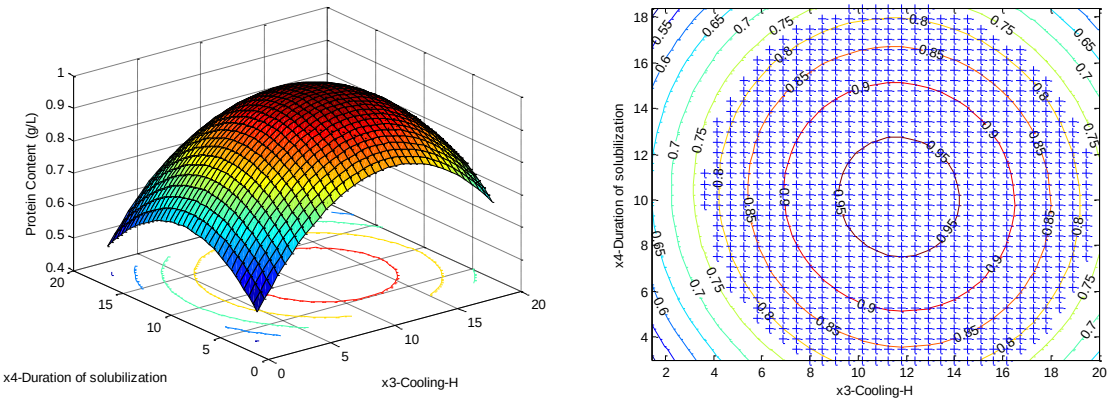
Surface response profiles and the contour plot with sweet spot area (+) for the binary interaction between the **pass number and cooling time**. The optimum is inside the operating range.

Figure 5-11: Surface Response Profile and Sweet Spot Plot (Pass Number and Solubilization Duration)



Surface response profiles and the contour plot with sweet spot area (+) for the binary interaction between the **pass number and solubilization** duration. The optimum is inside the operating range.

Figure 5-12: Surface Response Profile and Sweet Spot Plot (Cooling Time and Solubilization Duration)



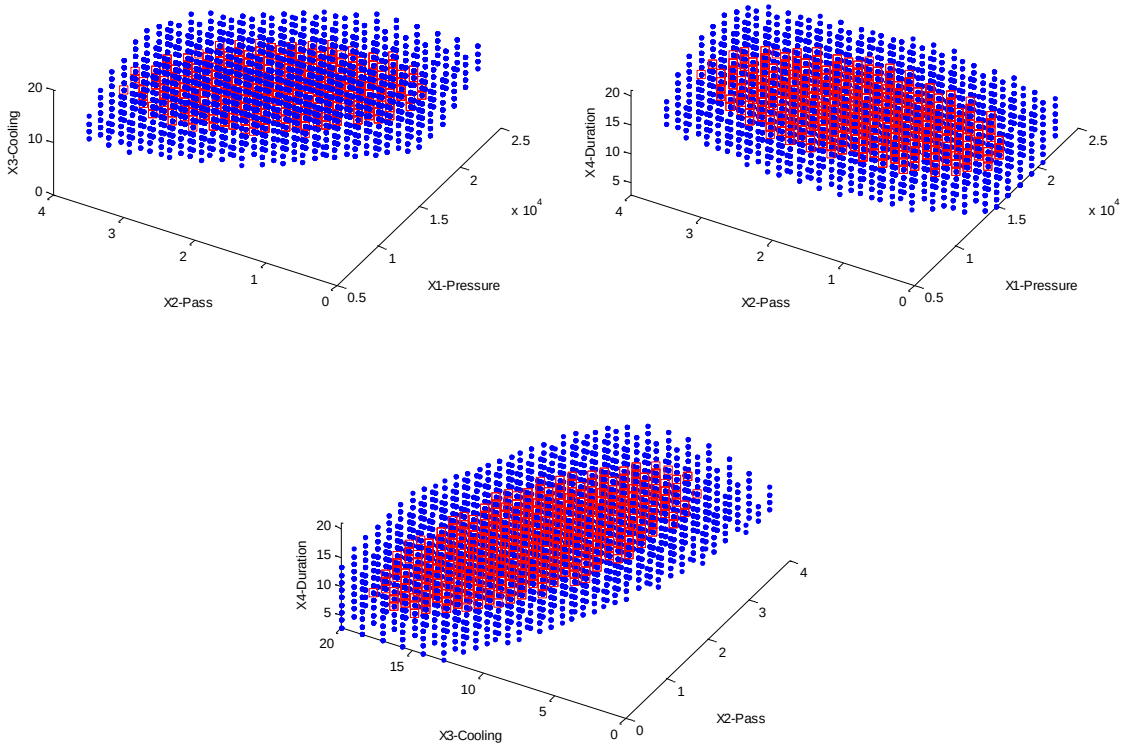
Surface response profiles and the contour plot with sweet spot area (+) for the binary interaction between the cooling time and solubilization duration. The optimum is inside the operating range.

5.9.1.1. Multivariate Interactions

To illustrate the multivariate interactions, 3D projections of all parameter combinations within the investigated space are shown below. Two space sets are shown. The wider range set represents the set corresponding to protein content better than or equal to 0.77**,** whereas the red square area represents a tighter space set that would result in a protein content better than or equal to 0.9.

The two sets are placed inside the investigated space. The RSM model was used to extract the two sets, and a design space based on the tighter space of 0.9 protein content is expected to have a more robust operation (one can extract a relaxed boxlike range around this tight space).

Figure 5-13: Multivariate Interactions



These are 3D projections of the multidimensional interaction space. The investigated space is represented by the entire axes range (white area), the 0.77-bounded space (+), and the 0.9-bounded space (□)

5.10. Summary of Criticality of E. coli VLP – Primary Recovery Step

From DOE #2 and the prior knowledge assessment shown in section 5.4, criticality of each parameter has been assessed as shown in Table 5-5.

Table 5-5: Summary of Criticality of E. coli VLP – Primary Recovery Step

Parameter	Current Target	Control Range	Criticality
Pass No. – Homogenization	2 times	1-3 times	CPP
Cooling – Homogenization	12 mins	7-16 mins	CPP
Number of Washes	2x	1-4 times	KPP
Urea Conc. – Washes	3M	1-5 M	KPP
Pressure	15,000psi	10,000-19,000 psi	CPP
Duration – Centrifugation	30 min	10-60 mins	Non-KPP
Temp. – Centrifugation	8	4-24 °C	Non-KPP
Urea Conc. – Solubilization	8	5-10 M	KPP
pH Solubilization	6	5-10	Non-KPP
Reducing Agent (ex. L-cys, DTT) – Solubilization	L-cys	DTT, L-cys	Non-KPP
Reducing Agent Conc. – Solubilization	10	0.5-50 mM	KPP
Duration – Solubilization	10 hrs	5-14 hrs	CPP

5.11. DOE #3: Model Verification at Target Conditions of the Control Space (Full-Scale Model [e.g., 20L Fermentor])

DOE #3 is a confirmation design from the analysis in DOE #2. The factors Pressure – Homogenization (15,000), Pass No. – Homogenization (2), Cooling – Homogenization (12), and Duration – Solubilization (10) were repeated for 16 runs.

5.11.1. Analysis of the Full Factorial Design

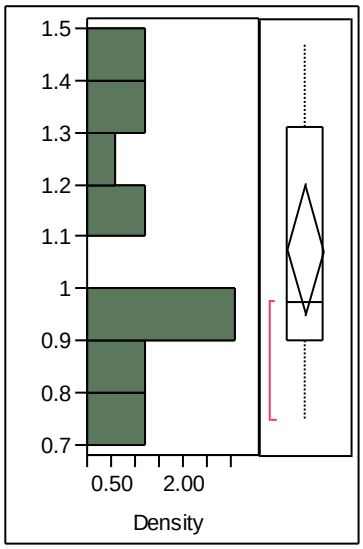
Run #	Pressure – Homogenization (psi)	Pass No. – Homogenization	Cooling – Homogenization (min)	Duration – Solubilization (min)	Specific Activity by ELISA -Protein Content
1	15000	2	12	10	0.86
2	15000	2	12	10	0.79
3	15000	2	12	10	0.89
4	15000	2	12	10	1.25
5	15000	2	12	10	1.14
6	15000	2	12	10	0.94
7	15000	2	12	10	0.94
8	15000	2	12	10	0.98
9	15000	2	12	10	0.95
10	15000	2	12	10	0.75
11	15000	2	12	10	0.97
12	15000	2	12	10	1.39
13	15000	2	12	10	1.15
14	15000	2	12	10	1.47
15	15000	2	12	10	1.42
16	15000	2	12	10	1.33

5.11.1.1. Error Estimation Attributable to Noise from the Control Space Analysis

The error estimate from the responses obtained from the repeated runs in DOE #3 is attributable to noise from the control space analysis. From DOE #3, the error estimate is calculated to be about 0.23. This means that the expected protein content value of 1.0 could lie anywhere between 0.77 and 1.23.

Distributions

Protein content



Moments	
Mean	1.07625
Std Dev	0.2336914
Std Err Mean	0.0584228
upper 95% Mean	1.2007754
lower 95% Mean	0.9517246
N	16

Based on the error estimation of the validated runs at 20L, subsequent scale-up scenarios by any factor should factor in this noise in assessing protein content limits at the primary recovery step. This means that the robustness of the yield recoveries should be expected to fluctuate around the error estimate since the repeated runs have shown some fluctuations of the yield recoveries under the same conditions.

5.11.1.2. Control Charts of the Responses from the Validation/Verification at Target Conditions for Routine Manufacturing

Figure 5-14: Control Chart – Individual Measurement of Protein Content (DOE #3)

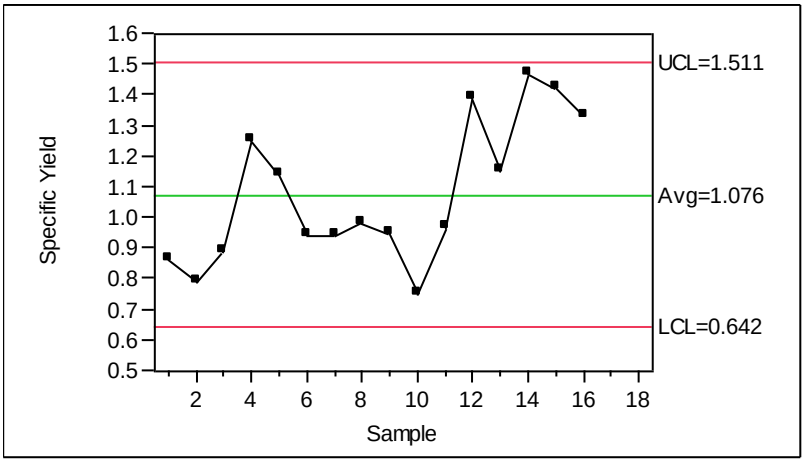
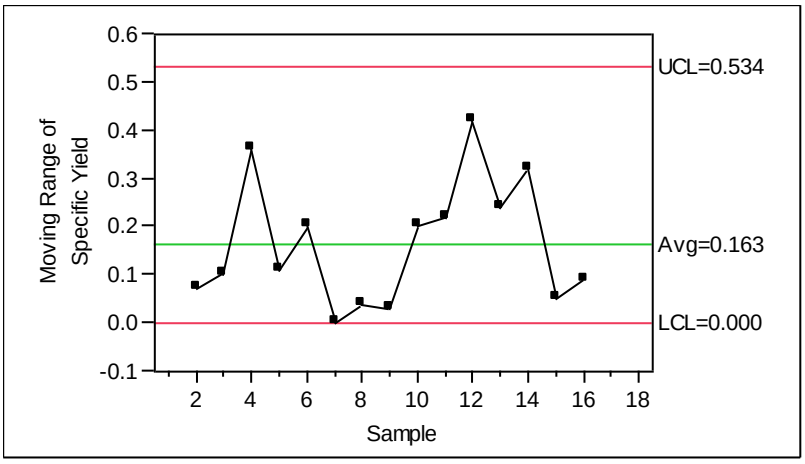


Figure 5-15: Moving Range of Protein Content (DOE #3)



5.11.1.3. Conclusions from DOE #3

Analysis of DOE #3 showed the following:

- The level settings of the input parameters for Pressure – Homogenization at 15,000, Pass No. – Homogenization at 2, Cooling – Homogenization at 8, and Duration – Solubilization at 7 are capable of obtaining a protein content response of 1.0. The error estimate from the control space analysis should be factored in, however.
- DOE #3 was also capable of estimating the error in the control space because of the 16 repeated runs. The degrees of freedom df for center points alone was 15 (n-1).
- The control charts of the model validation runs show that responses from the model are stable (range chart) and the individual measurements are in control, with the common cause of variation attributable to noise in the control space.
- The upper and lower limits will be used as the protein content specs at the end of the primary recovery step.
- The limits of the protein content values were used to drive the design space of the in-process parameters.

5.12. Post Validation

After completion of manufacturing process validation, additional changes may still be introduced during commercial production. Thus, an ongoing program should be established to collect and analyze product and process data that relate to product quality and to ensure the process remains in the validated state.

When a change is observed, it will be evaluated to determine if it results in changes outside the validated range of critical process parameters and/or quality attributes. If the change is within the validated range, no additional action is deemed necessary, other than conducting continued monitoring and trending analysis both of critical process parameters and quality attributes according to the established procedures. If the change falls outside the validated range but within the design space, a risk assessment-based approach (FMEA) will be undertaken. In this section, we use potential changes during the urea wash step as an example to illustrate the risk assessment and post-validation plans.

Urea is obtained as a raw material and is used at two steps during harvest. It is prepared, used as 3M solution to wash the VLP protein-containing inclusion bodies, and then used as 8M solution to solubilize the VLP proteins. When the solution is prepared at an incorrect concentration, it can prolong the VLP protein solubilization time; this can impact the performance of the validated process and subsequently affect the quality of the harvest protein such as its protein content, pellet mass, purity, and proportion of monomers. These quality attributes have been determined to impact the final purified drug substance.

When a change of urea is noticed, we shall go through the above two-step analysis. If key performance attributes of the harvest step are within the validated range, no actions will be taken other than continuing monitoring and trending analysis according to the validated procedures. If the performance attributes are observed to be outside the validated range, a root-cause investigation will be conducted, which may lead to re-optimizing the individual process step. In such a case, a new DOE may be required to confirm the impact of the change.

6. Downstream Section

6.1. Executive Summary

The “Downstream” manufacturing process development section comprises three parts. The first two cover the purification of the polysaccharides and virus-like particles (VLPs) produced by the upstream processes, and the third part addresses the conjugation of the polysaccharides and VLPs.

These processes are “platform-like” in that a common set of unit operations (i.e. process steps) can typically be employed to purify polysaccharides and VLPs and conjugate them. Therefore, experience with similar processes and products supplies knowledge to guide downstream manufacturing development. However, the processes are not truly “platform” because of differences specific to the polysaccharides and VLPs involved, which may require unique bioprocess conditions.

As with the “Upstream” section, the “Downstream” section will use select unit operations for the three parts to illustrate how Quality by Design (QbD) principles can be applied to vaccine process development. For conciseness, not all data mentioned as part of the examples are shown, but these data would be available at the time of license application.

The three parts of the “Downstream” section, polysaccharide (Ps) purification, VLP purification, and Ps-VLP conjugation, encompass: (1) a description of the overall process with an explanation for the selection of the representative process step used as an example; (2) a summary of prior process knowledge, an initial process risk assessment, and early stage process development for each representative process step; (3) a late development stage process risk assessment followed by (4) the development of a design space; and (5) a description of a post-licensure process change.

6.1.1. Key Points from Downstream Section

1. Multiple approaches to conducting risk assessments are applicable for evaluating vaccine processes.
2. Defining a design space ensures robust process operation.
3. Enhanced process understanding of linkages between process parameters and the vaccine's quality attributes and process performance is possible.
4. Post-licensure changes benefit from a defined design space and enhanced process knowledge through use of QbD development.

6.1.2. QbD Elements for Vaccine Downstream Processes

This section of the case study summarizes how process development can be performed using different approaches to specific unit operations to define downstream manufacturing process steps based on principles of Quality by Design. The "Downstream" section includes exemplification of the following QbD principles:

1. Prior knowledge for process scale-up and mixing during process steps impacts the QbD approach used, from risk assessment to optimal use of scale-down models.
2. Risk assessments identify process parameters to evaluate impact on quality attributes and process performance through experimentation.
3. Prior process knowledge is used to determine process parameter ranges for process evaluations.
4. Prioritized and focused experimental efforts supply the data to define the design space based on (1) critical quality assurance (QA); (2) mandatory process performance attributes; and (3) high-risk process parameters (i.e., multivariate design of experiment setup for high-criticality QA/process attributes and high-risk process parameters and OFAT [one factor at a time] for less critical parameters).
5. Integrated models from multivariate and univariate experiments define a design space that optimizes process performance and ensures product quality.
6. Scale-down process models are confirmed to be applicable to full-scale performance.
7. Continuous improvement can provide further understanding and optimization of the process.

6.2. Polysaccharide Process Description

6.2.1. Process Overview

The capsular polysaccharide is purified from inactivated fermentation broth after enzymatic extraction to release the Ps into the medium. Purification consists of a combination of precipitation, chromatographic, enzymatic, and ultrafiltration steps. The purified Ps is finally converted into a powder and stored at -70°C before conjugation to the VLP.

The downstream process flowsheet and the purpose of each step are summarized in Table 6-1.

6.2.2. Unit Operation Selected: Enzymatic Extraction

The enzymatic extraction step was selected as the Ps purification step to illustrate vaccine process development using QbD. For the sake of conciseness, other process steps were not addressed.

Step description

X. horrificus capsular polysaccharide is released in the medium by enzymatic treatment using horrificase, a specific endopeptidase that cleaves the peptide cross bridges found in *X. horrificus* peptidoglycans.

- Horrificase is a commercial, nonrecombinant enzyme purified from the bacterium *X. lyticus,* a species closely related to *X. horrificus.*
- After inactivation, X. horrificus culture is adjusted at pH 8.4 with 1M NaOH and treated with horrificase (100 U/ml) for 12 hours at 35°C under agitation in a stainless tank with marine impeller.
- The resulting extract is filtered on a composite filter and the capsular polysaccharide is recovered in the filtrate, which is further processed by precipitation.

Rationale for selecting the extraction step as an example

- Extraction conditions may impact several critical quality attributes (CQAs) and key process attributes (KPAs) such as residual peptidoglycan content, Ps size, O-acetyl content, step yield, and filterability of the extract. On the basis of prior knowledge, the optimal operating range of the enzyme may impact Ps stability in terms of size and O-acetyl content. It can therefore be anticipated that optimizing all the attributes simultaneously will require a trade-off, which further reinforces the added value of using a DOE approach.
- Uncontrolled sources of noise/**Error! Not a valid link.**variability arise at two levels:
  - Extraction is performed on a complex mixture subject to biological variability (fermentation broth).
  - The enzyme itself is a biological raw material. Background information on the stability and consistency of the enzyme is very limited since it is being used in an industrial process for the first time; there is no platform knowledge.
- Assessing the impact of extraction parameters requires further processing all the way to the last Ps purification step for some CQAs (ex: Ps size cannot be measured accurately on the extract). This feature is typical of vaccines, especially when the process steps are far upstream of the purified active ingredient.
- The quality of the extract can impact unit operations across several steps downstream in the Ps process. For example, extraction conditions leading to a small Ps size could impact the recovery at the ultrafiltration step (Ps leakage into the permeate). At the other extreme, suboptimal enzyme activity could result in large peptidoglycan fragments that will no longer be eliminated at the ultrafiltration step and will be poorly separated from the Ps in the subsequent size exclusion chromatography.

Subset of CQAs and KPAs used in example

Enzymatic extraction conditions most likely impact the following subset of CQAs and KPAs that will therefore be considered in the example (other CQAs and KPAs are not addressed for the sake of conciseness):

CQAs

- Residual peptidoglycan content, because peptidoglycan is the substrate of horrificase. Peptidoglycans are assayed by H-NMR or HPAEC-PAD on purified Ps. Note that this Ps attribute was not considered as a CQA in the “TPP-CQA” section. It was assigned a borderline severity score of 24 and was classified as LCQA (see “TPP-CQA” section XX) after the design space was defined.

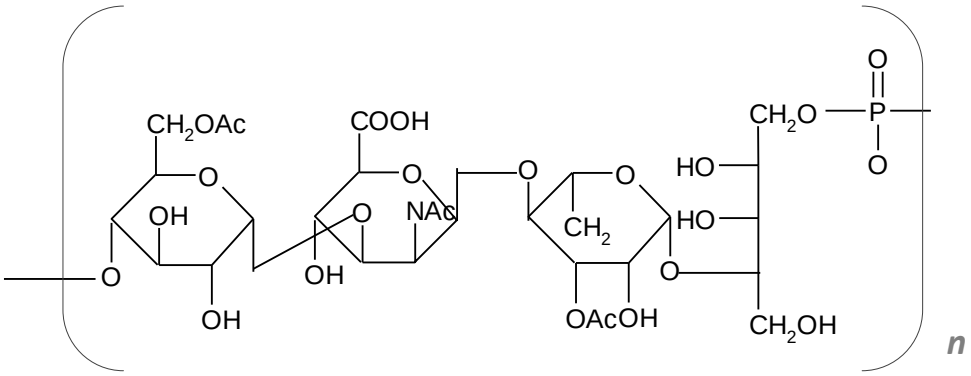
- Ps size, because all five Ps serotypes contain a phosphodiester bond that is prone to hydrolysis in alkaline conditions (extraction performed at pH 8.4 at 35°C). Size distribution is determined by HPSEC-MALLS on purified Ps.
- Ps structure (O-acetyl content), because de-O-acetylation could occur in the extraction conditions. O-acetyl content is assayed by H-NMR on the crude extract and on the purified Ps. The Ps structure is shown in Figure 6-1. The MW of the repetitive unit = 1530 g.mol-1 (without the counter-ion).

KPAs

- Extraction yield, because it is directly related to peptidoglycan digestion. Ps is quantified by HPAEC-PAD or ELISA.
- Filterability after extraction, because insufficient cell wall digestion leads to filter clogging. Filterability is assessed on small-scale filters in conditions that are qualified as representative of the large-scale process.

Figure 6-1: *X. horrificus* serotype 2 capsular polysaccharide structure

→4)-α-D-Glc*p*(6OAc)-(1→3)-β-D-ManNAcA-(1→4)-α-L-Rha*p*(3OAc)-(1→2)-D-Ribitol(5–P-(O→



2831 **Table 6-1: *X. horrificus* serotype 2 Ps flowsheet and objectives of the different steps**

Fermentation harvest	
↓	
Inactivation – 30 min at 65°C **24 h at room temperature**	
↓	
Transfer to downstream	
↓	
Enzymatic extraction with horrificase 100 U/ml – pH 8.4 – 12 h at 35°C	→ Releases Ps in the medium by cleaving peptidoglycan peptide cross-bridges
↓	
Clarification on composite filter	→ Removes cells and cell debris
↓	
Fractionated ethanol/$CaCl_2$ precipitation 25–75% – 10 mM $CaCl_2$ *(75% pellet resuspended in AEX buffer)*	→ Removes proteins, nucleic acids, peptidoglycans, and medium components
↓	
Anion exchange chromatography Elution with NaCl stepwise gradient	→ Removes proteins, nucleic acids, peptidoglycans, and horrificase
↓	
DNAse – RNAse treatment 100 U/ml – 3 h at 25°C	→ Digests residual nucleic acids
↓	
Ultrafiltration 100kD Concentration + Diafiltration	→ Removes digested nucleic acids, proteins, peptidoglycans, teichoic acids, and DNAse-RNAse → Concentrates for subsequent SEC
↓	
Size exclusion chromatography	→ Removes teichoic acids
↓	
0.22µm filtration	→ Controls bioburden
↓	
Lyophilization	
↓	
Freezing at -70°C	
↓	
Bulk polysaccharide	

6.3. Polysaccharide Extraction Early Process Development

6.3.1. Prior Knowledge

Most steps of the *X. horrificus* Ps purification process (ethanol precipitation, anion exchange chromatography, size exclusion chromatography, and nucleic acid digestion) have been used extensively in the manufacture of other bacterial polysaccharides and will not be further described here. Manufacture of *X. horrificus* capsular Ps requires enzymatic extraction or release, unlike other capsular polysaccharides that are spontaneously liberated into the medium upon bacterial inactivation. This enzymatic extraction is being used for the first time at an industrial scale. Early process development exploited prior knowledge gained from the following sources:

Literature: Six publications describe *X. horrificus* Ps extraction using horrificase. The operating ranges described in these articles are listed below:

- enzyme concentration 50 to 150 U/ml
- temperature 32 to 37°C
- pH 8.0 to 8.8
- duration 6 to 24 h

One of the papers also mentions that horrificase starts to denature at 38°C.

The horrificase enzyme manufacturer: The manufacturer specifies the optimal reaction conditions (based on a standardized assay using purified peptidoglycans). The manufacturer also stipulates that the enzyme should not be exposed to temperatures above 38°C.

Condition	Optimal (*)	Effective (**)
pH	8.4	8.0–8.8
Temperature	36°C	26°C–38°C

(*) operating range in which horrificase retains ≥ 90% of its activity in a standardized assay
(**) operating range in which horrificase retains ≥ 25% of its activity in a standardized assay

Polysaccharide structure: All five *X. horrificus* serotypes contain a labile phosphodiester bond that renders them prone to hydrolysis in mild alkaline conditions, especially at temperatures above 35°C–38°C (i.e., in conditions that are most suitable for horrificase activity). Therefore, stability data generated on purified polysaccharides in different pH and temperature conditions were used to define the testing ranges during early development and for the robustness DOE.

Other serotypes: Prior knowledge accumulated during development of the first serotype was leveraged to develop the others. For conciseness, only one serotype is discussed in this example.

6.3.2. Early Process Development

Prior knowledge gained from the different sources described above was used to set up extraction conditions for Phase 1 and 2 batches. Development proceeded in two steps:

- The time-course of extraction was studied at lab scale (0.5 L) at two pH levels and two temperatures at a fixed enzyme concentration of 100 U/ml. The reaction was followed using two readouts: the Ps extraction yield as determined by HPAEC-PAD and filterability of the extract. All conditions were tested on three different fermentation broths. The results were used to select four candidate conditions according to the following criteria: (1) maximum yield and (2) filterability of the extracts.
- The four sets of extraction parameters were tested at Phase 1 and 2 scale (15 L), and the complete purification process was performed on the resulting extracts. Data obtained on the purified polysaccharides are presented in Table 6-2. Ps size and O-Ac content met the criteria and were fairly consistent in all four conditions. Residual peptidoglycan appears as the most impacted CQA; therefore, it was used as the criterion to identify the reference conditions of 12 h treatment at pH 8.4 and 35°C because it lead to the lowest residual peptidoglycan content in the purified Ps.
- The other CQAs and KPAs were met for all four conditions. Although values for the residual peptidoglycan CQA were also within target for two other conditions, they were close to the limit and these conditions were deemed borderline, especially owing to the limited process knowledge at this early development stage.

Table 6-2: Results Obtained on Purified Ps Produced at 15 L Scale Using the Four Candidate Conditions Selected from the Extraction Time-Course Study

pH	Temperature	Resid. PG (%)	Mean MW (kD)	O-Ac (mol/mol)
8.0	30°C	3.3	211 kD	1.85
	35°C	1.7	236 kD	1.72
8.4	30°C	1.8	208 kD	2.09
	35°C	0.7	187 kD	1.94
Target		*≤2%*	*150–300 kD*	*≥1.6*

Reference conditions determined during early development and applied to Phase 1 and 2 batches

Enzyme concentration	100 U/ml
Temperature	35°C
pH	8.4

6.4. Polysaccharide Extraction Early Process Risk Assessment

A risk assessment approach is a useful way to categorize process variables and determine those that have an impact on product quality and process performance. This approach allows identification of parameters that require additional multivariate evaluation, those whose ranges can be supported by simpler univariate studies, and those that do not require additional experimental study but instead are supported by existing knowledge.

A variety of tools are suitable for risk assessment analysis. They can be broadly grouped into two categories: (1) basic tools including diagrammatic analysis, encompassing flowcharts, check sheets, process maps, and cause-and-effect diagrams; and (2) advanced tools including Fault Tree Analysis (FTA), hazard operability analysis (HAZOP), hazards analysis and critical control points (HACCP), and failure modes and effects analysis (FMEA). There is no single best choice among the available risk tools, but the methodology choice should be based on the complexity of the risk, depth of analysis required, and familiarity with the available tools. During early process development, basic tools such as risk rank and filtering and cause-and-effect analysis are generally adequate to differentiate parameters requiring multivariate or univariate evaluation. As the process matures and more process knowledge is available, a more sophisticated analysis is required to assess process risk (e.g., HACCP, FMEA).

A risk rank and filtering tool was used to screen the polysaccharide extraction parameters. The risk rank and filtering methodology classifies process variables based on their potential impact on quality and performance attributes. In addition to estimating the impact of individual process parameters, the method also assesses the potential interactive effects of multiple process parameters. This type of analysis is particularly useful in assessing situations where the risks and underlying consequences are diverse and difficult to characterize.

Risk Rank and Filtering

For the risk ranking and filtering analysis, a desired manufacturing range was identified for each process parameter and the impact on the presumptive CQAs (main effect) was measured over the parameter range. Any potential effect on other process parameters (interactive effect) was also assessed over the same parameter range.

The rankings for CQA impact (main effect and interaction effect) were weighted more severely than the impact to low-criticality quality attributes (LCQAs) or process attributes and Table 6-4). If no data or rationale were available to make an assessment, the parameter was ranked at the highest level.

2929 **Table 6-3: Impact Assessment of Attributes: Main Effect Ranking**

Impact Description	Impact Definition	Main Effect Ranking Based on Impact on Attributes	
		Critical Quality Attribute (CQA)	**Low-Criticality Quality Attribute or Process Attribute**
No Impact	Parameter is not expected to impact attribute – impact not detectable	1	1
Minor Impact	Expected parameter impact on attribute is within acceptable range	4	2
Major Impact	Expected parameter impact on attribute is outside acceptable range	8	4

2930
2931 **Table 6-4: Impact Assessment of Attributes: Interaction Effect Ranking**

Impact Description	Impact Definition	Interaction Effect Ranking Based on Impact on Attributes	
		Critical Quality Attribute (CQA)	**Low-Criticality Quality Attribute or Process Attribute**
No Impact	No parameter interaction; not expected to impact attribute – impact not detectable	1	1
Minor Impact	Expected parameter interaction; impact on attribute is within acceptable range	4	2
Major Impact	Expected parameter interaction; impact on attribute is outside acceptable range	8	4

2932
2933 Severity scores (Table 6-5)were determined by multiplying the potential for a parameter to impact a
2934 CQA or process attribute (main effect) by the potential of a parameter to impact a CQA or process
2935 attribute via interaction with another parameter (interaction effect). Only the largest main effect score

(either CQA or process attribute) was multiplied with the largest interaction score (either CQA or process attribute).

Severity score = Main effect x interaction effect

The severity score provided the basis for determining whether process parameters required additional multivariate or univariate analysis or whether prior knowledge provided adequate characterization of the parameters. This assessment was used to rank parameters within individual unit operations. No attempt was made to estimate interactive effects of parameters across multiple unit operations.

Table 6-5: Severity Score as a Function of Main and Interactive Rankings

		Main Effect Ranking			
		1	**2**	**3**	**4**
Interaction Effect Ranking	**8**	8	16	32	64
	4	4	8	16	32
	2	2	4	8	16
	1	1	2	4	8

Severity scores were ranked from a minimum of 1 to a maximum of 64. Categorization of severity scores into those requiring multivariate analysis, univariate analysis, or no additional studies was based on the following principles (Table 6-6). Severity scores that exceeded 32 represent the cumulative combination of parameters where minimally one parameter (main or interactive) was ranked to have a major impact on CQAs or process performance attributes (i.e., parameter impact outside the acceptable range of the CQA). Because of this risk, additional multivariate studies to more accurately characterize the design space are recommended.

Severity scores between 8 and 16 generally involve a combination of parameters that are expected to have a minor impact on CQAs or process performance attributes (i.e., impact of the parameters on CQAs is within an acceptable range). These parameters could be further evaluated by either multivariate or univariate studies, depending on prior knowledge or experience with these parameters.

Severity scores that are less than 4 are the result of a combination of parameters that are not expected to have a measurable impact on CQAs or process performance attributes. Simple univariate studies or in some instances the use of prior knowledge is often adequate to characterize these parameters.

Table 6-6: Severity Classification

Severity Score	Experimental Strategy
≥ 32	Multivariate study
8-16	Multivariate, or univariate with justification
4	Univariate acceptable
≤ 2	No additional study required

The process parameters evaluated by the risk ranking and filtering tool for this example (Table 6-7) were identified from prior knowledge (see Section 6.3.1), including experience with similar enzyme extractions. Otherwise, approaches such as those shown in the "Upstream" chapter (Section 5) would be used to identify the process parameters for the risk assessment.

970 **Table 6-7: Severity Scores**

	Testing Range		Rationale for Testing Range		Main Effect Rank[a]		Rationale for Main Effect Rank	Interaction Effect Rank[b]		Potential Interaction Parameters	Rationale for Interaction Rank	Severity Score (M x I)	Recommended Studies Based on Severity Score
Parameter	**Low**	**High**	**Low**	**High**	**CQA**	**KPA**		**CQA**	**KPA**				
pH (Reaction)	8.0	8.8	Insufficient pglycan clearance; Ps size distribution; low Ps yield and filterability	Insufficient pglycan clearance; Ps size distribution and O-acetyl content; low Ps yield and filterability	8	4	Reaction characterized by narrow pH optimum; Ps is prone to hydrolysis and de-O-acetylation in alkaline conditions	4	4	Enzyme conc., polysaccharide concentration, pglycan conc., incubation time, incubation temperature	Moderate additive impact expected based on known relationship among pH, enzyme conc., and temperature	32	Multivariate
Enzyme Concentration	25 U/mL	200 U/mL	Insufficient pglycan clearance; low Ps yield and filterability	Insufficient pglycan clearance; low Ps yield and filterability	8	4	Conc. impacts kinetics; optimum conc. influenced by kinetics vs. cost	4	4	Pglycan conc., incubation time, incubation temperature	Moderate additive impact expected based on known relationship between pH, enzyme conc., and temp	32	Multivariate
Incubation Temperature	20°C	37°C	Insufficient pglycan clearance; Ps size distribution; low Ps yield and filterability	Insufficient pglycan clearance; Ps size distribution and O-acetyl content; low Ps yield and filterability	8	4	Strong influence on reaction kinetics; Ps is prone to hydrolysis at higher temperatures	4	4	Pglycan conc., incubation time, incubation temperature, pH	Moderate additive impact expected based on known relationship between pH, enzyme conc., substrate conc., time, and temp	32	Multivariate
Incubation Time	10 hr	14 hr	Insufficient pglycan clearance; Ps size distribution and O-acetyl content; low Ps yield and filterability	Insufficient pglycan clearance; Ps size distribution and O-acetyl content; low Ps yield and filterability	4	4	Reaction most heavily influenced by pH, enzyme concentration, and incubation temperature	4	4	Pglycan conc., incubation time, incubation temperature, pH	Weak additive impact as pH, enzyme conc. and temperature drive Pglycan hydrolysis kinetics	16	Multivariate or univariate
Enzyme Batch	NA	NA	Variability among different batches of enzyme		4	1	Variability dependent on source	1	1	Pglycan conc., incubation time, incubation temperature	Weak additive impact as batch variability is expected to be small	4	Univariate

	Testing Range		Rationale for Testing Range		Main Effect Rank[a]		Rationale for Main Effect Rank	Interaction Effect Rank[b]		Potential Interaction Parameters	Rationale for Interaction Rank	Severity Score (M x I)	Recommended Studies Based on Severity Score
Parameter	**Low**	**High**	**Low**	**High**	**CQA**	**KPA**		**CQA**	**KPA**				
Fermentation Batch	?	?	Impact on kinetics	Impact on kinetics	1	1	Little impact on quality or recovery batch; variability is expected to be small	1	1	Pglycan conc., incubation time, incubation temperature	Weak additive impact as batch variability is expected to be small	1	Utilize prior knowledge
Filtration Rate	10 L/min	25 L/min	Recovery	Recovery	1	1	Little impact on quality or recovery	1	1	None expected	NA	1	Utilize prior knowledge
Mixing Rate	40 rpm	50 rpm	Reaction kinetics	Reaction kinetics	1	1	Reaction most heavily influenced by pH, enzyme concentration, and incubation temperature	1	1	None expected	NA	1	Utilize prior knowledge

[a, b] Rank based on impact to CQAs (peptidoglycan clearance, size distribution, O-Ac content) and process performance attributes (yield, filterability).

6.5. Polysaccharide Late Stage Risk Assessment

Process development following the early stage risk assessment seeks to understand the linkages between process parameters and both CQAs and KPAs so as to define an early design space and control strategy. A late development stage risk assessment is important to focus experimentation on characterizing the process and defining those parameters that will be most important for controlling process performance and product quality. A well-accepted tool to perform such a risk assessment is FMEA.

Failure Modes and Effects Analysis (FMEA)

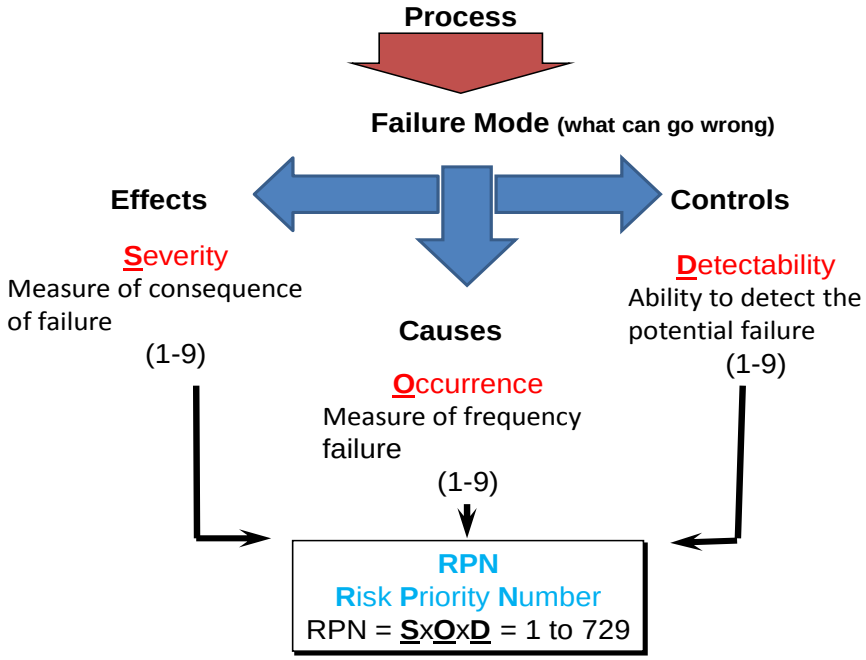
FMEA is a tool for methodically evaluating, understanding, and documenting the potential for risks to the process operation/consistency and product quality — in other words, “what can go wrong” (Figure 6-2).

- What is impacted
- How frequently the event occurs
- Detection of the event

The FMEA provides a framework for a methodical approach to evaluating, understanding, and documenting the potential for failure in a process that might pose a risk to process consistency and product quality. The FMEA is conducted by a multidisciplinary team comprising process experts familiar with process development and characterization and manufacturing site representatives with expertise in manufacturing operations, manufacturing procedures, and equipment capabilities and controls.

The application of FMEA can be throughout the product commercialization stages in an iterative approach. This allows the initial FMEA template to be developed and refined with improved process knowledge and greater understanding of manufacturing capabilities.

Figure 6-2: The FMEA Work Process



The first stage of the FMEA is to assign process parameter severity (S) scoring (Figure 6-2) based on the parameter's potential impact on quality attributes and process performance. Quality attributes specific to process intermediates, final drug substance/drug product specification, or quality targets are considered. Process performance should be focused primarily on important performance indicators (e.g., conjugation step yield). The severity assessment is conducted with the primary input from process experts using prior knowledge gained from process characterization (DOE), pilot scale, and full-scale process batches.

Note that severity scores for FMEA should be consistent with risk ranking and filtering (RR&F) or cause-and-effect (C&E) outputs. Ideally severity scores may be directly translated from RR&F or C&E, providing consistent scoring definitions were applied. Alternatively, RR&F or C&E severity output may be calibrated to fit FMEA scoring definitions

The potential severity impact should be assessed over process parameter ranges extended well beyond the normal operating range (NOR), and the ranges proposed below are supplied for team guidance. Where NORs are established, the process parameter range for severity consideration should be extended by about three times the delta of the NOR from the parameter setpoint or target. For example, with a temperature setpoint of 20°C and NOR of 20 ±1°C, the range for the severity assessment was established at 3 x, equating to 20 ±3°C (17–23°C).

In other cases where NORs are not established, a range of ±10% from the parameter setpoint or target value may be used. Using the temperature example below (Table 6-8), a range of ±10% equates to ±2°C (18–22°C).

In all cases, good scientific judgment should apply when establishing ranges for severity, and rationale should be fully documented.

Table 6-8: Process Example for Defining FMEA Severity Ranges

Temperature Setpoint	NOR	3 x NOR	±10% Setpoint
20°C	19–21°C (±1°C)	17–23°C (±3°C)	18–22°C (±2°C)

The highest severity score (9) is assigned to parameters with the greatest potential impact to product quality and process performance at the extended parameter ranges described. The scoring guidelines are listed in Table 6-9.

The second stage focuses on occurrence (O) (Figure 6-2). The scoring for occurrence (O) should focus on the likelihood of deviating beyond the specified NOR or setpoint/target for the process parameter assessed. The scoring scale is consistent with severity (1-9) with the highest occurrence assigned to parameters with the greatest likelihood of a deviation (Table 6-9). When considering occurrence, it's important to focus on common cause and not special cause events. Unexpected events (e.g., force majeure) are generally not considered. Other considerations may include prior knowledge, manufacturing history, equipment failure and human error and should be described in the FMEA worksheet.

The final stage of the FMEA is an assessment of detection (D) for detecting a potential deviation beyond the specified NOR or setpoint/target. The scoring range was consistent with scores assigned for severity and occurrence with the highest scores (7 and 9) assigned to process parameters with limited or no means of detection (Table 6-9). Considerations include equipment control capabilities, deviation alarms, and tracking procedures as described in the FMEA worksheet.

A final Risk Priority Number (RPN) number is assigned based on multiplying the scores for severity, occurrence, and detection (S x O x D) with appropriate rationales for each process parameter described. During the FMEA assessment, risk control or mitigation strategies are discussed and planned for implementation where appropriate. The RPN numbers for each unit operation are reviewed collectively and a cut off number (threshold) may be selected based on the data distribution to aid the selection of parameters for risk mitigation and/or criticality.

Table 6-9: FMEA Scoring Guidelines

Score	Severity	Occurrence	Detection
9 "HIGH risk"	Process failure potentially impacting one or more critical product quality attributes leading to product rejection	*> 20% (very frequent)*	*No way to detect excursion. Not tracked or alarmed.*
7	Potential impact on product quality or consistency (e.g., product related substances). Investigation needed prior to product release.	*~ 5-20% (frequent)*	*Difficult to detect excursion, and not until after it has impacted the process.*
5	No impact on product quality, but deviation from manufacturing procedures requires justification. Likely deterioration in process performance (e.g., yield or operability).	*~ 1-5% (occasional)*	*Excursion can be detected, but not until after it has impacted the process.*
3	No impact on product quality. Potential for minor deterioration in process performance (e.g., yield or operability).	*< 1% (rare)*	*Excursion is usually detected and corrected prior to impacting the process.*
1 "LOW risk"	No impact to product quality or process performance.	*0% (never observed)*	*Excursion is obvious and always detected prior to impacting the process.*

The impact of severity on the process and product depends on the step and proximity to the final drug substance or drug product. For example, upstream processes have few if any quality attributes; as a result, an assessment against quality targets or final release specifications is challenging. In such cases, the impact on the process step is more meaningful.

Table 6-10 and Table 6-11 describe an FMEA analysis performed to identify critical process parameters as well as potential steps to mitigate their criticality. The evaluation has been arbitrarily divided between process parameters (intrinsically related to the process) and operational parameters that are associated with the design and operation of the process in a specific manufacturing environment. Critical parameters were judged as those that exceeded an RPN value of 175. An RPN of 175 was chosen because it represented a severity that minimally impacted product quality (≥7), occurred with a minimal frequency of ≥5 (≥ 1–5%), and had a detection capability of ≥5 (excursion can be detected but not until it has impacted the process). This results in a minimal RPN score of 175. Based on this analysis, enzyme concentration was the only parameter identified as a critical process parameter.

3073 **Table 6-10: FMEA Process – Process Parameters**

Process Parameter	Operating Range	Potential Failure Mode	Potential Effect(s) of Failure	Severity	Potential Cause(s) of Failure	Occurrence	Current Controls and Prevention	Detection	Current Controls and Detection	RPN	Recommended Action
Enzyme Concentration	25–200 U/mL	Operational and equipment	• Low enzyme conc. limits pglycan digestion and decreases recovery and filterability	9	• Operator error • Balance calibration • Poor enzyme dissolution	5	• Batch record check	5	• Double sign-off on critical reagents	225	Classify as CPP, include in DOE
pH (Rxn)	8.0–8.8	Operational and equipment	• High pH results in phosphodiester cleavage and altered Ps size distribution • Low pH results in poor peptidoglycan cleavage, low Ps recovery, and poor filterability	9	• Probe failure • Calibration error	5	• pH check prior to rxn initiation • Training	3	• Automated pH output and alarming condition	135	Study in DOE
Incubation Temperature	20°–37°C	Equipment	• Low temperatures result in poor pglycan digestion and low recovery and filterability • High temperatures result in increased phosphodiester cleavage and altered Ps size distribution	9	• Equipment failure • Mixing failure • Operator error	5	• Automated temperature readout	3	• Automated readout and alarming condition	135	Study in DOE
Enzyme Batch	>100 U/g	Significant variability in specific activity among enzyme lots	• Inadequate peptidoglycan digestion results in low step yield and poor filterability	5	• Enzyme quality	1	• Specific activity assay prior to enzyme use	5	• Prequalification of enzyme lots	25	Study in OFAT
Incubation Time	10–14 h	Insufficient reaction time	• Insufficient reaction time results in poor Pglycan digestion and low recovery and filterability	7	• Operator error	3	• Batch record check	1	• Double sign-off	21	Study in OFAT

3074 **Table 6-11: FMEA Process – Operational Parameters**

Substeps	Process Parameter	Operating Range	Potential Failure Mode	Potential Effect(s) of Failure	Severity	Potential Cause(s) of Failure	Occurrence	Current Controls, Prevention	Detection	Current Controls and Detection	RPN	Recommended Action
Transfer to Reaction Vessel	Transfer Time	≤ 1 h	Operational or equipment	• Product stability	7	• Operator error • Equipment failure	3	• Batch record check	1	• Batch record recording	21	No Action Necessary
	Mass Transferred	22–26 kg	Operational or analytical	• Insufficient mass results in low step yield • Excessive mass results in high residual Pglycan, poor filterability and low yield	5	• Operator error • Equipment failure	3	• Batch record check	1	• Batch record recording	15	No Action Necessary
Raw Material Additions	Tank Tare Wt	200–210 kg	Equipment or calibration	• Incorrect reaction conditions	5	• Operator error • Equipment failure	1	• Batch record check	1	• Batch record recording	5	No Action Necessary
	Addition of Tris Base	1.5–1.7 kg	Operation or equipment	• Poor reaction kinetics and incomplete pglycan digestion	7	• Operator error • Equipment failure	3	• Batch record check	1	• Batch record recording and pH check	21	No Action Necessary
	Addition of Glycine	0.5–0.7 kg	Operation or equipment	• Poor reaction kinetics and incomplete pglycan digestion	7	• Operator error • Equipment failure	3	• Batch record check	1	• Batch record recording and pH check	21	No Action Necessary
	Addition of NaCl	0.1–0.2 kg	Operation or equipment	• Poor reaction kinetics and incomplete pglycan digestion	7	• Operator error • Equipment failure	3	• Batch record check	1	• Batch record recording	21	No Action Necessary
	Addition of Purified Water to Final Tare Wt	1,350–1,370 kg	Operation or equipment	• Poor reaction kinetics and incomplete pglycan digestion	7	• Operator error • Equipment failure	3	• Batch record check	1	• Batch record recording	21	No Action Necessary

Substeps	Process Parameter	Operating Range	Potential Failure Mode	Potential Effect(s) of Failure	Severity	Potential Cause(s) of Failure	Occurrence	Current Controls, Prevention	Detection	Current Controls and Detection	RPN	Recommended Action
	Agitation Rate	40–50 rpm	Operation or equipment	• Poor reaction kinetics and incomplete pglycan digestion	5	• Operator error • Equipment failure	3	• Batch record check	1	• Automated readout and alarming condition	15	No Action Necessary
Reaction Termination	Temperature Ramp	1 h	Operation or equipment	• Increased Ps hydrolysis	5	• Operator error • Equipment failure	3	• Batch record check	1	• Automated readout and alarming condition	15	No Action Necessary

6.6. Polysaccharide Extraction Design Space

6.6.1. Section Overview

This section describes the approach (outlined in Figure 6-3) used to define the design space for the Ps enzymatic extraction step. It comprises four subsections that can be summarized as follows:

- ***Experimental design:*** The outcome of risk assessment is combined with prior knowledge gained from different sources and from early development to establish a DOE. This DOE not only investigates the impact of critical parameters on CQAs and KPAs, but also targets process robustness.
- ***Optimization and determination of reference conditions:*** DOE results are used to create prediction models that allow understanding of factor effects and interactions. Optimal conditions are then identified using desirability functions. Reference conditions are finally optimized for robustness using overlay plots.
- ***Determination of design space:*** Based on simulations, the design space is defined using as criterion an upper limit for the simulated defect rate. Simulations within the design space are also used to gain more insight into how the different responses contribute to the predicted defect rate. Finally, this section shows how process knowledge within the design space can be advantageously combined with a simple univariate study to integrate the incubation time into the design space.
- ***Univariate studies:*** The way to study the possible impact of the enzyme batch is discussed along with the limitations linked to this specific investigation.

6.6.2. Experimental Design

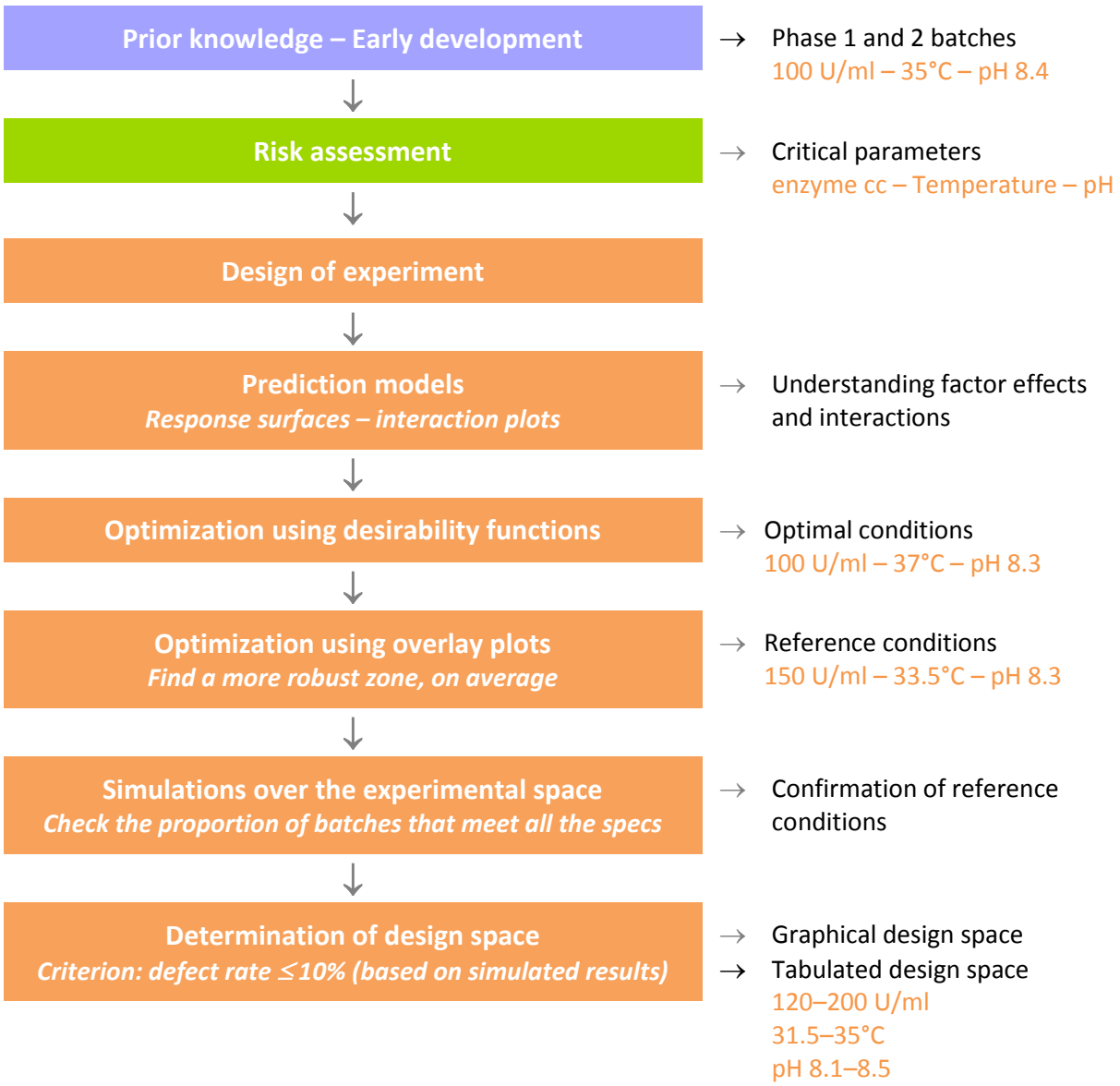
Factors to be investigated in a multivariate study

The three high-risk process parameters that were identified by risk assessment analysis (see previous section) are investigated in a multivariate study:

- pH
- enzyme concentration
- incubation temperature

The other key parameters (incubation time and enzyme batch) are investigated in univariate studies.

Figure 6-3: Overview of the Statistical Approach Applied to Define the Design Space



Type of design

At this late stage of process development, robustness is key and should be integrated into the optimization strategy. The experimental approach described in this section is therefore aimed at identifying optimal as well as robust extraction conditions. It is intended to determine the impact of process parameters on the variability of the output responses to select the combination of parameters that minimize variability while achieving the target responses.

Three approaches to robust design are commonly used: Taguchi, Dual Response, and Tolerance Analysis (compared in *Taylor, W.A (1996) Comparing three approaches to robust design: Taguchi versus Dual Response versus Tolerance Analysis, presented at 1996 Fall Technical Conference,* http://www.variation.com/anonftp/pub/ta-3.pdf). Among these, Dual Response Modeling was considered the most appropriate with respect to enzyme extraction optimization, chiefly because it is the only approach that addresses robustness versus unidentified sources of noise. Dual Response Modeling uses Response Surface Methodology (RSM): it is assumed that each studied response can be expressed as a mathematical function (second order polynomial) of the different factors investigated, thereby allowing calculation of the responses over the experimental space. The experimental structure of the Dual Response Modeling applied in the Quality by Design case study is illustrated in Figure 6-4:

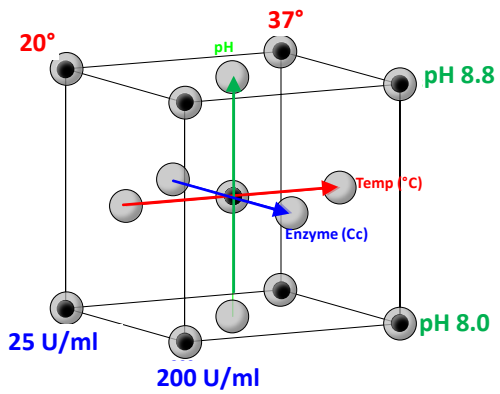
- A face-centered composite design is used; each studied factor (pH, temperature, and enzyme concentration) is tested at three levels (see table in Figure 6-4). The ranges investigated are based on early development results and prior process/product knowledge (enzyme brochure, literature data, Ps stability data) as detailed in Section 6.3.1 above. Based on this prior knowledge, a trade-off between horrificase activity and Ps stability should normally be found within these wide ranges covering both optimal enzyme operating ranges and Ps stability ranges.
- Repeats of the central point (triplicates) and of the entire factorial structure (duplicates) are performed and used to calculate the standard deviation of each response at these different places of the experimental domain (Figure 6-4). The repeats are done on different broths to account for broth-to-broth variability. The result is an economical, robust design compared to other experimental structures in which each point is repeated in duplicate or triplicate.
- The standard deviations are integrated in the model as secondary responses that will be used to optimize process robustness (minimize the impact of uncontrolled factors/noise).

Figure 6-4: Experimental Structure Selected to Optimize Extraction Parameters

Conditions marked with a black dot are repeated on different broths (duplicates at the vertices and triplicates at the central point).

Factor	High	Middle	Low
Temperature	37	28.5	20
pH	8.8	8.4	8.0
Enzyme cc	200	112.5	25

Fixed parameters
- incubation time
- enzyme batch
- mixing conditions



Design implementation

The 25 extraction conditions of the DOE were tested in random order at lab scale (starting from 0.5 L fermentation broth), and the resulting extracts were purified using a scaled-down process. Special care was taken to reproduce as closely as possible the conditions of the commercial scale process:

- All steps: carried out at the same temperature as the large-scale process.
- Vessels and agitation systems for enzymatic treatments and precipitations: same geometry, same sample volume/headspace ratio, same impeller type and impeller/vessel diameter ratio.
- Filtration steps: same sample volume/filter area ratio, scale-down factor applied to flow rate.
- Tangential flow filtration (TFF): same membranes (material, molecular weight cutoff, channel configuration, and path length), same sample volume/filter area ratio, same feed and retentate pressures, retentate flow rate proportional to scale-down factor, same sanitization procedures.
- Chromatographic steps: same sample/resin volume ratio, same bed height, same linear flow rate, buffer volumes proportional to column scale-down factor, same packing conditions and sanitization procedures.

The lab scale process was qualified as representative through comparison of process parameters, in-process data (clearance of contaminants, step yields), and Ps attributes obtained with the scaled-down process and at commercial scale.

Studied responses

The five responses that were studied to optimize the extraction conditions are discussed in Section 6.2.2. Four numerical outputs reflecting response variability are also analyzed using the standard deviations of the repeats as new outputs:

Responses (CQAs and KPAs)

- Residual peptidoglycan content (% w/w)
- Ps size (kDa)
- Ps O-acetyl content (mol/mol Ps)
- Ps extraction yield (%)
- Filterability after extraction value=1 if filterable and 0 if not filterable
  (filterability criterion: > 15 L/m² filter area)

Associated variability (SD = standard deviation)

- SD residual peptidoglycan content (% w/w)
- SD Ps size (kDa)
- SD Ps O-acetyl content (mol/mol Ps)
- SD Ps extraction yield (%)

6.6.3. Optimization and Determination of Reference Conditions

Prediction model creation

For each response, a reduced polynomial model is determined to reproduce output variation using a selection of factor effects and interactions. Based on an analysis of variance (ANOVA), factors and interactions having a ≥ 10% probability to influence the response are selected.

Analysis of response surfaces: factor effects and interactions

Using the prediction models, responses can be calculated over the entire experimental domain and represented as response surfaces to understand how the process parameters impact specific attributes and create variability in these attributes. An example of such prediction graphs is illustrated in Figure 6-5 for residual peptidoglycans. Full prediction results for the other responses can be found in the attached Excel file.

DoE Dual resp and results v2.xlsx

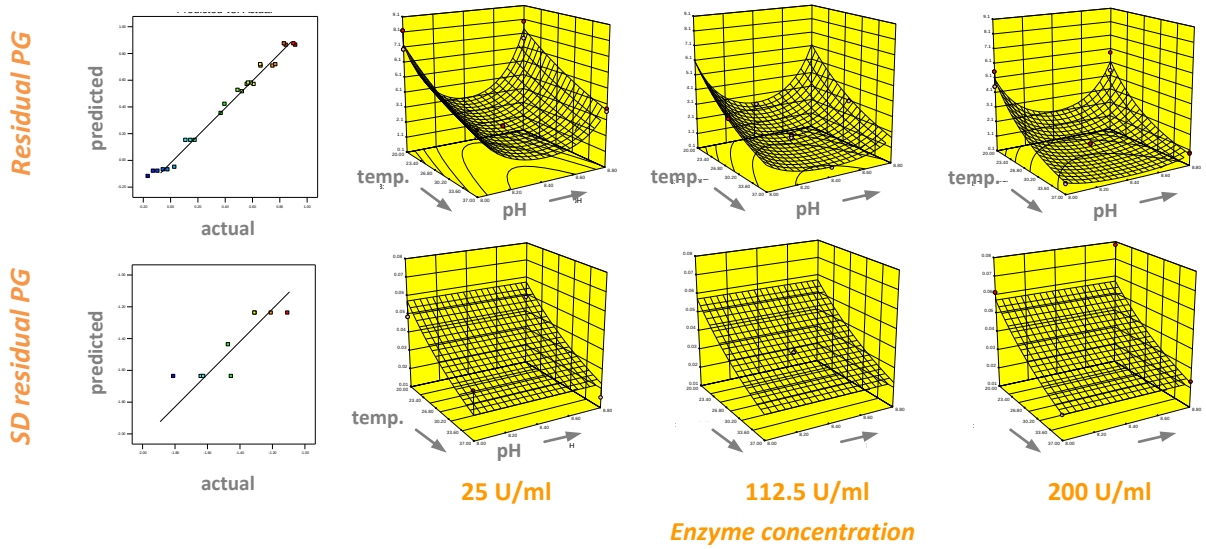
DoE Dual resp and results v2.xlsx

The response surfaces reveal that the selected product and process attributes are impacted by pH, temperature, and enzyme concentration as detailed below. The data are also used to identify factor interactions that can be best visualized on *interaction plots* as exemplified for Ps size (Figure 6-6).

Impact of process parameters on residual peptidoglycan content (Figure 6-5):

- Optimum (lowest content) at pH 8.4 reflects horrificase optimum pH.
- Improvement at higher temperatures and enzyme concentrations. The temperature effect is consistent with horrificase optimum temperature (36°C).
- Variability is higher at lower temperatures that are suboptimal for enzyme activity.

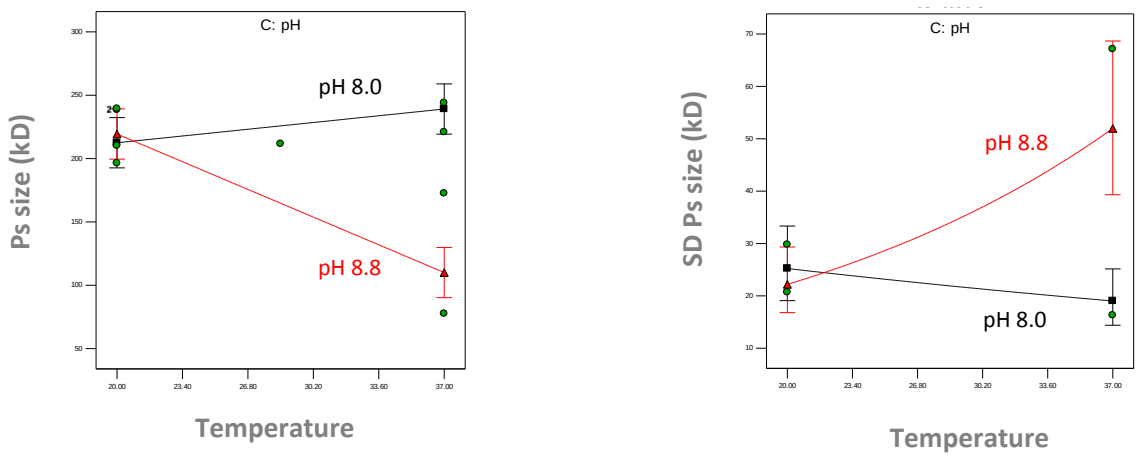
Figure 6-5: Predicted Response Surfaces of Residual Peptidoglycan (PG) Content as a Function of pH and Temperature at 3 Enzyme Concentrations



Impact of process parameters on Ps size:

- Size is fairly stable at low temperatures and pH, but decreases at higher temperatures and pH as a result of hydrolysis of the phosphodiester bond, which is prone to cleavage in alkaline conditions.
- This hydrolysis at high temperature and pH also impacts size variability.
- Interaction between pH and temperature is significant on Ps size and its associated variability, as evidenced by interaction plots (Figure 6-6).

Figure 6-6: pH-temperature Interaction Plots Show a Strong Interaction Between These Two Parameters in the Case of Ps Size and Its Associated Variability



Impact of process parameters on O-acetyl content:

- There is no impact from any of the factors over the entire experimental space. Any combination of the factors within the experimental domain leads to the expected value.

Impact of process parameters on step yield:

- Maximum yield is obtained at pH 8.4 reflecting the horrificase pH optimum.
- Yield is improved at higher temperatures, although to a lesser extent than residual peptidoglycan. The temperature effect is consistent with horrificase optimum temperature (36°C).
- Yield is improved by enzyme concentration between 25 and 112.5 U/ml.

- Variability decreases at higher enzyme concentrations.

Impact of process parameters on filterability:

- Filterability is lowest at low temperatures and pH, conditions in which horrificase is expected to be less efficient at digesting peptidoglycans and breaking the cell wall open. As a consequence, filter clogging is observed. Filterability also decreases at high temperature and pH, but in this case it is caused by a precipitate that starts to form under these conditions.
- Filterability is improved by increasing enzyme concentration to 25 - 112.5 U/ml.

Response optimization

Multi-response optimization frequently involves trade-offs: in most cases, one attribute is indeed optimized at the expense of another one. The desirability function, first introduced by Harrington in the mid-1960s (*Harrington, E.C., Jr. (1965) The Desirability Function, Industrial Quality Control **21**, 494-498*), is a widespread approach to balance multiple responses. A desirability function measures the adequacy of each response to the objective: it is defined by the developer and ranges from 0 (unacceptable response) to 1 (the response fits the objective). In this case, the objectives are defined as follows:

- Minimal residual peptidoglycan content
- Targeted molecular size of 200 kD
- O-acetyl content > 1.6 mole/mole RU
- Maximal Ps yield
- Filterable extract
- Minimal response variability
- Minimal enzyme concentration to reduce process costs

For each response, desirability is calculated over the experimental space. These desirability functions are then computed into one single desirability function (geometric mean of the individual desirabilities), which takes the entire selected product and process attributes into account and can thus be viewed as a global satisfaction index, enabling the conversion of the multi-response problem into a single response. The value of this overall desirability is 1 if all the objectives are met and 0 if at least one response is unacceptable.

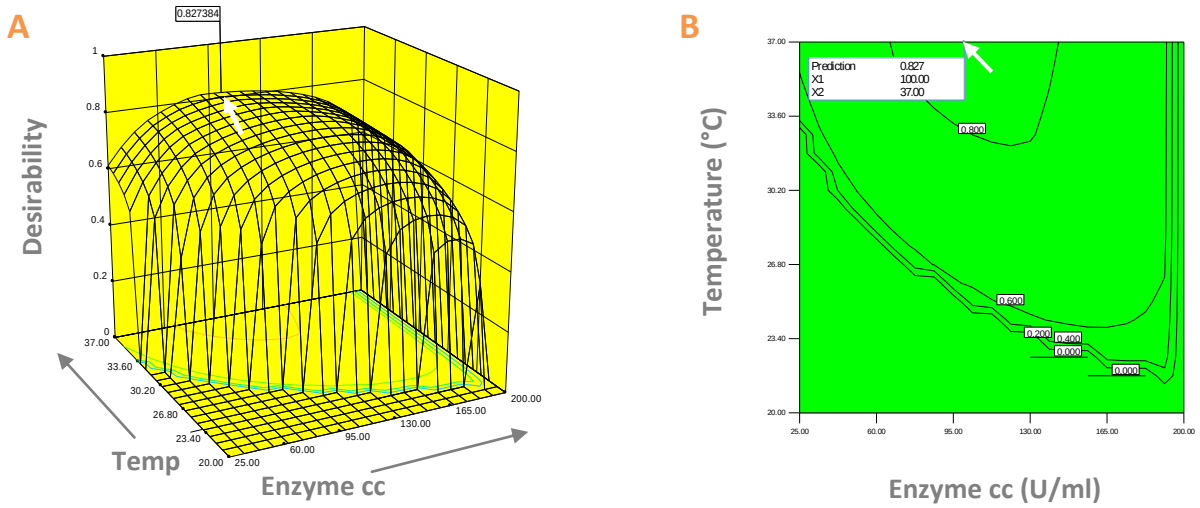
Starting from the predicted individual responses, desirability can be predicted over the experimental domain. Its representation as 3D-response surfaces or 2D-isoresponse plots (Figure 6-7) can be used to identify optimal conditions and evaluate the relative impact of the different factors. In this case, the optimal parameter combination is achieved for the following conditions:

Optimal conditions based on desirability response surfaces	
Enzyme concentration	100 U/ml
Temperature	37°C
pH	8.3

It must be noted that integrating the enzyme cost in desirability does not compromise any of the other CQAs/KPAs.

Figure 6-7: 3D-Response Surface (A) and 2D-Isoresponse Plot (B) of Desirability as a Function of Enzyme Concentration and Temperature at pH 8.3.

Enzyme cost was taken into account to calculate desirability. The arrows point to the optimal conditions.



Robustness

To avoid the selection of a satisfying but very sensitive combination of extraction parameters, the experimental space is studied from a robustness point of view. Ideal conditions should result in the desired attributes, but should also be located in the middle of a large area of conditions leading to acceptable responses. This area will allow departures from reference conditions (voluntarily or not) without affecting the process and product outputs.

A target range is specified for each response and for its associated coefficient of variation (Table 6-12).

Table 6-12: Target Ranges for Studied Responses

Response	Target range	
	Response	**Coefficient of variation (CV)**
Residual peptidoglycan content	< 2%	< 15%
Ps molecular size	150–300 kD	< 20%
Ps O-acetyl content	> 1.6 moles/moles RU	< 10%
Ps yield	> 75%	< 15%
Filterability	1	NA

Based on the prediction models, these target ranges are displayed simultaneously on an *overlay plot,* enabling discrimination among areas where all the criteria are met and those where one or more criteria are out of specification (Figure 6-8A):

- Green areas: all target values are met.
- Yellow areas: predicted responses comply with the target ranges, but one or more confidence interval(s) are out of range.
- White areas: one or more criteria are not met.

The overlay plot in Figure 6-8A shows that the optimal conditions are poorly situated in terms of process robustness. The combination of selected parameters is indeed located at the edge of the experimental domain with respect to temperature and close to borderline conditions with respect to enzyme concentration. Hence, the possibility of being close to unfavorable conditions cannot be ruled out. The optimal temperature (37°C) is of particular concern in this respect for two reasons:

- The Ps hydrolyzes readily above 37°C in mild alkaline conditions.
- Horrificase starts to denature at temperatures ≥ 38°C.

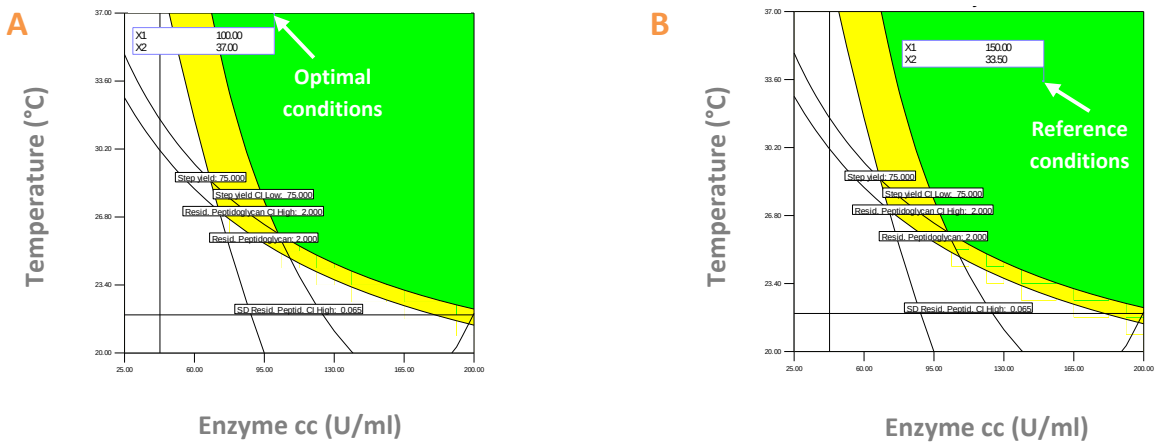
Because of these limitations, tests of additional conditions in an augmented DOE exploring temperature above 37°C were not considered. A better optimum that results in the desired process and product attributes, but is located in a more robust area of the design space should be evaluated. This can be done by decreasing the temperature and increasing the enzyme concentration as illustrated in Figure 6-8B. Thus, in this case, robustness is improved at the expense of enzyme cost.

Reference conditions based on overlay plots, optimized for responses and robustness

Enzyme concentration	150 U/ml
Temperature	33.5°C
pH	8.3

Figure 6-8: 2D-Overlay Plot

Ps compliance with specifications as a function of enzyme concentration and temperature at pH 8.3. Optimal conditions (white arrow) are located at the edge of the experimental domain (A). Reference conditions were therefore adapted to achieve a better robustness (B).



Predicted results with associated 95% confidence intervals and coefficient of variation can be calculated for these reference conditions (Table 6-13).

Table 6-13: Predicted Process Results at Reference Parameters

Response	Prediction	Lower 95% CI	Upper 95% CI	Predicted CV
Residual peptidoglycan content	0.79	0.73	0.86	6.4%
Ps molecular size	220	197	242	12.3%
Ps O-acetyl content	1.86	1.78	1.93	8.2%
Ps yield	91.5	84.9	98.2	8.2%
Filterability	1	1	1	NA

6.6.4. Determination of the Design Space

From predictions to simulations

Determination of the reference conditions was based on predicted responses and associated variability, which are actually predicted *averages*. For instance, a predicted molecular size of 200 kD means that 50% of future size responses will be below 200 kD and 50% above 200 kD. While the predicted results should, at the very least, meet the acceptance criteria *on average*, the proportion of future responses meeting the specifications (Table 6-14) is equally important information, of particular relevance to delineate a design space.

Table 6-14: Subset of Specifications Selected to Define the Design Space

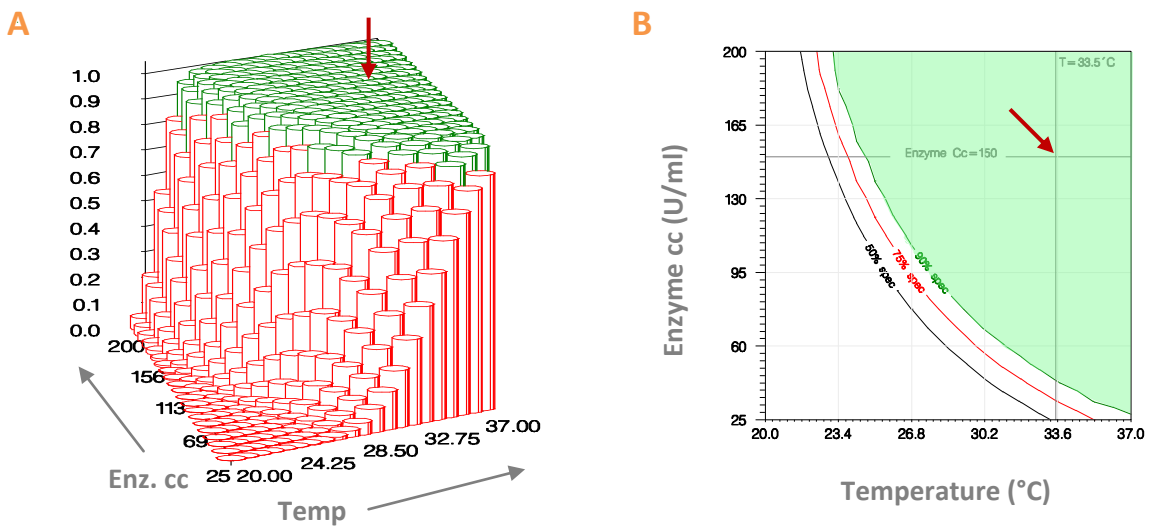
Response	Specification
Residual peptidoglycan content	< 2%
Ps molecular size	150–300 kD
Ps O-acetyl content	> 1.6 moles/moles RU
Filterability	1

To this end, a global model (e.g., Seemingly Unrelated Regression) synthesizing all individual prediction models is used. Monte-Carlo simulations, which reproduce process/measurement variability, can then be performed to mimic a huge number of experiments at numerous places of the experimental domain. Finally, the proportion of simulated results complying with the specifications can be graphically represented to generate a *3D-robustness surface plot* or its associated contour plot (Figure 6-9A).

In this case, the enzyme-temperature domain was subdivided into 20 x 20 intervals and 10,000 simulations were calculated for each response in each of these enzyme-temperature conditions at the reference pH (8.3). Conditions in which at least 90% of simulated results fall within the acceptance criteria listed in Table 6-14 are indicated in green in Figure B. Reference conditions (red arrow) are located within the optimal area with a prediction of 99% of future results meeting all the specifications.

Figure 6-9: Robustness Surface (A) and Contour Plots (B) Showing the Proportion of Simulated Results Meeting the Specifications as a Function of Temperature and Enzyme Concentration at pH 8.3

Conditions in which at least 90% of simulated results fall within the specifications are shaded in green. Reference conditions are indicated by the arrows.



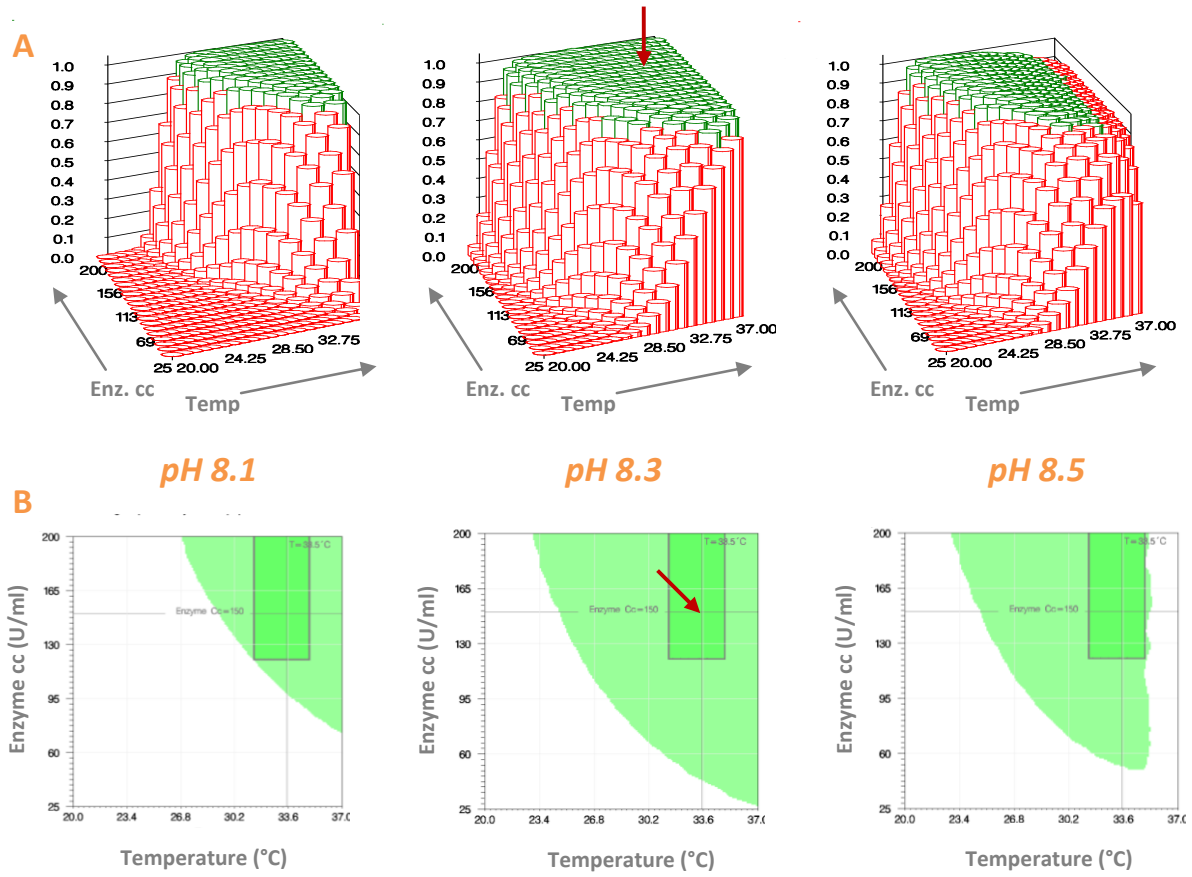
The arrows indicate the reference conditions.

Design space (enzyme concentration, pH, and temperature)

The design space, within which process parameters can deviate from reference conditions without leading to a critical increase in defect rate, can be determined graphically using simulations. Setting an upper limit of 10% for the defect rate, the design space (enzyme concentration x temperature) at pH 8.3 corresponds to the green area on the contour plot of Figure 6-9, which is only a slice of the design space. The same approach must indeed be repeated at other pH's to get a more complete visualization of the design space, as illustrated in Figure 6-10A. With three parameters as in this case, the design space could still be represented in three dimensions or under the form of different slices. With four or more parameters, however, graphical representation becomes increasingly complex. A more practical, intuitive alternative is to define the design space as a combination of ranges that can be easily tabulated. To this end, an iterative algorithm is used to determine the largest subdomain inscribed in the design space with constraints on minimal temperature and pH ranges ($\Delta T \geq 3°C$ and $\Delta pH \geq 0.4$). The obtained cuboid design space is represented as rectangular slices on the contour plots of Figure 6-10B. It can be seen that the practical aspects linked to this tabulated design space are gained at the expense of its size. Regarding the upper limit of 10% defect rate used as criterion to define the design space, it should be kept in mind that the approach is based on predictions

associated with an uncertainty of 5%. Therefore, targeting a lower defect rate would reflect an excessive confidence in the prediction models and could lead to a situation in which the uncertainty over the predicted responses would exceed the targeted defect rate. A too stringent defect rate could also lead ultimately to a narrow, unrealistic design space characterized by unaffordable operating ranges that are not in line with the accuracy of the standard equipment.
On the other hand, targeting a defect rate that is too high would extend the design space with conditions of little added value, corresponding to highly variable CQAs/KPAs. This is reflected by the steep red zone on Figure 6-9A, as opposed to the green flat surface delineated by the 90% cut-off defect rate limit and selected because of the robustness of the different responses toward process parameters.

Figure 6-10: (A) Robustness Surfaces Showing the Proportion of Simulated Results Meeting the Specifications as a Function of Temperature and Enzyme Concentration at pH 8.1, 8.3, and 8.5. (B) The Graphical Design Space, Represented As Green Areas, Is Significantly Larger Than the Tabulated Design Space (Rectangles)



Design space based on simulated results, targeting maximum 10% defect rate	
Enzyme concentration	120–200 U/ml
Temperature	31.5–35°C
pH	8.1–8.5

The tabulated design space can then be studied in further detail to acquire more process and product knowledge within the defined ranges. A useful tool in this respect is the *defect profiler*; relying once again on Monte-Carlo simulations, the defect rates for the different responses and the overall defect rates are graphically displayed diverging from the reference values as a function of each parameter (Figure 6-11). Such representations allow visualizing simultaneously the respective contributions of each response to the overall defect rate.

However, it should be kept in mind that the defect profiler is a univariate graphical representation. Hence, Monte-Carlo simulations are generated with process parameters randomly located in the design space, assuming a uniform distribution for each factor. These simulations therefore include the most unfavorable combinations of parameters. The individual and overall defect rates are then calculated from the simulated results at reference conditions and within the design space (Table 6-15); the overall defect rate at reference conditions amounts to 0.88%. It is slightly higher (1%) on average all over the tabulated design space and reaches a maximum of 8.08%.

Table 6-15 also confirms that Ps molecular size is the attribute that accounts for the major part of the defect rate, followed, locally, by residual peptidoglycans. The contribution of O-acetyl, if any, is marginal, and filterability is not a constraint. This type of information should be of great help to refine risk assessment and to design an appropriate control strategy.

Figure 6-11: The Defect Profiler Shows Defect Rates of Simulated Results as a Function of Enzyme Concentration, Temperature, and pH.

Defect rates refer to specifications of Table 6-15.

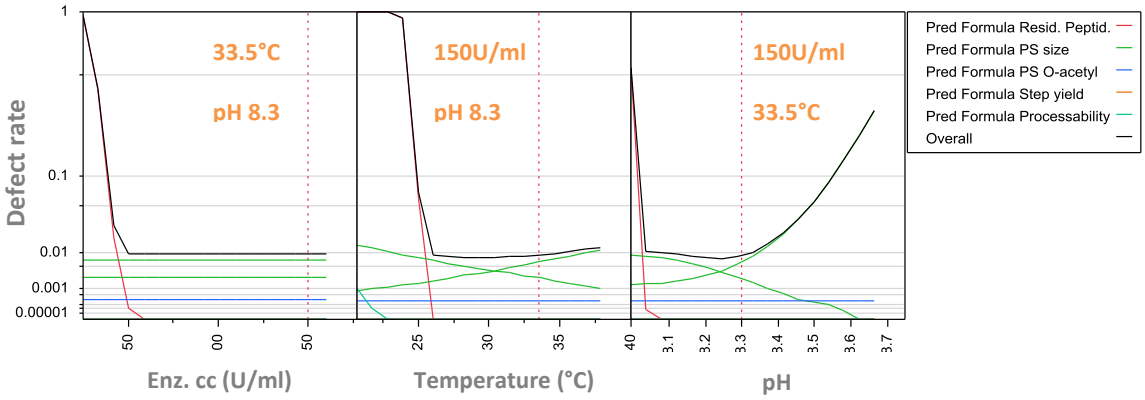


Table 6-15: Predicted Robustness Results at Reference Conditions and Into Design Space

Parameters	**Reference conditions**	**Design space**	**–**
Enzyme concentration (U/ml)	150	120–200	–
Temperature (°C)	33.5	31.5–35	–
pH	8.3	8.1–8.5	–
Defect rates	**Defect rate at ref. conditions**	**Average defect rate into design space**	**Maximum into design space**
Residual peptidoglycan	0%	0.001%	0.54%
Ps size	0.85%	0.98%	8.06%
Ps O-acetyl	0.02%	0.02%	0.02%
Filterability	0%	0%	0%
All	*0.88%*	*1.00%*	*8.08%*

Adding a parameter to the design space: incubation time

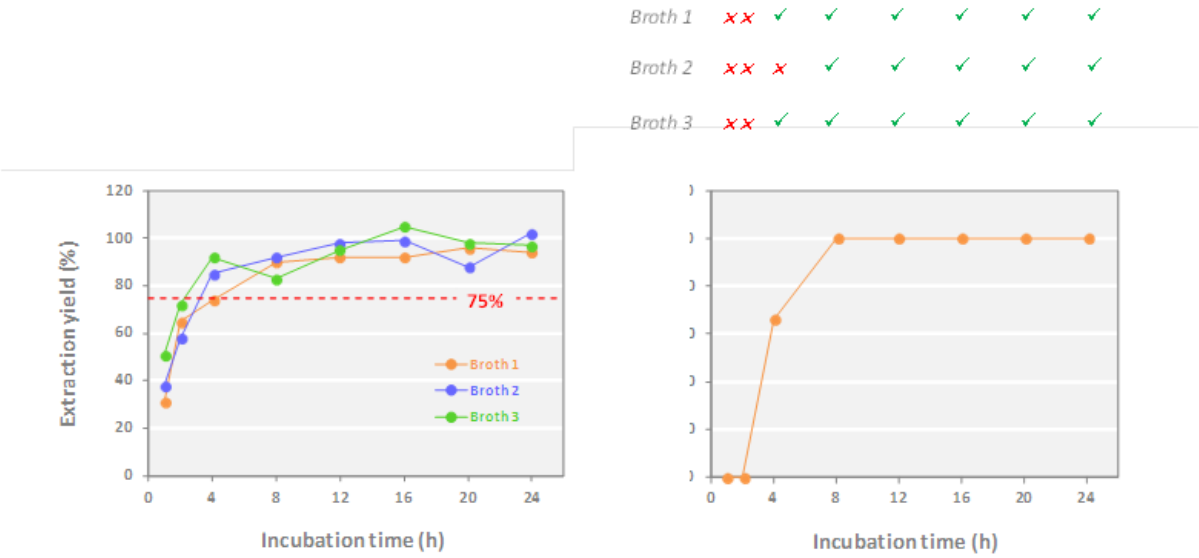
The time course of extraction was already investigated during early development (see Section 6.3) to define the incubation time (12 hours). Since the initial temperature and enzyme concentration were modified according to the results of the robustness DOE, the impact of incubation time is first re-explored in the new reference conditions:

- enzyme concentration 150 U/ml *(initial conditions : 100 U/ml)*
- temperature 33.5°C *(initial conditions : 35°C)*
- pH 8.3 *(initial conditions: 8.4)*

Each incubation time is tested at lab scale on three different fermentation broths, and two responses are studied: the Ps extraction yield as determined by HPAEC-PAD and filterability of the extract. As shown in Figure 6-12, two to four hours are required to achieve a Ps recovery of 75% in the extract, but it takes eight hours to ensure that all three extracts tested are filterable. The incubation time could therefore range from eight to 24 hours, which is advantageous in terms of organizational flexibility. A safety margin of two hours, however, is applied to the upper and lower limits, restricting the range to 10 to 22 hours.

Figure 6-12: Time Course of Extraction Step: The Target Yield Is Achieved Before the Filterability Criterion (Arrows)

✓ *= filterable extract -* ✗ *= nonfilterable extract*



To validate this range in the design space, the lower and upper incubation times are combined with worst-case conditions deduced from prior knowledge and from the design space limits:

- 10-h incubation combined with lowest pH (8.1), lowest temperature (31.5°C), and lowest enzyme concentration (120 U/ml) (i.e., conditions in which the reaction velocity is at a minimum and could thus lead to low extraction yields, poor filterability, and out-of-specification (OOS) levels of residual peptidoglycan).
- 22-h incubation combined with highest pH (8.5) and highest temperature (35°C) (i.e., conditions in which the Ps is most prone to hydrolysis). In this case, the enzyme concentration shouldn't have any impact and can be used at its reference concentration (150 U/ml).

If both extracts are filterable and the Ps yields exceed 75%, the full process is applied to check that the purified Ps complies with all CQAs and KPAs. If this is actually the case, and assuming that factor effects and interactions are not impacted by the incubation time, it suggests that the 10–22-h incubation range is applicable all over the design space.

In this approach, process and product knowledge captured from DOE studies is leveraged and combined with a simple univariate study to add a dimension to the design space with a limited number of experiments.

Design space based on simulated results, targeting maximum 10% defect rate

Enzyme concentration	120–200 U/ml
Temperature	31.5–35°C
pH	8.1–8.5
Incubation time	10–22 h

6.6.5. Univariate Studies

Incubation time

The univariate study of the incubation time was integrated in the design space study and is therefore included in the section dedicated to the design space.

Enzyme batch

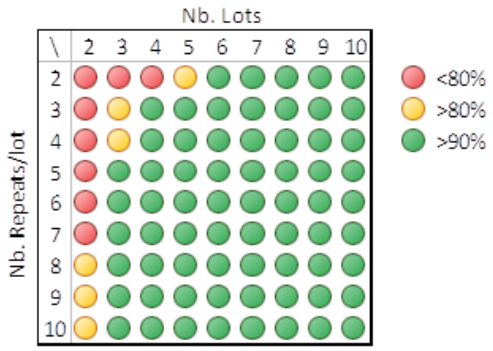
The study of this parameter is hampered by the limited availability of different enzyme batches at the time of process development (only two batches available). Indeed, to identify a possible batch-to-batch effect (three sigma) with a power >90%, a minimum of three batches are required and five repeats should be performed with each batch (Table 6-16); this would be unaffordable in terms of workload even if the batches were available.

Impact of the enzyme batch is therefore assessed through continuous monitoring as new batches are made available. If, despite passing all the QC tests, an enzyme batch is suspected to negatively impact CQAs/KPAs, its behavior could be checked at the vertices of the design space as described in the continuous improvement section for the shift to recombinant enzyme.

Table 6-16: Power to Detect a Three-Sigma Difference between Lots (F-test from a random one-way analysis of variance, α=5%)

Nb. Lots

Nb. Repeats/lot \	2	3	4	5	6	7	8	9	10
2	43%	65%	79%	88%	94%	96%	98%	99%	99%
3	63%	84%	93%	97%	99%	99%	100%	100%	100%
4	70%	89%	96%	99%	100%	100%	100%	100%	100%
5	74%	92%	97%	99%	100%	100%	100%	100%	100%
6	77%	94%	98%	99%	100%	100%	100%	100%	100%
7	79%	95%	99%	100%	100%	100%	100%	100%	100%
8	81%	95%	99%	100%	100%	100%	100%	100%	100%
9	82%	96%	99%	100%	100%	100%	100%	100%	100%
10	83%	96%	99%	100%	100%	100%	100%	100%	100%



6.7. Polysaccharide Extraction Scale-Up

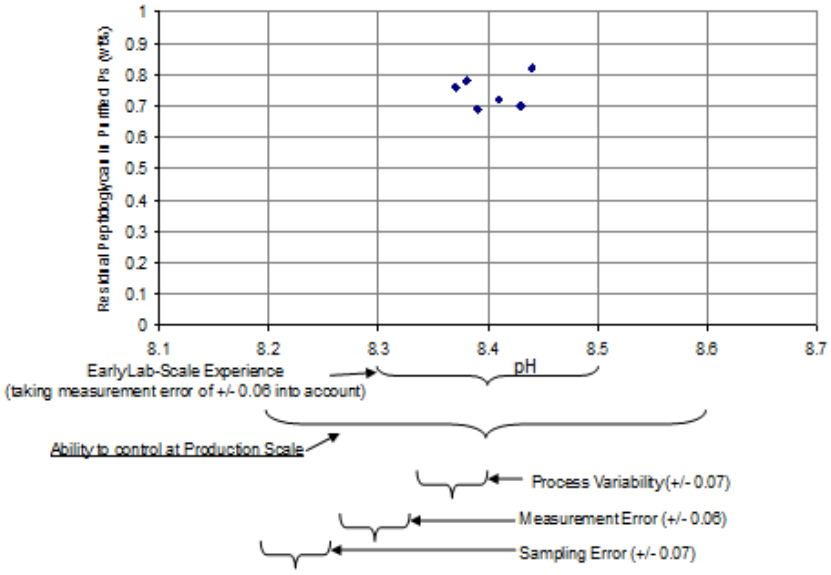
Knowledge and mechanistic understanding of the process serve as a foundation for developing a strategy for scale-up to manufacturing. Quality by Design tools and methodology help facilitate a systematic knowledge gain and process understanding. This knowledge is then coupled with thorough understanding of manufacturing-scale equipment (capabilities and limitations) to segregate process parameters considered important into scale-dependent and scale-independent parameters.

In the extraction step, parameters such as enzyme concentration, incubation time, and incubation temperature may be considered scale-independent parameters as long as confirming data using the full-scale equipment could be cited. With currently available technologies to ensure accurate reagent charges, ability to achieve a homogenous solution, and robust temperature control, maintaining control at manufacturing scale would not require any additional study. Acceptable ranges for these parameters would still need to be defined based on lab-scale studies, and ability to control these parameters at manufacturing scale would need to be confirmed. Confirmation also would be needed to demonstrate that dissolution characteristics of enzyme and other reagents are not sensitive to the type of mixing. This would be done through small-scale studies to evaluate dissolution rates using varying degrees of mixing (e.g., stir bar vs. overhead mixer).

In the event where the product is sensitive to shear or reaction rates are faster than a few seconds, parameters such as mixing, reagent addition methods, and variability in pH may be classified as scale-dependent parameters. Sensitivity to various types of mixing may need to be studied depending on mechanistic understanding of the process step and kinetics of reaction. In the extraction step, the type of mixing may be important to control shear on the molecule or prevent aggregation while ensuring good mixing. Reagent addition methods (dip-tube design, location, etc.) may impact reaction rates in cases where the kinetics of reaction is faster than the time it takes to achieve a homogenous solution. A reaction that takes place in a fraction of a second may require an exact scale-down version of manufacturing-scale equipment for development studies. In extraction for a 12-hour enzyme reaction, addition methods may not be as critical and may not require special equipment for development studies.

Lastly, variability in pH at manufacturing scale may negatively impact process performance. Ability to control pH at manufacturing scale would need to be well understood, including variability introduced by the pH measurement system. In the extraction step, enzyme efficiency may be optimal at the target pH and diminish quickly for a pH lower or higher than target pH. Assuming that manufacturing-scale equipment and pH control strategy are able to achieve control over only ±0.2 pH units and lab-scale process was developed by controlling pH within ±0.1 pH units, additional lab-scale studies may be required to show acceptable performance over this wider range of pH. Ideally, lab-scale process should be demonstrated over a pH range of ±0.3 pH units, slightly wider than the ability to control at manufacturing scale. See Figure 6-13 for an example of this data.

Figure 6-13: Residual Peptidoglycan in Purified Ps vs. pH during Extraction at Lab Scale



In an unlikely event where a wider pH range of ±0.3 pH units does not show acceptable process performance, additional development work and process changes may be required to ensure successful scale-up to manufacturing.

In an ideal scenario, knowledge related to the ability to control at production scale should be incorporated early in process development and generate data to support a wider pH range. In other words, design space work done early would be sufficient and no additional work would be required for scale-up. But in a typical process development scenario, it may be difficult to perform a large number of DOE studies early on to evaluate the impact of pH, and it may be desirable to tightly control pH around a known optimum to minimize risk of failure and stay on track for Phase 1 or 2 clinical timelines.

As the program progresses and probability of scale-up increases, a risk assessment exercise should be planned to identify scale-dependent parameters and ability to control them at production scale. These design reviews involving process, manufacturing, and equipment experts early in the development process will ensure “right the first time” DOE design. They will minimize the number of experiments required at lab scale for Phase 3 process development while ensuring high probability of success at manufacturing scale. Early design reviews also help confirm applicability of the scale-down model and facilitate work to qualify the model.

Parallel processing of the same starting material through lab, pilot, and manufacturing-scale equipment would be one way to confirm applicability of the scale-down model. These experiments would be evaluated through appropriate CQA, product, and process characterization testing (data not shown).

In summary, early design space work using Quality by Design methodology can help ensure sufficient data is collected to properly define the manufacturing process and list of important parameters to be controlled. This early characterization work helps minimize the number of additional small-scale studies required during scale-up and tech-transfer activities. It also helps ensure that manufacturing-scale equipment is designed to best fit the process.

6.8. Polysaccharide Extraction Post-Licensure Change

***Shift to recombinant enzyme expressed in* E. coli**

During the life cycle of a commercial product, changes in raw materials (e.g., source, vendor) often occur. Manufacturers must have processes in place to accommodate these changes without compromising product quality based on established critical quality attributes. A risk assessment is usually performed to assess the impact of a change such as a different raw material on the critical quality attributes. Using risk assessment tools, a severity score can be assigned based on the main and interaction effects (see Section 6.5, Polysaccharide Late Stage Risk Assessment). Based on the outcome of the risk assessment, manufacturers must develop a strategy to evaluate the change.

A change in raw material merits a number of considerations. As raw materials are usually product contact, the safety and consistency of the raw material are essential. Raw material qualification should be part of a company's GMP procedures and change control. The process typically involves qualification/audit of the vendor and qualification of the specific raw material (*Shadle, P.J., BioPharm, February 2004*). Raw material testing is also a key part of change control when a new raw material is introduced into the manufacturing process.

In the current case study, A-VAX, nonrecombinant enzyme (horrificase) that is purified from the bacterium *X. lyticus* is replaced with a new recombinant horrificase that is expressed in *E. coli* as part of a post-launch change. Because horrificase is a critical raw material, a change in expression source requires qualification and testing. It is expected that the vendor manufactures the raw material using a controlled process that is documented and personnel are trained to perform the manufacturing process. The following discussion addresses only the anticipated change in enzyme source. All other steps in the extraction process will be performed as developed, and thus no changes in impurity levels (e.g., DNA) are expected.

Raw material testing is performed to ensure that the new enzyme acts as expected in the vaccine manufacturing process. This qualification includes a comparison of the performance of the original enzyme (nonrecombinant purified from *X. lyticus*) with the new enzyme (recombinant purified from *E. coli*) against performance criteria that have been established for the specific unit operation (release of the capsular polysaccharide from *X. horrificus)*. For the new enzyme, testing of different lots (or batches) is performed to ensure consistency of the new raw material (refer to ICH guidance Q7: Good Manufacturing Practice Guide for active pharmaceutical ingredients (API)). Verification that the new enzyme also meets the specifications stated in the vendor's certificate of analysis is performed and involves evaluation of the enzyme activity and purity as noted in the specifications provided by the manufacturer.

In this case study, the change in enzyme was made because the recombinant enzyme had better purity compared with the nonrecombinant horrificase Table 6-17.

Table 6-17: Horrificase Batch Specifications

Attribute	Specification (non recombinant)	Specification (recombinant)	Method
Purity	>90%	>95%	RP-HPLC
Specific activity	>5,000 U/mg	>5,000 U/mg	Turbidimetric assay Ref. lot: horrificase (manufacture A) as standard
Absence of contaminant glycosidase activity	Pass (no size decrease of ref. Ps in predefined conditions)	Pass (no size decrease of ref. Ps in predefined conditions)	SEC-RI Ref. lot: Ps bulk

The impact of this raw material change can be evaluated using a traditional or enhanced approach. The traditional approach relies on "confirm and verify," and the process would be run at a small scale using the setpoints (input parameters) previously established. The "output parameters" are measured and must meet the responses (CQAs) established. For A-VAX, the specifications are shown in Table 6-18. Finally, comparability studies would be performed to assess the conformance and behavior of the Ps bulks at commercial scale (see Comparability Section X.Y).

Table 6-18: CQAs and Methods for Drug Substance (Extraction Step)

Parameter	Specification	Method
Peptidoglycan content (%, w/w)	< 2	H-NMR
Ps size (kDa)	150–300	SEC-MALS
Ps O-acetylation (mol/mol Ps)	≥1.6	HPLC
Ps purity (%, w/w)	≥80	H-NMR
Ps yield (%)	>75	HPAEC-PAD

Enhanced approach

The enhanced approach relies on application of product and process knowledge from the DOE used to determine the design space for the nonrecombinant horrificase (Table 6-19). Rather than checking the equivalence of the current and new enzymes at reference process conditions, the enhanced approach addresses whether the design spaces for the two enzymes overlap. To this end, a mini-DOE is performed at lab scale (0.5 L) to evaluate the behavior of the recombinant horrificase at the vertices (extremes) of the design space determined for the nonrecombinant horrificase. The lab-scale model was qualified as representative of the commercial-scale process during development with the nonrecombinant enzyme (see "Design Space" Section 6.6). It is assumed that the representativeness of the lab-scale model can be extended to the recombinant enzyme. This assumption relies on a risk assessment exercise based on product and process knowledge (not reported in this case study). Recall that the validity of the lab-scale model was verified using a commercial-scale batch produced with the nonrecombinant enzyme (extraction and purification at lab-scale run in parallel from the same commercial batch and comparison of in-process, QC, and characterization data).

The aspects of process performance and product quality are addressed as follows (Figure 6-14):

- Process performance (equivalence of KPAs): Extraction yield and filterability of the extract are checked at reference conditions and eight conditions representing the extremes of the design space. The clarified extract is not further processed.
- Product quality can be assessed only on the purified Ps: The full purification process is applied (in duplicates) to clarified extracts obtained in two worst-case conditions of the design space. The resulting Ps is submitted to the full QC and characterization plan, including accelerated stability testing. Worst-case conditions are identified through risk analysis based on product and process knowledge:
  - Condition 1 is the worst case for enzyme activity. It corresponds to the lowest enzyme concentration and shortest incubation time combined with the lowest pH and temperature (suboptimal conditions for enzyme activity).
  - Condition 8 is the worst case for Ps stability. It corresponds to the longest incubation time combined with the highest pH and temperature (risk of Ps hydrolysis).

Table 6-19: Reference Conditions and Design Space for Extraction Step (Nonrecombinant Horrificase)[a]

Parameter		Design space range	Reference cond.
Enzyme concentration	*(U/ml)*	120 – 200	150
Temperature	*(°C)*	31.5 – 35.0	33.5
pH		8.1 – 8.5	8.3
Incubation time	*(h)*	10 – 22	12

a. See "Design Space" Section 6.6.

Figure 6-14: Experimental Setup to Demonstrate the Design Space Equivalence for Current and New Enzyme. All Experiments Are Performed at Lab Scale.

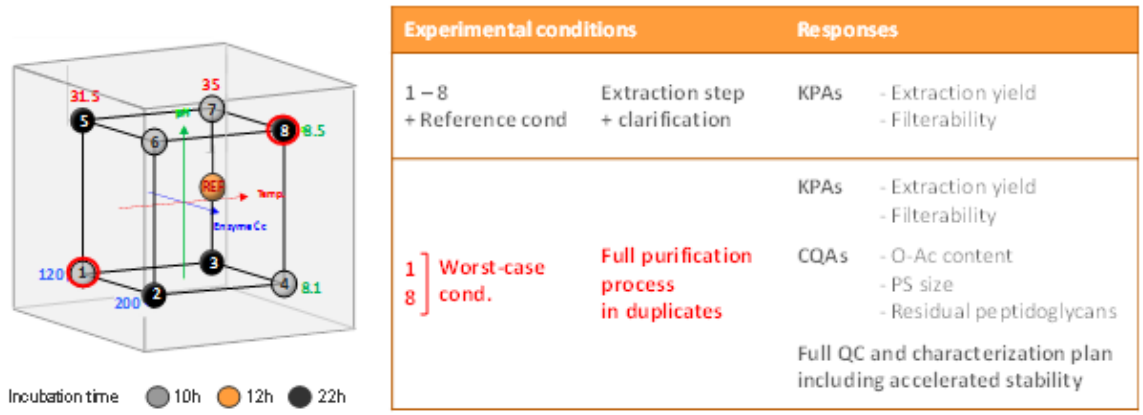


Figure 6-15 (KPAs) and Table 6-20 (CQAs) show the results of the mini-DOE; all the responses meet the following acceptance criteria:

For KPAs (Figure 6-15):

- Extraction yields with the recombinant enzyme fall within ranges in which 95% of the results are expected with the nonrecombinant enzyme.
- All the extracts are filterable (> 15 L/m² filter area).

For Ps quality attributes (Table 6-20):

- All Ps CQAs and other QC data meet the specifications (T=0 and accelerated stability).
- All Ps CQAs, QC, and characterization data fall within ranges in which 95% of the results are expected in reference conditions with the nonrecombinant enzyme (T=0 and accelerated stability). For the sake of conciseness, only the three Ps CQAs used as responses in the initial DOE on the current enzyme are listed in Table 6-20.

It is concluded that the design space defined for the nonrecombinant enzyme applies to the recombinant enzyme, which is therefore deemed equivalent to the current enzyme.

Figure 6-15: Extraction Experiment Design and Results Using the Nonrecombinant Enzyme. The extraction and clarification steps are performed at reference conditions and at the eight extremes of the design space with the new enzyme. The responses meet the acceptance criteria: Extraction yields are in the expected ranges, and all the extracts are filterable.

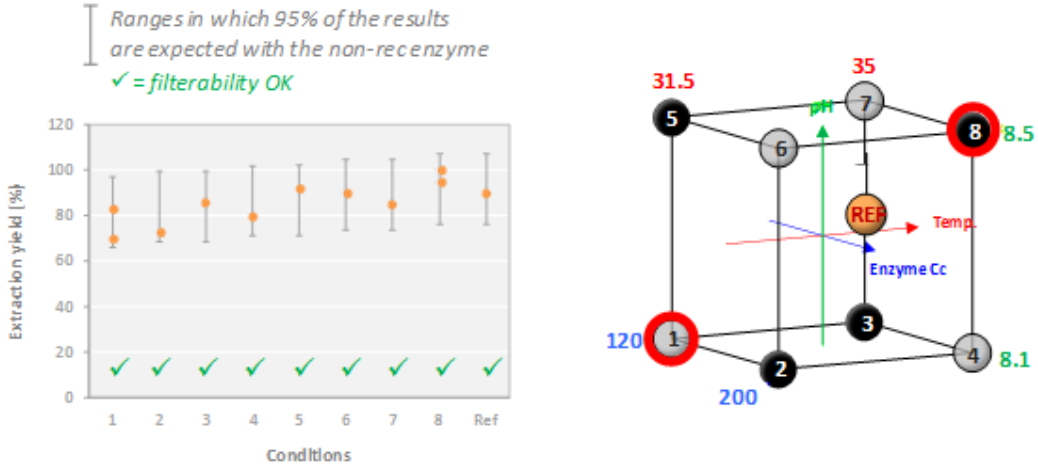


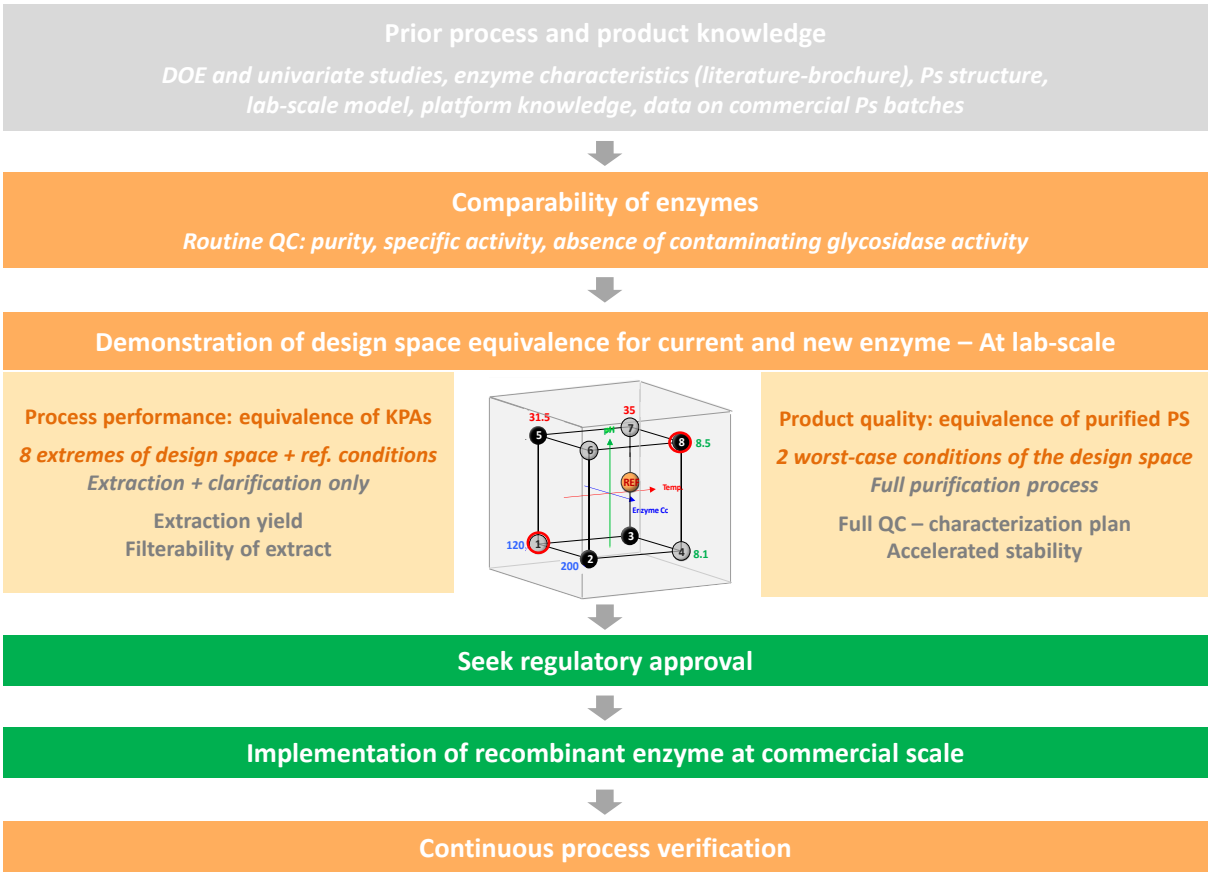
Table 6-20: Extraction Plus Purification Experimental Results with Nonrecombinant Enzyme. The full process is applied in duplicates to clarified extracts obtained in two worst-case conditions with the new enzyme. The four purified Ps meet the acceptance criteria: They comply with the specifications, and all the attributes fall within the expected ranges.

CQA	Spec	Expected range*	Cond 1	Cond 8
Ps size	150-300 kD	180 – 260	189 – 248	211 – 191
Resid PG	< 2%	0.3 – 1.0	0.7 – 0.9	0.4 – 0.4
O-ac	> 1.6 mol/mol	1.7 – 2.0	1.92 – 1.89	1.95 – 1.73
All other acceptance criteria were met (QC/characterization data at T=0 and upon accelerated stability)				
* With nonrecombinant enzyme				

In the case of biological products with process improvements that have low-level impact and high process robustness based on well-defined CQAs and design space, the process is in a state of control and meets the predetermined quality requirements. As such, the requirement to complete three validation runs at full scale would not apply, and data from the DOE studies described in the enhanced approach could be used to support this change. Continued process verification is sufficient to show that at full scale, the purified Ps extracted with the new enzyme complies with all CQAs and KPAs and is comparable to the Ps produced with the current enzyme. In this case, establishing the

comparability is facilitated by the high degree of physico-chemical characterization that can be achieved on polysaccharides. The enhanced approach is outlined in Figure 6-16. As explained in the regulatory section, a comparability protocol can be filed to seek regulatory approval. Comparability would be demonstrated at small scale; i.e., demonstration of design space equivalence between the current and the new enzyme, including the processing of two small scale lots to purified polysaccharide utilizing two worst-case conditions (see Figure 6-15 and Table 6-20). As laid out in Figure 6-16, full quality control including characterization and accelerated stability data are generated on the material at lab scale. This regulatory package should be satisfactory to seek regulatory approval; no commercial scale data are deemed necessary as the small scale model was demonstrated representative of commercial scale. Continuous process verification data on commercial scale lots, confirming process consistency within pre-set control limits, would be available post-registration and can be reviewed by the authorities as part of the Company's Quality Management System.

Figure 6-16: Overview of the Enhanced Approach



In a worst-case scenario where the recombinant horrificase did not perform as observed in the previous design space for the nonrecombinant horrificase, the results would be exploited to extend the DOE with relevant conditions to recalculate a new design space. Wherever possible, prior knowledge should be used to reduce the work. The new design space must provide a process that yields a purified Ps that complies with all CQAs and KPAs and should correspond to operating ranges that are compatible with the existing equipment.

Whether the design spaces for the current and new enzymes are equivalent or not, the enhanced approach offers several advantages in terms of process understanding and control. In a traditional approach, the current reference conditions would be applied to the new enzyme on three consistency batches at commercial scale, and the success criteria would be met if the three Ps batches comply with the usual QC requirements.
The enhanced approach, however, provides information on process robustness and determines if the new enzyme is more sensitive than the current enzyme to the process parameters. In addition,

in the event of problems with performance at scale, the enhanced approach provides important information for how to adapt the process parameters. In the case that the design spaces are not equivalent, the results of the mini-DOE can help orient an extended DOE and ultimately delineate a new design space – and perhaps new reference conditions – ensuring better process robustness and control.

Thus, in both cases (design spaces are equivalent or not), the enhanced approach reduces the failure risk of the first Ps batches produced at commercial scale with the new enzyme, assuming that the lab-scale model is predictive.

Regulatory Filing Strategy

To utilize product knowledge captured in the design space to achieve a lowered change reporting category at the time of change implementation (at a later time), the design space pertinent to assessing future changes must be captured in the regulatory filings and approved as a sanctioned approach for regulatory change management. To accomplish this in US and EU filings, a protocol would need to be placed into the regulatory filings for each of the changes envisioned in the future that would merit the effort of seeking a lowered regulatory reporting category. In the arena of downstream processing, this could include a change in the type of process step (e.g., change in tangential flow cartridge, chromatography resin, change in critical raw material, change in process parameters).

The change in source of horrificase (nonrecombinant to recombinant) as presented in this case study is an anticipated change. A DOE approach would be used to determine whether the polysaccharide extraction process performs in the existing design space or whether a new design space is needed. To support the process change, the data from these studies would be used, as well as data from comparability studies performed to assess the conformance and behavior of the Ps bulks and compared against reference batches. The purified Ps bulk must meet all CQAs and KPAs established.

The initial US filing would be in the form of a “Comparability Protocol” (CP), and the initial EU filing would be in the form of a “Change Management Protocol” (CMP). These filings would require approval prior to their use in assessing a change (i.e., the US filing would be a Prior-Approval Supplement, and the EU filing would be a Type II variation). The protocol may be incorporated either at the time of the initial filing of the product for marketing approval or added after initiation of commercial marketing during later product life cycle management through the use of a post-approval update to the regulatory filings (see “Regulatory” section for more detail). In instances where a change control matrix has been established within the product marketing application, the initial filing of the update would also include the revised overall change control matrix table.

The protocol (CP or CMP) adds value for the sponsor by providing an agreement with the regulatory health authorities on the content of the filing that supports the change in advance of making the change. This mitigates the risk of delayed regulatory approval and provides additional control over timing and speed of implementing change for product distribution.

The initial protocols captured in the regulatory filing would fully describe how the change would be evaluated prior to distribution at commercial sale. The filing would contain a description of the change and the protocol for product comparability assessment, including prospectively defined acceptance criteria. The design space data would be provided as background and used to justify the acceptance criteria that are proposed for the evaluation of product comparability.

The regulatory health authorities would evaluate the filing, and once they approve, it should be granted a lowered category for reporting. The categorization will depend on the degree to which the regulatory health authorities find the information sufficient to provide them with confidence that the change will be assessed in a manner that minimizes the potential for adverse impact on product safety, purity, potency, and effectiveness.

In general, the US FDA would lower the second report to the CBE30, CBE, or annual report reporting category level; and the European Union would be expected to reduce the second report to a Type $1A_{IN}$ or IB variation. The reporting category for the second filing would be proposed in the initial filing, and the specific second filing category found acceptable to the regulatory health authorities would be defined in the approval notification.

At the time of implementing the change, the assessment of change would have to be performed without significant deviation using the specific protocol that was approved by the regulatory health authorities, and reported using the method specified in the protocol approval notification received from the regulatory health authorities. Deviations from the protocol should be justified and discussed with regulatory health authorities to ensure that they do not see the potential for upgrading the change to a prior approval or Type II submission.

6.9. Virus-Like Particle Freezing Process Description

6.9.1. Process Overview

The virus-like particles (VLPs) are purified after disrupting the *E. coli* cells in the harvested fermentation broth. Purification consists of a combination of filtration, chromatographic, enzymatic, and ultrafiltration steps. The purified VLP solution is frozen and stored at -70°C before conjugation with activated polysaccharides.
The downstream process flowsheet and the purpose of each step are summarized in Figure 6-17.

6.9.2. Unit Operation Selected

For the sake of conciseness, purified VLP solution freezing is the only VLP downstream step that will be covered in this case study.

Step description
Purified VLP solution is transferred to containers for freezing and storage at -70°C.

- VLP solution is dispensed into containers that can withstand the freezing process as well as physical handling in the frozen state while maintaining integrity.
- The VLP solution is frozen by placing the containers in a -70°C blast freezer. Afterwards, the containers are transferred to -70°C freezers for long-term storage.
- The VLP solution in the containers will eventually be thawed and filtered at 0.2 microns prior to use in the conjugation process.

Rationale for selecting the freezing step as an example

- The step is likely to impact the key CQA of average VLP size, an indirect measure of the extent of aggregation.

Subset of CQAs and KPAs used in example
VLP solution freezing conditions most likely impact the following CQA and KPA, which will be considered in the example:

CQA

- VLP size: Aggregation of the VLPs may influence the average VLP size and therefore the average size of the resulting Ps-VLP conjugate.

KPA

- VLP concentration following thaw and filtration (yield): Because aggregation can lead to VLP losses upon filtration of the thawed VLP solution. Measured by UV or BCA protein assay.

Figure 6-17: Virus-Like Particle Flowsheet and Objectives of the Different Steps

Fermentation harvest	
↓	*Transfer to downstream*
Cell disruption	→ Releases VLPs
↓	
DNase treatment	→ Digests residual nucleic acids
↓	
Clarification by centrifugation	→ Removes cells and cell debris
↓	
Cation exchange chromatography	→ Removes proteins, host cell impurities
↓	
Hydroxyapatite chromatography	→ Removes proteins and nucleic acids
↓	
Anion exchange chromatography	→ Removes proteins
↓	
Detoxification	→ Removes endotoxin
↓	
Ultrafiltration 100kD Concentration + diafiltration	→ Buffer exchange and concentration
↓	
0.22µm filtration	→ Control bioburden
↓	
Freezing at -70°C	
↓	
Bulk Virus-Like Particles	

6.10. Virus-Like Particle Freezing Early Process Development

Following purification, the purified VLPs are transferred to storage containers, frozen, and stored at -70°C. During downstream conjugation, the bulk containers are thawed at 2–8°C prior to use. Although the product is stable at the listed temperatures, limited information is available to characterize the impact to product quality of the freezing and thawing process.

Literature *(S.D. Webb, J.N. Webb, T.G Hughes, D.F. Sesin, and A.C. Kincaid, "Freezing Biopharmaceuticals Using Common Techniques and the Magnitude of Bulk-Scale Freeze Concentration," Biopharm 15(5) 2-8 (*2002)) suggests that freezing processes can affect the properties of proteins and other biopharmaceutical intermediates via various mechanisms. One mechanism, cryo-concentration, has been evidenced through data showing a greater than eight-fold increase in bulk Bovine Serum Albumin (BSA) concentration and a 20-fold range of BSA concentrations within frozen 1-liter bottles *(S.D. Webb, et. al.).* During cryo-concentration, salts and other large molecules diffuse from the ice front that forms as the bulk solvent freezes. Slower freezing kinetics will increase the degree of cryo-concentration, as the solutes have more time to diffuse.

Early development: target storage conditions

The scale and container for early development work were chosen to minimize freezing path length and potential reactions with materials of construction. This work was done in a 1 mL glass cryovial. Freezing and thawing rates at this scale will be much greater than the practical freezing rate at final manufacturing scale. The scale/container was chosen to represent "ideal" rates of change (i.e., minimization of container path length). A very small container (1 mL) was selected to maximize rates of freezing and thawing. Analytical confirmation (size by dynamic light scattering (DLS) and concentration using BCA as the referenced standard) during the freeze/thaw developmental work confirmed suitability of frozen storage conditions and that the VLP was stable through the freezing and thawing process.

Seven 1 mL glass cryovials were filled to 800 μL with VLP; one cryovial was placed in a 2–8°C refrigerator (control), and six cryovials were placed in a -70 °C freezer. After five days of storage, the vials were thawed at 2–8°C and tested for VLP size using DLS and concentration using BCA as the reference standard.

Results from the early development work indicate that there were no appreciable changes in VLP size or concentration following the freeze-thaw process. Measurements of size and concentration were within 3% of the control value, indicating no significant changes in the attributes.

Early Development: Establishment of Glass Transition Temperature to Determine Storage Conditions

Controlled temperature units (CTUs) typically have a tolerance of +/- 10–15°C around the setpoint. It is essential that the VLP is stored at a temperature where natural CTU temperature oscillations do not cause constant transition across the T_g'. Additionally, because the bulk will be kept in inventory for ~10 years, storage conditions will be chosen so that the VLP is below the T_g'.

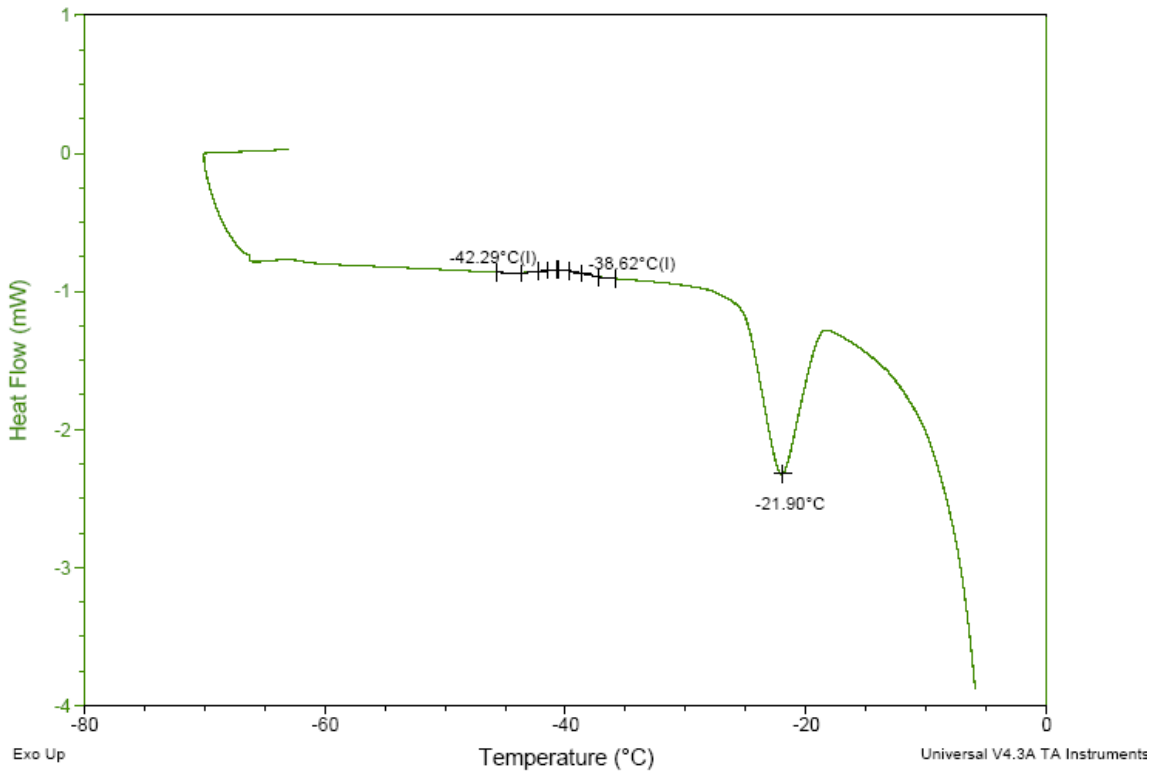
Results from differential scanning calorimetry (DSC) are presented in Table 6-21. An example of the DSC plot is shown in Figure 6-18. The average T_g' value by DSC analysis for three lots of VLP was calculated as -40.8°C.

Standard freezer design requirements are intended for storage at -20, -40, or -70°C. Ideally, the VLP would be frozen at -20 or -40°C; however, the T_g' determined by DSC indicates that selection of a -20°C freezer would be above the glass transition temperature and a -40°C freezer would cause continuous oscillations across the transition temperature because of freezer cycling. The T_g' data suggests -70°C storage is more appropriate for the VLP.

Table 6-21: VLP in 200 mM NaCl, 30 mM Histidine pH 7.2

Description	N	Tg' (deg C)	Onset (deg C)	Heat flow reduction (deg C)
VLP Run 1	1	-38.62	-42.29	-21.9
VLP Run 2	2		-40.99	-21.71
VLP Run 3	3		-39.26	-21.49
		AVERAGE	**-40.85**	**-21.7**

Figure 6-18: Example Glass Transition Temperature and Heat Flow Onset for VLP



6.11. Virus-Like Particle Freezing Risk Assessment

Because the VLP will be stored as a bioburden-reduced bulk, sterilized containers will be required. Additionally, container closure integrity (CCI) must be demonstrated to prevent potential extrinsic contamination during the container life cycle. Following container selection, freezing conditions, and determination of fill volume, torque specifications and CCI for the closure will be established as part of a separate validation study. All of these will be taken into account when selecting the final VLP container.

At a VLP concentration of ~ 1 g/L, approximately 100 L of purified bulk will be generated per lot. It is assumed that minimizing path length is critical to prevent impact on the bulk attributes during freezing. The appropriate container size will minimize the number of containers while maximizing the fill volume (typically 60–80% of container volume). This balance also will consider the greater path length with increasing container size. The bottle cannot be so large that the kinetic rate of freezing/thawing as a result of path length impacts the bulk attributes.

A cause-and-effect matrix risk assessment (Table 6-22) was performed to categorize the operating parameters that may impact VLP attributes during freezing and thawing. The parameters were

placed into two groups: (i) parameters warranting experimental evaluation and (ii) parameters that are considered low risk and would not require evaluation. The category (ii) parameters would employ ranges based on prior knowledge. Each process parameter was assessed based on the potential impact on VLP size and VLP concentration.

The scoring of process parameters and quality attributes is described in Section **Error! Reference source not found.** and outlined in Table 6-30. The cumulative score is determined by Σ*(Impact of parameter x weight of quality or process performance attribute).* The cumulative score represents the relative importance of the parameter on VLP storage considerations. Parameters with scores exceeding 50 were considered to be high risk with the potential to impact product quality or process performance and were candidates for further experimental evaluation. Those with scores less than 50 were considered low risk and were not further evaluated.

Table 6-22: Cause-and-Effect Matrix for VLP Storage Conditions

	Quality Attribute Weight				
Parameter	**VLP Size**		**VLP Concentration**		**Cumulative Score**
	Impact	*Weight*	*Impact*	*Weight*	
Container Size	7	7	7	7	98
Fill Volume	1	7	1	7	14
Rate of Thawing	5	7	7	7	84
Rate of Freezing	7	7	7	7	98
Material of Construction	5	7	5	7	70
Initial Temperature	1	7	1	7	14
Initial [VLP]	1	7	5	7	42

6.12. Virus-Like Particle Freezing Design Space

Using the cause-and-effect matrix cumulative scores, three design criteria were assessed during developmental work: the rates of freezing and thawing and the container size. A full-factorial DOE (n=3, 3 levels) covering three freezing and thawing conditions and three container sizes was conducted for various materials of construction.

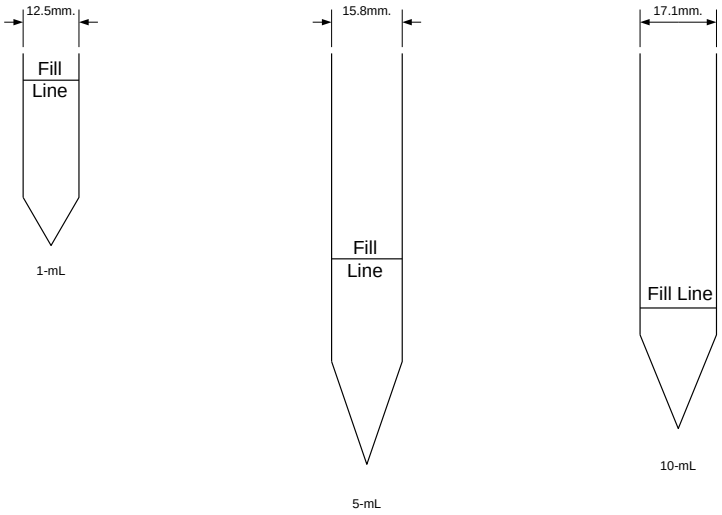
Freezing levels were on dry ice, in a -70°C upright freezer, and at 0.1°C/min. The 0.1°C rate of change was mediated through a temperature-controlled chamber (TCC). Rheostat control was used to adjust the TCC between -80 and 8°C to achieve the predefined freezing rate. The three freezing rates represent fast, medium, and slow freezing, respectively. Thawing was initiated two days after freezing. Table 6-23 lists the full-factorial design conducted per container.

Thawing levels were evaluated using a 30°C water bath, 2–8°C CTU, and 0.1°C/min, representing fast, medium, and slow thawing, respectively. Following thawing, samples were kept at 2–8°C before testing. Container size was modeled by scaling the final targeted containers (1 L, 2 L, and 3 L) to cryovials of increasing size. Samples were filled to 0.80 mL in a 1 mL cryovial, 1.09 mL in a 5 mL cryovial (26% increase in path length), and 1.27 mL in a 10 mL cryovial (37% increase in path length), illustrated in Figure 6-19.

All samples were tested against the 1 x 1 mL cryovial control. Since a cryovial is much smaller than the final manufacturing container, concentration and size effects may not be observed. The intent of varying path length during developmental work was to determine if any sensitivity exists when

tested at a minimized scale. If attribute changes related to changing path length are observed at a small scale, the opportunity for freezing the VLP in larger containers may be limited.

Figure 6-19: Increasing Path-Length Modeling Varying Container Sizes



Because material of construction also scored high, a variety of materials were also evaluated experimentally. Because the Tg′ studies indicated frozen bulk storage would be required, a subset of materials was chosen because of the materials' thermostability at -70°C and previously demonstrated CCI validation. The three materials selected were polypropylene, perfluoroalkoxy (PFA), and fluorinated ethylene propylene (FEP).

An additional FMEA (not shown) was conducted to identify failure modes during the freezing process. The highest-scoring RPN out of that assessment resulted from pulling a "half-frozen" container out of the freezer, thawing, and then re-freezing it. A one-factor-at-a-time study was conducted to evaluate multiple freeze/thaws. The results of this study showed no statistically significant ($p < 0.05$) differences against an unfrozen control.

Table 6-23: Freeze-Thaw Study Arm Description

Factor	High	Middle	Low
Thawing	30 °C	2–8 °C	0.1 °C/min
Freezing	Dry ice	-70 °C	0.1 °C/min

Fixed parameter: fill volume

Data analysis identified PFA as the material showing the least change in VLP attributes. The analytical summary of PFA results is presented in Table 6-24.

The VLP was insensitive to freezing or thawing rates and container size within the bounds of the study at all but one condition. When the VLP was frozen at the slowest and thawed at the fastest kinetic rates, there was a statistically significant increase in VLP size ($P < 0.05$).

The experimentally evaluated design space encompassed a broad range of kinetic rates. Although no failure limits were identified within the selected ranges, the design space would suggest there is an impact on VLP size when a slow rate of freezing is combined with a high rate of thawing, regardless of path length. This interaction was not seen when the main effects were evaluated for each individual condition. Additionally, the effect was noted only for VLP size.

Table 6-24: Percent Change Against 2–8 °C Reference for PFA Container DOE

Fill volume	Freezing	Thawing	% change after freeze/thaw against 2–8 °C reference	
			VLP size	VLP conc.
+	+	+	+ 2.7	+ 2.5
+	+	(1)	+ 1.0	+ 0.2
+	+	-	+ 0.3	- 1.9
+	(1)	+	+ 2.1	+ 2.1
+	(1)	(1)	- 2.1	- 2.7
+	(1)	-	+ 1.9	- 2.2
+	-	+	+ 8.2	+ 0.4
+	-	(1)	- 0.4	+ 0.0
+	-	-	- 0.1	+ 1.0
(1)	+	+	- 0.5	+0.1
(1)	+	(1)	+0.1	+0.2
(1)	+	-	+0.0	+0.5
(1)	(1)	+	-0.4	+2.4
(1)	(1)	(1)	-0.2	- 0.5
(1)	(1)	-	+ 2.7	+ 2.5
(1)	-	+	+ 13.0	+ 0.2
(1)	-	(1)	+ 0.3	- 1.9
(1)	-	-	+ 2.1	+ 2.1
-	+	+	- 2.1	- 2.7
-	+	(1)	+ 1.9	- 2.2
-	+	-	+0.3	-2.1
-	(1)	+	-1.8	+0.2
-	(1)	(1)	- 0.5	+0.3
-	(1)	-	-0.7	+.08
-	-	+	+ 9.6	+1.4
-	-	(1)	-1.4	+2.1
-	-	-	+1.3	+2.2

(1) represents center, (+) represents high, (-) represents low

Lab-scale model:

Six batches of VLP drug substance lots in 200 mM NaCl, 30 mM histidine (pH 7.2) were aseptically transferred to autoclaved, 1 L PFA bottles with c-flex tubing and closures. A 1 L container was selected to determine if the early development work was reproducible at a larger scale. After filling, the PFA bottles were placed in an upright freezer (<-60°C) for at least 15 hours. Frozen VLP lots were

thawed either in an approximately 20°C water bath with periodic swirling or in an approximately 4°C cold vault without swirling.

Samples taken from VLP lots before and after the freeze/thaw cycle were assayed using DLS for size and BCA for concentration. The ID number and fill weight of each lot are listed in Table 6-25. Also listed in Table 6-25 is the approximate thaw temperature used for each VLP lot.

Table 6-25: ID Numbers, Fill Weights, and Thaw Temperatures used in 1 L PFA Freeze/Thaw Studies

VLP ID number	Fill weight (g)	Approximate thaw temperature (°C)
VLP 1	507	20
VLP 2	293	20
VLP 3	455	4
VLP 4	429	20
VLP 5	510	20
VLP 6	443	4

The percentage (%) change in VLP size and concentration after the freeze/thaw in 1-L PFA bottles is listed in Table 6-26. Based on the results shown in Table 6-26, there were no statistically significant changes in properties measured by the DLS or BCA assays ($p < 0.05$).

Table 6-26: Percent Change in VLP Properties after Freeze/Thaw

VLP ID number	% change after freeze/thaw	
	VLP Size	VLP conc.
VLP 1	-2.7	-2.5
VLP 2	-1.0	-0.2
VLP 3	-0.3	1.9
VLP 4	-2.1	-2.1
VLP 5	2.1	2.7
VLP 6	-1.9	2.2

Results of the 1 L PFA bottle scale-down confirmed that the VLP attributes remain unchanged when compared with the early development work. The 500 mL fill in a 1 L PFA bottle will be used to model the rate of freezing. This rate will be used to specify the large-scale design requirements. Because thawing rates have not shown an impact on conjugate attributes at 1 mL and 500 mL scale, a fixed 2–8°C thaw will be used for the final process.

Static freezing temperature profiles

Experiments were performed to determine the freezing profiles of 500 mL of VLP buffer (200 mM NaCl, 30 mM histidine, pH 7.2) in a 1 L PFA bottle. Studies were conducted within a <-60°C upright static freezer (Forma Scientific). A single bottle filled with room-temperature buffer was placed in the middle of the second shelf from the top (in the four-shelf freezer). Temperatures were collected during the freezing process. Three independent experiments were performed, each collecting temperatures at three different positions along a horizontal plane in the PFA bottle.

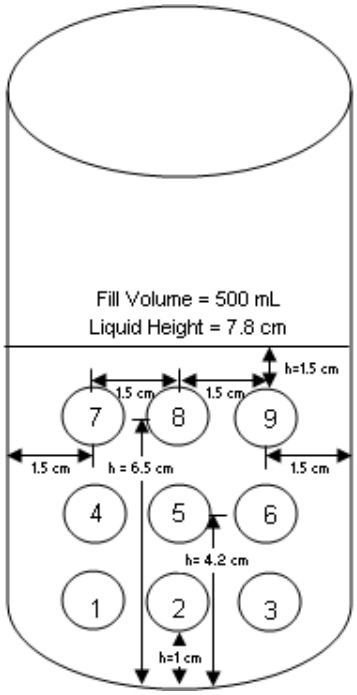
Depicted in

Figure , thermocouples 1, 3, 4, 6, 7, and 9 were positioned 1.5 cm from the vertical wall of the bottle; thermocouples 1, 2, and 3 were positioned 1 cm from the bottom of the bottle; and thermocouples 7, 8, and 9 were positioned 1.5 cm below the buffer surface. The 500 mL buffer volume was measured to have a liquid height of 7.8 cm in the 1 L PFA bottle. Thermocouples were equally spaced along the horizontal plane at 1.5 cm apart.

Temperatures were recorded for thermocouple positions 1 through 3 for freezing experiment 1, positions 4 through 6 for freezing experiment 2, and positions 7 through 9 for freezing experiment 3.

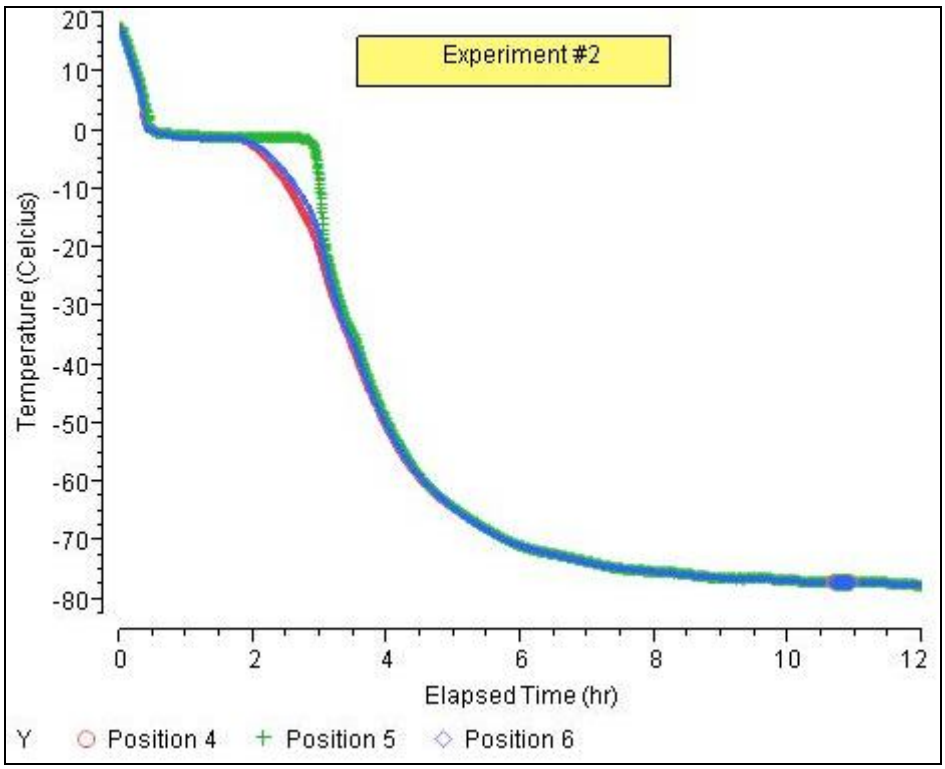
Figure 6-20: Position of Thermocouples

Experiment 1: Thermocouple Positions 1 through 3,
Experiment 2: Thermocouple Positions 4 through 6, Experiment 3: Thermocouple Positions 7 through 9



Refer to Figure 6-21 for the static freezer temperature profiles for a single set of experimental conditions (worst-case freezing positions shown). Experiment 1 evaluated the bottom-most container plane (positions 1–3). Experiment 2 evaluated the mid-plane (positions 4–6). Experiment 3 evaluated the top-most plane (positions 7–9). Thermocouple position 5 (experiment 2, position 5) was identified as the worst-case location for freezing, and the maximum pull-down time to the onset of the glass transition temperature, -41°C, was determined to be 3.7 hours. Each thermocouple position within the 1 L bottle reached -70°C after six hours of storage. Because the 500 mL lab-scale work showed no impact to VLP attributes at the same rate of freezing, the 3.7-hour pull-down time was used to set the large-scale user requirements.

Figure 6-21: Experiment 2 Temperature Profiles: Thermocouple Positions 4 through 6



These studies were completed in 1 L PFA bottles (d=92 mm). The rate of freezing at the 1 L scale is presented as a worst case and will be used to justify the maximum allowable drop-down to the glass transition temperature onset in larger-capacity bottles. Table 6-27 indicates the bottle specifications for the 1 L, 2 L, and 3 L narrow-mouth PFA bottles. Because path length is critical in freezing phenomena such as cryo-concentration, maintaining or reducing the pull-down time of 3.7 hours for the 1 L bottle (worst-case condition) assures that the overall rate of freezing is faster than the 1 L scale-down study.

For the final manufacturing facility, the blast freezer user requirements specify a pull-down time of 3.7 hours for a 3 L bottle with a 146 mm diameter or a 2 L bottle with a 125 mm diameter. Since a maximum of 100 L purified bulk will be generated per batch, approximately 65 containers will be generated. The blast freezer and associated trolley should be designed to allow all 65 containers to be frozen at once.

Table 6-27: Narrow-Mouth PFA Bottle Specifications

Part No.	Neck ID (mm)	Filled Capacity (mL)	Body Diameter (mm)
1 L bottles	25.5	1,060	92
2 L bottles	36.5	2,080	125
3 L bottles	26.5	3,350	146

<u>Cryo-concentration and blast freezer evaluation:</u>
In a liquid nitrogen blast freezer, a fine spray of liquid nitrogen is directed on the product containers. Two internal “turbulence fans” circulate the cold gas generated by the evaporation of the liquid nitrogen. This freezing method takes advantage of both a high temperature gradient (ΔT) for the entire freeze cycle and an increased overall heat transfer coefficient achieved by the convection enhanced by the turbulence fans.

Because the pull-down time specified in the static freezing experiment is not achievable in a conventional <-60°C upright freezer, a blast freezer will be used.

The maximum pull-down time of 3.7 hours was used to set blast freezer design criteria.

The 2 L production-scale containers were filled with 1.6 L of VLP in 200 mM NaCl, 30 mM histidine, pH 7.2. Increasing to a 3 L bottle increases the diameter by 15%. The 2 L bottle was chosen because of blast freezer design considerations. The addition of 15% in bottle diameter would drive the purchase of an additional 8.5 kW of condensing requirements. This increase would result in an additional upfront capital cost of ~35%. It is always possible to increase the height of the 2-L bottle and maintain the path length. The containers selected here are currently available from an approved vendor and were selected to minimize additional vendor qualification activities.

During blast freezer operational qualification (OQ), the unit was temperature mapped using minimum and maximum bottle loads within the freezer trolley. This study was to identify which position in the chamber was the fastest and slowest to reach the glass transition temperature.

Following blast freezing to -70°C, one bottle from the center of each shelf and the slowest and fastest freezing locations was physically cut into three discs (top, middle, and bottom). The top and middle discs were cut into nine segments. The bottom disc was cut into two concentric circle segments. After the segments were thawed, the conductivity and VLP size of each sample were tested to determine whether stratification or cryo-concentration had occurred at the final design condition and to determine if there were any impacts on the VLP size. As expected, the maximum observed conductivity and size difference was at the center of the bottle. The difference at the center was within 5% and 3% of the average conductivity and size values, respectively (acceptance criteria is < 10% and < 5% against control for conductivity and size, respectively. These results confirm cryo-concentration was successfully minimized upon scale-up.

Establishing a design space for VLP bulk storage requirements demonstrated that the VLP attributes of size and concentration can be preserved within the ranges tested. The ranges were used to select the final container and design the final freezer requirements needed to maintain the maximum pull-down rate. The design space data also showed that the VLP attributes were relatively unaffected within the ranges tested. Use of the blast freezer and a 2 L PFA container, regardless of the thawing rate, will be acceptable during the final manufacturing process. Table 6-28 shows the target and acceptable ranges based on the design space.

Table 6-28: Target and Acceptable Ranges for VLP Freezing Design Space

Parameter	Target	Acceptable Range
Material of construction	Perfluoroalkoxy (PFA)	N/A
Container diameter (mm)	125	+/- 20 mm
Fill volume (L)	1.6	+/- 0.5 L
Average rate of freezing (°C/min)	- 0.64	</= - 0.64
Average rate of thawing (°C/min)	0.03	</= 0.03

Post-licensure change

Changes in material availability are a common occurrence during a product life cycle. If the current 2 L container is no longer available, a change will be required to continue manufacturing activities. If a comparable 2 L PFA container is not available, any container within the acceptable diameter range can be considered. The design of the blast freezer was chosen to achieve frozen conditions at all container locations using a 125 mm-diameter container. A decrease in size below the target

diameter would decrease the path length and maintain the acceptable rate of freezing. Increases in diameter are acceptable; however, modifications to the blast freezer may be required to ensure acceptable rates of freezing. To support this change, freezing rates would be confirmed using temperature mapping during the blast freezing process.

If a change in VLP mass is required on a per-container basis, the fill may be increased or decreased within the acceptable range. Since volume change does not alter path length, the rate of freezing will not alter at the core locations (worst-case location). If a fill volume change and a container diameter increase are required, the same consideration for blast freezer design will be evaluated.

If needed, the listed change would occur after initiation of commercial marketing during later product life cycle management through the use of a post-approval update to the regulatory filings (see "Regulatory" section for more detail).

6.13. Ps-VLP Conjugation Process Description

Unit operations selected

A-VAX provides an enhanced cellular (Th1) and humoral (Th2), antigen-specific, protective immune response when compared to a natural *X. horrificus* infection. The exact mechanism by which A-VAX stimulates the cellular and humoral immune response is not known; however, only the Ps-VLP conjugate can initiate a protective immune response to Ps in the target age group. The effectiveness of this conjugate in vaccination depends on the activation and conjugation steps since they determine the chemical structure of the product.

Process description

The conjugation process is summarized in Table 6-29.

Activation

The rationale for the activation design was to increase the number of polysaccharide chains and attachment sites, more specifically the number of available aldehyde groups on a polysaccharide chain that could be used for conjugation. The target mean molecular size for the depolymerized polysaccharides was based in part on literature precedence, intellectual property, and the target density of the reducing end sugar groups.

Dissolved polysaccharide is treated with base to reduce the O-Ac content and create more vicinal diols for oxidation to aldehydes (Figure 6-1). Oxidation is accomplished with sodium meta-periodate. Conditions were optimized for decreasing polysaccharide chain length to an average MW between 10,000 and 15,000 Da, and for the activated polysaccharide to contain an average concentration of reducing groups of 30 mol/mol of Ps. Size is monitored at-line by HPSEC. Activation is closely monitored and controlled: pH is monitored in-line and molecular size is monitored at-line.

Conjugation

The conjugation was designed to link aldehyde groups on the activated polysaccharide directly to amino groups on the VLP via reductive amination. Conjugation was optimized to produce a loading ratio of activated polysaccharide to VLP of 0.3–0.7 based on the results of animal studies for maximum immunological response. Reductive amination is accomplished using sodium cyanoborohydride. The number of available aldehydes is controlled by time and pH of conjugation, and the conjugation reaction is stopped by a "capping" reaction with sodium borohydride to reduce unreacted aldehydes to alcohol. Unreacted Ps is separated from the conjugated VLPs using tangential flow filtration and chromatography unit operations (Table 6-29).

Subset of CQAs and KPAs

- Activation and conjugation parameters can be critical as these steps determine the chemical structure of the product.
- Conjugation performance is linked with the outcome of the activation step.
- Conjugation can Impact downstream steps (e.g., aggregate from conjugation step could result in fouling of TFF membrane).

CQAs

Activation

- Activated Ps size: There is a general relationship between immunogenicity and Ps size. Size is monitored at-line by HPSEC.

Conjugation

- Free Ps: The presence of free unbound Ps could modify the immune response produced by the immunization with the Ps-VLP. Also, a conjugate vaccine with less unconjugated Ps is preferable since it contains more active ingredient. Free Ps is monitored by HPAEC-PED.
- Ps-VLP ratio: The ratio of Ps to protein was found to be critical for optimal antibody responses in other Ps-protein conjugate vaccines. The ratio is calculated from extent-of-conjugation data.
- Ps-VLP size: The molecular size of the conjugate is considered important for the potency of the targeted product. Ps-VLP size is monitored by dynamic light scattering (DLS).
- Potency: Conjugation reaction completes the formation of the Ps-VLP molecule that is the active ingredient inducing immunologic response.

KPAs

- Reducing activity after activation: Ps cannot be chemically linked to a protein without first undergoing activation.
- O-acetyl concentration after activation: It could be linked with the immunogenic epitope of the Ps. The concentration is calculated by H-NMR or the Hestrin method.
- Activation and conjugation step yields.

Impact of conjugation on potency

The premise behind the example in this case study is unique. Though differences in the nature of the conjugated Ps-VLP product could impact its potency, we cite prior experience and claim that results of *in vivo* testing of Ps-VLP product made using worst-case conjugation conditions (at extremes of the targeted design space) show that differences in conjugate structure in this example do not impact its potency. However, even if this were true, a typical vaccine candidate would not have a potency assay that had been correlated with human performance as is claimed for four of the serotypes in this case study. Therefore, a typical vaccine candidate might be handled as the fifth serotype in this case study, and only minor changes within the design space might be considered acceptable without clinical confirmation.

4192 **Table 6-29: Process Flow Diagram**

Dissolution of the Polysaccharide Bulk Time 8–12 hr Mix speed 200–250rpm Temperature 2°C–8°C	The powder is dissolved in 75 mM sodium acetate to a concentration of 10 g/L.
↓	
Activation of the dissolved Ps a. Add 80 mM NaOH and incubate 15 min ± 5 min at 35±5 °C, pH 11 b. Adjust pH to 5.5 ± 0.1 with HCl and adjust temp to 15°C ± 2°C c. Adjust the Ps solution to 25 mM sodium meta-periodate, pH 5.5, and stir at 15°C in dark d. Allow the reaction to mix until the mean molecular size is less than 15,000 Da determined by HPSEC e. Quench the reaction by adding 0.5 mL of glycerol per gram Ps.	Ps is depolymerized and oxidized using periodate to introduce terminal reactive aldehydes. Monitoring testing: - Sampling for Ps size (HPLC) - pH
↓	
Concentration and diafiltration of the depolymerized/activated polysaccharide Adjust pH 6.3 ± 0.1 (pH adjusted with 1N NaOH and 1N HCl) and concentrate to 20g L. Diafilter against PBS and 0.1M PIPES (MWCO 1000 Da).	Remove activation reactants/residuals and exchange buffer for preparation of conjugation.
↓	
Conjugation of depolymerized/activated polysaccharide (DAPS) to VLP a. Target 10 gL-1 VLP and 20 gL-1 DAPS b. Adjust pH to 8.0–8.5 c. Add NaCNBH4 at excess 10–20 mg mL-1 d. Mix 18–24 hr @ 200 ± 50 rpm @ 15–35 °C e. Dilute with saline 1:2 f. Add NaBH4 at excess 10–20 mg mL-1 g. Mix 15–25 min@ 200 ± 50 rpm	
↓	
Tangential Flow Filtration Diafilter, 10 vol physiological saline, 50 kDa MWCO membrane.	Remove unreacted components and conjugation residuals.
↓	

Hydroxylapatite Chromatography Elution with phosphate buffer in isocratic gradient.	Remove unreacted components and conjugation residuals.
↓	
Tangential Flow Filtration Diafilter, 15 vol PBS, pH 6.3, 100 kDa MWCO membrane.	
↓	
0.22 µm filtration	

6.14. Ps-VLP Conjugation Early Process Development

6.14.1. Prior Knowledge

General process steps and conditions were defined based on two licensed conjugated polysaccharide vaccines and general conditions described in literature.

6.14.2. Activation

Literature reference
The rationale for developing a depolymerization process of the purified capsular polysaccharide was to decrease the Ps size and increase the number of activation sites per polysaccharide chain that could be used for conjugation *(Silveira et al, Vaccine 25 (2007), 7261–7270)*.

The operating ranges mentioned in the literature cover the following ranges:

Process parameter	Min	Max
Sodium meta-periodate concentration (mM)	10	25
Activation time (hr.)	0.5	4
Temperature (°C)	15	40
pH	9	12

However, the literature does not show a consistent relationship between Ps size and immunogenicity (*C.H. Lee, et al, Vaccine, 27, 2009; T. Carmenate et al, FEMS Immunology and Medical Microbiology, 40, 2004*).

Early process development
To determine the minimum chain length of the Ps that can be used to elicit a specific anti-polysaccharide immune response in laboratory animals and define a working range for temperature and sodium meta-periodate concentration, the following was performed with four lots of Ps.

Two levels of temperature and sodium meta-periodate were selected while keeping all other variables at target values (see process flow diagram in Table 6-29).

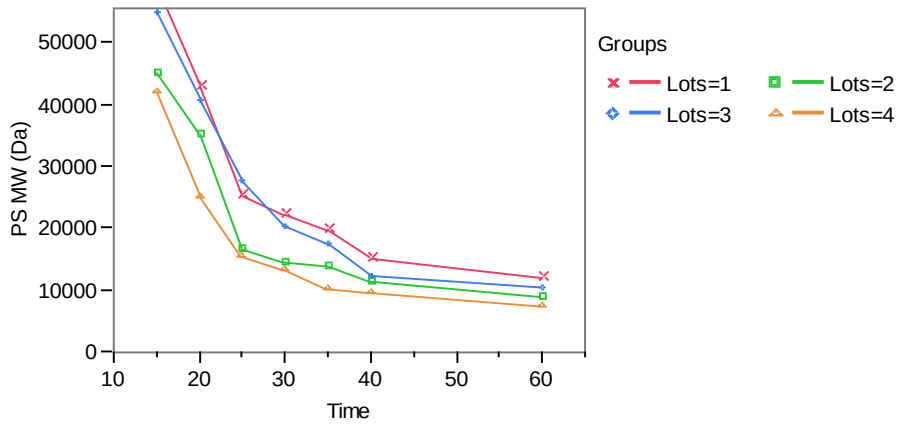
Lot	Temperature (°C)	Sodium meta-periodate (mM)
1 2	15	10 25
3 4	40	10 25

The rate of depolymerization was evaluated by sampling at different times and size fractions evaluated by HPSEC (Figure 6-22).

Based on the results, it can be observed that:

- The rates of the depolymerization reactions are faster at higher concentrations of sodium meta-periodate (groups 2&4).
- There was no apparent relationship between reaction temperature and the rate of depolymerization (group 1 vs. 3 and group 2 vs. 4).
- The reducing activity content of the four lots was considered comparable to one another based on the assay variability (data not show).

Figure 6-22: Mean Ps MW (Da) by Reaction Time (min)



Fractions of small, medium, and large size were conjugated accordingly for further study of their immunogenicity.

Immunogen	Ps MW (Da)	Titer*
Low	5,000–10,000	4.9
Medium	10,000–15,000	4.3
Large	15,000–25,000	3.5
Initial Ps	> 40,000	1.6

* Mean ELISA titers were calculated using arbitrary unit of ELISA (EU/mL).

Although these studies were successful in confirming that all of the conjugates developed greater response than the initial polysaccharide, no significant response was observed among the Ps size tested. Also, determining the minimum chain length requirement to elicit immunogenicity of the polysaccharide-protein conjugate in lab animals is a risk because these relatively short chain lengths may not necessarily be the optimal chain length that maximizes the immune response in humans. Thus, taking into account literature precedence, intellectual property, and the target density of the reducing end sugar groups, a final range of 10,000 to 15,000 Da was defined.

In addition, the primary structure of the depolymerized Ps purified from the reaction was evaluated by NMR spectroscopy. The ^{1}H NMR spectra of DAPS presents the same assignments as the Ps, showing that the polysaccharide structure remains unchanged. However, after de-O-acetylation and periodate treatment, chemical shifts are present that correspond to the novel end groups. These chemicals shifts are consistent with the aldehydic group.

As a result, the following conditions were defined.

Temperature (°C)	**35 ± 5**
Sodium meta-periodate (mM)	25
Ps size (Da)	10,000–15,000

6.14.3. Conjugation

The process that was evaluated during the design phase attempted to yield more than one reactive site per polysaccharide chain, and this in turn led to multi-site attachment of the Ps to the VLP. Furthermore, the process while maintaining antigenic consideration must also be applicable to conjugate the five different serotypes.

Literature reference

Typically, the ratio DAPS:VLP could change among the serotypes, leading to adjustments in the DAPS and VLP concentration. In addition, increasing the VLP concentration while keeping the DAPS concentration constant normally results in an increase in VLP-VLP cross-link, which has a potential impact on filterability. Also, conjugation reaction could be affected by the charge density associated with each serotype polysaccharide and the reactivity of the amino groups of VLP *(Joshi et al, Carbohydrate Polymers 75 (2009), 553–565)*.

Early process development

Concentration ratios from about 1:2 to about 2:1 were used for the other serotypes. Based on that previous experience, 2:1 conditions for the Ps:VLP concentrations were selected. To define pH conditions, different pHs were evaluated at lab scale while keeping constant other reaction conditions. Conjugate molecules were further purified by dialysis.

Ps molecular weight (MW)	**Ps:VLP concentration**	**pH**	**Free Ps (%)**	**Conjugate ratio (0.3–0.7)**
10,000–15,000	2:1	7.0	10.2	0.28
10,000–15,000	2:1	7.5	9.5	0.32
10,000–15,000	2:1	8.0	11.3	0.53
10,000–15,000	2:1	8.5	10.8	0.49

Working in a pH range of 8.0 to 8.5, there did not appear to be a significant impact on either the polysaccharide-to-protein ratio or the extent of free Ps. The other attributes met their criteria. As a result, the following conditions were defined.

Conjugation pH	**8.0–8.5**
Concentration ratio Ps:VLP	2:1

6.15. Ps-VLP Conjugation Early Process Risk Assessment

A cause-and-effect matrix (C&E) was the risk assessment (RA) tool used to identify processes parameters for creation of the design space. The C&E matrix provides a mechanism to assess process parameters (inputs) against quality and process attributes (outputs) to prioritize parameters for experimental studies. However, the matrix does not provide manufacturing control boundaries (process parameter ranges) to assess the potential severity impact of the factors assessed.

The goals of the C&E matrices are to capture the current knowledge and the relationships among inputs and outputs, to prioritize areas for further study and experimental design, and to evaluate the completeness of the process understanding.

The key deliverable is the prioritization of high-risk process parameters for designed process characterization experiments. As knowledge of the commercial manufacturing process and facility becomes available, facility control and procedural capabilities may also be evaluated with failure modes and knowledge gaps identified.

Cause-and-effect matrices

To create a cause-and-effect matrix, the following steps are necessary:

3. Create a process flow map (prerequisite as described above).
4. Define focus areas/unit operations (prerequisite).
5. Identify and rank attributes (quality and process) for each focus area/unit operations.
6. Identify and rank the relationship between process parameters and attributes.
7. Calculate cumulative parameter scores.

The CQAs for the final drug substance and drug product should be determined prior to the creation of a C&E matrix.

Each process parameter (input) is assessed based on the potential impact on the outputs of a particular focus area, including quality attributes or process performance attributes. The inputs are process parameters that can be people, equipment, measurements, process, materials environment etc., while the outputs are VLP-poly conjugates, aggregates, biopotency, endotoxins, free VLP, free poly, excess reagents, contaminants, product degradants, step yield, etc. A subset of CQAs was considered for risk assessment (e.g., free Ps, Ps/VLP ratio, Ps-VLP size, potency). The objective is to establish the functional relationship between quality attributes (y) and process parameters (x). Each quality attribute is assigned a “weight” score based on its potential impact on product quality, safety, or efficacy (Table 6-30).

For example, QAs that are deemed to be critical will fall into the 10 or 7 scores, while QAs that are borderline regarding criticality would score a 5 (Table 6-30).

A cumulative score is then calculated for each parameter using Equation 6-1.

Equation 6-1: Cumulative Score for Parameter in C&E Matrix

Cumulative score = ∑ (Impact of parameter x weight of quality or process performance attribute)

The cumulative scores in the (C&E) matrix are used to identify the process parameters and the experimental approach for process understanding studies. The maximum cumulative score will vary by focus area and will depend on the number of attributes scored.

The cumulative score represents the relative importance of a process parameter for the focus area (or unit operations), so parameters with high scores could potentially be of high risk to product quality or process performance and should have supporting process understanding. The process

parameter prioritization for experimentation is subject to the team's interpretation and may be governed by statistical approaches, prior knowledge, or specific product safety concerns.

For those parameters requiring study, a combination of univariate and multivariate experimental studies may be performed to identify significant effects and to characterize the process design space. The justification for parameters requiring no new studies may be complemented by the consideration of prior knowledge established for the same or related products (platform data) or of literature information.

The process parameters evaluated in the risk assessment for the activation and conjugation steps in Table 6-31 and Table 6-32, respectively, were identified based on prior experience.

Table 6-30: Scoring of Process Parameters and Quality Attributes

Process Parameters		Attributes[1]	
Impact Score	**Ranking Criteria**	**Weight Score**	**Ranking Criteria**
10	Strong relationship known based on available data and experience	*10*	Established or expected direct impact on safety and/or efficacy of product.[2]
7	Strong relationship is expected	*7*	Moderate or indirect impact on safety and/or efficacy. Direct impact on efficiency.
5	Not-so-strong relationship expected or unknown	*5*	Low or unlikely impact on product safety and/or efficacy. Moderate or indirect impact on efficiency.
1	Known to not have a relationship	*1*	No impact to product safety and/or efficacy. Low or unlikely to impact efficiency.

[1] Process performance attributes may have no direct impact on product quality, safety, or efficacy but are assessed where they are important indicators of focus area function or performance consistency. Examples include step recoveries and overall yield.
[2] May include efficiency/process attributes, but most efficiency attributes are not a 10 unless they significantly impact product viability.

Table 6-31: Cause-and-Effect Matrix for Activation of Polysaccharide

	Reducing activity	[O-Ac]	Activated poly size	Yield	Total score
Quality attribute scores	10	7	7	7	
Parameter					
Activation temp	10	7	10	5	254
Activation pH	10	10	7	5	254
Activation time	10	7	10	5	254
Poly concentration	1	5	5	1	87
Total grams of poly added	1	5	5	1	87
Concentration of meta-periodate [1]	5	5	1	1	87

	Reducing activity	[O-Ac]	Activated poly size	Yield	Total score
Addition rate of meta-periodate	1	5	1	1	59
Activation reaction agitation rate	1	5	1	1	59
Ratio of glycerol to poly for quenching	1	1	1	1	31
Quenching reaction time	1	1	1	1	31
Post-quench hold temperature	1	1	1	1	31
Post-quench hold time	1	1	1	1	31

[1] Parameter known to not have an impact on activated Ps size at the range to be used in this process based on prior experience.

The highlighted scores signify grouping of parameters with similar scores. In this example, parameters with scores of 254, highlighted in red in the C&E table, are deemed to be of high priority for process characterization studies. The color grouping of parameters is based on the natural breaks in the scores. For example, parameter scores of 87 are highlighted yellow, and the remaining parameters with scores from 59 through 31 are not highlighted. The parameters highlighted in yellow have lower cumulative scores and have ample prior knowledge/literature, thus do not require further studies. The parameters in the no-shaded box were deemed to be of low risk, and no further study was undertaken.

Table 6-32: Cause-and-Effect Matrix for Conjugation

	Free Ps	Ps/VLP ratio	Ps-VLP size	Yield	Potency	Total score
Quality attribute scores	10	10	10	7	10	
Parameters						
VLP and poly concentration	10	10	10	10	10	470
Conjugation reaction incubation temp	10	10	10	10	7	440
Agitation rate during VLP addition	10	5	5	10	7	370
NaCNBH4 excess ratio	10	10	5	5	7	370
VLP addition rate	5	1	1	5	5	155
Conjugation reaction time	1	1	1	1	5	87
Conjugation reaction agitation rate	1	1	1	1	1	47
$NaBH^4$ excess ratio	1	1	1	1	1	47

	Free Ps	Ps/VLP ratio	Ps-VLP size	Yield	Potency	Total score
Capping reaction time	1	1	1	1	1	47
Capping reaction temp	1	1	1	1	1	47

The process parameters identified (highlighted in red in the C&E table) after RA for further study are: activation temperature, time, and pH for the activation of polysaccharide step. For the conjugation reaction: VLP/poly stoichiometry, incubation temperature, agitation rate during VLP addition, and $NaCNBH_4$ excess ratio. All of these parameters were selected for their relative high scores when compared with the other parameters assessed in their respective unit operations. The parameters highlighted in yellow have lower cumulative scores and have ample prior knowledge/literature, thus do not require further studies. The parameters in the no-shaded box were deemed to be of low risk, and no further study was undertaken.

6.16. Ps-VLP Conjugation Late Stage Risk Assessment

The second-round RA is conducted prior to process validation. For this evaluation, the large-scale manufacturing process normal operating ranges (NORs) are known or estimated based on prior experience. The DOE studies have identified potential NORs and proven acceptable ranges (PARs) within which consistent process performance and acceptable product quality are expected.
The FMEA is conducted to evaluate the drug substance manufacturing processes and the potential impact on process performance and product quality.
The goals of the FMEA are focused on assessing the potential severity impact in relation to manufacturing process, site capabilities, and operational experience. Other outcomes from the second RA include process parameter risk identification/mitigation and potential parameter criticality classification.

Failure Modes Effects Analysis (FMEA)

The principles of FMEA were previously described in Section 6.5.

Table 6-33 and Table 6-34 describe FMEA analyses performed to identify critical process parameters and potential actions to mitigate their criticality for the activation and conjugation steps, respectively.

4394 **Table 6-33: Activation Step FMEA Scores**

Process parameter	NOR	Failure mode	Cause	Effect on quality attributes	Effect on process attributes	Severity	Occurrence	Detectibility	Risk score	Rationale	Action if required
Ps concentration (g/L)	5-15 g/L	Ps concentration < NOR	Mixing conditions during dissolution of the bulk powder (agitation 200-250 rpm & time 8-12 hr)	Possible impact on free Ps and ratio Ps/VLP if correlation with reducing activity is confirmed		9	3	3	81	Ensure dissolution consistency	Mixing ranges are to be validated concurrent with process validation batches. Also a monitoring test before activation step to control Ps concentration may be added.
			Moisture content of the purified polysaccharide bulk powders is variable			9	1	1	9		Moisture test for Ps release and validated Ps container closure
Temperature (ªC)	30-40°C	Overheating	Heating transfer issues		Possible yield impact due to suboptimal level of reducing activity	5	3	1	15	Vessel design was considered during scale-up definition.	
			Equipment-dependant failure		Possible yield impact due to suboptimal level of reducing activity	5	3	3	45	Cover by equipment & instruments qualification.	Temperature monitored during activation. Tank Maintenance plan.
pH	10-12	pH outside NOR	NaOH preparation	May impact Ps size. Degree of de-Oacetilation is pH dependent		7	3	5	105	Range is suitable for control of the Ps size.	pH monitoring during activation
			NaOH addition			7	3	3	63	Cover by equipment & instruments qualification.	

Process parameter	NOR	Failure mode	Cause	Effect on quality attributes	Effect on process attributes	Severity	Occurrence	Detectibility	Risk score	Rationale	Action if required
Time (min)	10-12	Under time limit	Human error		Deviation below this range may impact overall yield by decreasing the level of reducing activity	5	1	1	5		Kaizen criteria in SOP description to reduce risk of human error.

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4397 **Table 6-34: Conjugation Step FMEA Scores**

Process parameter	NOR/PAR	Failure	Cause	Effect on quality attributes	Effect on process attributes	Severity	Occurrence	Detectibility	Risk score	Rationale	Action if required
Ps concentration (g/L)	5-15 g/L	Concentration range outside PAR	Mixing conditions during dissolution of the bulk powder (agitation 200-250 rpm & time 8-12 hr), error in analysis	Possible impact on free Ps and ratio Ps/VLP if correlation with reducing activity is confirmed		9	3	3	81	Ensure dissolution consistency.	Mixing ranges are to be validated concurrent with process validation batches. Also a monitoring test before activation step to control Ps concentration may be added.
Temperature (ªC)	30-40°C	Overheating	Heating transfer issues Equipment-dependant failure		Possible yield impact due to suboptimal level of reducing activity	5	3	1	15	Vessel design was considered during scale-up definition.	
					Possible yield impact due to suboptimal level of reducing activity	5	3	3	45	Cover by equipment & instruments qualification.	Temperature monitored during activation. Tk Maintenance plan.
pH	10-12	pH outside PAR	NaOH preparation	May impact Ps size		7	3	5	105	Range is suitable for control of the Ps size.	pH monitoring during activation
			NaOH addition			7	3	3	63	Cover by equipment & instruments qualification.	
Time (min)	20-Oct	Under time limit	Human error?		Deviation below this range may impact overall yield by decreasing the level of reducing activity	5	1	1	5		

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6.17. Ps-VLP Conjugation Design Space

6.17.1. Objective

Given that activation and conjugation steps were considered most significant in potentially impacting CQAs of A-VAX based on prior knowledge, a multivariate experimental design was employed to understand the effect of process parameters on those steps.

To allow an optimal and economic transition between the screening phase and optimization phase, experiments have the following objective and structure:

a. Screening design: Parameters and ranges are selected based on risk assessment and prior knowledge with the objective to identify main effects on the selected attributes. Two levels of fractional-factorial central composite design plus two central points are used. Each parameter was represented at the levels (minimum and maximum) indicated below. As a result, main effects are identified between the parameters and the attributes.
b. Optimization design: Augment the screening results by adding axial and central points considering only those parameters with an effect on attributes. The final design matrix is a fractional-factorial central composite design combined with central points and axial points, where one parameter is set at an extreme level while the other parameters are set at their central point level ($\alpha=\pm1$). Thus, experimental-based ranges can be defined to ensure CQA acceptability.

Multivariate techniques such as partial least square can handle large numbers of variables simultaneously, while DOE deals with a limited numbers of variables because of limited experimental runs.

The use of fewer experimental runs, particularly during the screening phase, could underestimate the impact of any particular parameter on the evaluated attributes. To reduce this risk whenever possible, prior knowledge will be used to select parameters.

Also, the results obtained through these DOE studies can be used as complementary information when the process is established, allowing a better understanding of its inherent complexity.

All experiments were performed at lab scale considering scalable requirements.

DOE definition and analysis were performed using the software package: JMP v7.0 (SAS).

6.17.2. Activation Step

Factors

Four critical process parameters were identified as design factors based on the risk assessment analysis.

Ranges (Table 6-35) were selected based on prior knowledge and realistic manufacturing operating ranges.

Table 6-35: Activation Parameters

Parameters	Unit	Min (-1)	Max (+1)
Ps concentration	g/L	5	15
Temperature	°C	30	40
pH	pH unit	10	12
Time	Min	10	20

Attributes

The activation process responses or attributes (Table 6-36) were selected based on risk assessment analysis.

Table 6-36: Activation Attributes

Attributes	Category	Unit	Min	Max	Analytical Procedure
Reducing activity	KPA	mol/mol Ps	18	30	BCA (using glucose as a reference)
O-Ac	CQA	mol/mol Ps	–	1.8	H-NMR/Hestrin
Ps size	CQA	Da	10,000	15,000	HPSEC-MALS-RI
Ps yield	KPA	%	75	–	High-pH HPAEX-PAD

Screening design

To identify which parameters have significant effects on the selected attributes, a two-level factorial design including two central points was employed in which each parameter was represented at the levels (minimum and maximum) indicated above.

Taking into account the previous knowledge gained through production of other conjugate vaccines and the risk assessment, a fractional-factorial design was chosen; it ignores interactions among parameters (resolution III) to minimize the number of runs. Only parameters with high significant levels will be selected for optimization studies.

Table 6-37 shows the results obtained after the first set of experiments.

Table 6-37: Activation Screening Design Matrix and Results

Run	Temperature (°C)	pH	Time (min)	Ps concentration (g/L)	O-Ac	Reducing activity (mol/mol Ps)	Ps size (Da)	Ps yield (%)
1	40	12	10	5	0.14	34.77	13598.89	59.15
2	30	10	10	15	1.27	12.23	16616.15	93.7
3	30	10	20	5	0.25	32.64	17195.17	78.12
4	30	12	10	5	0.34	32.69	12685.52	60.76
5	40	10	10	15	1.32	13.66	16182.89	90.59
6	35	11	15	10	0.8	23.89	14879.44	74.11
7	30	12	20	15	0.89	18.72	13178.31	74.5
8	35	11	15	10	0.64	22.02	15135.85	76.56
9	40	10	20	5	0.1	34.53	14548.39	69.18
10	40	12	20	15	0.94	19.26	14328.87	67.12

The analysis of variance was performed for all attributes. Table 6-38 shows for each studied attribute the p value and the estimate value for each of the parameters.

Parameters that were significant at a 95% confidence interval (p-value < 0.05) were selected for further evaluation.

However, the estimated value of each parameter could also be used to support the selection of parameters. For example, the temperature effect on yield is not significant, but the effect is large enough to be further evaluated.

For this exercise, only results on reducing activity will be discussed.

Table 6-38: Summary of Results for Screening Design on Activation Step

Attribute	Temperature (°C)		pH		Time (min)		Ps concentration (g/L)	
	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate
O-Ac	0.3954	-0.03125	0.0662	-0.07875	***0.0213***	-0.11125	***<0.0001***	0.44875
Reducing activity (mol/mol Ps)	0.1572	0.7425	***0.0179***	1.5475	***0.0214***	1.475	***<0.0001***	-8.845
Ps size (Da)	0.7249	-127.0138	***0.0110***	-1343.876	0.9535	20.91125	0.4418	284.78125
Ps yield (%)	0.1432	-10.13	0.8379	-1.2575	0.3822	5.59	0.9789	-0.1625

Results

Table 6-39 shows sorted parameter estimates for reducing activity. It can be seen that Ps concentration, activation time, and pH have p values <0.05 and thus are significant for reducing activity levels.

The activation temperature results are neither significant at 0.05 nor have high estimated value; therefore, temperature is not expected to have a significant impact on reducing activity.

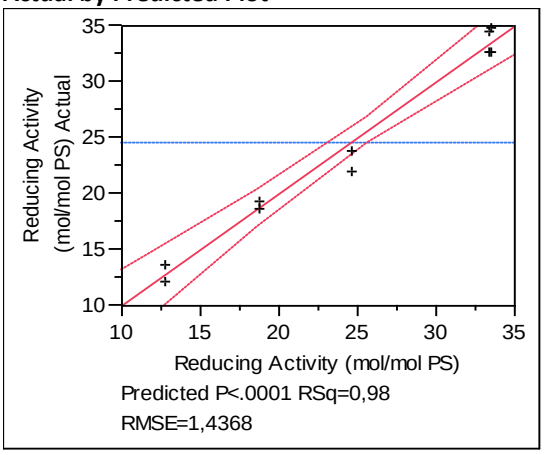
Table 6-39: Sorted Parameter Estimates for Reducing Activity (Screening)

Term	Estimate	Std Error	t-ratio	t-ratio	Prob>|t|
Ps (g/L) (5,15)	-8.845	0.446542	-19.81		<.0001
Activation pH (10.12)	1.5475	0.446542	3.47		0.0179
Activation Time (10.20)	1.475	0.446542	3.30		0.0214
Activation Temperature (°C)(30.40)	0.7425	0.446542	1.66		0.1572

After removing the insignificant term (activation temperature), a model fit was performed (Figure 6-23). The ANOVA table shows that the model as a whole is significant (p= 0.0001).

Figure 6-23: Model Fit and ANOVA for Reducing Activity

Actual by Predicted Plot



Analysis of Variance

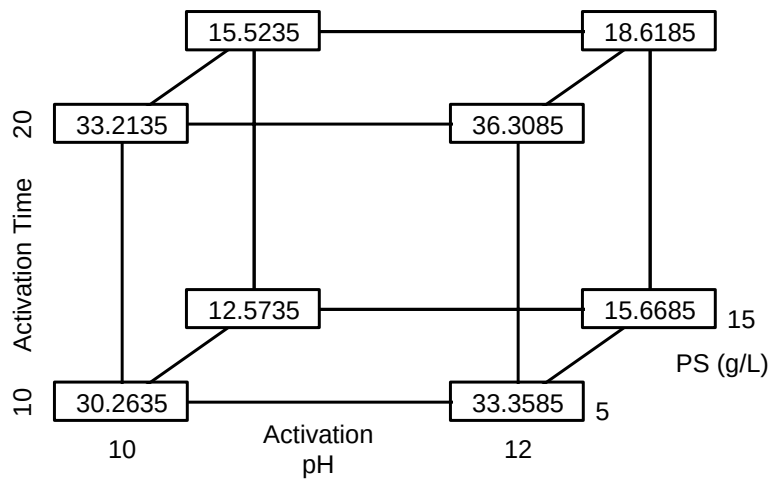
Source	DF	Sum of Squares	Mean Square	F-Ratio
Model	3	662.43525	220.812	106.9614
Error	6	12.38644	2.064	**Prob > F**
C. Total	9	674.82169		<.0001

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F-Ratio
Lack of Fit	1	5.520490	5.52049	4.0202
Pure Error	5	6.865950	1.37319	**Prob > F**
Total Error	6	12.386440		0.1013
				Max RSq
				0.9898

The following figure displays a set of predicted values for reducing activity for the extremes of the parameter ranges (vertices of a cube). It can be seen that some process conditions could lead to values outside the criteria for reducing activity (18-30 mol/mol Ps). Thus, process ranges for the selected parameters must be adjusted to meet the criteria for reducing activity.

Figure 6-24: Box Plot on Reducing Activity



Conclusion on screening design

The results for the screening design show the following conclusions:

- There is no apparent relationship between temperature and the attributes in the evaluated range; therefore, it is considered to not be a critical process parameter. In addition, because of the high estimated value obtained for yield, the target value could be further optimized.
- Significant interaction among activation time, pH, and Ps concentration on the evaluated attributes was found. Thus, these parameters must be considered as critical process parameters and their ranges adjusted to guarantee process robustness.
- There is a significant impact of pH and Ps concentration on yield; however, caution must be taken to optimize yield based on these parameters as they have an impact on a CQA.

Optimization design

Results obtained during the screening phase show that some process conditions could lead to values out of acceptance criteria for reducing activity (18-30 mol/mol Ps). They also allow identification of the process parameters that have significant impact on reducing activity.

Taking into account the screening results, an augment design is proposed to test intermediate process conditions and also to evaluate second-order interactions.

The final design (Table 6-40) matrix is a fractional-factorial central composite design. It combines four central points and six axial points where one parameter is set at an extreme level while the other parameters are set at their center point (α=±1). The values of the parameters are given in Table 6-37.

Table 6-40: Activation Optimization Design Matrix

Run	pH	Time (min)	Ps concentration (g/L)
1	1	-1	-1
2	-1	-1	1
3	-1	1	-1
4	1	-1	-1
5	-1	-1	1
6	0	0	0
7	1	1	1
8	0	0	0
9	-1	1	-1
10	1	1	1
11	0	0	0
12	0	0	0
13	1	0	0
14	-1	0	0
15	0	1	0
16	0	-1	0
17	0	0	1
18	0	0	-1

Results

A preliminary evaluation of the parameters and their interactions is performed to identify the strongest effects.

Table 6-41 shows that only Ps concentration has a significant effect on reducing activity (p-value < 0.005). However, the estimate values are comparable between parameters and parameter interactions. Specifically, the second-order interaction "Ps concentration*activation time" has a comparable value of estimate and a borderline p-value. The results suggest that Ps concentration and the second-order interaction "Ps concentration*activation time" should be further evaluated (Ps concentration must be included as it is involved in the second-order interaction).

Table 6-41: Contrasts for Reducing Activity (mol/mol Ps)

Term	Contrast	Plot of t-ratio	Length t-ratio	Individual p-value
Ps (g/L)	-5.69005		-4.37	*0.0039*
Activation Time	2.00799		1.54	0.1286
Activation pH	0.04472		0.03	0.9726
Ps (g/L)*Ps (g/L)	-2.01731		-1.55	0.1275
Ps (g/L)*Activation Time	2.21743		1.70	0.0982
Activation Time*Activation Time	0.98267		0.75	0.4271
Ps (g/L)*Activation pH	-1.81718		-1.40	0.1651
Activation Time*Activation pH	-1.80525		-1.39	0.1671
Activation pH*Activation pH	2.03553		1.56	0.1246
Ps (g/L)*Activation Time*Activation pH	0.20767		0.16	0.8814

The ANOVA analysis shows that the model as a whole is significant (data not show). However, only Ps concentration has p-values <0.05 and thus is significant (Table 6-42).

Table 6-42: Sorted Parameter Estimates for Optimization Design

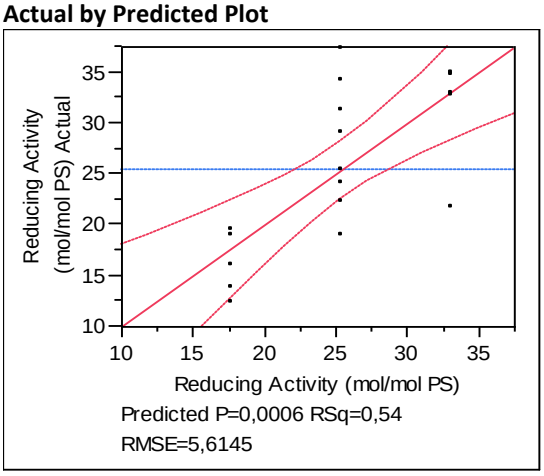
Term	Estimate	Std Error	t-ratio	t-ratio	Prob>|t|
Ps (g/L) (5.15)	-7.634	1.716786	-4.45		0.0006
Activation Time (10.20)	2.694	1.716786	1.57		0.1389
Ps (g/L)*Activation Time	1.5475	1.919425	0.81		0.4336

Despite the fact that only Ps concentration was found to be significant, a new analysis was performed. It considered both Ps concentration and activation time because of the high estimated value obtained for activation time (2.694). Second-order interactions are considered negligible.

The ANOVA analysis for the resulting model is significant at p-values <0.05.

Figure 6-25: Model Fit and ANOVA for Reducing Activity

Actual by Predicted Plot



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F-ratio
Model	1	582.7796	582.780	18.4876
Error	16	504.3641	31.523	**Prob > F**
C. Total	17	1087.1437		0.0006

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F-ratio
Lack of Fit	1	73.25142	73.2514	2.5487
Pure Error	15	431.11267	28.7408	**Prob > F**
Total Error	16	504.36409		0.1312
				Max RSq
				0.6034

The sized Ps has a MW of ~10–15 kD, which corresponds to six to ten repetitive units.

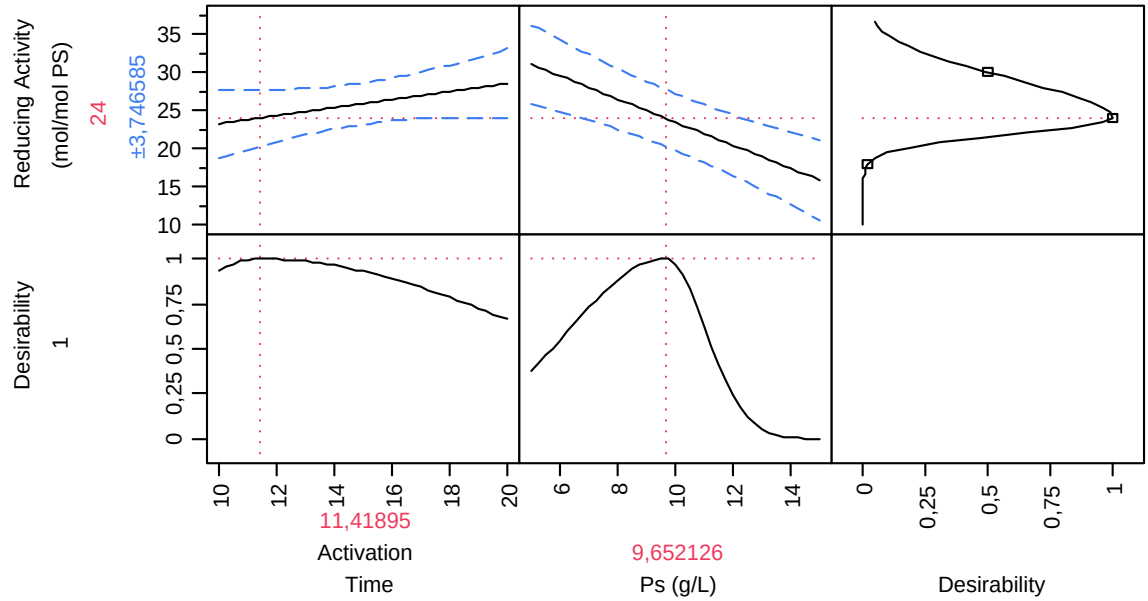
According to the *X. horrificus* serotype 2 capsular polysaccharide structure, after the activation step, three activated sites per repetitive unit are expected, resulting in multipoint attachment to the VLP (Figure 6-1).

However, the 2 OH on Glc could also be oxidized to render five activated sites per unit. This could lead to increased Ps-VLP conjugation sites, which may have an undesirable impact on Ps/VLP ratio and Ps-VLP size. Therefore, the range for reducing activity has been defined as 18-30 mol/mol Ps.

In an attempt to increase confidence about the degree of multipoint attachment of the Ps-VLP, the target value for reducing activity was defined as 24 mol/mol Ps.

Using the desirability function where a value of 1 represents 24 mol/mol Ps, the target values for activation time and Ps concentration are estimated as 11.4 min. and 9.65 g/L, respectively (Figure 6-26).

Figure 6-26: Desirability Function for Reducing Activity vs. Activation Time and Ps Concentration



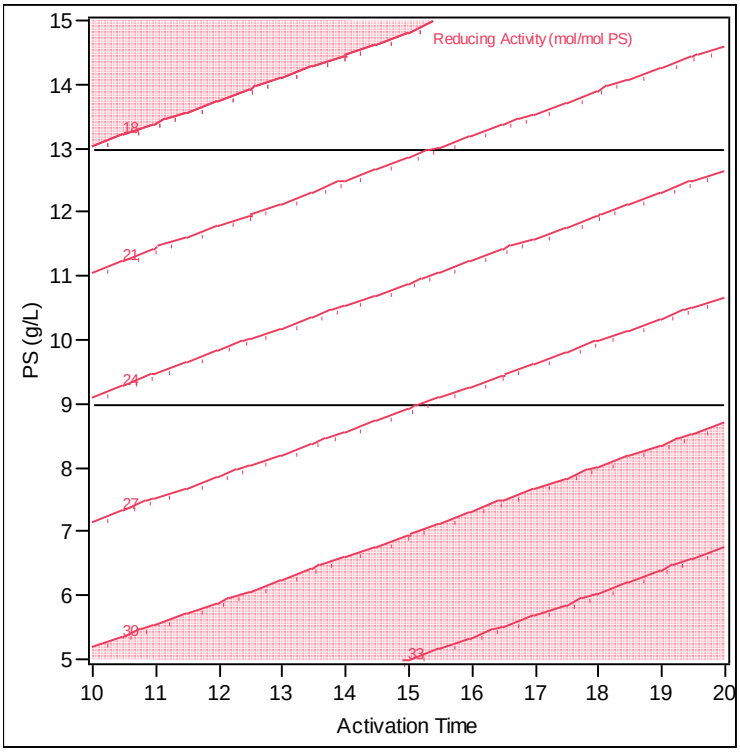
When the reducing activity is plotted against the activation time and the Ps concentration, it can be observed that between Ps concentrations of 9 and 13 g/L, the variation of time within the established range does not lead to out-of-specification values of reducing activity (Figure 6-27).

Thus, the Ps concentration range can be narrowed from 5–15 g/L to 9–13 g/L with a target value of 11 g/L. The range could even be tightened to 9.0–12.0 g/L to prevent a low level of reducing activity.

Table 6-43: Inverse Prediction for Reducing Activity

Reducing Activity (mol/mol Ps)	Predicted Ps (g/L)	Lower Limit	Upper Limit	1-Alpha
18	14.8456467	12.6040951	20.1995286	0.9500
24	10.9158589	9.0153857	13.4045828	
30	6.9860711	3.1346763	8.9016371	

Figure 6-27: Reducing Activity Values Plots vs. Ps Concentration and Time



Conclusion on activation

The pH range was also adjusted because it has a correlation to Ps size. However, because of the on-line HPSEC monitoring of the Ps size during sodium meta-periodate treatment, no further tightening of the pH ranges was considered necessary. No adjustment was found necessary for time and temperature ranges. Based on these conclusions, the design space for activation is defined as follows:

Table 6-44: Process Parameter Ranges for Activation Step

Parameters	Unit	Min	Max
Ps concentration	g/L	9.0	12.0
Temperature	°C	30	40
pH	pH unit	11	12
Time	min	10	20

6.17.3. Conjugation Step

Parameters

Five critical process parameters were identified as design factors based on the risk assessment analysis (Table 6-45). Incubation time for the conjugation step has been identified as a process improvement opportunity and therefore is included in the design. Ranges were selected based on prior knowledge and realistic manufacturing operability.

Table 6-45: Conjugation Parameters

Parameters	Unit	Min	Max
VLP concentration	g/L	8	12
DAPS concentration	g/L	15	25
Incubation temperature	°C	15	35
Agitation rate during VLP addition	rpm	150	250
NaCNBH4	mg/mL	10	20
Incubation time	hr	12	24

Attributes

The product and process attributes were selected (Table 6-46) based on the risk assessment analysis. Yield is included for a comprehensive evaluation of the design space.

Table 6-46: Conjugation Attributes

Attributes	Category	Unit	Min	Max	Analytical Procedure
Free Ps	CQA	%	–	10	High-pH HPAEX-PAD
Ps/VLP ratio	CQA	–	0.3	0.7	HPLC/BCA protein assay
Ps-VLP size	CQA	nm	20	50	DLS
Ps-VLP yield	KPA	%	50	–	HPAEC-PAD or ELISA

Screening design

A two-level factorial design including two center points was employed. Each parameter was represented at two levels (minimum and maximum) in ten runs (Table 6-47). The result is a resolution-three screening design. All the main effects are estimable, but they are confounded with two-parameter interactions as was mentioned in the screening design for the activation step. The runs were performed in random order, and results are displayed in Table 6-48.

Table 6-47: Conjugation Screening Design Matrix

Run	DAPS (g/L)	VLP (g/L)	Incubation temperature (°C)	Agitation during VLP addition (rpm)	$NaCNBH_4$ (mg/mL)	Time (hr)
1	25	12	35	250	20	24
2	25	8	15	250	20	12
3	15	8	15	250	10	24
4	15	12	15	150	20	12
5	15	8	35	150	20	24
6	25	12	15	150	10	24
7	25	8	35	150	10	12
8	20	10	25	200	15	18
9	15	12	35	250	10	12
10	20	10	25	200	15	18

Table 6-48: Conjugation Screening Design Results

Run	Free Ps (%)	Ps/VLP ratio	Ps-VLP size	Yield (%)
1	11.58	0.59	54.36	53
2	12.78	0.49	31.31	45
3	7.58	0.24	27.57	44
4	7.13	0.28	48.32	35
5	8.31	0.26	26.85	57
6	10.19	0.25	59.2	35
7	13.33	0.58	32.84	53
8	9.4	0.49	41.21	47
9	7.35	0.22	46.24	58
10	11.24	0.40	37.73	56

The analysis of variance was performed for all attributes. Table 6-49 shows for each studied attribute which parameters are significant at a 95% confidence interval. However, only results on Ps-VLP size will be discussed.

The estimated value for attributes could be also used to support the selection of parameters. For example, the DAPS concentration effect on Ps/VLP ratio is not significant, but the effect is large enough for further evaluation. A similar situation can be expected for the effect on yield of VLP concentration and agitation during VLP addition.

Table 6-49: Summarized Results for Screening Design on Conjugation Step

Parameter	Free Ps (%)		Ps/VLP ratio		Ps-VLP size		Yield (%)	
	Estimate	Prob>|t|	Estimate	Prob>|t|	Estimate	Prob>|t|	Estimate	Prob>|t|
DAPS (g/L)	2.18875	0.0057	0.11375	0.0730	3.59125	0.0100	-1.08375	0.5923
VLP (g/L)	-0.71875	0.1020	-0.02875	0.5423	11.19375	0.0004	-2.13875	0.3234
Conjugation incubation temperature (°C)	0.36125	0.3258	0.04875	0.3292	-0.76375	0.3025	7.64375	0.0244
Agitation during VLP addition (rpm)	0.04125	0.9020	0.02125	0.6473	-0.96625	0.2141	2.50125	0.2618
$NaCNBH_4$ (mg/mL)	0.16875	0.6221	0.04125	0.3979	-0.62625	0.3834	0.13375	0.9459
Incubation time (hr)	-0.36625	0.3202	-0.02875	0.5423	1.15875	0.1560	-0.21625	0.9126

Results

Table 6-50 shows sorted parameter estimates for Ps-VLP size. Both VLP and DAPS have p-values <0.05 and thus are significant on Ps-VLP size. Also they account for the higher estimated values.

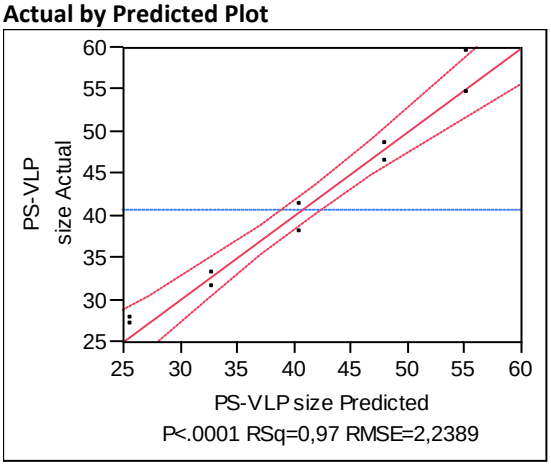
Table 6-50: Sorted Parameter Estimates for Ps-VLP Size

Parameter	Estimate	Std Error	t-ratio	t-ratio	Prob>|t|
VLP (g/L) (8,12)	11.19375	0.614896	18.20		0.0004
DAPS (g/L) (15.25)	3.59125	0.614896	5.84		0.0100
Incubation time (Hs) (12.24)	1.15875	0.614896	1.88		0.1560
Agitation during VLP addition (rpm) (150.250)	-0.96625	0.614896	-1.57		0.2141
Conjugation incubation temperature (°C) (15.35)	-0.76375	0.614896	-1.24		0.3025
$NaCNBH_4$ (mg/mL) (10.20)	-0.62625	0.614896	-1.02		0.3834

After removal of the insignificant terms (incubation time, agitation during VLP addition, conjugation incubation temperature, and $NaCNBH_4$ concentration), a model fit was performed (Figure 6-28). The ANOVA table shows that the model as a whole is significant (p= 0.0001).

Figure 6-28: Model Fit and ANOVA for Ps-VLP Size

Actual by Predicted Plot



Analysis of Variance

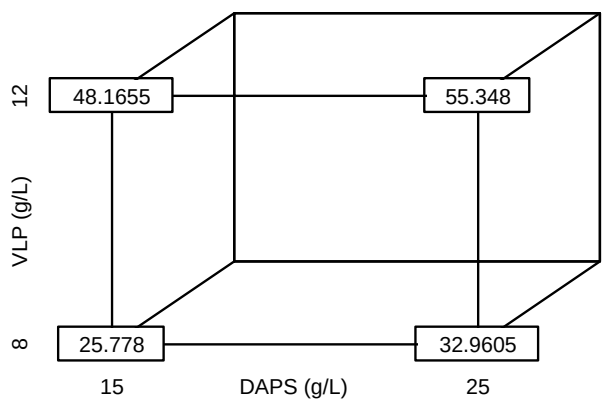
Source	DF	Sum of Squares	Mean Square	F-ratio
Model	2	1105.5769	552.788	110.2770
Error	7	35.0891	5.013	**Prob > F**
C. Total	9	1140.6660		<.0001

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F-ratio
Lack of Fit	2	13.728235	6.86412	1.6067
Pure Error	5	21.360850	4.27217	**Prob > F**
Total Error	7	35.089085		0.2891
				Max RSq
				0.9813

Figure 6-29 displays a set of predicted values for Ps-VLP size for the extremes of the parameter ranges (vertices of the cube). Based on these preliminary results, some process conditions could result in values outside of the acceptance criteria for Ps-VLP size (20–50 nm). Thus, process ranges for the selected parameters (VLP and DAPS concentration) must be adjusted to meet the criteria for Ps-VLP size.

Figure 6-29: Box Plot on Ps-VLP Size



Conclusion on screening design

The results for the screening design show the following conclusions:

- No correlation for $NaCNBH_4$ concentration was found. This is an expected result considering that it is added in excess.
- There is no apparent relationship between incubation time and the evaluated attributes.
- Agitation rate at this scale has no significant effect on the evaluated attributes.
- A positive correlation of incubation temperature on yield allows for optimizing the process conditions. Also, VLP concentration and agitation during VLP addition should be taken into account as they reach high estimated values.
- Process ranges for VLP and DAPS concentrations require further evaluation because of their correlation with Ps-VLP size.
- DAPS concentration has a significant effect on free Ps. Other parameters were found not to be significant and had low estimated values.

Optimization design

Results

Considering the screening results, some combination of values for DAPS and VLP concentration could lead to unacceptable values for Ps-VLP size (20–50 nm). Thus, a reevaluation of the preliminary ranges was required. An augmented design is proposed based on the screening results. The final design matrix (Table 6-51) is a full-factorial central composite design of two parameters, including four center points and four axial points on the face for each design factor ($\alpha=\pm1$). Free Ps is also included in the evaluation since a correlation with DAPS concentration was found.

Table 6-51: Optimization Matrix and Results for Conjugation Step

Run	DAPS (g/L)	VLP (g/L)	Free Ps (%)	Ps-VLP size
1	25	12	11.58	54.36
2	25	8	12.78	31.31
3	15	8	7.58	27.57
4	15	12	7.13	48.32
5	15	8	8.31	26.85
6	25	12	10.19	59.2
7	25	8	13.33	32.84
8	20	10	9.4	41.21
9	15	12	7.35	46.24
10	20	10	11.24	37.73
11	20	10	8.42	39.04
12	20	10	10.22	41.5
13	25	10	13.02	40.7
14	15	10	7.88	39.99
15	20	12	10.65	49.12
16	20	8	9.37	30.66

Table 6-52 shows the sorted parameter estimates for Ps-VLP size. The results confirm the correlation observed for DAPS and VLP concentration on Ps-VLP size (p-values <0.05), but second-order interactions were not found to be significant. Also, the estimated values of VLP and DAPS concentration are large, thus supporting the selection.

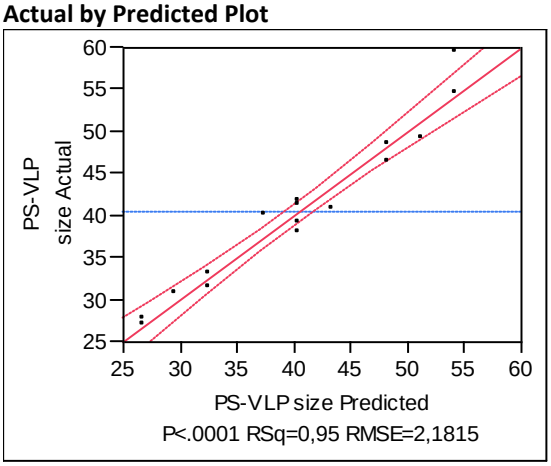
Table 6-52: Sorted Parameter Estimates

Parameter	Estimate	Std Error	t-ratio	t-ratio	Prob>|t|
VLP (g/L) (8.12)	10.801	0.693654	15.57		<.0001
DAPS (g/L) (15.25)	2.944	0.693654	4.24		0.0017
VLP (g/L)*DAPS (g/L)	1.15875	0.775529	1.49		0.1660
DAPS (g/L)*DAPS (g/L)	0.7320455	1.280743	0.57		0.5802
VLP (g/L)*VLP (g/L)	0.2770455	1.280743	0.22		0.8331

After removal of the insignificant parameters (second-order interactions), a model fit was performed (Figure 6-30). The ANOVA table shows that the model as a whole is significant (p= 0.0001).

Figure 6-30: Model Fit and ANOVA for Ps-VLP Size

Actual by Predicted Plot



Analysis of Variance

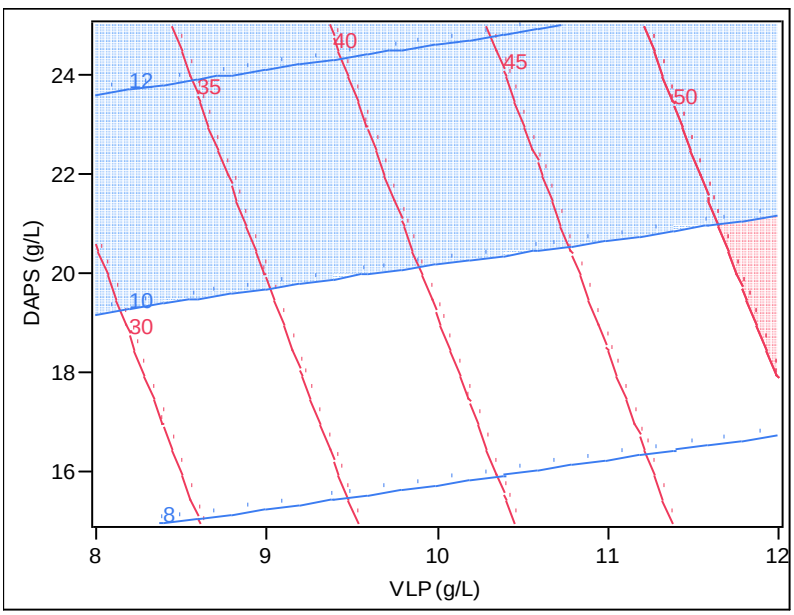
Source	DF	Sum of Squares	Mean Square	F-ratio
Model	2	1253.2874	626.644	131.6810
Error	13	61.8644	4.759	**Prob > F**
C. Total	15	1315.1518		<.0001

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F-ratio
Lack of Fit	6	36.837780	6.13963	1.7173
Pure Error	7	25.026650	3.57524	**Prob > F**
Total Error	13	61.864430		0.2474
				Max RSq
				0.9810

The same analysis was performed on free Ps where DAPS concentration was found to be the only parameter to have a significant interaction. Figure 6-31 represents the free Ps (blue lines) and Ps-VLPs size (red lines) results as a function of VLP and DAPS concentrations. To reduce the level of free Ps (<10%) and maintain the Ps-VLP size within the acceptance criteria (20–50 nm), the process conditions should be adjusted.

Figure 6-31: Counter Plots as a Function of VLP and DAPS Concentrations. Shadow Areas Indicate Condition With Results Out of Specifications.



Despite the fact that DAPS concentration has a major impact on free Ps, the following points should be taken into account to define the range for the process at manufacturing scale:

- Lowering the value of DAPS concentration reduces the level of free Ps; however, process constraints such as large working volumes should be considered.
- 90% free Ps removal is expected to be obtained through diafiltration in a tangential flow filtration mode.

Based on the inverse prediction values (Table 6-53 and Table 6-54), ranges were defined for VLP and DAPS concentrations.

Table 6-53: Inverse Prediction Response on Ps-VLP size

Ps-VLP size	Predicted DAPS (g/L)	Lower Limit	Upper Limit	1-Alpha
35.000000	10.8033288	0.958786539	14.3103961	0.9500
Ps-VLP size	**Predicted VLP (g/L)**	**Lower Limit**	**Upper Limit**	**1-Alpha**
35.000000	8.99731506	8.71630246	9.23940811	0.9500

Table 6-54: Inverse Prediction Response Free Ps (%)

Free Ps (%)	Predicted DAPS (g/L)	Lower Limit	Upper Limit	1-Alpha
7.700000	15.1365894	12.9104875	16.5716027	0.9500
10.000000	20.2138521	19.1021685	21.3603211	

Considering the preliminary work (see prior knowledge Section 6.14.1), no impact on conjugate potency is expected while moving within the preliminarily selected ranges. However, to confirm this and provide a complementary confirmation of the selected ranges for VLP and DAPS, the following extreme conditions were evaluated.

Table 6-55: Complementary Evaluation on DAPS and VLP Ranges

DAPS (g/l)	VLP (g/l)	Free Ps (%)	Ps-VLP size	Potency*
12.9	8.7	6.98	29.21	4.5
14.3	9.2	7.50	32.74	4.9
12.9	9.2	6.87	31.91	5.1
14.3	8.7	7.61	30.04	4.1

* Mean ELISA titers were calculated using arbitrary unit of ELISA (EU/mL).

The results of this study confirmed that the selected ranges have no impact on quality attributes of the conjugate.

Conclusion on conjugation

Based on the aforementioned, the conditions for the conjugation process design space are defined in Table 6-56.

Table 6-56: Process Parameter Suggested Ranges for Conjugation Step

Factors	Unit	Min	Max
VLP concentration	g/L	8.7	9.2
DAPS concentration	g/L	12.9	14.3
Incubation temperature	°C	30	35
Agitation rate during VLP addition	rpm	150	250
NaCNBH4	mg/mL	10	20
Incubation time	hr	12	24

Also, since no correlation was observed between incubation time and the evaluated attributes, it is advisable to further evaluate this factor to optimize process cycle time. Though scalable requirements were employed during the designs, the applicability of the design space should be assessed.

6.18. Ps-VLP Conjugation Scale-Up

6.18.1. Sensitivity of Activation and Conjugation to Mixing

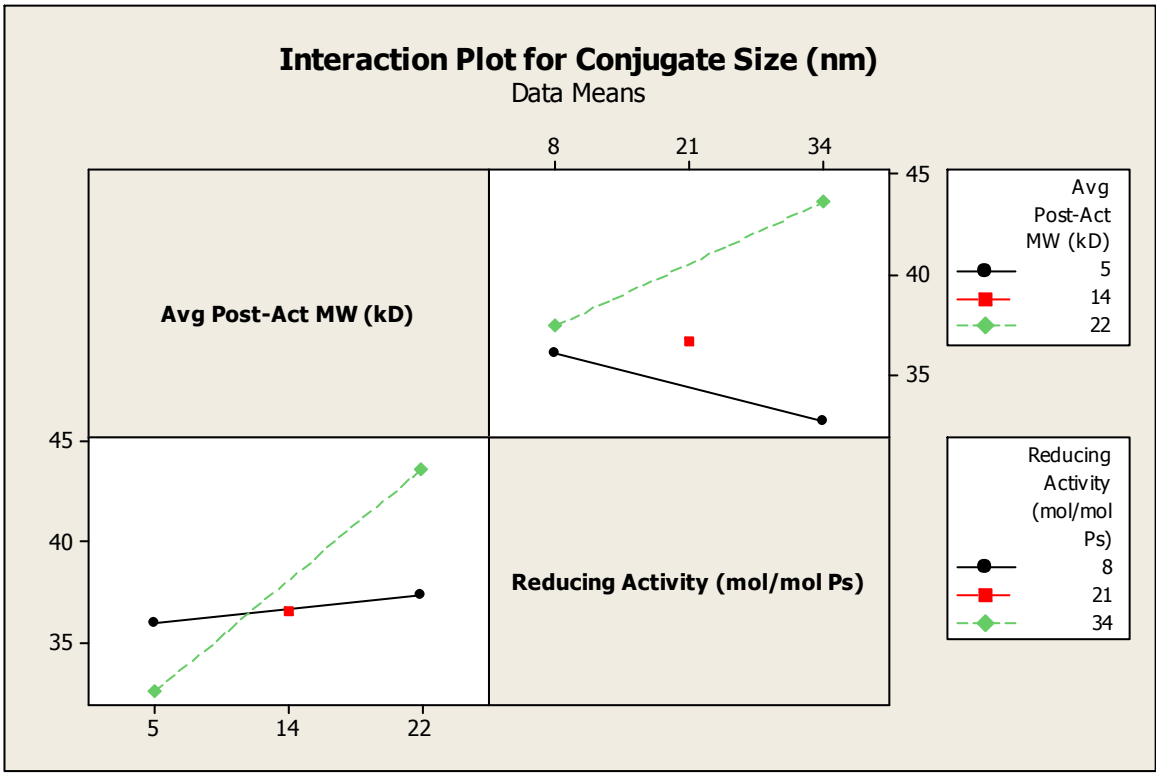
Addition of sodium meta-periodate to the reaction vessel may lead to nonrobust activation outputs during manufacturing by inducing conformational changes within the polysaccharide ring or creating a heterogeneous distribution of aldehydes within the Ps backbone. Quality by Design tools can be used to prevent inconsistent levels of activation or heterogeneous distributions of aldehydes during the oxidation reaction — manufacturing variability that could impact the conjugation reaction and ultimately the final drug substance's potency.

Heterogeneous activation may directly impact the conjugation chemistry and the resulting conjugate attributes including molecular weight, free polysaccharide, unconjugated VLP, and the Ps-to-VLP ratio. Furthermore, heterogeneous activation may lead to multiple covalent attachments between the Ps and VLP or may lead to VLPs cross-linked by Ps.

The level of activation achieved and the size reduction of the Ps during the activation reaction may be influenced by the temperature, pH, and amount of sodium meta-periodate added to the dissolved polysaccharide solution. The data in Figure 6-32 shows the impact of post-activation average molecular weight (expressed in kilodaltons) and activation level on a key conjugate attribute: the conjugate molecular weight. Activation level is represented as a ratio of the mols of aldehyde formed during the oxidation reaction to the polysaccharide molar mass per repeating unit. The data in Figure 6-32 was generated from three experiments with all variables held constant except for the quality of mixing during sodium meta-periodate addition. Although the same amount of sodium meta-periodate was added to the reaction vessel for each experiment, the resulting reducing activity (activation level) varied from 8 to 34 mol of aldehyde per mol of polysaccharide as the mixing quality decreased. In addition, the resulting molecular weight of the activated polysaccharide correspondingly measured 5 to 22 kilodaltons after a fixed reaction time of 15 minutes.

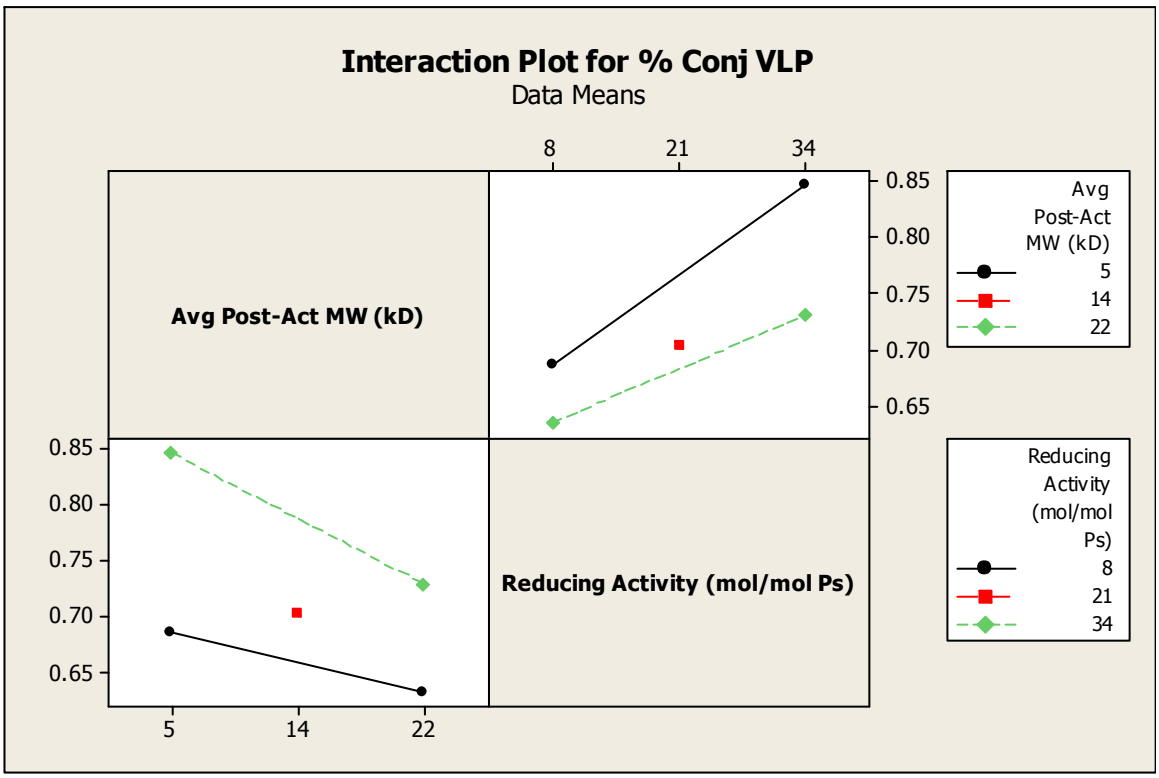
The activated process intermediates from the three activation experiments were then analyzed by H-NMR to show different distributions of aldehydes along the Ps chain. Such variability in activation level directly impacted the conjugate attributes. The Ps-VLP conjugates that were generated from the three activation experiments ranged from 20 to 50 nanometers as shown in Figure 6-32. The conjugate molecular size has been classified as a CQA, important for potency of the targeted product, and must be controlled within 20-50 nanometers.

Figure 6-32: Effect of Polysaccharide Reducing Activity and Molecular Weight Inputs on Ps-VLP Conjugate Molecular Size



The data in Figure 6-33 shows how the variability in activation level and Ps molecular weight can directly impact the fraction of conjugated or reacted VLP in the conjugate. The fraction of reacted VLP varied from 0.45 to 0.85 and was affected by two factors: (1) the different distributions of aldehydes along the Ps chain and (2) the Ps molecular weight of the activated polysaccharide intermediate. Since the Ps molecular weight of the activated intermediate is controlled by on-line HPSEC monitoring, the distribution of aldehydes must be controlled by optimizing the mixing in the activation vessel.

Figure 6-33: Effect of Polysaccharide Reducing Activity and Molecular Weight Inputs on Percentage of Conjugated VLP in Ps-VLP Conjugate



6.18.2. Scale-Dependent Issues

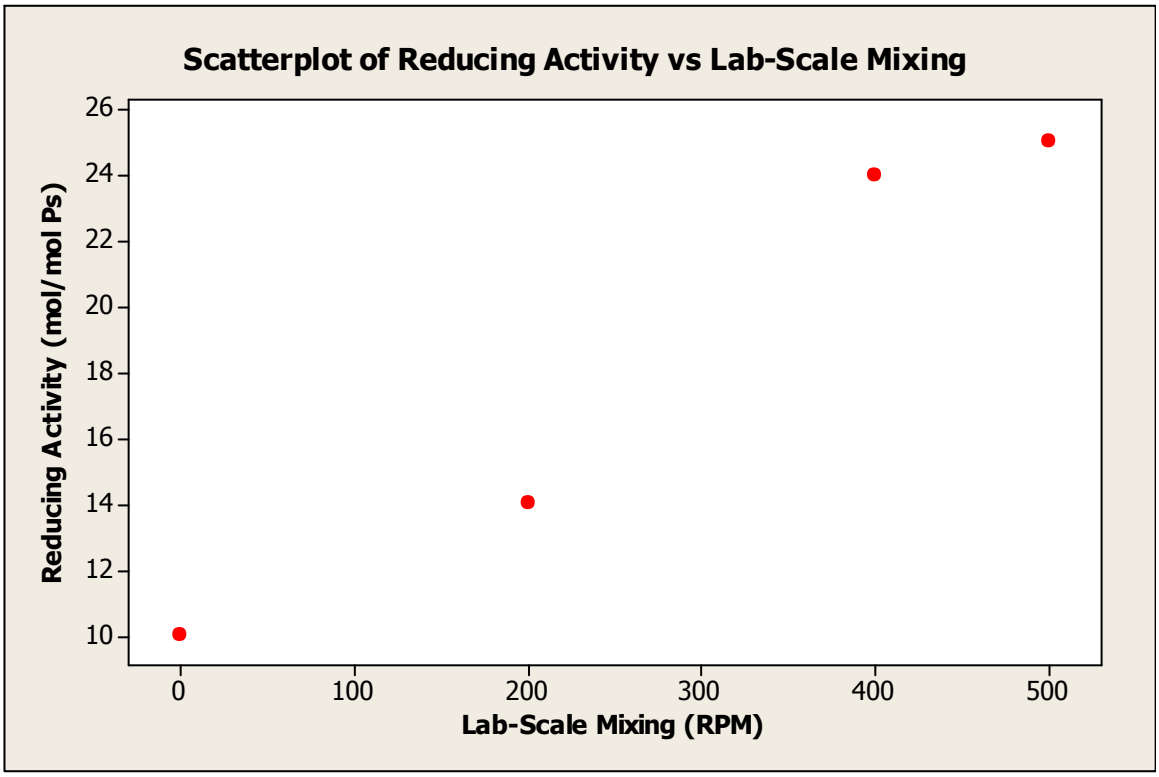
For the chemistry steps of activation and conjugation, process parameters may be classified as either scale independent or scale dependent. Temperature and reagent concentrations are readily scalable based on full-scale equipment capabilities and defined as scale-independent parameters for the A-VAX case study. Lab-scale experiments are still required to determine failure points and define acceptable ranges for manufacturing. Engineering studies utilizing the manufacturing-scale equipment to determine parameter controllability are also required. For example, a Kaye validator would be used to ensure that the temperature distribution in the manufacturing-scale vessel can be maintained within the process specification.

Although temperature and reagent concentrations are readily scalable, the chemical activation step of the Ps with sodium meta-periodate has been identified as a mixing-sensitive, scale-dependent reaction. Activation vessel geometry and impeller design are critical for scale-up. The kinetics of the oxidation reaction for each of the five serotypes have been quantified on the order of minutes, approximately one minute for the fastest-reacting serotype (“A”) and 20 minutes for the slowest-reacting serotype (“E”), which has trans-vicinal diols in the Ps structure. Scale-up of mixing is most critical for serotype “A,” in which approximately 2% of the total aldehydes are formed per second during the oxidation reaction.

The activation reaction kinetics suggest that the quality of mixing of the Ps solution during sodium meta-periodate addition will impact conjugate attributes and ultimately the quality of the drug substance upon scale-up. By scaling the manufacturing-scale vessel to conserve the mixing successfully demonstrated at lab scale, these quality implications can be reduced or eliminated.

The sensitivity of lab-scale mixing on activation is illustrated in Figure 6-34 using an example of power per volume. By increasing the impeller RPM setpoint during sodium meta-periodate addition, the power per unit volume also increases. The homogeneity of the aldehyde distribution within the polysaccharide chain and the average activation level within the polysaccharide chain are directly impacted by the RPM setting, which influences the axial and radial flow vectors within the vessel. The time constants for the activation reaction at a molecular level can be calculated to predict scale-up performance.

Figure 6-34: Effect of Bulk Mixing in Reaction Vessel During Sodium Meta-periodate Addition



Three mixing regimes must be considered for scale-up of the activation reaction: macro-, meso-, and micro-mixing. Macro-mixing, or bulk blend time, occurs at the scale of the reactor and is a critical parameter for suspension of particles larger than 1,000 microns (note that the target activated polysaccharide chain length is 10 to 15 kDa or less than 10 nanometers). Meso-mixing is a critical parameter for reagent addition into a stirred tank through a diptube. Turbulent and inertial driving forces influence how the reagent bolus from a diptube is incorporated into the bulk liquid. Micro-mixing is a function of kinematic viscosity and energy dissipation at the molecular scale and is maximal near the impeller.

Micro-mixing and meso-mixing are competing mechanisms. When the time constant for meso-mixing is smaller than the time constant for micro-mixing, micro-mixing is the limiting mechanism of diffusion in a reaction, and mixing at the molecular scale (power per volume) is important. When the time constant for meso-mixing is larger than the time constant for micro-mixing, meso-mixing impacts the reaction occurring near the impeller, and the final reaction product is sensitive to how reagents are added to the bulk solution. Process modeling tools, such as Dynochem software, may be used to calculate the local mixing timescales at the impeller to determine the dominant mixing regimes for the reaction system. For the reaction system in the A-VAX case study, both meso-mixing and micro-mixing effects were determined to be most important for scale-up.

6.18.3. Process Model

Scale-up of mixing for the activation reaction from the 0.1 L lab-scale development model to the 100 L manufacturing scale depends on the vessel and impeller geometry. Two ratios must be maintained for a geometric scale-up of the system:

(1) $d_{impeller}/d_{tank}$, where d = diameter

(2) $h_{liquid_level}/d_{tank}$, where h=height

Recommendations for common ratios of impeller-to-tank diameters and the location of the impeller in the vessel can be found in literature. The guidelines cited in the Handbook of Industrial Mixing by Ed Paul et al. for a liquid-liquid mixing system (which applies to the sodium meta-periodate addition to a Ps solution in this case study) are included in Table 6-57. Note that other impeller equipment designs (e.g., bottom-mounted) may be evaluated.

Table 6-57: Impeller Clearance and Spacing Guidelines

Mixing System	Maximum Liquid Height hliquid_level/dtank	Number of Impellers	Impeller Elevation from Tank Bottom	
			Bottom Impeller	Top Impeller
Liquid-Liquid	1.4	1	$h_{liquid_level}/3$	
	2.1	2	$d_{tank}/3$	$2\ h_{liquid_level}/3$

Polysaccharide concentration, activation reaction temperature, and pH are scale-independent parameters and can be controlled within the same ranges at lab scale and manufacturing scale. Therefore, it is assumed for the A-VAX case study that the fluid parameters (density, viscosity, and kinematic viscosity) will remain constant at both scales (data to confirm this assumption could be obtained).

For the A-VAX case study, scaling by power-per-unit volume in the stirred reaction vessel will reduce undesirable effects on activation level and conjugate attributes caused by mixing. Scaling by power-per-unit volume assumes that the feed location is the most turbulent location in the vessel (e.g., not shielded by baffles) and that geometry similarity is maintained. The more precise criterion is to scale by holding constant the local rate of turbulent energy dissipation per unit mass in the region of most intense mixing. For geometrically similar vessels, the local rate of turbulent energy dissipation is proportional to the overall power-per-unit volume. Therefore, for this case study, scaling by power-per-unit volume is specified.

A fundamental understanding of mixing within the Ps reaction vessel is critical for ensuring activation homogeneity, robustness, and consistent process performance upon scale-up. For this process, a feed pipe or diptube is utilized for subsurface addition of sodium meta-periodate to the Ps solution at the region of highest turbulence in the vessel, just above the radial edge of the impeller blade. The parameters for feed addition are critical to maintain the meso-mixing and micro-mixing upon scale-up. The linear velocity of the sodium periodate must be fast enough to prevent backmixing but slow enough to prevent the reagent from jetting past the turbulent impeller zone to the bottom of the vessel.

A test chemistry, such as the ioidide-iodate system proposed by Guichardon et al. (2000), may be used to establish a scale-down mixing model to define manufacturing-scale processing parameters for the fast chemical reactions between the Ps and the sodium meta-periodate. Reagent linear velocity,

impeller type and dimensions, baffling, and power-per-unit volume were optimized in a DOE in the manufacturing-scale vessel using the test chemistry reagents instead of valuable product.

The scale-down model must be qualified to ensure application of process development results to manufacturing scale. Parallel activations should be performed in the scale-down system and manufacturing-scale vessels. The activation kinetics should be characterized at both reaction scales to demonstrate that the same degree of activation is achieved in the 10–20-minute activation time at both scales.

For the A-VAX case study, serotype A exhibits the fastest reaction kinetics and will be most sensitive to mixing during sodium periodate addition. Furthermore, the decrease in O-acetate concentration should be measured by H-NMR or the Hestrin colorimetric assay before and after the activation reaction at both reaction scales to confirm the same percentage of decrease. If geometric similarity is maintained and power per volume is conserved upon scale-up, a comparison of scale at centerpoint conditions alone is sufficient to qualify the activation scale-down model. Assuming similarity in process performance as measured by CQA and characterization testing (data not shown here for conciseness), additional full-scale studies at extremes of the design space are unnecessary.

After activation, scale-up of conjugation can be confirmed by mixing VLP with the depolymerized polysaccharide (DAPS) at centerpoint conditions. The reaction time, pH, and concentrations of DAPS, VLP, and sodium cyanoborohydride in the conjugation reaction mixture are scale-independent parameters that can be controlled within the same range at lab scale and manufacturing scale. Unlike the activation reaction, the conjugation reaction is less sensitive to mixing because the conjugation reaction kinetics are characterized to be much slower, on the order of hours instead of several minutes. Therefore, meso- and micro-mixing do not control the extent of reaction for conjugation. Instead, macro-mixing is most important for uniform heat transfer throughout the bulk reaction mixture during the 18- to 24-hour conjugation incubation period.

Since temperature influences the rate of reaction and ultimately the final molecular weight and conjugate attributes, an engineering study should be performed in the manufacturing-scale vessel to ensure that the mixing is defined to provide a uniform temperature distribution in the vessel. If the scale-independent parameters are controlled within acceptable ranges and uniform temperature distribution is maintained in the conjugation reaction vessel, then the resulting Ps-VLP attributes will be measured within the design space regardless of scale. Conjugate molecular weight, free Ps, unconjugated VLP, Ps to VLP ratio, potency, and impurity assays can be used to gauge equivalency of scale.

Note: Refer to the “Drug Product” Section 7 for additional discussion on mixing scale-up design.

6.19. Ps-VLP Conjugation Post-Licensure Change

6.19.1. Rationale for Change

The conjugation step has a target incubation time of 23+/-0.5 hours, with a proven acceptable range of 18–24 hours. To increase capacity in the manufacturing facility, the incubation time will be reduced to 18.5+/-0.5 hours. The reduction of incubation time will allow an additional capacity of 20% for this critical vaccine.

6.19.2. Approach

The incubation time is required to ensure the attachment of polysaccharides to the VLP in the presence of $NaCNBH_4$. The conjugation incubation step has a wide design space, and process characterization data shows it to be quite robust (Section 0). The DOE studies indicate the incubation time has no impact on the CQAs (e.g., Ps/VLP ratio, Ps-VLP size, free Ps, and step yield). Therefore, a change in setpoint would not require an update to the file as it might in a traditional development and filing approach. Step yield data at 18.5 hours of incubation time will be generated for five lots at manufacturing scale to ensure there is no reduction in the step yield. In addition, any other CQAs that might be impacted by this change would be tested for these five manufacturing-scale lots.

As this change is within the filed design space, the proposed change in the incubation time for this step will be administered by the Change Control process.

7. Drug Product Section

7.1. Target Product Profile

A-VAX drug product) is a lyophilized presentation of a pentavalent vaccine containing the capsular polysaccharide (Ps) of X. horrificus serotypes 1, 2, 3, 4, and 5 individually linked to a recombinant, noninfectious virus-like particle (VLP). The vaccine is reconstituted with aluminum phosphate adjuvant prior to immunization. The target profile of the vaccine is shown in Table 7-1.

Table 7-1: Quality Target Product Profile (QTPP) for A-VAX Drug Product

Product attribute	Target
Dosage form	Sterile product lyophilized, single use. To be reconstituted with aluminum phosphate diluents.
Dose	50 µg each of polysaccharides from serotypes 1–4 and 5 µg polysaccharide 5, each individually conjugated to VLP and adsorbed to 300 µg aluminum as aluminum phosphate adjuvant following reconstitution.
Label volume	0.5 mL filled (actual fill volume will be greater than the label volume to account for losses)
Concentration	100 µg/mL of active polysaccharide for serotypes 1–4 and 10 µg/mL for serotype 5
Mode of administration	IM
Dose administration	3 doses administered 2 months apart (preferably two, four, and fix months or based on pediatric vaccine schedule)
Dose volume	0.5 mL nominal dose
Viscosity	1–3 cP
Container	Single-dose vial (ISO2R vial, clear, Type I glass), latex-free stopper, and flip-off seal
Shelf life	≥ 3 years at 2—8°C VVM14 required for developing world and emerging-market supply (14 days at 37ºC, and 90 days at 25ºC)
Secondary packaging and shipping	Allowed shipping-excursion temperature 2–40°C for three days in a carton (10 vials/carton)

7.2. Drug Product Critical Product Attributes

Refer to the *Target Product Profile, Critical Quality Attributes, and Product Risk Assessment* section for drug product CQAs.

7.3. Initial Formulation and Process Development

Prior to initiation of development studies on the A-VAX vaccine, some assumptions have been made to ensure appropriate formulation and process efforts are resourced effectively.

Drug substance assumptions

- Based on early development work in the downstream drug substance area, the stability of each of the Ps-VLPs has been established. The stability has been based on biophysical analysis and the optimum pH and buffer for the five serotypes established based both on long-term and accelerated stability studies.
- Additional efforts by the downstream drug substance team have led to understanding the freeze-thaw ability, as well as light sensitivity (photostability) of the drug substance, to ensure appropriate process handling parameters were followed during formulation and filling processes.
- Each of the Ps-VLPs can be stored frozen and then thawed without aggregation events. The frozen concentrated drug substance is stored in a similar formulation composition of buffer and excipients as the final drug product and is stored at a final concentration of 1–2 mg/mL.
- All five drug substances have demonstrated acceptable stability during accelerated stress conditions, allowing for formulation and filling activities to be completed at room temperature for up to one week.

Drug product assumptions

- The use of a platform formulation for initial formulation development was implemented. This platform formulation is based on past experience developing conjugated vaccines associated with aluminum-based adjuvants.
- Through initial formulation screening activities at both accelerated and long-term conditions, it was observed that a product that was liquid would not be able to meet the World Health Organization Vaccine Vial Monitor 14 (VVM14) requirements.
- To align with prior knowledge and other marketed vaccines, the drug product will be lyophilized. The final formulation will be designed to enable lyophilization; acceptable glass transition and collapse temperatures will be achieved during in-process conditions and at targeted storage conditions.
- To monitor long-term and accelerated stability, the key stability indicating assay will be based on either nephelometry or an ELISA format. For serotypes 1, 2, 3, and 4, the ELISA-based assay is stability indicating. For serotype 5, the rate nephelometry assay is used, but there is no correlation between in vivo and in vitro. However, for design of experiment (DOE) work, the rate nephelometry assay is utilized.
- To enhance the immunogenic effect, multiple adjuvants were examined in preclinical models and in early stability studies. Through these investigations, an aluminum adjuvant was required for enhanced immunogenicity. Based on the stability profile for the different serotypes, an aluminum phosphate adjuvant was selected with a pI of 5.0 to 5.5.
- Histidine buffer was chosen based on three factors:

4999 – pH for maximizing binding to the antigens
5000 – Optimal for lyophilization because this buffer minimizes the chances for pH shifts during freezing
5001 and lyophilization
5002 – Stability of the drug substances under frozen conditions and freeze-thaw prior to formulation
5003 • All serotypes do not bind to the aluminum phosphate adjuvant in a similar manner. The formulation
5004 will be designed to optimize binding of all five serotypes. Additionally, it is expected that during
5005 formulation screening and optimization, lyophilization of the Ps-VLPs will not impact their ability to
5006 bind to adjuvant in post-lyophilization and storage.
5007 • The adjuvant will not be part of the drug product matrices that are lyophilized but will be
5008 incorporated into the diluent, and similar binding as observed in liquid will occur.
5009 • Overall adsorption to aluminum occurs within seconds of reconstitution of the drug product with
5010 the diluent and allows for administration soon after reconstitution.
5011
5012 **Process flow for A-VAX vaccine**
5013
5014 Based on the assumptions outlined above, the overall high-level flow diagram for how to manufacture
5015 the A-VAX vaccine is outlined in Table 7-2.
5016
5017 **Table 7-2: Process Flow for Production of the Drug Product**

Step	Process
1	Addition of WFI, buffer, sucrose, and polysorbate to obtain final desired concentration Volume to be between 50% and 60% of final drug product formulation Adjustment of formulation pH to desired condition
2	Mixing of buffer components to ensure homogeneity
3	Thaw of individual antigen components in specified water bath Dilution calculation of antigens to ensure proper amount added to formulation tank Addition of antigens to conjugate blend tank Volume between 50% and 40% of final batch
4	Addition of conjugate blend to final formulation tank Mixing of product to ensure homogeneity Filtration of final formulated bulk through 0.22 um PVDF membranes; two filters in sequence Filtered FFB filled into respective vials and half-stoppered for lyophilization
5	Lyophilization of A-VAX vaccine Sealing and inspection
6	Packaging of A-VAX vaccine Lyophilized A-VAX vaccine combined with aluminum-containing diluent

5018

5019 **Table 7-3: Process Flow for Production of the Aluminum Diluent**

Steps	Process
1	Reception of aluminum adjuvant material
2	Homogenization and transfer of aluminum suspension to sterilization vessel
3	Heat-sterilization of aluminum adjuvant • Sterilization with mixing during 30 minutes at 121,5°C
4	Transfer to storage containers
5	Formulation of aluminum diluent • Re-pooling of aluminum containers in formulation tank • Resuspension of aluminum suspension and dilution with 150 mM NaCl under mixing Transfer to filling tank
6	Filling of aluminum diluent in prefilled syringes Sealing and inspection
7	Packaging of A-VAX vaccine • Combine lyophilized A-VAX vaccine with aluminum-containing diluent

5020

5021 ### 7.3.1. Formulation Process Development

5022 During early development of the formulation for the A-VAX vaccine, initial time and investment were
5023 spent examining multiple formulation conditions in the liquid state. During the early development, a
5024 platform formulation strategy was employed. This platform formulation effort was based on other
5025 programs that have been worked on in the past to expedite development. Through the developmental
5026 efforts, it was determined that the use of an adjuvant would be necessary to ensure a robust
5027 immunogenic response was achieved.
5028
5029 In addition to demonstrating the need for aluminum adjuvant multiple, preclinical immunogenicity
5030 studies would be required to support the desired target product profile.
5031
5032 Completing initial stability studies at both long-term and accelerated conditions clearly indicated that
5033 12-month shelf life stability at 2–8ºC could be achieved. This stability followed by decreased stability
5034 under accelerated temperatures of 25 ºC and 37ºC suggested that to obtain a 36-month shelf life and be
5035 able to penetrate the developing world and emerging markets, the vaccine must be a lyophilized
5036 presentation.
5037
5038 Because of the inability to achieve a liquid formulation, efforts in the formulation centered on using past
5039 lyophilization experience, and they identified an initial formulation consisting of sucrose, histidine, and
5040 polysorbate 80. These formulation excipients have been successfully used in vaccines, and there is
5041 significant prior knowledge on the formulation and potential issues with lyophilization.
5042

5043 The target formulation for the lyophilized pentavalent vaccine containing the capsular Ps of *X. horrificus*
5044 serotypes 1, 2, 3, 4, and 5 individually linked to a recombinant, noninfectious VLP is assumed to be the
5045 following, as shown in Table 7-4.
5046

5047 **Table 7-4: Assumptions on Platform Formulation for Lyophilized Vaccine**

		Concentration/mL	Amount/dose
Sucrose	Bulking agent/stabilizer	50 mg	25 mg
Histidine	Buffer	10 mM - pH 6.0	
Polysorbate 80	Surfactant	0.01%	0.025mg
Ps 1-VLP	Active	100 µg/mL	50 µg
Ps 2-VLP	Active	100 µg/mL	50 µg
Ps 3-VLP	Active	100 µg/mL	50 µg
Ps 4-VLP	Active	100 µg/mL	50 µg
Ps 5-VLP	Active	10 µg/mL	5 µg

5048

7.3.2. Lyophilization Process Development

After understanding that a liquid platform formulation for A-VAX did not allow the desired target product profile (TPP) to be achieved, the team devoted efforts to evaluate lyophilization as a means to achieve the necessary VVM14 required for both the developing world and emerging markets. Upon reconstitution with aluminum phosphate adjuvant, all five serotypes readily bind to aluminum within two minutes; this data supported lyophilization as a viable option.

The data became the basis of supporting data that allowed the team to lyophilize the Ps-VLP conjugates, and then reconstitute the vaccine with the aluminum-containing diluent and achieve similar adsorption conditions as observed for liquid material following reconstitution and mixing by inverting the vials three to five times prior to administration.

Although the team did consider lyophilizing the A-VAX vaccine in the presence of aluminum, it has not been demonstrated to this point with any currently marketed products. Thus, to minimize delays to the timeline, the approach of the aluminum phosphate diluent was employed. The starting point for formulation development associated with a lyophilized formulation, much like the liquid development efforts, used a platform formulation.

Once the initial matrix of sucrose, histidine, and polysorbate 80 was determined, two techniques to help shape the initial lyophilization cycle were used to better characterize the formulation. The first was the use of modulated differential scanning calorimetry to determine the glass transition temperature (Tg') for the formulation of choice. Second, the collapse temperature (Tc) was measured. These biophysical techniques resulted in a Tg' value of ~ -33ºC and a Tc value of ~ -30ºC. Both are well in line with past knowledge associated with sucrose-containing formulations.

Using Manometric Temperature Measurements (MTM), the initial lyophilization development was expedited. MTM is one of many lyophilization development technologies that has truly benefited early stage development and lyophilization robustness. Using the Tg' value, along with the formulation composition (glassy or amorphous), fill volumes, and the vial configuration, the identification of primary drying conditions can be obtained in a few runs rather than multiple interactions of development. The lyophilization cycle based on MTM was defined for early stage development (Table 7-5).

After establishment of the initial primary drying conditions using MTM, lab-scale runs utilized temperature probe data to monitor cycle progress. This was completed to ensure that throughout the development, the product temperature was staying below the Tg' and collapse temperature during primary drying. As development of the cycle for ramp rates, pressure, and secondary drying continued and was optimized, the cycle shifted from use of temperature probes to a time-/pressure-driven cycle.

Table 7-5: Preliminary Lyophilization Cycle for A-VAX Vaccine

Lyophilization Stage	Initial Cycle
Loading/Freezing Temperature	-50ºC
Freeze Time Post-load	60 minutes
Ramp to Primary Drying	1ºC/minute
Primary Drying Temperature	-25ºC
Primary Drying Time	1,500 minutes
Ramp to Secondary Drying	0.5ºC/minute
Secondary Drying Temperature	20ºC
Secondary Drying Time	420 minutes
Final Stage Postsecondary Drying	4ºC
Stoppering Pressure	800 mBarr
Stoppering Gas	Nitrogen
All conditions during lyophilization used 130 µbar pressure, based on past experience.	

7.3.3. Adjuvant Development

Early preclinical development needed to determine whether an adjuvant would be required for the vaccine. Based on experience from other conjugate vaccines on the market and in our portfolio, it was expected that an adjuvant would be required to ensure robust immunogenicity in the patient population.

As a starting point for choosing the adjuvant, the team assessed aluminum phosphate and aluminum hydroxide adjuvants. A main consideration in choosing the adjuvant was the robustness of adsorption as well as ensuring that the stability of the Ps-VLP conjugates was preserved post-reconstitution.

Results from the early work indicated that both adjuvants showed robust adsorption kinetics; however, the stability of the Ps-VLP conjugates was better with the aluminum phosphate adjuvant. The aluminum phosphate adjuvant significantly increased the anti-capsular Ps antibody levels, and the adjuvant mitigated ligand exchange between the Ps and the aluminum hydroxide. This ligand exchange impaired the immune response in animal models.

A correlation between the preclinical results and the clinical studies was observed, and the aluminum phosphate adjuvant dose level was selected during the Phase II clinical studies.

Aluminum phosphate is supplied from a commercial manufacturer and then pooled and sterilized prior to use as a diluent for the lyophilized drug product. Other vaccines in the pipeline have used aluminum-containing diluents with a standard formulation and filling process. As a start for development, the team decided to use the standard image (Table 7-6).

Table 7-6: Adjuvant Formulation

Component	Concentration

Aluminum phosphate	600 µg/mL
NaCl	150 mM
pH	5.0–8.0

7.4. Initial Risk Assessment: Cause and Effect

To prioritize the design space work early in program development, a preliminary risk assessment was performed. It utilized cause-and-effect matrices to examine the different process steps that could impact the critical quality attributes of the product (Table 7-8).

Utilizing knowledge gained from other Ps-VLP vaccines with formulation compositions similar to A-VAX, each of the specific process steps was examined. The scoring for the overall cause-and-effect matrices is outlined in Table 7-7.

Table 7-7: Scoring Approach for Initial Risk Assessment

Scoring of Process Parameters	
Impact Score	Ranking Criteria
10	Strong relationship known based on available data and experience
7	Strong relationship is expected
5	Not-so-strong relationship expected or unknown
1	Known to not have a relationship

Utilizing the approach from Table 7-7, the manufacturing unit operations associated with the product were scored to determine the risk/level of potential interaction (Table 7-8). The individual scores were assigned based on prior knowledge from other vaccine programs in the company's product line and on literature review.

After scoring for individual interactions, a total score was determined for each unit operation and quality attribute. These total scores were determined by summing the respective individual scores horizontally against the specific unit operation and vertically for individual quality attributes. The total scores were then used to assess relative risk for individual quality attributes and to prioritize development work for specific unit operations.

Using this scoring, the highest-risk unit operations of formulation (including drug substance (DS)/buffer quality) and lyophilization were selected for further investigation during initial development efforts using design of experiment (DOE) studies. Scoring vertically allowed the team to better understand which parameters would appear to have the most significant impact on the product moving forward (i.e., potency, moisture, sterility, adsorption).

Table 7-8: Cause-and-Effect Matrix

Cause-and-Effect Matrix

Process Parameters	Potency	Purity	Identity	Dose	pH	Moisture	Appearance (Lyo)	Appearance (Recon)	Recon Time	Endotoxin /LAL	Sterility	General Safety	Sub-Visible Particulates	Adsorption	Formulation Composition	Score
Raw Material (DS)	10	10	10	5	5	1	1	5	1	5	5	5	5	10	5	83
Raw Material (Buffer)	1	5	1	1	10	7	7	7	5	5	5	5	1	7	10	77
Raw Material (Vial/Stopper)	1	1	1	1	1	10	5	5	1	5	10	1	5	1	1	49
DS Thaw/ Handling	5	5	1	1	1	1	1	1	1	1	1	1	5	5	1	31
Formulation Compounding & Mixing	10	1	1	10	5	5	5	1	5	5	5	1	5	7	7	73
Filtration	5	5	1	5	1	1	1	1	1	1	10	1	7	5	1	46
Filling	7	1	1	10	1	1	5	1	5	5	5	1	7	5	1	56
Lyophilization	1	5	1	1	1	10	10	5	10	5	5	1	5	1	1	62
Capping	1	1	1	1	1	5	1	1	1	1	7	1	1	1	1	25
Visual Inspection	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
	42	35	19	36	27	42	37	28	31	34	54	18	42	43	29	

Since lyophilization of the drug product is required to ensure the TPP profile is achieved for the vaccine, the aluminum adjuvant is provided in a separate diluent and has its own manufacturing process and COA. Because the aluminum adjuvant has its own process and quality attributes, an additional cause-and-effect matrix was generated (Table 7-9).

Prior knowledge learned through other vaccines similar to A-VAX and use of an aluminum diluent aided in the initial risk assessment. Aluminum adjuvants' characteristics were studied in several publications. S.L. Hem and collaborators have widely published on aluminum hydroxide and aluminum phosphate adjuvants. The effect of autoclaving on aluminum phosphate adjuvant was addressed in an article2; its impacts on pH (decreased by deprotonation and dehydration), point of zero charge (decreased), and protein adsorption capacity (lyzozyme model, decreased) were demonstrated. The amorphous structure was not affected by 30 or 60 minutes of autoclaving. History with other sterilizable-in-place equipment shows that sterility will be guaranteed if Fo of a minimum 20 minutes is reached during SIP operations.

Table 7-9: Cause-and-Effect Matrix for Aluminum Adjuvant

Quality Attribute Parameter	Sterility (Fo)	Homogeneity	Alum. Size	Alum. PZC	Alum. Adsorption Capacity	Score
Heating profile	5	1	5	5	5	21
Sterilization temperature	10	1	10	10	10	41
Sterilization duration	10	1	10	10	10	41
Cooling profile	5	1	5	5	5	21
Mixing speed	5	10	5	3	3	26
Impeller configuration and vessel geometry	1	5	5	1	1	13
	36	19	40	34	34	

Similar to Table 7-8, scores were added vertically (quality attributes) and horizontally (process parameters) to determine which should be examined during design space development. Higher scores were associated with the sterilization temperature and duration, which can be extended to the heating/cooling profile, and with the mixing speed. These parameters will be evaluated during design of experiment studies described for aluminum in the next section.

[2] Burrel, Lindblad, White, Hem, Stability of aluminum-containing adjuvants to autoclaving, Vaccine 17, 2599–2603, 1999

7.5. Design Space Development

To complete the initial risk assessment tool, the steps to be further studied during developmental work included the formulation compounding step and examination of the levels of excipients and pH associated with the product. Thawing and handling of the DS will not be further studied as a result of information learned from the downstream DS team, as well as experience with thawing and handling of the DS for related vaccines in the pipeline.

Moving into the lyophilization process for the product, the parameters to be investigated include: primary and secondary drying, ramp rate, and chamber pressure during the lyophilization run. Although the freezing process may impact the product's quality attributes, knowledge gained from past lyophilized vaccines shows the risks associated with freezing are minimal, and they will not be extensively examined early in product development. However, if issues arise during scale-up to commercial scale, additional development efforts will focus on examining the freezing process associated with the A-VAX vaccine.

Although in the example outlined in this document freezing was not investigated as part of the lyophilization process, it should be noted that freezing would be part of the process to examine. It is known that different methods of freezing can significantly impact the overall crystal structure (i.e., faster freezing (LN2 blast freezing) and can lead to smaller ice crystal structure vs. shelf freezing or controlled freezing with larger ice crystal structure. These differences in ice structure can impact the overall drying properties of the drug product and should be examined. It should not be assumed that freezing would not impact the lyophilization process, and it should be examined during routine development.

For aluminum adjuvants, two major factors will be further investigated during early development. These factors are the impact of mixing shear and the impact of thermal treatment on the aluminum particle characteristics.

7.5.1. Leveraging Prior Knowledge: Parameters That Will Not Be Studied

Based on the C&E matrix and prior knowledge, the following parameters will not be explored further in the case study. The reason is either the risk is low to the final drug product or prior knowledge has been gained through literature reviews or experience with other vaccines similar to A-VAX.

7.5.1.1. Hold Time Studies

Once the formulation for the lyophilization is determined, hold studies will be conducted to determine acceptable hold times in the selected formulation. The data will demonstrate that this process intermediate can be held at 2–8°C and 25°C for seven days without significant degradation or impact to product quality. The parameters for assessment will be based on the following criteria: pH, appearance, total protein, antigenicity, and other characterization assays such as DLS.

7.5.1.2. Drug Substance Preparation and Handling

An assumption has been made that all the Ps-VLPs are maintained frozen. Each of the drug substances will be thawed using standard procedures and will be discussed in this document.

7.5.1.3. Sterile Filtration

An assumption has been made that the appropriate filter membrane, size, and membrane housing have been chosen based on experimental data, which will be discussed.

7.5.1.4. Vial Filling

Vial filling is a standard platform process, with the respective vial and stopper configuration. Required filling tolerances have been previously demonstrated for similar formulation compositions.

7.5.2. Formulation Experiments and DOE

Following the completion of the initial risk assessment tool, it was identified that a better understanding of the formulation composition was necessary to ensure that a robust process and product were achieved. To accomplish these activities, the formulation development studies will be conducted in two phases utilizing design of experiments.

- Determine the optimal final formulation matrix following the reconstitution process with the aluminum phosphate adjuvant. This will be determined based on liquid studies showing the conditions of the formulation necessary to ensure robust stability for a short period of time and rapid adsorption to the aluminum phosphate adjuvant.
- Identify the lyophilization matrix, and complete design space studies on the actives and excipients associated with the formulation. The overall adjuvant formulation matrix would be defined based on the overall formulation matrix required to support lyophilization.

Formulation Optimization DOE

1.5.2.1 Design Space for Formulation Matrix Following Reconstitution

To initiate formulation development, it was necessary to identify a formulation that would ensure that all five serotypes would bind to the adjuvant in a timely manner and consistently absorb to aluminum so that the immunogenicity of the vaccine was maintained.

In the first set of formulation DOE studies, the formulation excipient levels along with pH and aluminum adjuvant concentrations were varied to determine an optimal formulation for the pentavalent Ps-VLP vaccine. The optimal binding for all five serotypes will be determined for the product along with the respective design space. Factors investigated included the concentration of sucrose, salt, and adjuvant along with a pH range from 5.0–8.0 (Table 7-10).

102 **Table 7-10: Factors Determining Binding of Antigens to Aluminum**

Excipients	Range
Sucrose	4%–10%
NaCl	0–150 mM
pH	5.0–8.0
Aluminum phosphate	0.4–0.6 mg/mL as aluminum
Antigens constant factor	Ps 1-VLP to PS 4-VLP at 100 µg/mL and Ps 5-VLP at 10 µg/mL

103
104 Using the ranges listed in Table 7-10, the first DOE study was determined to investigate four
105 factors.
106
107 **Table 7-11: Study Design for DOE to Determine the Drug Product Matrix**

Run	Aluminum Concentration (mg/mL)	pH	Sucrose (%)	NaCl (mM)
1	0.4	5.0	4.0	150.0
2	0.8	8.0	10.0	0.0
3	0.4	5.0	10.0	150.0
4	0.4	5.0	10.0	0.0
5	0.4	5.0	4.0	0.0
6	0.8	8.0	4.0	0.0
7	0.8	5.0	4.0	150.0
8	0.4	8.0	4.0	150.0
9	0.8	5.0	10.0	150.0
10	0.8	8.0	4.0	150.0
11	0.8	5.0	4.0	0.0
12	0.4	8.0	10.0	150.0
13	0.4	8.0	4.0	0.0
14	0.6	6.5	7.0	75.0
15	0.6	6.5	7.0	75.0
16	0.8	5.0	10.0	0.0
17	0.4	8.0	10.0	0.0
18	0.8	8.0	10.0	150.0
19	0.6	6.5	7.0	75.0
Response: Polysaccharide binding to aluminum phosphate based on immunoassay such as ELISA or nephelometry.				

One of the important aspects of formulation will be quantitation of the individual antigens. Given that an immunoassay will be utilized for quantitation, that may lead to variability associated with the analytical methods following formulations.

An immunoassay specific for each serotype was used to measure the overall binding of the serotype-specific antigen to the aluminum fraction. The result from the DOE for percent of binding for each serotype was captured and presented. As expected, the binding of conjugates to aluminum did vary depending on the formulation investigated (Table 7-12).

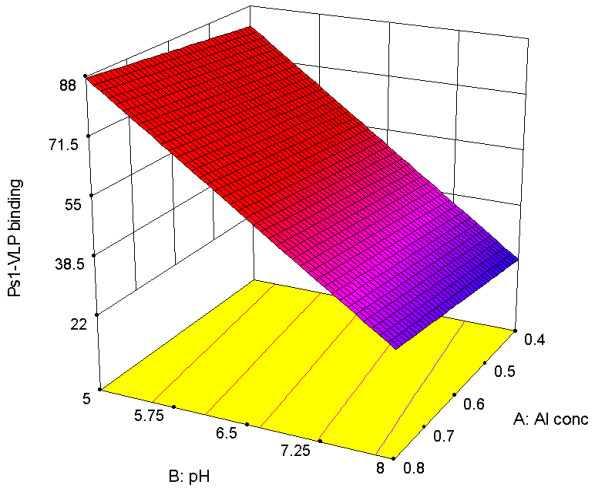
Table 7-12: Example % of Binding of Ps-VLP Conjugates

Run					
	Ps 1-VLP	Ps 2-VLP	Ps 3-VLP	Ps 4-VLP	Ps 5-VLP
1	80	85	98	98	98
2	30	30	55	49	50
3	82	78	80	78	97
4	83	79	97	45	96
5	82	78	55	42	99
6	20	20	30	30	95
7	98	98	95	95	98
8	30	30	70	70	82
9	85	85	99	99	95
10	40	40	80	80	86
11	82	78	80	49	50
12	20	20	50	50	50
13	20	20	50	50	50
14	59	56	95	95	95
15	57	57	100	100	100
16	85	85	50	50	89
17	20	20	30	30	90
18	30	30	60	60	90
19	57	55	93	93	93
Data in table shows the impact of salt, aluminum concentration, and pH on the binding.					

Through the DOE work completed examining the impact of pH and aluminum concentration on binding, as expected, there appeared to be a strong correlation between pH and adsorption.

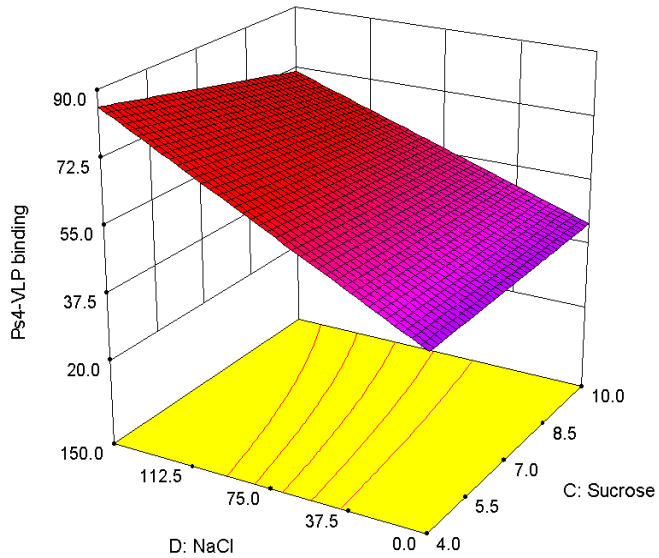
Additionally, it has been observed the overall concentration of aluminum did not have a significant impact on binding. The Ps 1-VLP conjugate appeared to show the best example for the impact of pH on binding (Figure 7-1). As a result, utilizing Ps 1-VLP to optimize the pH range would ensure a robust formulation is achieved.

Figure 7-1: Ps 1-VLP Binding as a Function of pH and Aluminum Concentration



In addition to examining the impact of pH and aluminum concentration on Ps-VLP binding to aluminum, the DOE also examined the impact of salt and sucrose concentrations on adjuvant binding. Four of the serotypes (1, 2, 3, and 5) indicated that there was no significant impact to binding when varying the concentrations of the excipients. However, for serotype 4, there was a strong correlation observed related to the concentration of sucrose and salt (Figure 7-2). As with the impact of pH, future formulation development will center on ensuring that serotype 4 would adsorb to aluminum and meet the required TPP.

Figure 7-2: Ps 4-VLP Binding as a Function of NaCl and Sucrose



7.5.2.1. Determination of the Lyophilized Matrix

Once the matrix for the final formulation is determined, the antigen formulation and the adjuvant formulation will be separately evaluated. The next phase is to lyophilize the antigens in the matrix and determine that the binding is maintained following lyophilization.

The parameters would mainly be the limits of adsorption to aluminum for the five Ps serotypes within the wider design space of the lyo matrix. The factors for design space and the DOE for these optimizations are shown in Table 7-13.

Table 7-13: Factors Determining Edges of Formulation to Ensure Binding Is Maintained

Excipients	Range
Sucrose	3–7%
Histidine	5–15 mM
Polysorbate 80	0%–0.03%
pH	6.0
Antigens constant factor	Ps 1-VLP to 4 at 100 µg/mL and Ps 5-VLP at 10 µg/mL
Response: Cake cosmetics, moisture, and binding to aluminum phosphate diluent on reconstitution as determined as optimal in the first study.	

For simplicity, the second DOE evaluating the lyophilization matrix is not discussed here. However, it was observed that under the optimal conditions, there is consistency of binding and the necessary stability profile was achieved.

Using the ranges from Table 7-13, a DOE experiment was initiated to vary the different factors to understand if there are any issues related to the sucrose, histidine, and PS 80 concentration on cake appearance, moisture, and overall binding to aluminum following reconstitution.

Table 7-14: Optimization of Formulation for the Lyophilized Matrix

Run	Histidine (mM)	PS 80 (%)	Sucrose (%)
1	15.0	0.30	7.0
2	15.0	0.00	3.0
3	5.0	0.30	3.0
4	5.0	0.30	7.0
5	10.0	0.15	5.0
6	10.0	0.15	5.0
7	15.0	0.30	3.0
8	5.0	0.00	3.0
9	15.0	0.00	7.0
10	10.0	0.15	5.0
11	5.0	0.00	7.0

The data in Table 7-15 is an example showing that the drug product is robust based on the key parameters of the lyophilized cake, moisture, reconstitution time, and binding of each of the Ps-VLPs within the limits of the excipients, which are histidine, PS 80, and sucrose.

Table 7-15: Binding Analysis within the Drug Product

Run	Moisture %	Recon time sec	% Binding of Ps-VLP conjugates				
			Ps 1-VLP	Ps 2-VLP	Ps 3-VLP	Ps 4-VLP	Ps 5-VLP
1	1	10	55	50	90	90	90
2	1.2	125	56	55	92	92	92
3	1.4	24	59	56	95	95	95
4	0.9	16	46	49	98	98	98
5	1.1	18	59	69	100	100	100
6	1.4	17	57	57	100	100	100
7	1.1	20	57	55	93	93	93

Run	Moisture %	Recon time sec	% Binding of Ps-VLP conjugates				
			Ps 1-VLP	Ps 2-VLP	Ps 3-VLP	Ps 4-VLP	Ps 5-VLP
8	0.8	20	55	56	91	91	91
9	1.0	22	54	59	99	99	99
10	1.1	25	59	46	97	97	97
11	0.9	29	61	59	98	98	98

7.5.2.2. Plcaeholder for text

The samples that were prepared were placed on stability and monitored under accelerated conditions to ensure alignment with the TPP and VVM14 requirements. The conditions investigated included one month of 50ºC thermal stress as well as both refrigerated and room temperature stability through 24 months to support shelf life.

Following the completion of the development, the lead formulation was identified based on both the adsorption and stability results associated with the design of experiments. The lead formulation was XX.

7.6. Dosage Administration and Stability

This section focuses on the dosage administration instructions at the clinic for delivering the vaccine. It is assumed that the vaccine will be administered by medical personnel. The vaccine will be supplied as two components: the lyophilized vaccine, packaged with an aluminum phosphate adjuvant for reconstitution.

To reconstitute the vaccine, personnel first will withdraw the aluminum phosphate with a syringe and inject it into the lyophilized vial. They will mix the vial well, and the instructions will be based on data generated by monitoring the uniformity of the vaccine as measured by product uniformity. Although ideally the vaccine should be given soon after reconstitution, it may need to be held for a time. To support the period of use following reconstitution of the vaccine, stability data will be used. An example of the experimental design is shown in Table 7-16, used to measure the quality attributes associated with the reconstituted vaccine.

Table 7-16: Stability of Vaccine Following Reconstitution

	Time in hours							
	0	2	4	6	12	24	48	72
Appearance								
pH								
Subvisible particles								
Total protein								

Saccharide concentration								
Protein adsorption to aluminum								
Saccharide adsorption to al								

The data will support the in-use period. However, because the vaccine is a preservative-free product, the time that the vaccine is held post-reconstitution should be limited.

7.7. Lyophilization Process Development and DOE

Lyophilization process development

Based on prior knowledge of the lyophilization process development and scale-up, primary drying is one of the most critical process steps in terms of scale dependency and product quality. Many models are available in the literature to calculate product temperature, sublimation kinetics, and sublimation time once the heat and mass transfer for a given equipment and a given product are known (*Pikal. J. Parentr. Drug Assoc.. 1985, 39 (No. 3), 115–138; Mascarenhas et al. Comput. Methods. Appl. Mech. Eng. 1997, 148, 105–124*). Models are also available to calculate all shelf temperature and chamber pressure combinations that would ensure that the product temperature remains below the collapse temperature throughout primary drying (*Chang et al. 1995. Pharm. Res. 12:831–837; Nail et al. 2008. Biopharm Int. 21:44–52; Giordano et al J. Pham. Sci. 2011.100(1),311–24*).

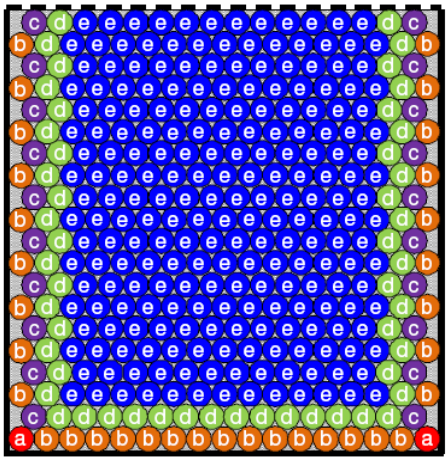
More recently, more advanced approaches have been published. They propose to take into account intrinsic batch heterogeneity and transfer parameters' uncertainty (*Fissore et al. Advanced approach to build the design space for the primary drying of a pharmaceutical freeze-drying process. J. Pharm. Sci. Vol. 100 (11), pgs 4922–4933*). For the lyophilization cycle development of the A-VAX vaccine, a standard approach of experimental measurement of heat (Kv) and mass (Rp) transfer coefficients coupled with a monodimensional model (for example, *Giordano et al J. Pham. Sci. 2011.100 (1), 311–24*) was used to define optimal freeze-drying conditions during primary drying.

Experimental determination of heat transfer coefficient Kv at lab scale

A gravimetric method was used to determine the heat transfer coefficient Kv values throughout the shelf for vials directly loaded in the shelf, as described in *Brülls M, Rasmuson A. Int. J. Pharm. 2002; 246(1-2):1–16.* Other averaging spectroscopic methods can be used (*Kuu et al. 2009. J. Pharm. Sci. 98:1136–1154*); however, they do not provide information about the heat transfer heterogeneity resulting from radiation phenomena at the edges of the shelf. Figure 7-3 below details the different five locations of vials on the heating shelf of the freeze dryer.

Figure 7-3: Various Zones of the Heating Shelf in Terms of Heat Transfer

Named from (a) to (e). Half a shelf is represented, and vials are in direct contact. (a) type vials represent 1.3% of the total number of vials, (b) type vials represent 9.6%, (c) represent 5.6%, (d) represent 15.3%, and (e) represent 68.2%.



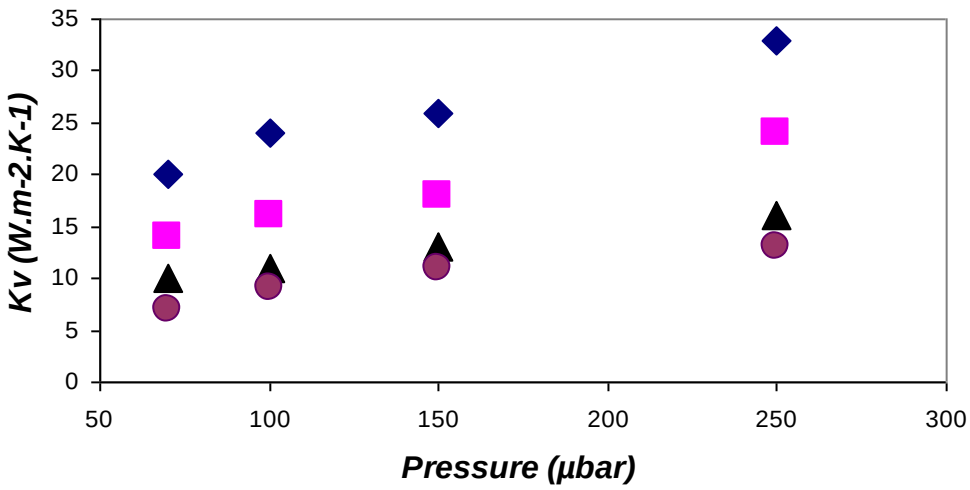
The table below gives an example of overall heat transfer values measured at 100 µbar as a function of vial locations:

Vial location	Kv, W.m-2.K-1
(a)	35.2±3.4
(b)	24.5±2.0
(c)	16.3±0.9
(d)	11.8±1.0
(e)	9.3±0.7

Kv is pressure dependent and was therefore calculated at different pressure for each vial location identified above in the different zones of the heating shelves.

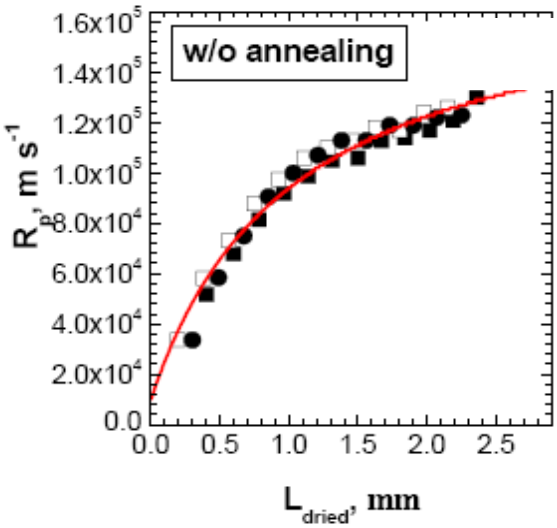
Figure 7-4: Kv Values As a Function of Pressure for Different Vial Locations in the Lab-Scale Freeze Dryer

Diamonds for (b) vials, squares for (c) vials, triangles for (d) vials, and circles for (e) vials



Experimental determination of resistance to mass transfer coefficient Rp of the product

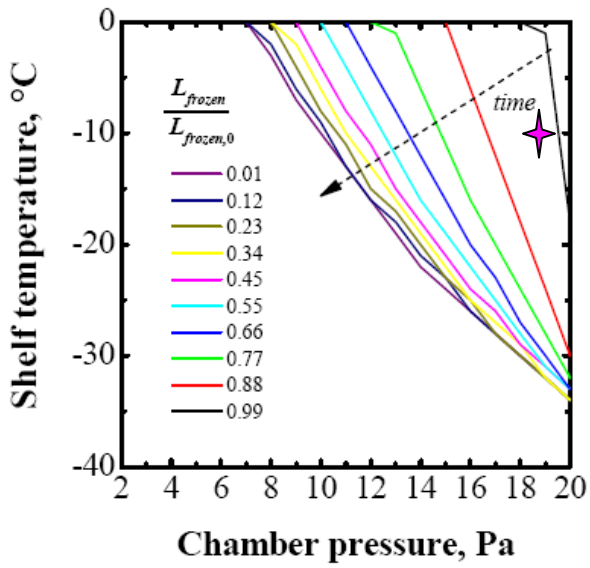
A pressure rise method was used to determine Rp values as a function of the dry layer thickness (L_{dried}) in the freeze-dried cake (*Milton et al. 1997. PDA J. Pharm. Sci. Technol. 5:7–16*). The measurement was repeated three times to evaluate an average and the variability associated with the Rp values.



Definition of the design space for primary drying using mathematical modeling

For the A-VAX vaccine, the modeling and the design space representation described in (*Fissore et al. Advanced approach to build the design space for the primary drying of a pharmaceutical*

freeze-drying process. J. Pharm. Sci. Vol. 100 (11), pgs 4922–4933) was chosen. An example of this representation is given in the figure below:

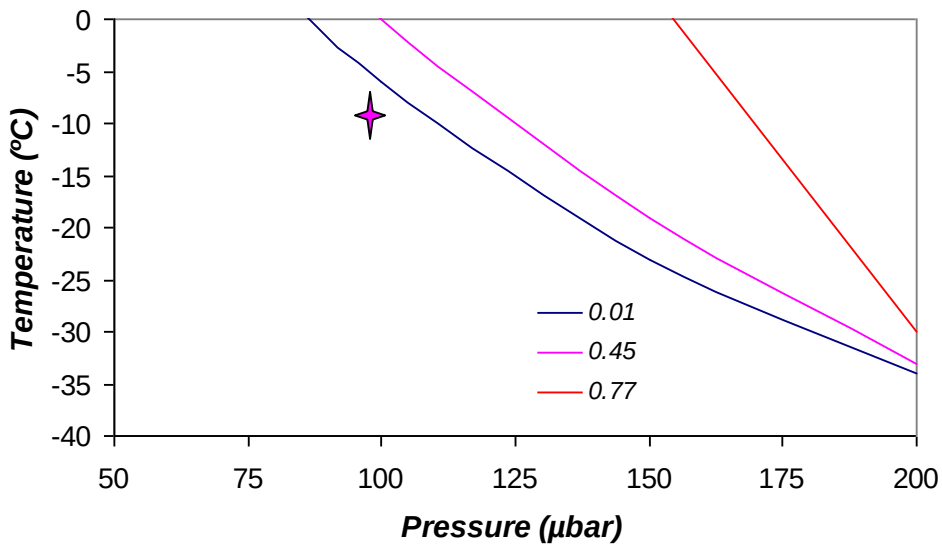


In this design space representation, chamber pressure is represented in the abscise axis and shelf temperature is represented in the ordinate axis. A quasi-steady state monodimensional model was used to calculate at a given value of *Rp* (i.e., at a given value of the dry layer thickness) all combinations of shelf temperature and chamber pressure values that would set the product temperature at the sublimation interface equal to the collapse temperature. These combinations are represented by the color solid lines in the graph, each color corresponding to a given dry layer thickness.

For example, for a frozen layer thickness equal to 88% of its initial value (prior to sublimation), the combinations of T_{shelf} and P_c are represented by the red solid line. For a frozen layer thickness equal to 1% of its initial value, they are represented by the purple solid line.

Above this solid line, the product temperature at the sublimation interface is above the collapse temperature. Below this line, the product temperature at the sublimation interface is below the collapse temperature; and the area below this line represents the design space for primary drying conditions at a given *Rp* value.

The figure below represents the calculation of the primary drying design space of our A-VAX vaccine, for different values of the frozen layer thickness compared to the initial thickness (77%, 45%, and 1%) and the (b) type locations. The (b) type locations were selected to define T_{shelf} and P_c values because they have the higher Kv values after (a) type locations, whose number was considered negligible, and therefore are the vial location at risk to exceed collapse temperature during primary drying.



The selected conditions are represented by the purple star on the graph. T_{shelf} = -10C and *Pc*=100 µbar will ensure product temperature below the collapse temperature for all vial locations (except (a) location) and throughout the duration of primary drying.

Primary drying time was selected by calculating its value in these conditions for (e) type locations, which have lower Kv value and therefore the longer sublimation time. The calculated sublimation time is 620 minutes. The selected sublimation time is 720 minutes, to include a 100-minute safety margin. The maximum calculated sublimation rate during primary drying in these conditions is 0.34 kg $h^{-1}m^{-2}$.

Experimental validation of the model at lab scale

The table below gives, for important process parameters, the maximum difference (*Δmax*) between calculated and measured values at the pilot scale throughout the duration of the primary drying. The measurement system is specified in the table:

Process parameter	**Δmax during primary drying**
Product temperature	*Average of 5 t-type Thermocouple: ΔTmax=0.7°C* *Pressure rise test measurement: ΔTmax=0.9°C*
End of sublimation time	*MKS/Pirani gauge ratio: Δtsublimation=30min*
Maximum sublimation flow rate	*TDLAS: Δ(max sublimation rate)= 0.05 kg h-1m-2*
Rejection rate based on cake appearance	*Δ(rejection rate)=0.2%*

The good agreement between calculated and measured important process parameters validated the use of this model and this design space approach to define primary drying process conditions.

Definition of freezing and secondary drying conditions

Based on prior knowledge, it was demonstrated that the freezing rate, within the capabilities of an industrial freeze dryer, did not have any impact on product quality. As mentioned earlier, it would be important for the team to investigate the impact of freezing early in development to determine the impact of freezing. In this example, only a few different parameters were examined, but freezing should be examined routinely since it can impact the overall lyophilization process substantially.

Shelf temperature ramp rate was then set to 0.3°C/min. Similarly, previous data demonstrated that up to 40°C was an acceptable product temperature for secondary drying for all serogroups. The setpoint was then set at 30°C for 10 hours to achieve moisture levels lower than 2%.

Table 7-17: Pilot Scale Optimized Lyophilization Cycle for A-VAX Vaccine

Lyophilization Stage	Initial Cycle
Loading/Freezing Temperature	-50ºC
Freeze Time Post-load	60 minutes
Ramp to Primary Drying	1ºC/minute
Primary Drying Temperature	-10ºC
Primary Drying Time	720 minutes
Ramp to Secondary Drying	0.3ºC/minute
Secondary Drying Temperature	30ºC
Secondary Drying Time	600 minutes
Final Stage Post-secondary Drying	4ºC
All conditions during lyophilization utilized 100 µbar pressure.	

Lab-scale lyophilization DOE

Based on initial risk assessment, the main areas to examine include freezing, primary and secondary drying, pressure control, and ramp rate.

Applying the output of the initial cause-and-effect risk assessment, a series of development activities were executed to further understand the sensitivity of product quality attributes to process parameters. The first of these activities was a screening DOE, whereby the potential impact of high-risk process parameters could be further assessed.

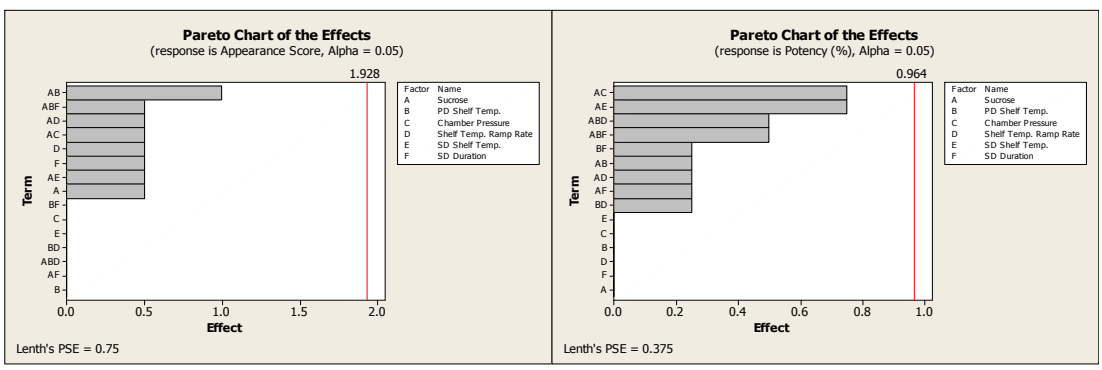
The screening study was designed as a one-quarter fractional two-level DOE on the parameters outlined in Table 7-18. This design was selected to allow direct evaluation of main effects while screening for the potential presence of two-way interactions. Lyophilization-related quality attributes of potency, moisture, appearance, and reconstitution time were evaluated across all runs. The ranges explored for each parameter were selected to be >3X NOR for expected performance in similar commercial equipment.

Table 7-18: Design of Experiment to Screen Lyophilization Parameters

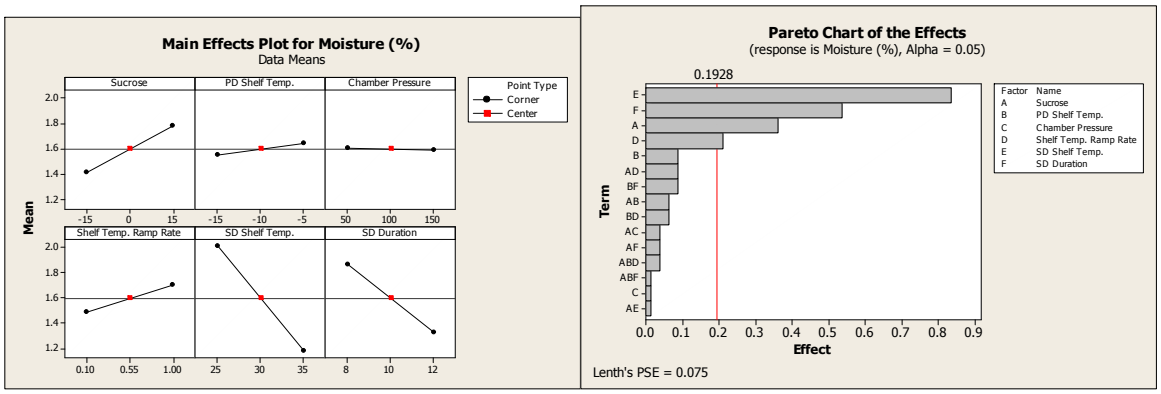
Factor	Low	Set point	High
Sucrose	-15%	0%	+15%
Chamber Pressure	50 µbar	100 µbar	150 µbar
1° Drying Shelf Temperature	-15°C	-10°C	-5°C
Shelf Temperature Ramp Rate	0.1°C/min	0.3°C/min	1.0°C/min
2° Drying Shelf Temperature	25°C	30°C	35°C
2° Drying Duration	8 hr	10 hr	12 hr

The results of this DOE suggest the following:

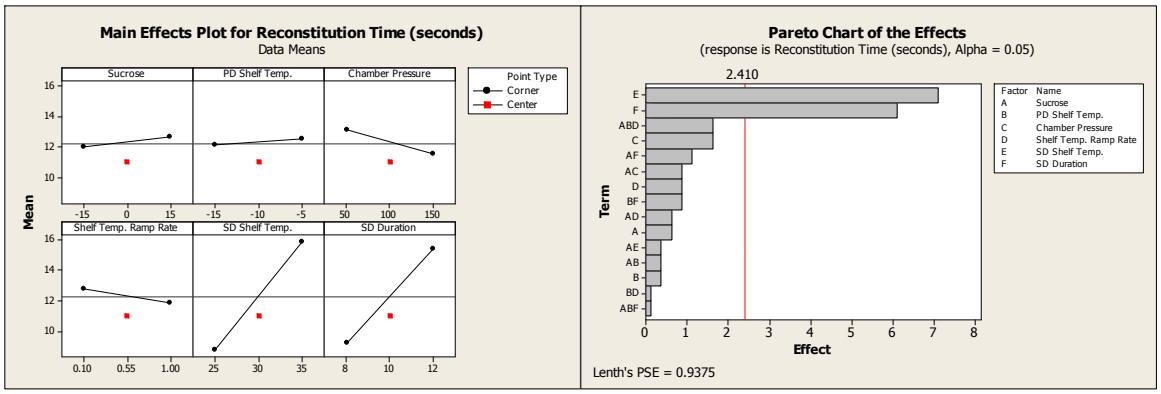
- The process parameters explored did not have a statistically significant response (95% CI) on cake appearance or potency.



- Statistically significant effects were observed for product moisture, specifically sucrose, shelf temperature ramp rate, secondary drying temperature, and secondary drying duration. Additional work should be performed to understand specific risks for this quality attribute and the parameters indicated.



- Statistically significant effects were also observed for reconstitution time, specifically secondary drying temperature and secondary drying duration. It is important to note that while the effects were statistically significant, the range of values observed (2–20 seconds) is well below the maximum specification of 120 seconds.



- Two-way interactions were not observed for the conditions explored in this DOE for any of the lyophilization-related critical quality attributes.
- Over the range of conditions evaluated, primary drying shelf temperature and chamber pressure did not have a statistically significant response on any of the quality attributes. While this would suggest that these parameters are not important to the process, it is well documented that these parameters are important to maintaining appropriate product temperature during sublimation and successful removal of ice from the product prior to removal of bound water in secondary drying. If controls can be implemented to ensure that primary drying is completed at the commercial scale and product temperature is monitored during scale-up, this data may be used to justify a reduced criticality for these parameters.

With statistically significant, but not functionally meaningful, effects on reconstitution time and no statistically significant effects on potency and appearance, future development efforts during scale-up should focus on product moisture (and associated parameters) and implementation of a control strategy to ensure successful primary drying completion and product temperature.

7.7.1. Adjuvant Sterilization Process Development

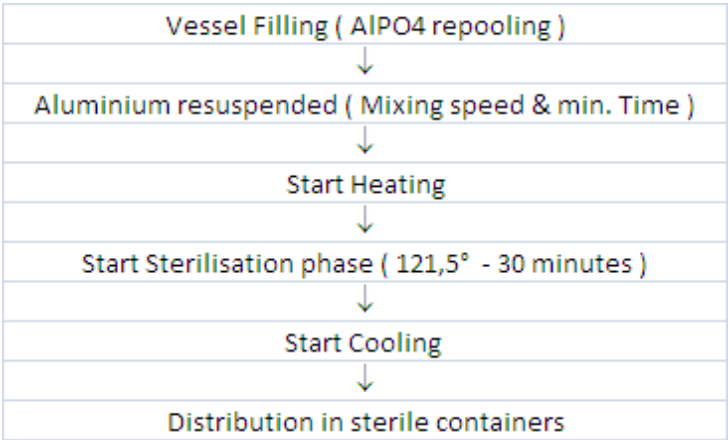
Based on the initial risk assessment, the main focus of development efforts will be on the sterilization process of the aluminum suspension.

The sterilization step must guarantee product sterility, while delivering a homogeneous aluminum suspension of consistent characteristics. The sterilization step is performed in a jacketed vessel under mixing. (FIGURE XX to show process investigated.)

7.7.2. Aluminum Sterilization DOE

Since the sterilization process will be examined, a flow diagram (Figure 7-5) indicates the overall process associated with sterilization.

Figure 7-5: Aluminum Sterilization Process



With knowledge of the process and the cause-and-effect matrix, the parameters of sterilization temperature, mixing speed, and cycle duration (Table 7-19) were examined using a design of experiment.

Table 7-19: Adjuvant Sterilization DOE

Parameter Investigated	Range
Sterilization Temperature	119.5 ºC–123.5ºC (target 121.5°)
Sterilization Duration	30 minutes
Cycle Duration	100–250 min (target 160 min)
Mixing Speed	104–310 rpm (target 210 rpm)
Quality Attributes Evaluated	pH, Zeta Potential, PZC, Particle Size, Adsorption Capacity, Viscosity, Settling Velocity, Turbidity, Free Phosphate

Because aluminum phosphate production is already in place for other vaccines, a lot of historical information and prior knowledge is already available. The system is well understood, critical process parameters are identified, and the design of experiment will focus on the demonstration of the process robustness of the sterilization step performed in a new stainless steel vessel, associated with scale-up considerations.

Two factors will be evaluated, split in three quantitative factors studied through a full two-level factorial design with three additional central points (reference conditions):

- The impact of mixing speed: A boundary condition is the minimal speed defined to guarantee aluminum suspension homogeneity. The maximal speed is defined from the scaling-up studies and will cover the worst-case conditions identified for larger-scale vessels that will be used in the future. Scale-up considerations for mixing are described in chapter 7.4, Adjuvant Scale-up Considerations.
- The temperature profile: It is the combination of the sterilization temperature and the kinetics of heating/cooling. Sterilization temperature is fixed at 121.5°C, and the variation range around the target value is fixed to 2°C, taking into account the overall precision of the temperature probes and the process control system. Sterilization step duration is fixed to 30 minutes. Sterilization duration starts automatically (PID) when sterilization temperature is reached and ends automatically after 30 minutes (PLC-controlled).

Overall process duration is composed of the heating, sterilization, and cooling steps. Extremes' profiles will be evaluated (short to long heating/cooling kinetics). The short temperature profile is associated with the lowest temperature (119.5°C) and must guarantee a minimal Fo value for sterility assurance. The long temperature profile is associated with the highest temperature and is the worst case for temperature impact on aluminum properties.

The main output of the DOE will be the PZC, the particle size, and the adsorption capacity. Adsorption capacity can be measured with a model protein (allow to make the link with previous aluminum phosphate-based vaccine development), and in the case of the A-VAX vaccine development, the impact on the binding of the worst-case serotype will also be evaluated.

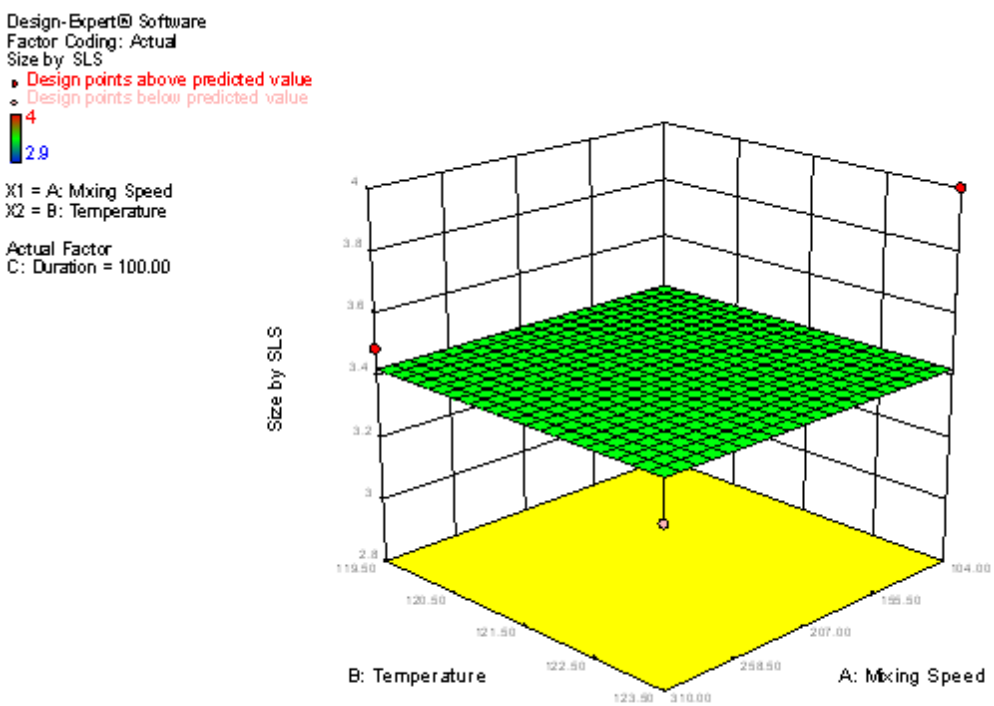
The DOE is based on an equivalence approach, with a target of robustness demonstration.

Objective of the DOE is demonstration that evaluated changes do not impact aluminum quality.

441 **Table 7-20: DOE for Aluminum Sterilization and Responses for Measured Quality Attributes**

			PZC	Adsorpt. Capacity	Size by SLS	Remarks
Mixing Speed	Temperature	Duration		mg Lyz / mg Al	µm (D [v, 0,5])	
210	121.5	160	5.3	0.99	2.9	Central Point
210	121.5	160	5.4	0.87	3.6	Central Point
210	121.5	160	5.2	0.91	3.1	Central Point
104	119.5	100	5.5	1.06	3	
104	119.5	100	5.5	0.97	3.5	
104	123.5	100	5.3	0.96	4	
310	123.5	100	5.4	0.88	3.3	
104	119.5	250	5.1	0.86	3.8	
310	119.5	250	5.4	1.01	3.5	
104	123.5	250	5.1	0.82	3.2	
310	123.5	250	5.1	0.79	3.9	

442
443 Based on manufacturing experience with aluminum phosphate and on characteristics of
444 aluminum adjuvant used in A-VAX preclinical and clinical development, acceptance ranges are
445 defined for PZC, particle size, and adsorption/binding:
446
447 5,0 ≤ PZC ≤ 5,6
448 0,7 ≤ Ads. Capacity Lyz. ≤ 5,6
449 2,5 ≤ Size by SLS ≤ 5,0
450
451 Particle size by Static Light Scattering (SLS) is not affected by mixing speed, sterilization
452 temperature, or temperature profile:



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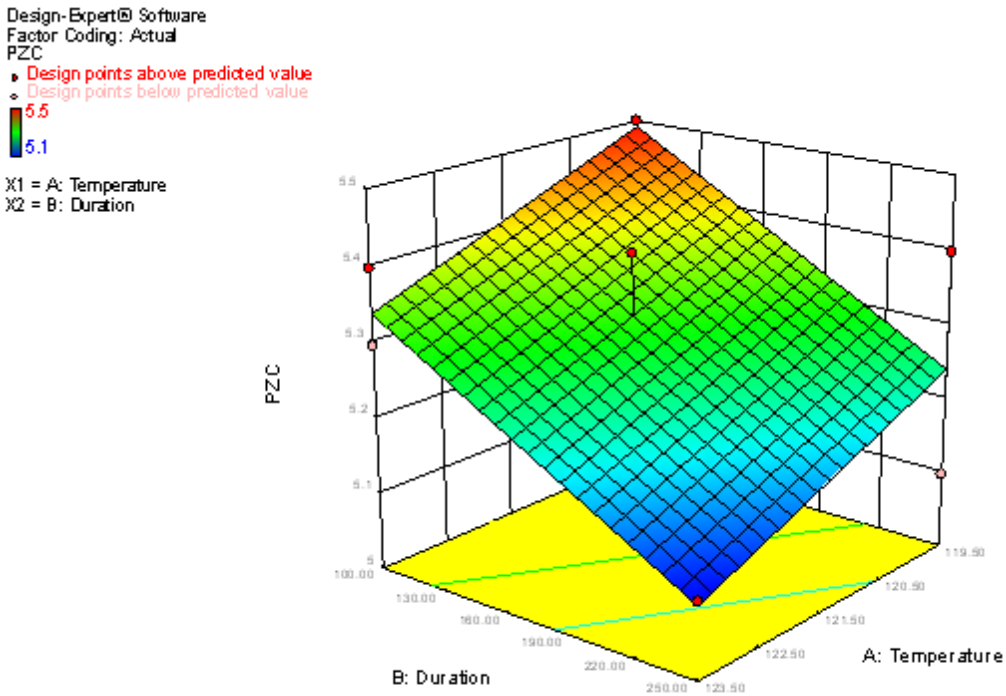
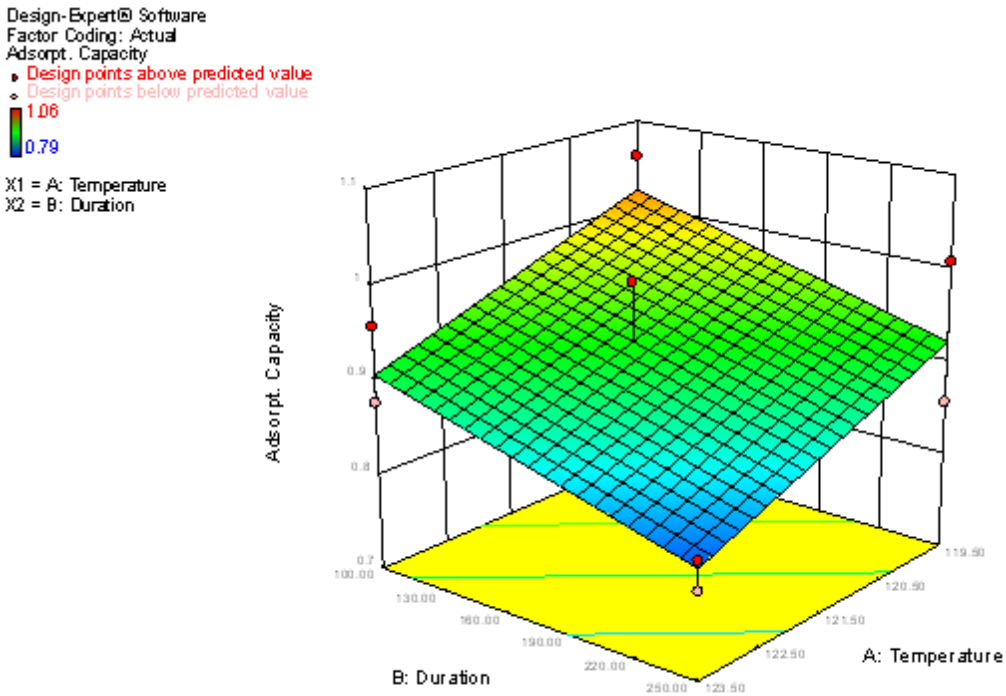
Experimental data are all in the range of acceptance criteria. However, DOE analysis allows us to understand the impact of some parameters on aluminum characteristics:

PZC is impacted by the temperature and the duration of the complete sterilization cycle (p-value < 5%, ANOVA analysis).



Adsorption capacity is also affected by the temperature and the cycle duration (p-value < 5%, ANOVA analysis).



Two-way interactions were not observed for the conditions explored in this DOE.

This kind of experimental plan does not allow us to model curvature effects. However, it is possible to check with the central points that the model is not affected by the absence of curvature modeling.

A lack of fit parameter is calculated by comparing the measured values and the predicted values.

The lack of fit is found not to be significant, and it is not necessary to add experimental points to take into account curvature in the modeling.

From the prediction model build from the DOE, it is possible to determine potential risk of failures.

At reference (target) conditions or for the most critical parameter combinations, predicted responses and associated 95% confidence intervals are inside acceptance criteria:

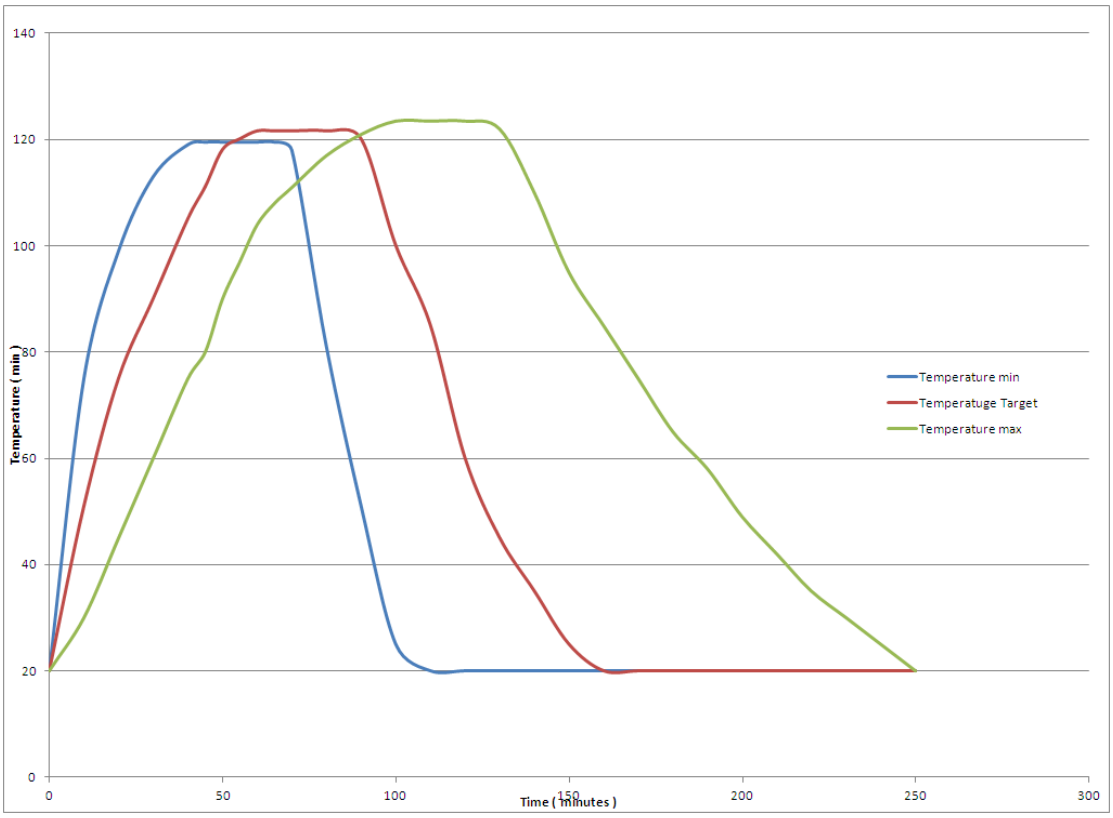
Table 7-21: Impact of Process Parameters on Quality Attributes: Predicted Response Based on Model from DOE

							95%CI	
Response	Mix. speed	Temp.	Duration	Low spec	High spec.	Prediction	low	high
PZC	*	121.5	160	5	5.6	5.3	5.3	5.4
PZC		119.5	100			5.5	5.4	5.6
PZC		123.5	250			5.2	5.1	5.3
Adsorpt. Capacity	*	121.5	160	0.7	1.2	0.9	0.9	1.0
Adsorpt. Capacity		119.5	100			1.0	0.9	1.1
Adsorpt. Capacity		123.5	250			0.8	0.8	0.9
Size by SLS	*	*	*	2.5	5	3.4	3.2	3.7

*: Not significant factor, predictions valid for the whole studied range

Conclusion of the DOE is demonstration of process robustness, but attention must be paid to the heat treatment conditions (duration and temperature).

Figure 7-6: Aluminum Sterilization



7.8. Scale-up Risk Assessment: FMEA Analysis

Moving into scale-up, additional learnings obtained from design space activities were applied along with known scale-up risks to perform a higher-rigor-level risk assessment (FMEA).

7.8.1. Failure Modes and Effect Analysis

Following the completion of initial lab-scale DOE work and continued development into the commercialization space, a second-stage risk assessment was conducted based on failure modes and effect analysis (FMEA). The analysis leverages process understanding and the known risks associated with different unit operations during the formulation and filling processes. The FMEA aids in the determination of potential failures that could occur within the process and helps to identify critical process parameters (CPPs). Once the CPPs are identified, adequate control strategies can be utilized to ensure a robust process is achieved. For each unit operation, scores of severity (S), occurrence (O), and detectability (D) are assigned. For the A-VAX study, the scoring system is listed below (2).

Table 7-22: Scoring System for FMEA

Score	Severity	Occurrence	Detection
9 "HIGH risk"	Process failure potentially impacting one or more critical product quality attribute(s) leading to product rejection.	> 20% (very frequent)	No way to detect excursion. Not tracked and not alarmed.
7	Potential impact on product quality or consistency (e.g. product-related substances). Investigation needed prior to product release.	~ 5-20% (frequent)	Difficult to detect excursion, and not until after it has impacted the process.
5	No impact on product quality, but deviation from manufacturing procedures that requires justification. Likely deterioration in process performance attributes (e.g. yield) or ease of process operability.	~ 1-5% (occasional)	Excursion can be detected, but not until after it has impacted the process.
3	No impact on product quality. Potential for minor deterioration in process performance attributes (e.g. yield) or ease of process operability.	< 1% (rare)	Excursion is usually detected and corrected prior to impacting the process.
1 "LOW risk"	No impact to process performance attributes or product quality.	0% (never observed)	Excursion is obvious and always detected prior to impacting process.

Process Step	Key Process Inputs	Ranges Evaluated	Potential Failure Mode	Potential Failure Effects	SEVERITY	OCCURRENCE	DETECTION	Current RPN
What is the process step	What are the Key Process Inputs? (KPIV's)	What are the ranges expected to experienced during normal operations?	In what ways can Key Inputs go wrong? (Process fail to meet requirements)	What is the impact on the Key Output Variables (customer requirements) or internal requirements?	How Severe is effect ? (9,7,5,3,1)	How frequent is cause likely to Occur? 9, 7,5,3,1	How probable is Detection of cause? 9, 7, 5, 3, 1	Risk Priority # to rank order concerns
Raw Materials (DS)	Sucrose concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	pH	Set point: 6.5 Range: 5.5 - 7.5	Improper formulation of Buffer, or incomplete buffer transfer, pH probe calibration or pH instrument issues	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	NaCl Concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Histidine Concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Polysorbate Concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, adsorption, recon time, potency	3	3	3	27
	Antigen Concentration		Problem with dilution, analytical testing issue, aggregation / degradation of DS	Potency, concentration, stability,	9	3	5	135
	Bag Volume	+/- 15%	Under filled bag	Potency, concentration, stability,	9	5	1	45
	Storage Temperature	Set point: 4ºC Range: 0 - 10ºC	Wrong with cold chain, shipping deviation, cold storage equipment deviation, wrong TOR reporting	Stability, potency	1	3	3	9
Formulation / Mixing	Mixing Time	Set point: 15 minutes Range: 10 - 20 minutes	Don't mix product, not mixed long enough, too long mixing time	Stability, potency, concentration	5	3	1	15
	Mixing Speed	Set Point: 200rpm Range: 150rpm - 250rpm	Too fast or too slow, no stirring at all	Stability, potency, concentration, Free Ps,	5	3	1	15
	Mixing temperature	Set point: 4ºC Range: 0 - 10ºC	Too warm, too cold, loss of temperature control	Stability, potency, concentration	3	5	1	15
	Dilution Buffer Volume Added	+/- 15%	Weighing error, under filled bags, overfilled due to error, Calculation error for dilutions, Line losses	Concentration, potency, stability	7	3	5	105
	DS Volume added	+/- 15%	Weighing error, under filled bags, overfilled due to error, Calculation error for dilutions, Line losses	Concentration, potency, stability	7	3	5	105
	Dilution Buffer Sucrose concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Dilution Buffer pH	Set point: 6.5 Range: 5.5 - 7.5	Improper formulation of Buffer, or incomplete buffer transfer, pH probe calibration or pH instrument issues	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Dilution Bufffer NaCl Concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Dilution Buffer Histidine Concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Dilution Buffer Polysorbate Concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Loading temperature	Set point: -50ºC Range: -45 - -55ºC	loss of temperature control of cabinet, improper recipe	Stability and Potency	1	3	1	3
	Freezing time (Duration)	Set Point: 60 minutes Range: 50 - 70 minutes	too short	Cake Appearance, Moisture, Stability, Potency, Recon Time	7	3	1	21
	Initial Pressure	Set Point: 100µBarr Range: 75µBarr - 125µBarr	too high	Cake Appearance, Moisture, Stability, Potency, Recon Time	1	3	3	9
	Ramp rate to 1º Drying	Set Point: 1ºC / minute Range: 0.5 - 1.5ºC / minute	too fast or slow ramp	None Identified	1	3	3	9
	Primary Drying temperature	Set Point: -10ºC Range: -15ºC - -5ºC	too high or low	Cake Appearance, Moisture, Stability, Potency, Recon Time	9	3	5	135
	Primary Drying duration	Set Point: 720 minutes Range: 660 - 780 minutes	too short	Cake Appearance, Moisture, Stability, Potency, Recon Time	9	3	5	135
	Primary Drying Pressure	Set Point: 100µBarr Range: 75µBarr - 125µBarr	too high or low	Cake Appearance, Moisture, Stability, Potency, Recon Time	9	3	5	135
	Ramp rate to Secondary Drying	Set Point: 0.3ºC / minute Range: 0.1 - 0.5ºC / minute	too fast or slow ramp	Cake Appearance, Moisture, Stability, Potency, Recon Time	7	3	5	105
	Secondary Temperature	Set Point: 30ºC Range: 25 - 35ºC	too high or low	Cake Appearance, Moisture, Stability, Potency, Recon Time	7	3	5	105
	Secondary Duration	Set Point: 600minutes Range: 540 - 660 minutes	too short or long	Cake Appearance, Moisture, Stability, Potency, Recon Time	5	3	5	75
	Secondary Pressure	Set Point: 100µBarr Range: 75µBarr - 125µBarr	too high or low	Cake Appearance, Moisture, Stability, Potency, Recon Time	3	3	5	45
	Stoppering Temperature	Set Point: 5ºC Range: 0 - 10ºC	too high or low	Sterility, Stability, Moisture	1	3	5	15
	Stoppering Pressure	Set Point: 800mBarr Range: 750 - 850mBarr	too high or low	None Identified	1	3	5	15
	Stoppering Gas	Nitrogen	wrong gas utilized, leak in gas line	Stability, Moisture	9	1	5	45
	Stoppering Force		too high or low	Sterility, Stability, Moisture	9	3	3	81
Aluminum	**Sterilization**							
	Temperature	Set-Point : 121.5 °C Range : 119.5 - 123.5 °C	too high (impact on Alum) or low (Impact on Sterility)	Sterility, Adsorption Capacity, PZC	9	3	1	27
	Duration	Set-Point : 30 min.	too short or long	Sterility, Adsorption Capacity, PZC	9	3	1	27
	pressure	Set-Point : 0.5 bar	too high (bursting disc) or low (risk of sterility failure)	Sterility	9	3	1	27
	Mixing time	Continuous mixing during heat./ster./cool.	too high (risk of shearing) or none (temperature non-homogeneity)	Sterility, Particle Size	9	3	1	27
	Mixing Speed	Scale dependent param. DOE-20L scale Set-Point : 210 rpm Range : 104 - 310 rpm	too high (risk of shearing) or low or none (temperature non-homogeneity)	Sterility, Particle Size	9	3	1	27
	Heating & Cooling time	Range : 100 - 250 min.	too long or short	Adsorption Capacity, PZC	3	3	1	9
	Distribution of Product							
	Mixing speed	Scale dependent param. DOE-20L scale Set-Point : 210 rpm	not enough speed to stop settling	Non-Homogeneity	7	3	1	21
	Mixing time	Min. 15 minutes	not enough time to ensure homogenous	Non-Homogeneity	7	1	1	7
	Speed of filling		Settling can occur if filling speed is too low	Non-Homogeneity	7	3	1	21

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7.9. Scale-up Considerations and Site Transfer Activities

7.9.1. Formulation Scale-up Considerations

During early development activities, the formulation was shown to be highly robust as regards the serotypes binding to the aluminum adjuvant. Two factors shown to have an impact on binding (pH and sucrose concentrations) can be readily controlled during scale-up and commercialization. As a result, scale-specific considerations are not expected to be high risk.

7.9.2. Freeze-drying Scale-up Considerations

A successful scale-up and transfer of a freeze-drying cycle imply that their performances are equivalent between lab or pilot scale and industrial scale (i.e., that product temperature: time profiles are identical). By performance, one should consider cycle robustness, cycle time, and product quality (potency, residual moisture, dissolution time).

To ensure successful scale-up, several aspects need to be taken into account during cycle development and process transfer. The ones listed below are a subset of the aspects that would be examined during scale-up and process transfer:

- Industrial process configuration: trays configuration, heat transfer map, sublimation rates within the design space, door placement, temperature jacketed units, gas injection (single or multi port, continuous), sensor type (Pirani vs. mks), and condenser location
- Industrial equipment performance: choke flow, shelf temperature homogeneity, radiative effects, and condenser capacity

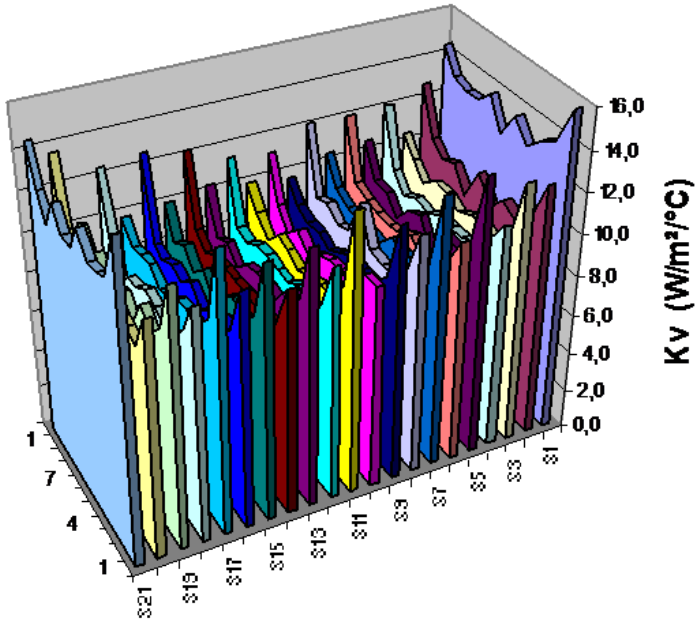
Details of these considerations are available in the study guide appendix.

7.9.2.1. Industrial Equipment Configuration Vs. Pilot:

The basic rule is to ensure that all components in the process that influence the heat and/or mass transfer characteristics, and therefore the sublimation rate during primary drying, are identical between the pilot and the industrial scale. In other words, identify and implement what can be identical between the pilot and the industrial scale. These components include:

- **Freeze-drying trays (if any)**: Figure 7-7 below represents the level of heterogeneity in overall vial heat transfer coefficient Kv, as a function of its location on an aluminum tray. Vials on the edges of the tray can receive up to 60% more energy than vials located in the middle of the tray. The level of heterogeneity can vary as a function of the tray's material (aluminum, stainless steel, plastic) and its configuration (bottomless vs. standard).

Figure 7-7: Heat Transfer Profile in Commercial Lyophilization Unit



- **Componentry:** As with trays, it is important to use identical vials during freeze-drying cycle development because they can have very different overall heat transfer coefficient values Kv, as demonstrated by Pikal et al. For example, important differences have been demonstrated in the literature. (*Pikal M.J., Roy M.L., Shah S., 1984: Mass and heat transfer in the freeze-drying of pharmaceuticals: Role of the vial. Journal of Pharmaceutical Sciences, 73, 1224–1237.)*
- Similarly, stoppers used for development should be the same, and moisture uptake studies at the lab scale should be done with residual moisture levels in the stopper equivalent to what the industrial process delivers.
- Design of the condenser: The position of the condenser (i.e., inside the freeze-drying chamber vs. external condenser with a separating valve) can significantly impact mass-transfer characteristics, and similar design should be used during development when possible.
- Two types of pressure gauge are commonly used for pressure control during primary and secondary drying: Pirani type (heated wire sensor; reading is impacted by the gas composition) and MKS type (capacitance sensor; reading independent of the gas composition). Using the same type of sensor is critical because there is a ~1.6 ratio between the two when atmosphere in the chamber is saturated with water vapor, which is the case during primary drying.

7.9.2.2. Industrial Equipment Performance Vs. Pilot:

Equipment-imposed boundaries and intrinsic heat and mass transfer differences must be identified, measured, and taken into account during cycle development. In other words, identify and control what cannot be identical between the two scales. These parameters include:

- Shelf temperature homogeneity is critical and is verified during commissioning of the equipment. But it has also been shown that the difference between the shelf temperature setpoint and the actual surface temperature of the shelves during primary drying can be important and significantly different, as a function of scale and sometimes equipment itself. Reported values in the literature are in the 2°C to 7°C range, as a function of sublimation rates.

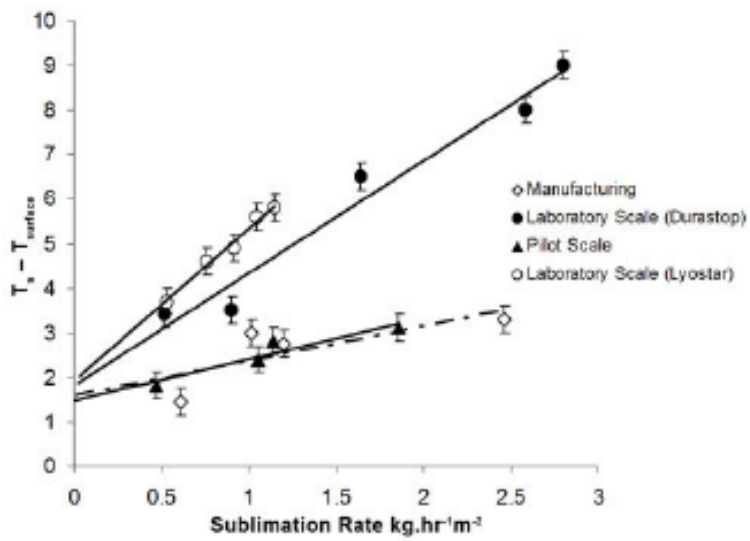


Figure 1. Difference between shelf temperature (T_s, fluid inlet) and shelf surface temperature ($T_{surface}$) obtained as a function of sublimation rate for different lyophilizers. Error bars represent estimated errors (±0.2°C) in thermocouple measurements.

AAPS PharmSciTech 2006; 7 (2) Article 39 (http://www.aapspharmscitech.org).

- Radiative contribution to overall sublimation heat transfer coefficient often depends on equipment scale and design. This "edge effect" is mainly related to differences in chamber parts emissivity values and potentially chamber wall temperature difference as a function of equipment size and cooling technology used after sterilization. In most cases, radiative contribution is higher in pilot scale equipment, leading to shorter primary drying times for the identical freeze-drying recipe.

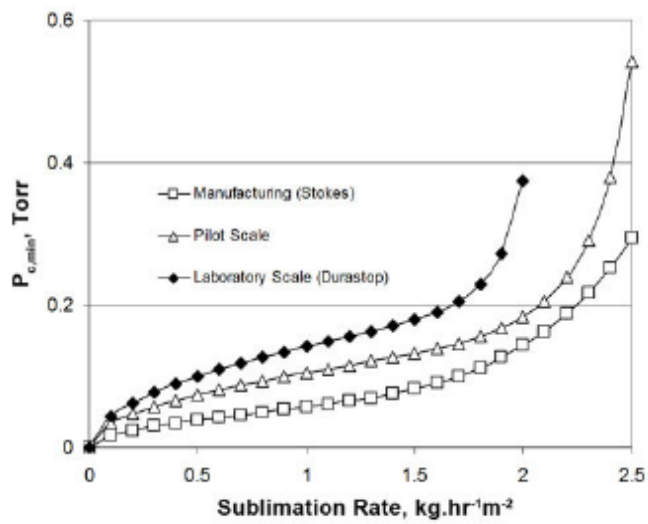


Figure 4. Comparison of minimum chamber pressure ($P_{c,min}$) as a function of sublimation rate.

AAPS PharmSciTech 2006; 7 (2) Article 39 (http://www.aapspharmscitech.org).

Primary drying time should be carefully monitored during scale-up, and adequate safety margin should be applied to primary drying time to compensate for this edge effect.

- The choke flow corresponds to the maximum water vapor flow rate that can pass through the spool toward the condenser. This value is a function of equipment design and pressure and should be measured to ensure that for any cycle scaled-up and transfered to industrial equipment, instantaneous sublimation rate is always lower than choke flow value at the corresponding pressure, to avoid loss of pressure control in the drying chamber.
- Similarly, maximum condenser capacity (expressed in $g.min^{-1}$) should be measured to secure primary drying and avoid loss of temperature control of the condenser.
- Freeze-drying cycle design should be compatible with heating and cooling performance of the industrial equipment at full load.

The freeze-drying cycle should be robust enough to absorb all these intrinsic differences, keeping the product temperature always below its collapse temperature throughout primary and secondary drying.

7.9.3. Lyophilization Process Scale-up and Transfer from Pilot Scale to Industrial Scale

Industrial freeze-dryer characteristics vs. pilot scale

Table 7-23 below compares the main characteristics of the industrial freeze-dryer in which the product is transferred with the ones of the pilot scale equipment in which the lyo cycle was developed.

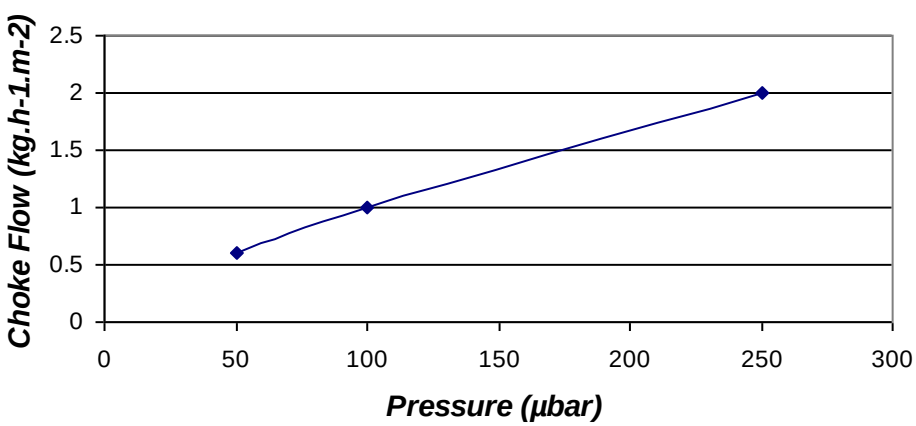
Table 7-23 Industrial Freeze-dryer Characteristics vs. Pilot Scale

Characteristics	Pilot scale equipment	Industrial scale equipment
Shelf area	*1m2*	*40m2*
Batch size	*4,000 vials*	*160,000 vials*
Condenser	*External*	*External*
Trays	*Bottomless trays*	*No trays-direct contact-Automatic Loading System (ALS)*
Pressure gauge	Capacitance	Capacitance

During the cycle development, bottomless trays were used to mimic direct loading on the shelves, and the same pressure gauge, same vials, and same stoppers were selected.

Choke flow measurement in the industrial unit at 100 µbar

Methodologies to accurately measure the choke flow in a freeze dryer are described in the literature. As an example, a simple protocol is described by *Patel et al., Chemical Engineering Science, Volume 65, Issue 21, 1 November 2010, pages 5716–5727*.



The choke flow for the industrial unit was measured at approximately 1 $kg.h^{-1}.m^{-2}$ at 100 µbar, the operating pressure of our freeze-drying cycle. This value is way above the 0.34 kg $h^{-1}m^{-2}$ calculated by the model at pilot scale and, therefore, choke flow was not considered as a concern for our vaccine in this unit.

Heat transfer measurement in the industrial unit at 100 µbar

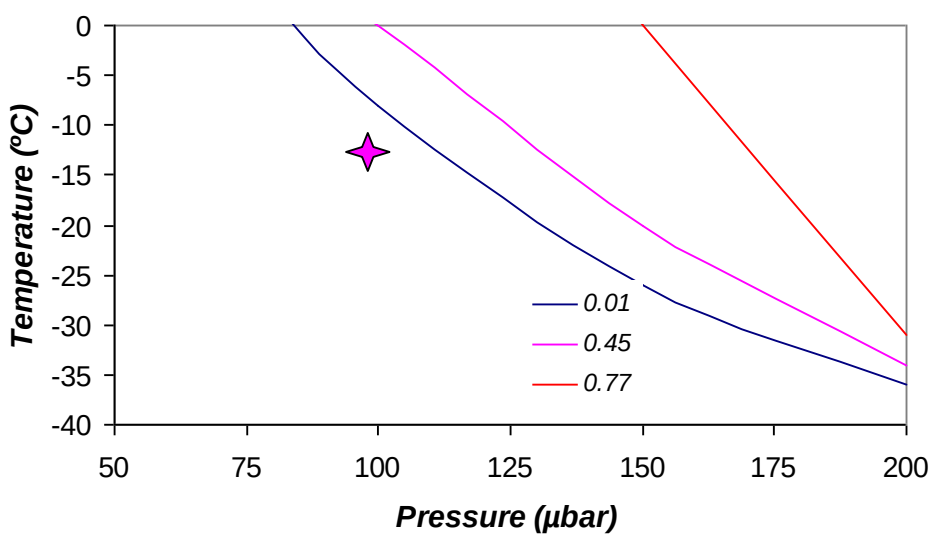
The table below gives the overall heat transfer values measured at 100 µbar as a function of vial locations in the industrial unit; as a reminder, the Kv values obtained in the pilot unit are reported in the right column:

Vial location	Kv, W.m-2.K-1 Industrial scale	Kv, W.m-2.K-1 Pilot scale
(a)	40.2±3.4	35.2±3.4
(b)	29.5±2.0	24.5±2.0
(c)	21.3±0.9	16.3±0.9
(d)	10.8±1.0	11.8±1.0
(e)	8.1±0.7	9.3±0.7

Moreover, the proportion of each vial location is changed in the industrial unit compared to the pilot unit. It was evaluated that (a) location vials represent 0.05% of the total number of vials, (b) location vials represent 5.2%, (c) represent 2.1%, (d) represent 9.4%, and (e) represent 68.2%.

Freeze-drying cycle parameters adjustment for scale-up

The design space was redefined for the industrial-scale process, taking into account these difference in Kv values for different vial locations, and is represented in the figure below.



The shelf temperature was set 3°C lower compared with the pilot-scale conditions because of higher Kv value of (b) location vials. The primary drying time was therefore increased to 960 minutes to compensate for the lower (e) location vials' Kv value, the decreased shelf temperature value, and to include a 120-minute calculated safety margin. In these conditions, the calculated maximum flow rate during primary drying is equal to 0.32 $kg.h^{-1}.m^{-2}$ and remains far below the choke flow of the industrial equipment. Choke flow is therefore not a concern for this process.

The selected cycle for the industrial-scale process is given in Table 7-24 below:

Table 7-24: Industrial-Scale Lyophilization Cycle for A-VAX Vaccine

Lyophilization Stage	Initial Cycle
Loading/Freezing Temperature	-50ºC
Freeze Time Post-Load	60 minutes
Ramp to Primary Drying	1ºC/minute
Primary Drying Temperature	-13ºC
Primary Drying Time	960 minutes
Ramp to Secondary Drying	0.3ºC/minute
Secondary Drying Temperature	30ºC
Secondary Drying Time	600 minutes
Final Stage Post-secondary Drying	4ºC
All conditions during lyophilization utilized 100 µbar.	

Freeze-drying process scale-up

Engineering runs at full scale were performed prior to process qualification and validation to check for cycle suitability at industrial scale. In some cases, the active ingredient is not available and a proper placebo has to be identified. This is the case for our cooties vaccine; a placebo formulation containing (everything but active ingredient) was characterized and demonstrated similar freeze-drying characteristics as the actual drug product: glass transition at maximum cryoconcentration Tg', collapse temperature Tc, and resistance to mass transfer Rp as a function of dry-layer thickness during primary drying.

The following attributes were measured during these runs; additional attributes may be measured as well during transfer (i.e., product temperature, pressure):

- Actual primary drying duration vs. setpoint for primary drying duration $\delta t_{sublimation}$: It was determined considering completion when the Pirani value meets and equals the capacitance value. An example is described in the figure below:

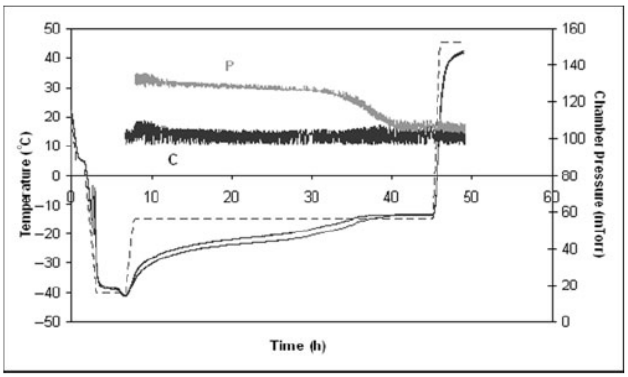


Figure 1. Temperature and pressure profiles of a freeze-drying cycle. The broken line represents the shelf setpoint temperature, the smooth lines represent thermocouple product temperatures, and line P and line C represent the chamber pressure measured by Pirani gauge and capacitance manometer, respectively.

Controlled nucleation in freeze drying: effect of pore size in the dried product layer, mass transfer resistance, and primary drying rate. Konstantinidis et al. 2011. J. Pharm. Sci. Apr 4.

- Residual moisture: samples were taken at corners and center of each shelf
- Cake appearance and associated rejection rate

Results are gathered in Table 7-25 below:

Table 7-25: Scale-up Results

	δtsublimation (min)	Residual moisture (%±σ)	Rejection rate %
Engineering run 1	45	0.7±0.4	0.8
Engineering run 2	60	0.8±0.3	0.4

Engineering runs were successful and confirmed the cycle adjustment performed with the model. Primary drying time was about one hour longer than calculated, but the remaining safety margin was considered acceptable to proceed with process validation.

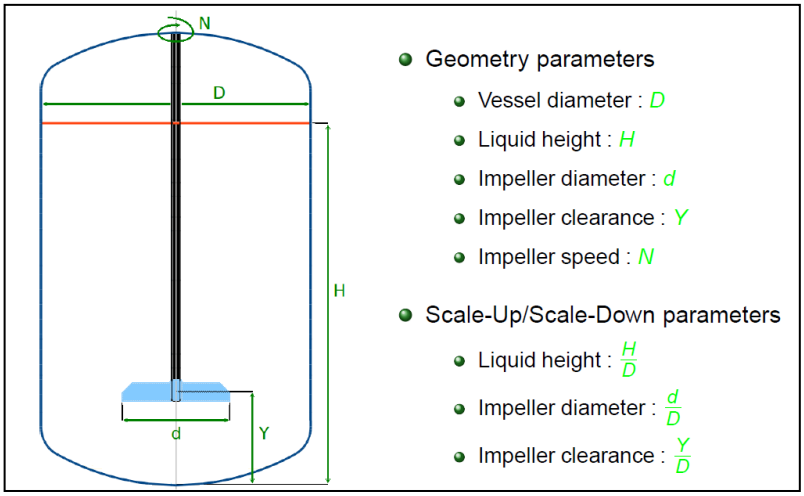
7.9.4. Adjuvant Scale-up Considerations

Sterilization will occur through jacketed vessel (no direct steam injection to avoid product dilution). Design of the vessel must guarantee efficiency of SIP process.

Characterization of aluminum particles' rheological properties (mass per unit volume, apparent viscosity, settling velocity) have allowed appropriate impeller configuration selection to guarantee homogeneity through mixing. It also helps to build scale-up models regarding agitation. Particularly, it will allow defining per vessel size a minimal mixing speed for homogeneity. The scale model for the sterilization vessel is discussed below, and aided in defining a scale-independent process.

The vessels at intermediate scale (DOE scale) (20 L) and commercial scale (500 L) are in geometric similitude. That means that they have the same shape, one being a uniform scaling (enlarging or shrinking) of the other; in other words, the ratio of all corresponding dimensions is equal. Main characteristics of the vessels are:

- Torispherical bottom
- 1 axial flow impeller
- No baffles
- H/D = 1
- d/D = 0.4
- Y/D = 0.2



Scale-up of agitation speed:
The scale-up is performed at constant volumetric power dissipated in the vessel (P/V). It allows to reproduce at both scales the particle attrition and breakage rate resulting from fluid stress and mechanical impacts of the particles (mainly particle-impeller collisions). In turbulent regime, the power dissipated by the mixing in the liquid is given by:

$$P = \rho \, Np \, N^3 \, d^5$$

Where:

- P is the dissipated power (W)
- ρ is the density (kg/m3)
- Np is the power number (-), Np = 0.32 for our axial impeller
- N is the agitation speed (rps)
- d is the impeller diameter (m)

Calculation of minimal agitation speed:

For the DOE and for the process operated at large scale, the suspension must remain homogeneous during sterilization. The minimal speed required for homogeneous suspension is measured at small scale; the extrapolation to larger scale uses the Grenville law (one level of impeller).

$$N_{min} * cst = N_{js} = \frac{x'}{N_P^{\frac{1}{3}} D^{\frac{2}{3}}} \left(\frac{g\Delta\rho}{\rho}\right)^{0.5} X_v^{0.141} d^{0.166} \left(\frac{Y}{D}\right)^{0.243}$$

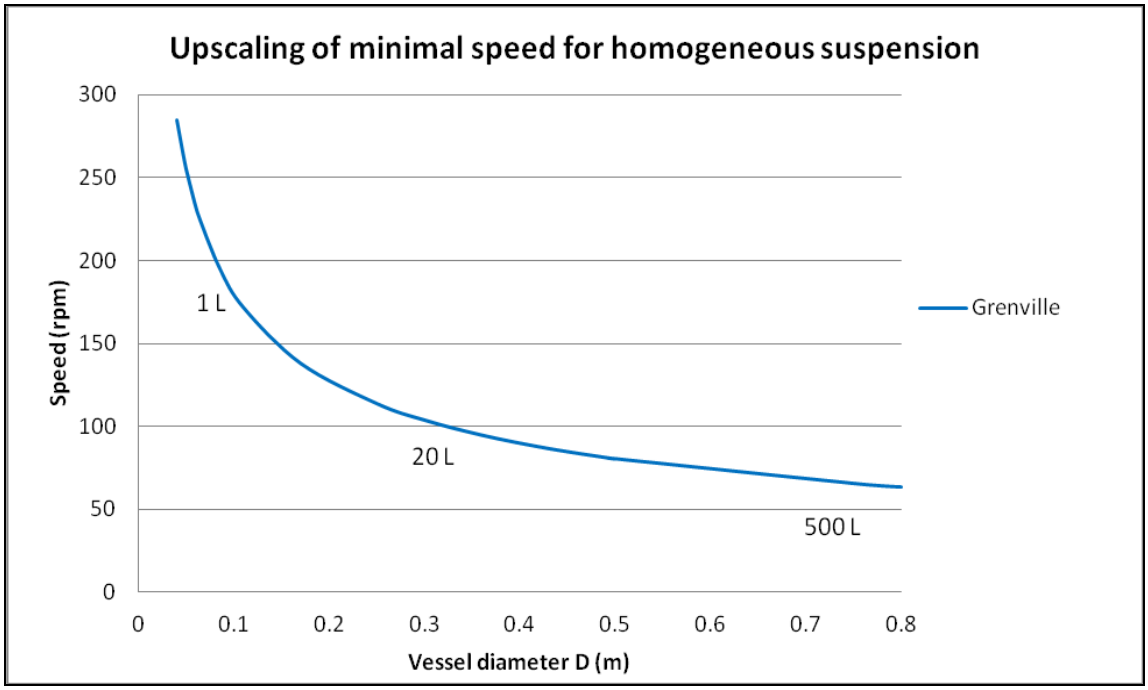
Where:

- Njs is the minimal speed to get just suspended particles; nonhomogeneous (rps)
- x' is a constant depending of the impeller type (-)
- Np is the power number (-)
- D is the vessel diameter (m)
- d is the impeller diameter (m)
- Xv is the solid fraction (-)
- Y is the distance of the impeller from the bottom (m)

For equipment in geometric similarity, this law can be simplified to:

Nmin ~ $D^{-0.5}$.

Experimental studies confirmed this dependency on scale-up; the experimental curve is shown below.



Thermal transfer feasibility check

The thermal transfer is scaled up maintaining a constant volumetric heat transfer rate (Q/V).

$Q = U A \Delta T$

- Q = heat transfer rate (W)
- ΔT = temperature difference (K)
- A = heat transfer area (m2)
- U = overall heat transfer coefficient (W/m2K)

The overall heat transfer coefficient takes into account the convective resistance of the jacket, the resistance of the vessel wall, the fouling of the jacket and vessel surface, and the convective resistance of the process. In most applications, the heat transfer rate from the process side is the limiting step (convection in the vessel).

Then :

$$Q = h_{process} A \Delta T$$

where $h_{process}$ is the heat transfer coefficient on the process side.

It can be shown that for a stirred vessel, with double jacket:

$$h_{process} \sim Re^{2/3}/D$$

Re being the Reynolds number

$$\Rightarrow \quad Q/V \sim (Re^{2/3} A \Delta T)/(D V)$$

For equipment in geometric similarity, with a same fluid, the expression is simplified to:

$$Q/V \sim (N/D)^{2/3}$$

It was shown at 20 L scale that an agitation speed of 10 rpm was sufficient to assure a nonlimiting heat transfer during sterilization.

7.9.5. DOE Range

- The minimal value is fixed to assure that the aluminum suspension is homogeneous in the vessel.
- The maximal value is calculated to reproduce the maximal shear produced at large scale.

a. Minimal speed of DOE:

The minimal speed required to get homogeneous suspension was measured at 1 L scale (D= 0.11 m) and is 170 rpm.

=> the extrapolation (see law above)

- to 20 L scale (D = 0.30 m): Nmin = 104 rpm
- to 500 L scale (D = 0.88 m): Nmin = 81 rpm

b. Maximal speed of DOE:

The maximal speed is calculated to cover the maximal particles' damages encountered at commercial scale; this is a function of P/V.

Commercial scale:
The existing 500 L vessel has a maximal speed of 150 rpm.

=> $P_{max} = \rho\, Np\, N^3\, d^5 = 27$ W
$P_{max}/V = 54$ W/m3

20 L scale: P/V = 54 W/m3 => P = 1.1 W

=> $$N = \left[\frac{P}{Np * \rho * d^5}\right]^{1/3} * 60 = 310 \text{ rpm}$$

c. DOE range:

104 < N < 310 rpm

Reference (target) mixing speed for the DOE is placed at the middle of the range (210 rpm).

7.9.6. Extrapolation of the Optimal Speed Determined by DOE

The extrapolation of the optimal speed determined at 20 L scale to the commercial scale is performed at constant P/V using the formula:

$$P = \rho\ Np\ N^3\ d^5$$

Example:

- If the optimal speed in the DOE is 210 rpm

  => P = 0.3 W
  P/V = 17 W/m3

  At 500 L scale: P/V = 17 W/m3

  => P = 8.5 W

$$N = \left[\frac{P}{Np * \rho * d^5}\right]^{1/3} * 60 = 103 \text{ rpm}$$

- Homogeneity check:
  This speed is superior of the minimum speed required to maintain the suspension as homogeneous (81 rpm). The setpoint can then be fixed at 100 rpm.

- Thermal transfer check:
  $Q/V \sim (N/D)^{2/3}$

  It was shown at 20 L scale that an agitation speed of 10 rpm was sufficient to assure a nonlimiting heat transfer during sterilization. Extrapolation to 500 L scale at constant Q/V:
  $N_{500L} = N_{20L} * D_{500L}/D_{20L}$

  => N_{500L} = 10*088/0.3 = 30 rpm

  The setpoint of 100 rpm is superior to this lower limit.

7.9.7. Adjuvant Scale-up Transfer:

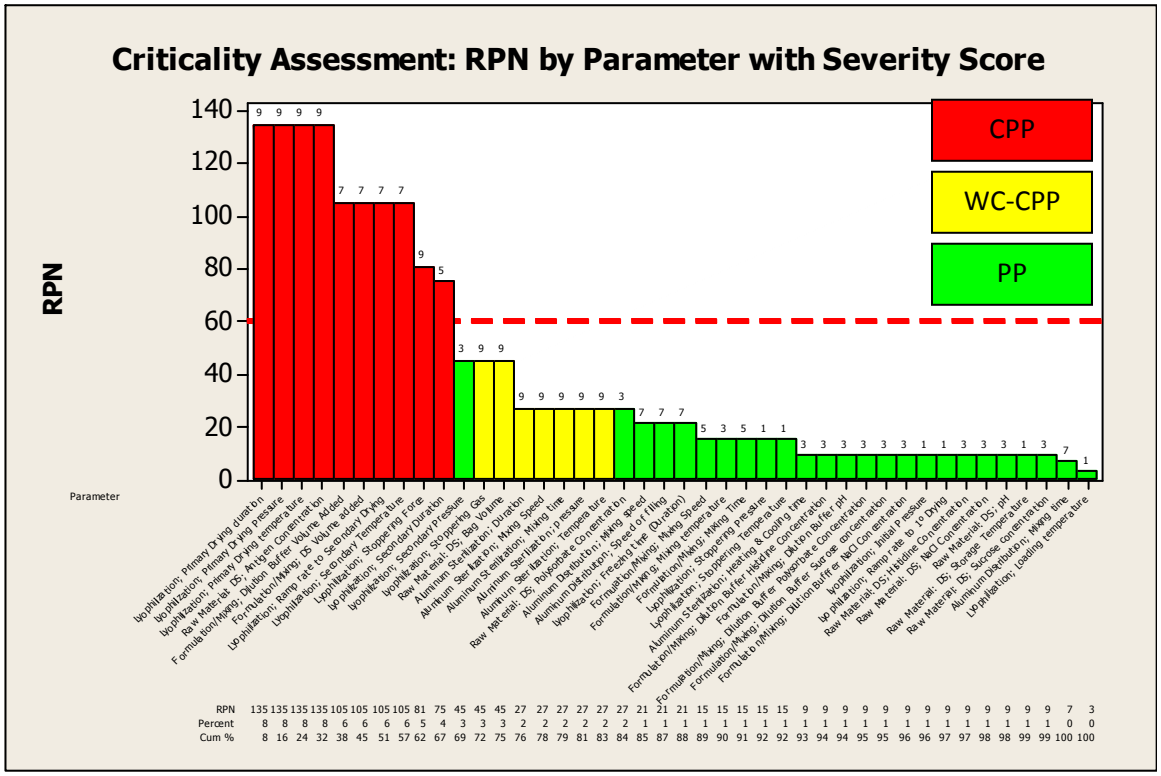
Confirmation runs were performed with same steps' duration and mixing speed defined by the scale-up model.

Homogeneity was checked by temperature profiles in different points of the vessel.
Homogeneity is also checked by Alum sampling and Al content measurement (+ turbidity as IPC).

7.10. Control Strategy

7.10.1. Parameter Criticality Assessment

Critical parameters were identified using a Pareto-type analysis of the FMEA results. The RPN cut-off for criticality was selected at an RPN of 60, above which parameters would be considered critical. In addition, all parameters with RPN <60 and a severity score of 9 were considered well-controlled CPPs. The outcome of this assessment is shown below:



871 **Table 7-26: Operating Ranges for CPPs**

Parameter	Classification	Control Limits	Proven Acceptance Ranges	Control Strategy
Lyophilization Primary Drying Duration	CPPs	> 960 minutes	960 minutes	Lyo cycle automation and recipe selection, alarms, in-process monitoring
Lyophilization Primary Drying Pressure		75–125 µBarr	50–150 µBarr	
Lyophilization Primary Drying Temperature		-11ºC to -15ºC	-5ºC to -15ºC	
Raw Material DS Ag Concentration		1.35–1.65 mg/mL	1–2 mg/mL	CoA, downstream process controls
Formulation Mixing/ Dilution Buffer Added		+/- 5%	NA	Batch record, gravimetric checks
Formulation Mixing/DS Added		+/- 5%	NA	
Lyophilization Ramp Rate to Secondary Drying		< 0.5ºC/min	0.1–1.0ºC/min	Lyo cycle automation and recipe selection, alarms, in-process monitoring
Lyophilization Secondary Drying Temperature		28ºC–32ºC	25ºC–35ºC	
Lyophilization Stoppering Force		> 1,000 psi	NA	Equipment setup and routine preventative maintenance and qualification
Lyophilization Secondary Drying Duration		> 600 minutes	480–720 minutes	Lyo cycle automation and recipe selection, alarms, in-process monitoring
Lyophilization Stoppering Gas	WC-CPPs	Nitrogen	Nitrogen	Equipment setup, facility design
Raw Material DS Bag Volume		+/- 5%	NA	Batch-record calculations, CoA, container labels, gravimetric checks, downstream dispensing controls
Aluminum Sterilization Duration		30 minutes	NA	Batch-record procedures/eqt PID
Aluminum Sterilization Mix Speed		100 rpm	80–150rpm	Batch-record procedures
Aluminum Sterilization Mix Time		Defined by PID	NA	Eqt PID
Aluminum Sterilization Pressure				
Aluminum Sterilization Temperature		121.5ºC	199.5ºC–123.5ºC	Batch-record procedures/eqt PID

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7.11. Comparability Protocols for DP Lyophilization Site Change

7.11.1. Introduction

It is anticipated that during the post-file life cycle of A-VAX, the site of drug product manufacturing will be changed. The purpose of this comparability protocol is to describe the process demonstrations that will be required to support such a change, specifically for the lyophilization process. Other process changes or quality system reviews potentially associated with a change in lyophilization site or equipment are out of scope. The purpose of this protocol is to describe the scientific justification for the change, not necessarily the regulatory mechanics to support the change. In actual execution, this approach could be supported through multiple protocols.

7.11.2. Description of a Planned Change

The definition of a site change will range from the addition of similar lyophilization units in the current facility to transfer of the product to a new or existing facility in the same or different location with either comparable or noncomparable lyophilization units, which may or may not include process changes to maintain comparable product quality. In the case of a new facility, the reporting categories suggested may not apply because of the need for quality system reviews.

Site changes can be executed for a range of reasons, including:

- Enable manufacturing flexibility in multiple units
- Increase manufacturing capacity
- Support local manufacturing in emerging markets
- Distribute capacity to balance facility utilization across manufacturing network
- Increase reliability/uptime
- Improve/maintain existing equipment

Table 7-27: Lyophilization Cycle Description

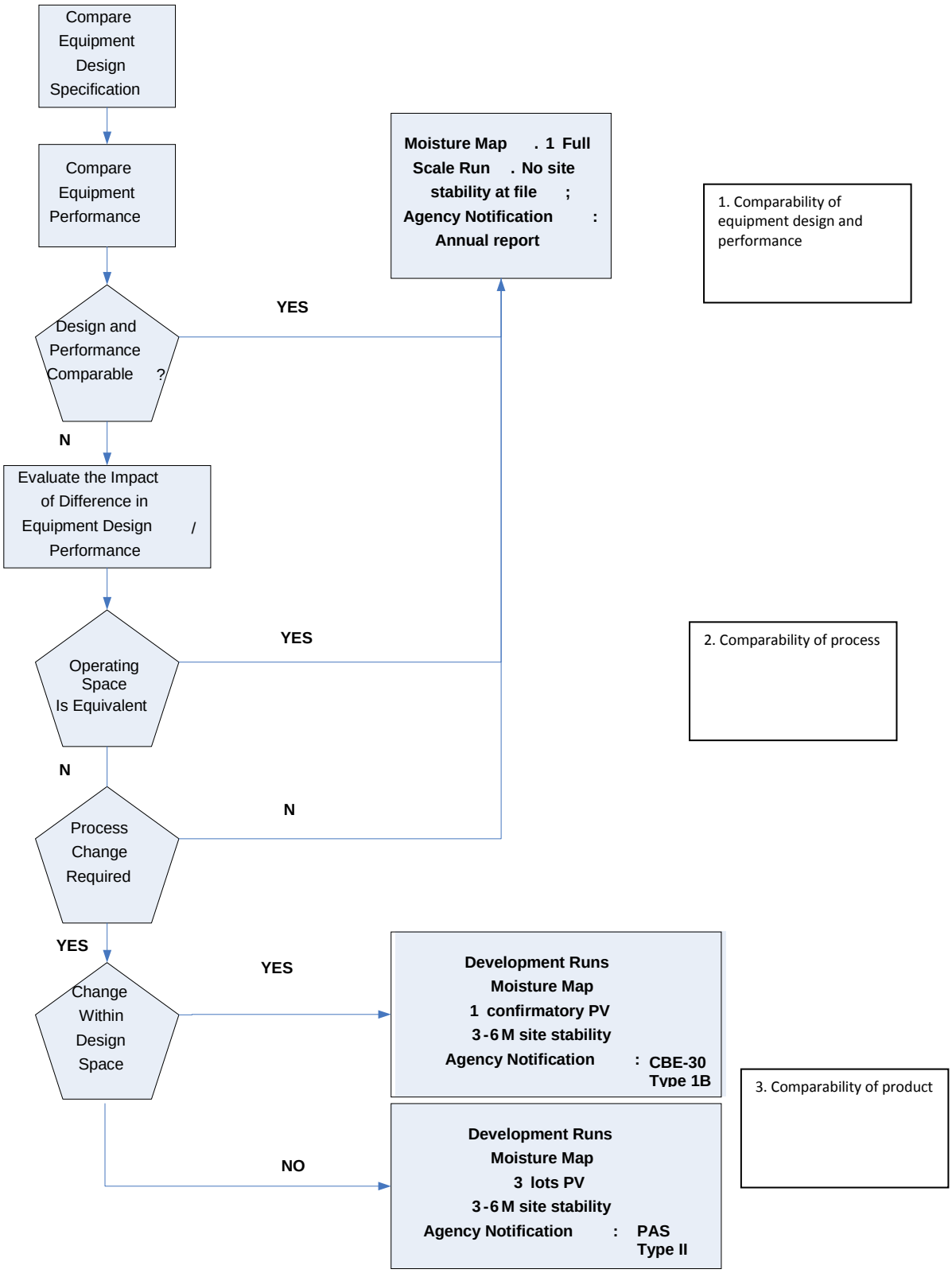
Lyophilization Stage	Initial Cycle
Loading/Freezing Temperature	-50ºC
Freeze Time Post-load	60 minutes
Ramp to Primary Drying	1ºC/minute
Primary Drying Temperature	-10ºC
Primary Drying Time	960 minutes
Ramp to Secondary Drying	0.3ºC/minute
Secondary Drying Temperature	30ºC
Secondary Drying Time	600 minutes
Final Stage Post-secondary Drying	5ºC
All conditions during lyophilization utilized 100 µbar.	

The potential impact of the lyophilization process on critical quality attributes is described in the attached risk assessment (see cause-and-effect matrix). A statistically designed experiment was executed based on this risk assessment, and it concluded that the primary impacts of the lyophilization cycle were on moisture and reconstitution time quality attributes.

7.11.3. Justification of Equivalency

The information required to support equivalency for the site changes described above will be determined based on lyophilization and equipment performance comparability, with the information required increasing with decreasing comparability, as shown in Figure 7-8. In the scenarios outlined in Figure 7-8, the rationale for completing only one process validation lot is based on knowing that during routine manufacturing, additional data would be captured and utilized to monitor performance. This would be part of the continuous verification process.

915 **Figure 7-8: Equivalency Demonstration Decision Tree**



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Equipment Design Specification Comparability

The equipment design specification comparability will be determined by a detailed evaluation of various equipment elements that are known to impact lyophilization performance. This may include the following:

- Manufacturer
- Automation system and system architecture
  - SCADA sampling
- Chamber design
  - Material of construction of internal shell, similar finish
  - Dimensions/volume
  - Door placement (mechanical, loading slot, etc.)
  - Post-SIP cooling mechanism (jacketed or not jacketed)
    - Insulative controls
    - Consistency/limits
- Pressure control mechanism
  - Gas injection (single or multiple point, continuous, location)
  - Capacitance manometer vs. Pirani gauge, location
- Shelf design
  - Number of shelves
  - Use/type of trays
  - Surface finish (similar)
  - Number of trays/product vials per shelf
  - Loading sequence (by row or tray/shelf)
  - Shelf construction material
  - Spacing between shelves
  - Distance between silicone oil in shelves and vial (shelf thickness)
  - Flow pattern/rate of flow of silicone oil in shelves, flow meter/control
  - Working shelf area
  - Shelf-by-shelf cooling capability
  - Counter plate at top of chamber
- Shelf temperature control
  - Heat transfer fluid used for shelf temperature control
  - Location of probe for shelf temperature control
  - Temperature control mechanism, algorithm, design
- Condenser configuration
  - Above, below, beside
  - Isolation valve (diameter, length, type)
  - Deflector design
  - Spool piece design (diameter, length)
  - Construction type (coil, plate, internal, external)

- Maximum ice capacity (kg/)
- Ratio of usable shelf surface to ice capacity
- Number of compressors
- Refrigeration system type
- Cooling mechanism, compressor type
- Number of coils
- Backup system
- Number of vacuum pumps
- Number of vacuum boosters

There is a very broad range of potential equipment designs and possible differences in specifications. Because of this range, the specification evaluation will need to be risk based and dependent on the magnitude of the difference observed and the potential impact to process parameter control and product quality. If significant differences in the equipment design are identified, the design specifications will be deemed to be noncomparable and an equipment performance evaluation will be conducted.

Equipment performance evaluation

As described above, if the equipment design is deemed to be noncomparable, a more detailed comparison of the equipment performance must be performed. This shall include statistical comparability of the following:

Performance Comparison	Acceptance Criteria
Pressure Control	+/- 10 ubar
Temperature Control	+/- 1°C
Chamber Leak Rate	<25 ubar-L/sec
Condenser Ice Capacity	> Reference cabinet
Shelf Temperature Uniformity	+/-1°C between and across all shelves
Heat Flux Studies	Range of target facility inside range of current facility

Again the assessment of comparability for equipment performance should be risk based, including an assessment of the magnitude of difference and potential impact on process parameters and product quality. This will include an assessment of the impact on CQAs and determination of the necessity for a process change. If significant differences in these elements are observed, the equipment performance will be deemed to be noncomparable and a process change evaluation will be conducted.

Process change evaluation

If equipment performance is deemed to be noncomparable, an assessment will be performed to determine whether a process change is required to accommodate the change in performance. The approach to evaluate process changes will depend on the difference observed.

If the change in performance is observed in temperature control/uniformity, pressure control, or heat flux, the change will be evaluated using the first principles mathematical model described in the tech transfer section of the document. Using this approach, product temperature and moisture responses can be predicted based on the observed differences in temperature, pressure, or heat transfer.

If the change in equipment performance compared with the existing facility is small enough that it will not have a significant impact on the ability of the process to deliver product within defined the specifications and design space identified, no changes will be made to the process. A development run will be performed to confirm acceptable product performance, followed by a single process validation lot to demonstrate process/product comparability; this will include full CQA testing per release, extended characterization protocols, and three months of stability data. Additional data will be collected as manufacturing lots are completed. The data will be utilized for continuous verification that the process and site-to-site changes are acceptable.

If the change in equipment performance compared with the existing facility is large enough to suggest a process change outside the design space based on scale-down model predictions, development runs will be performed. The runs will support the new process prior to execution of a full series of three process validation lots to demonstrate process/product comparability, again including full CQA testing per release, extended characterization protocols, and three months of stability data.

7.11.4. Proposed Regulatory Reporting Categories

The present example of lyophilization DP site change can be submitted as part of an initial marketing authorization application (as Post-approval change management plan/protocol) or submitted after licensure as a change management/comparability protocol. The current change is an example that can be managed via a comparability protocol, which has been written to be independent of the manufacturing location; in this way, subsequent sites can be introduced with reference to the same comparability protocol using lower reporting categories.

With the application of QbD, the expanded process and product understanding serve to support the sponsor's ability to assess the change according to the decision tree and apply a risk-based approach as described above (process change evaluation).
(Under the paradigm for post-approval change, the introduction of a new facility for a previously approved product requires regulatory review and approval.)

This type of change generally poses little risk of impact on product quality when the manufacturing site is a multi-product facility with established quality systems and a successful inspection history. In the European Union, a new secondary manufacturing site can be introduced without a specific product-related preapproval inspection. This is the case provided that the site is authorized for the type of pharmaceutical form and a manufacturing

authorization and/or GMP certificate is provided with the application. A possibility to waive the Pre-Approval Inspection (PAI) is based on successful inspection history or recent PAI for a similar type of product.

Therefore, provided the inspection status is compliant and a comparability protocol has been approved, it could be expected that for the introduction of each new site, the following reporting categories might be proposed:

1. In the case the comparability assessment confirms the equipment design or performance is comparable, the change falls within the initial design space. Therefore, the change could be reported as a minor notification (EU-Type IB; US-Annual report). This will be the case if the equipment performance difference is small enough that it will not require a significant change to deliver product within defined specifications and therefore no process changes will be necessary. For intra-site changes, if the site has already been approved for the EMA, no notification would be necessary and changing equipment within a site would be possible.
2. In the case of a process change, but where the process is comparable and remains within the design space, the change could be reported as a minor notification with agency review (EU-Type 1B; US-CBE-30).
3. In the case of a significant process change moving outside the design space, the change would be submitted as a regular variation (EU–Type II; US–PAS) and a modification to the design space/protocol should be considered.

7.11.5. Long-term Protocol Maintenance

- Update and/or withdraw this comparability protocol should the protocol become obsolete as a result of changes in the regulatory environment, identification of a new safety or scientific issue, and/or changes in technology.

8. Regulatory Section

The regulatory environment for incorporating design space into regulatory filings for vaccines is expected to evolve in coming years as regulators and vaccine companies gain more experience. This section of the case study explores the application of Quality by Design (QbD) concepts to the content of regulatory filings. These examples were developed in the absence of significant precedents; the applications will continue to evolve as experience is gained. The regulatory section concludes with a section on future challenges.

The section was created to introduce topics where there is tremendous potential value from applying the principles. However, there are also enough unanswered questions that it is important to emphasize the fluid and exploratory nature of the discussion. The additional product knowledge gained through the application of QbD concepts is expected to: 1) provide more strength to the data set supporting operational ranges and control strategy elements described for the product; and 2) justify management of change in a manner that increases the assurance of maintaining product quality while ensuring appropriate assessment across the spectrum, from gaining full prior-approval board of health review to empowering companies' quality systems to oversee that change.

To utilize product and/or process knowledge captured in the design space, the design space must be captured in the regulatory filings and approved. Given the limited experience to date in managing change in the context of a design space, to accomplish this in the EU and US filings today, a change management plan could be submitted to clarify the anticipated treatment of changes envisioned for the product life cycle. Examples are provided.

The case study is a scientific document addressing the application of Quality by Design to vaccine development and product life cycle management. It is intended to serve as an example of potential ways that scientific principles and tools described under ICH documents Q8, Q9, Q10, and Q11 could be applied seamlessly during vaccine development and through post-approval life cycle management. The examples have been created as a teaching tool and as an opportunity to encourage stakeholder discussions on the application of these concepts.

These examples are not presented as a mock submission, nor is there any expectation that the combination of illustrative examples would represent a realistic filing. The scientific principles are discussed and data are provided to demonstrate how the assignment of quality attributes, conduct of risk assessments, performance of experiments, and development of design space and control strategy could be utilized in regulatory filings to enhance the depth of product knowledge, increase the robustness of process control, and facilitate continuous improvement. We have indicated what data could be presented to support the analysis, where summary information is appropriate, and how the data would be analyzed in each of the process sections.

The focus of discussion in this document is on US and EU approaches. There are potential applications in multiple other regions; however, they are not addressed given the regional regulations.

This section will address the following regulatory aspects:

- Incorporating prior knowledge and design space information into initial regulatory filings.
- Applying the scientific principles behind the FDA PV guidance throughout the product life cycle. Proposals for change management are based on existing precedents and exploration of emerging opportunities.

8.1. Assessing Change Within the Context of the Life Cycle of a Vaccine

Throughout the development and commercial phases of a vaccine's life cycle, changes in the starting materials, manufacturing process, process control strategy, and analytical control strategy are inevitable. Drivers for these changes may include external influences, such as availability of material supplies and new technologies, and internal influences such as a need to improve productivity, decrease variability, or respond to changes in a company's supply network.

The spectrum of changes and the reasons for them are similar throughout all of the pharmaceutical and biotech industry, across small molecules, biotherapeutics, and vaccines. However, the implications of such changes and the tools employed to manage and assess the impact of these changes vary significantly between these product classes. Boards of health have generated specific guidance (or detailed sections within guidance) pertaining to these subclasses individually.

Generally speaking, the requirements for managing and assessing changes for vaccines have been among the most restrictive or conservative. The reasons for this conservative stance include the diversity of products in the class, the complexities of their manufacturing processes, the challenges of analytical characterization of the drug substances and products, limited specific knowledge of mechanisms of action, and a high demand for safety given that vaccines are typically given to healthy individuals and often to infants.

The expected contribution from this case study to the field of vaccine development is to illustrate how application of product and/or process knowledge as captured in the enhanced process understanding, design space, and control strategy can enhance continuous improvement, change management, and the assurance of product quality.

A robust process development program will study the effects of variation in material inputs, independent process parameters, and upstream quality attributes. These variables will have been assessed on the basis of their effect on the downstream process parameters, intermediate quality attributes, and critical quality attributes (CQAs) of the drug substance and drug product. This development program will drive the definition of design space, process control strategies, and analytical control strategies. The availability of the enhanced data set provides the underpinning for improved life cycle management.

Among the most significant contributions and benefits of QbD are decreasing the potential for unanticipated impact on CQAs and more objectively (less subjectively) defining the ranges for critical process parameters (CPPs) and non-CPPs.

8.1.1. Changes During the Development Phase

Throughout vaccine development, there will be changes made to the manufacturing process, including the modification of processing steps, scale-up of unit operations, and revisions to formulation and container-closure systems. While these must be handled on a case-by-case basis, data gathered at a smaller scale make a significant contribution to the design of protocols to demonstrate product comparability. For those operations where product and/or process understanding has sufficient depth, it may be possible to build arguments for utilizing analytical and nonclinical bridging data in lieu of collecting clinical bridging data.

During development, companies describe a manufacturing process and control strategy in an investigational filing (IND, IMPD, or equivalent document in other countries as required) and, depending on the significance of a change, report changes as development continues if required by the boards of health. As these changes would be followed up with additional testing in clinical trials, where safety and immune response, or even efficacy, are subsequently evaluated, generally the burden of proving comparability before and after a change at this stage is relatively low. Indeed, companies' concerns about observing clinical results inconsistent with earlier preclinical and clinical findings or confounded with the main objectives of the clinical study discourage companies from making large changes during this phase of the life cycle. The concerns drive companies to lock down major product and process design decisions relatively early in the development of vaccines compared with other product classes.

The case study provides examples of changes that could be justified largely through design qualification, process evaluation, and product characterization. In some situations, the subsequent clinical data are robust with respect to yielding acceptable clinical response even after moderate process changes and variability in the CQAs that are used to characterize the vaccine product. In such cases, the robustness to process change begins to illustrate that the historical paradigm for vaccine development that "… the product is the process …" can, in fact, within at least some ranges and for some moderate changes, be shown to be overly conservative.

8.1.2. Post-approval Changes

Companies are responsible for assessing, prior to distribution of a product, the effect of any post-approval chemistry, manufacturing, and controls (CMC) changes on the identity, strength, quality, purity, and potency of the product as they may relate to the product's safety or efficacy. Such an assessment generally includes data that demonstrate that the pre- and post-change products (i.e., the products manufactured prior to and subsequent to a manufacturing change) are comparable. In a QbD environment, the analysis is facilitated because of the available enhanced process and product knowledge. The company must report significant post-approval CMC changes to regulatory agencies, in one of the reporting categories described by each regulatory body.

8.2. Regulatory Applications Would Contain a Hybrid of Traditional and QbD Filing Content

- Industry will generally implement QbD for vaccines in certain process steps ("Targeted QbD Implementation" for vaccines), and filings with the enhanced approach applied to targeted steps will be standard.
- QbD implementation for vaccines may be limited to those areas that would benefit most from QbD and where the strength of the product characterization capability and process equipment understanding is consistent with the enhanced approach. Most likely areas for application are those that require changes post-licensure (e.g., equipment changes, process changes, process optimization, site changes).
- Comparability protocols (post-approval change management protocols/expanded change protocols) provide a flexible mechanism to implement QbD across the product life cycle (e.g., by including comparability protocols in initial marketing authorization or submitting these post approval).

Today a company can apply both traditional and enhanced development approaches, based on QbD principles, to different aspects of the production process in developing a drug substance and drug product, as the approaches are not mutually exclusive. Both approaches may generally be used in a single vaccine submission, giving rise to a hybrid submission.

The focus areas/process steps chosen for QbD study are driven by the individual project expectations. In the first instance, implementation may be limited to those areas that would benefit most from QbD, most likely areas that require most of the changes post licensure, such as equipment changes, process changes, process optimization, and site changes. It is highly likely that the extent of application of QbD will vary among process steps. Steps are chosen for evaluation based on impact on the QTPP, prior knowledge, reproducibility, yield, and expected process changes such as site/scale. The outcome of these choices for a filing is a submission where a complete arsenal of QbD principles is applied to a subset of the process steps and an approach that is primarily traditional is applied for the remainder of the process. In summary, industry will most likely implement QbD for vaccines in certain process steps; hence, "Targeted QbD Implementation" for vaccines will result in filings with a combination of enhanced and traditional elements.

8.3. Guidance on Dossier Content for QbD Regulatory Submissions

- ICH Q11 lists expectations in terms of dossier content (S.2 Drug Substance) for the traditional and enhanced approaches. Points to Consider for ICH Q8/9/10 implementation provide considerations for development of the control strategy and its life cycle.

ICH Q11 lists expectations in terms of dossier content (S.2 Drug Substance) for the traditional and enhanced approach. The key elements for QbD files are: the linkage between material attributes and process parameters and the CQAs, and also the control strategy, which can include a proposal for a design space. The quality target product profile (QTPP) and potential CQAs of a drug product are discussed in ICH Q8R.

Points to Consider for ICH Q8/9/10 implementation provide considerations for development of the control strategy and its life cycle. They also provide guidance regarding the level of information that is expected in an enhanced regulatory filing. Not all studies performed/data generated during product development needs to be submitted; however, sufficient information should be provided to address the following:

- The scientific justification of the proposed control strategy
- The scientific rationale for the DOE studies conducted
- A concise description of methodologies used to conduct these studies and to analyze the generated data
- The summary of results and conclusions drawn from these studies

The sections of the case study lay out appropriate packages to summarize the analysis performed and enable appropriate review in line with the proposals in the Points to Consider.

As highlighted in Q11, the minimal requirements for manufacturing process development in the traditional approach are as follows:

- Identifying potential CQAs associated with the drug substance so that those characteristics having an impact on product quality can be studied and controlled
- Defining an appropriate manufacturing process
- Defining a control strategy to ensure process performance and drug substance quality

An enhanced approach to manufacturing process development would additionally include the following elements:

- Identifying, through prior knowledge, experimentation, and risk assessment, the material attributes and process parameters that can have an effect on drug substance CQAs
- Determining the functional relationships that link material attributes and process parameters to CQAs
- Developing an appropriate control strategy using the enhanced approach in combination with QRM (quality risk management); for example, the strategy can include a proposal for a design space(s) and/or real-time release testing (RTRT) or potentially reduced end-product testing

In either the traditional or enhanced approach, there is an expectation that CQAs will be identified. This remains a particular challenge in vaccine development. Examples of the range of options for different polysaccharides are provided.

Understanding the appropriate level of documentation for enhanced regulatory submissions is evolving as submissions are made. The level of detail in a QbD filing should be sufficient for a regulatory reviewer to understand how conclusions were derived. Cited studies should be summarized with detail that is sufficient to convey an understanding of the purpose of the study, the data collected, how it was analyzed, the conclusions reached, and the impact of the study on the manufacturing process. The risk assessment tools and study results, on which a design space is based, should be adequately described. However, it is important to note that not

all the studies performed and/or data generated during product development are expected in the submission.

This case study includes examples of ways to present in the dossier risk assessments, results of DOEs, and design spaces to facilitate understanding of the conclusions drawn and enable health authority reviews. A related analysis is also applied to the treatment of prior knowledge.

For initial filings or post-approval QbD submissions, guidance suggests the dossier contains a statement by the applicant describing the proposed regulatory outcome and expectations. For post-approval changes this can be presented in the form of a post-approval change management plan.

8.3.1. Use of Prior Knowledge

Prior knowledge is information gained from experience and may come from production of previous products, literature searches, and/or experiments on related products. Prior knowledge is a key component in making appropriate risk assessments of critical quality attributes (CQAs), process parameters, and process inputs and outputs (as per the ICH guidance Q11).

Prior knowledge can be applied for multiple purposes such analyzing potential risks of a process step, doing design of experiments based on historical understanding of the strengths and limitations of a process step, and ensuring that the design of process steps is based on a contemporary understanding of the technology.

The application of prior knowledge is clearest when dealing with platform processes, as has been seen with the development of monoclonal antibodies where a number of unit operations can be covered by the platform. However, there are numerous applications in vaccine development that can utilize this springboard concept. Platform processes in vaccines can cover single unit operations such as conjugation and lyophilization; there are also wider applications such as polysaccharide production, the development of new drug delivery systems, introduction of formulation excipients, inclusion of adjuvants, and the manufacture of a drug substance without further process optimization. Such platform processes will be based on extensive prior knowledge with other vaccines and other large molecules. The extent of the use of prior knowledge is limited by the scientific strength and presentation of the platform and the options to demonstrate the relevance of the cited scientific data.

Companies may choose not to cross-reference data between products. There are real challenges to be addressed to facilitate incorporating information from another filing, although doing so can have significant payoff and should be considered. Deciding how to incorporate prior knowledge into an application is not at all trivial because 1) it may include an extremely large data set if referring to production data, 2) both CMC and clinical data may be required to support relevance, and 3) relevance of historical data must be justified.

The key point to consider with regard to prior knowledge is the ability to adequately document the information and relate it with good rationale to the contemporary situation. Prior knowledge can be applied extensively as long as the arguments made based on the data are

scientifically sound, clear relationships exist between the scaled-down models and commercial scale, and appropriate supporting information can be provided for reference.

8.3.2. Design Space

Establishing a design space can be done by linking the process inputs and variables to the CQAs through design of experiments (DOEs), failure modes and effects analysis (FMEA), and life cycle knowledge. A design space can be determined operationally through a combination of proven acceptable ranges derived from multivariant experiments and/or through modeling. The rationale for the inclusion of these parameters in the design space should be provided in the dossier, and in some cases it is helpful to provide a rationale as to why some parameters were excluded.

In the QbD paradigm, movement within an approved design space is not viewed as a change and will not require review or approval, but will be managed in the company quality system. As manufacturing experience grows and opportunities for process improvement are identified, the operating parameters could be revised within the design space without the need for post-approval submission. The same is true for design spaces built with mathematical models. In all cases, continuous process verification can help to verify performance within the design space.

Presentation in the dossier can include a description of the design space in tabular format, including the variables (material attributes and process parameters, as appropriate) and their proposed ranges. Examples of how to present the design space in a QbD submission can be found in the Annex 2c of ICH Q8. The present case study also includes examples of ways to present design spaces/modeling in a regulatory submission.

8.3.3. Control Strategy

The control strategy can include a number of interacting elements that assure full control of the product to be marketed. In the dossier the control strategy should be summarized in Module 3, Section P.5.6 with a scientific justification provided for the strategy. Additional information can be presented in other sections of the dossier (refer to Q8, Q11). Consideration should be given to the identification of potential residual risk that might remain after the implementation of the proposed control strategy and proposals for managing these residual risks.

Continual improvement of the control strategy through such methods as continuous process verification might be introduced into an application using a post-approval change management plan, which would set out the applicant's proposed regulatory outcome and expectations.

8.3.4. Process Validation

- Traditionally, process validation has been used to prove that the manufacturing process can consistently produce the product meeting specifications. The process validation exercise has traditionally encompassed production of three consecutive lots of product that met the specifications. In the context of Quality by Design, the same objectives of process validation may be established through a life cycle approach leveraging process development and data from studies at commercial scale along with continuous process monitoring. This section describes a potential application of this approach along with its impact on the regulatory

submission. The validation discussion is an important element of the case study because of the potential to utilize small-scale data.

Managing variability is one of the key ideas for managing a process. A QbD development effort will define the interrelatedness of process variables. FDA's Process Validation guidance is evaluated here because the paradigm presented for process validation is based on Quality by Design and the application of multiple guidance documents that have been developed and authored in the last decade. These guidance documents include Q8, Q9, Q10, Q11, and the associated Q&A for the first three ICH guidelines. Further guidance is expected as the QbD concept matures.

There are two guidance documents that discuss the impact of the enhanced approach on process validation. Question 2 (under "For General Clarification") in the ICH "Q8, Q9, and Q10 Question and Answer" document states the following regarding the process validation methodology using the enhanced approach:

The objectives of process validation are unchanged when using ICH Q8, Q9, and Q10. The main objective of process validation remains that a process design yields a product meeting its predefined quality criteria. ICH Q8, Q9, and Q10 provide a structured way to define product critical quality attributes, design space, the manufacturing process, and the control strategy. This information can be used to identify the type and focus of studies to be performed prior to and on initial commercial production batches.

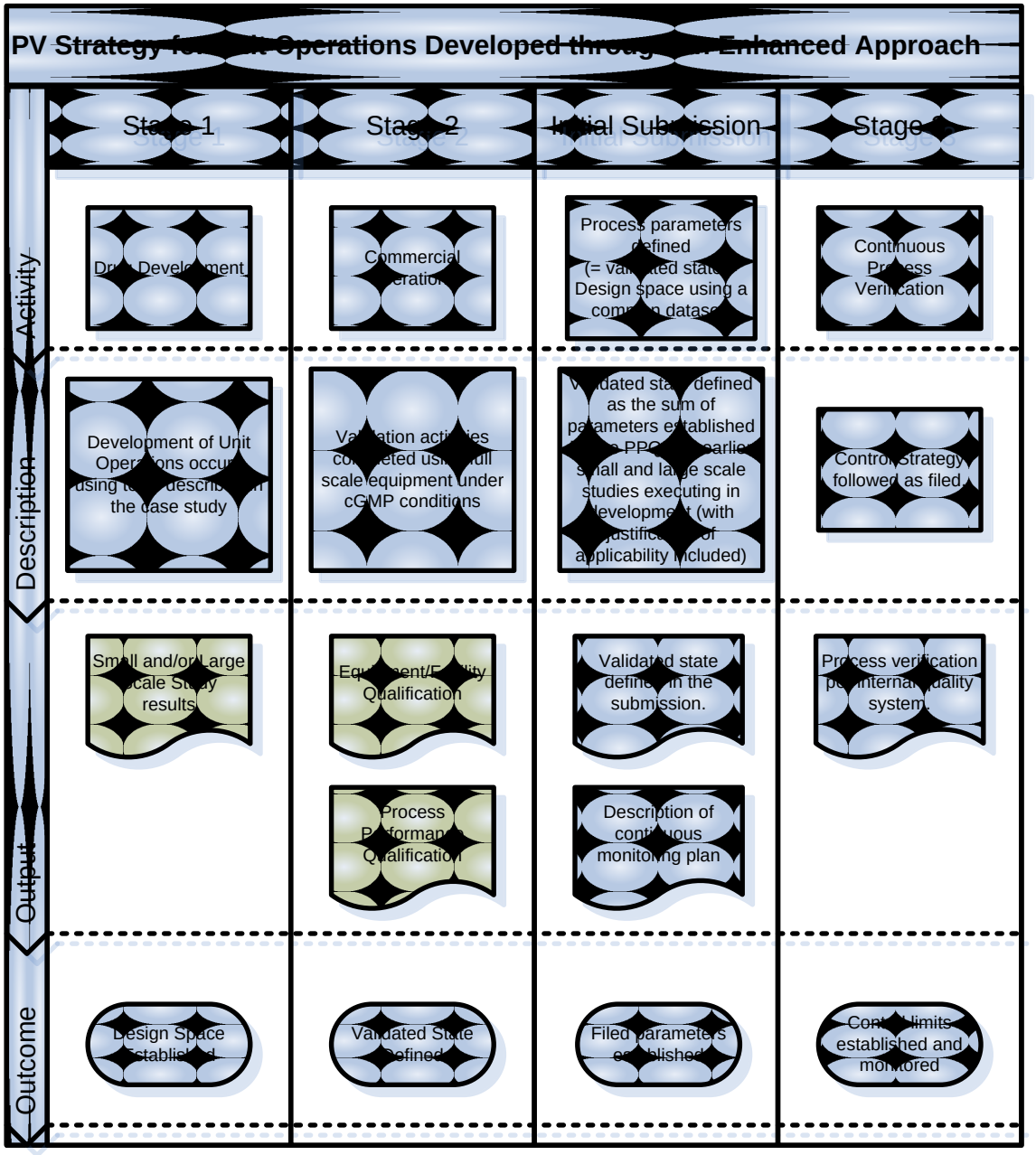
The answer to the next question from the same document (Question 3 under "For General Clarification") recognizes that "process validation also has a lifecycle (process design, process qualification, and ongoing process verification)." This approach describing these same stages of the process validation life cycle is further elucidated in the second guidance document, FDA's "Guidance for Industry: Process Validation: General Principles and Practices." This document was written as an application of the subject ICH documents. In the absence of similar guidance from other industry groups or health authorities, the terms and concepts from the latter document are utilized in the remainder of the section.

The life cycle approach to process validation described in the guidance should be utilized for unit operations where QbD concepts have been applied in development. This section will not repeat the concepts outlined in the guidance, but will give additional suggestions toward application of the concepts contained therein.

Those unit operations where development has occurred through a traditional approach would be expected to have process validation conducted in the traditional fashion and filed as such. CTD Section 3.2.S.2.5 should clearly delineate different validation approaches for different unit operations based on the differences in process development approaches to facilitate the understanding of this section by the reviewer. Only process validation through an enhanced approach is discussed throughout the remainder of this section.

A graphical description of the suggested approach is captured below in Figure 8-1. Note that the figure does not include all outputs from each stage of the process validation, but focuses on those pertinent to the process validation approach described hereafter.

Figure 8-1: PV Strategy for Unit Operations Developed through an Enhanced Approach



Stage 1: Process Design

With the new guidance, the process validation life cycle begins in process design. Identification and quantification of process parameters critical to product quality need to be discussed in the submission. The guidance allows for limits of quantification to be established at either small-scale or full-scale development lots or during Stage 2 process qualification.

In the traditional approach to process validation, all parameters were frequently challenged during the process validation study itself; therefore, this data was often provided in 3.2.S.2.5 Process Validation and/or Evaluation. With the new guidance, much of the data developed during process design will ultimately define the validated state, and as such may be described in other sections based upon early data collection efforts with robustness studies using models when scientifically justified. Discussion of Stage 1 in 3.2.S.2.5 should establish the design space as the basis for the validated process referencing ranges based on earlier data collection efforts. Portions of the process that must be validated during Stage 2 should also be highlighted.

Stage 2: Process Performance Qualification

Stage 2 has two elements: i) the facility and equipment design and the qualification of both to support the full-scale manufacturing process; and ii) the process performance qualification used to establish that a process is in a state of control and capable of reliably producing product with the desired specifications.

Per the guidance, the Process performance qualification (PPQ) “combines the actual facility, utilities, equipment (each now qualified), and the trained personnel with the commercial manufacturing process, control procedures, and components to produce commercial batches.”

As most ranges are established during Stage 1, the PPQ would be expected to be run at set points within normal operating ranges. However, some operations and studies require execution under all conditions required to produce commercial batches (e.g., aseptic processing simulation) or concurrently with commercial manufacturing (e.g., column resin or TFF filter re-use studies). These studies are executed in the traditional approach and, as such, their description is not impacted by the QbD approach.

With this approach to process validation, the validated state may be described as the culmination of parameters established during both PPQ and process development. As the design space is created from the same data set, the design space submitted should be equivalent to the process description and to the validated parameters. This approach greatly simplifies evaluation of changes post approval.

Stage 3: Continued Process Verification

Although continuous verification is routinely part of GMP monitoring, Stage 3 represents a stage in the process validation life cycle not typically described in a product dossier developed solely with the traditional approach,. It should describe establishment of a continuous verification plan. It would not be expected to submit control limits because a statistically significant data set is not typically available at the time of submission. Additionally, control limits are expected to change as a result of continuous process improvement throughout the product life cycle. As a

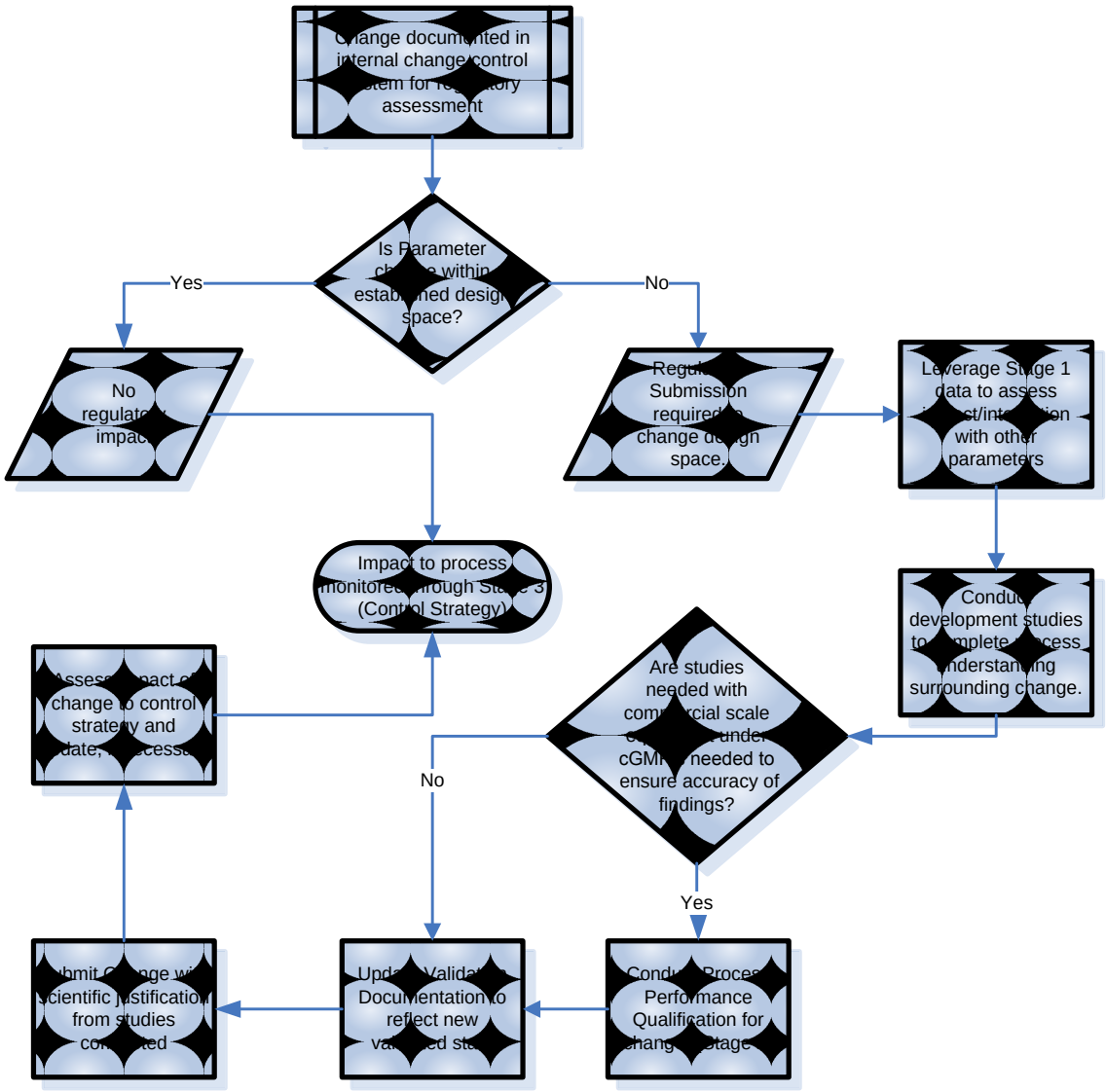
result, the dynamic nature of these values along with their periodic review during inspection negates the value in their submission.

A description of the continuous monitoring plan and potential for establishing control limits based on data collected over time should be discussed.

Post-licensure evaluation of changes to the validated state of the process

A potential work flow for evaluating changes to a unit operation validated under an enhanced approach is shown in Figure 8-2:

Figure 8-2: Potential Work Flow for Evaluating Changes to a Unit Operation Validated Under an Enhanced Approach



It should be noted that although it is not explicitly stated, it is expected that cGMP and quality assessments of all changes are an intrinsic part of an internal change control system and would occur throughout the process listed above.

Evaluation of the first question is critical for the remainder of the work flow to be accurate. The established design space may be described for these purposes as the design space submitted to and approved by the health authority. The classic example would be to create a new parameter set point and allowable ranges outside the normal operating range, but within the design space previously filed and therefore validated parameters. As these changes affect neither the filing nor the validated state, there is no regulatory impact from a design space perspective. Internal quality systems ensure that cGMP concerns (e.g., documentation) are addressed. In addition, the established continuous monitoring program provides assurance post change that there has not been a negative impact to the process. However, note that movement within the design space may still constitute a regulatory impact based on cGMP and statutory considerations.

In the case where the parameter change is outside the design space, the process is more complex. Often, this would involve movement of a set point or ranges outside the ranges previously filed and validated. Other changes could involve use of a parameter not previously considered (e.g., introduction of PAT), expansion of a range based on new process understanding, or other change to the process outside what has been observed or reported as part of process development. All of these changes would be handled under a prior approval mechanism or at a lower reporting category if they have been appropriately downgraded based on approval of a change protocol. The appropriate data required to support these will leverage prior knowledge already generated.

The overall approach in the flow chart is to leverage previous process development data and supplement with additional studies to create new data in the design space using the same risk assessments and approaches as in the initial filing. The need to reexecute PPQ should be based on the same rationale included in the original process development and validation approach that determined whether the parameter needed to be demonstrated under commercial conditions or had been sufficiently demonstrated in stage 1 studies. In either case, the new validated state is defined, and the impact to the process is confirmed via continuous process monitoring.

The variation should clearly explain how previously filed data and risk assessments were leveraged with supplemental data and how the filed validation approach was applied to create the package needed. Additionally, the change to the process description and stage 1 and 2 process validation should be clearly documented. A statement should also be included that justifies that the stage 3 continuous monitoring plan is sufficiently robust to capture any impact of the change on the process.

8.4. Appropriate Regulatory and Quality Oversight

- Understanding of CQAs and their linkage to critical process parameters and the design space allows clear identification of the parameters that may affect product safety or effectiveness and thus require the most stringent regulatory approval and oversight.
- Only a limited number of lots can be tested in clinical trials. One role of the case study is to illustrate examples where it is scientifically sound to establish criticality and process understanding beyond the information provided by clinical experience.
- Based on ICH Q8, working within a design space, which will have been approved in the initial license application, is *not considered a change* from a license perspective. Movement within the design space would not require regulatory notification because the space has already been assessed and approved. However, based on ICH Q10, *all changes* should be evaluated by a company's change management system.

Identification of critical quality attributes and linkages with process parameters provides a strong rationale for making risk-based decisions about the appropriate oversight. Those process parameters with a potentially significant impact on CQA(s) are expected to be subject to the most stringent levels of oversight. Design spaces are composed of acceptable ranges for the CPPs (critical process parameters) identified for each unit operation. The design space may also require regulatory control of critical raw materials. Other parameters not associated with CQAs are controlled and monitored in the quality system to ensure process and product consistency.

The case study cannot provide a definitive treatment with regard to the designation of regulatory commitments. As highlighted in the validation guidance, "all attributes and parameters should be evaluated in terms of their roles in the process and impact on the product or in-process material, and re-evaluated as new information becomes available. The degree of control over those attributes or parameters should be commensurate with their risk to the process and process output."

The case study is also important to demonstrate how the targeted experimentation guided by risk assessment and data collected through the DOE expands the available knowledge about process scale and reproducibility. Based on the knowledge generated, there is increased confidence in the understanding of the appropriate parameters to monitor and control throughout the process. There is also additional clarity about the appropriate point to execute testing to ensure the most critical attributes are appropriately controlled.

Movement within a design space does require an assessment of the risks associated with the particular move. This assessment would be performed within a company's quality system (as per ICH Q10), and a conclusion that the proposed change is supported by the existing product and process knowledge would be required.

8.5. Procedural Framework for Enhanced/QbD Filings

- The provision of the data and information for the design space and control strategy can be submitted either at the time of the initial application or during post-approval submissions.
- ICH guidance introduces the concept of a change management plan in the *Q8/9/10-Implementation* paper *"Q8/9/10 Points to Consider,"* finalized in June 2011, where it is proposed that the plan can be incorporated into regulatory submissions as part of the manufacturing process description.
- The purpose of a change management plan is to facilitate more effective and proactive management of future changes resulting from business or technical reasons, and the plan would be part of continuous improvement of the manufacturing and control processes. For products already licensed, the EU Variation Regulation 1234/2008 was revised in January 2009 and introduced the option to file a design space as variation application. The Classification Guideline refers to the introduction of a new design space or an extension of an approved design space for the active substance or finished product. In the United States, a new design space will be introduced as a PAS.
- In addition to the introduction of design space, the concept of a Post-approval Change Management Protocol (CMP) was introduced through the revised EU Variation Regulation 1234/2008 that went into effect in January 2010. The CMP concept provides a flexible mechanism to implement enhanced/QbD principles across the life cycle of a product and occurs in two steps (Figure 8-3). The CMP concept can also be included in the initial marketing authorization application and then follow the variation procedures for the implementation step.

8.5.1. How QbD Can Facilitate Stronger Control Strategies and Defined Pathways for Continuous Improvement

The enhanced (QbD) approach brings opportunities to include information on increased knowledge of the product and process that can be used to support the range of available regulatory approaches. The appropriate extent of regulatory oversight depends on how the design space and control strategy are defined and approved.

A robust process development program will study the effects of variation in material inputs, independent process parameters, and upstream quality attributes. These variables will have been assessed on the basis of their effect on the downstream process parameters, intermediate quality attributes, and CQAs of the drug substance and drug product. A number of potential scenarios are envisioned for life cycle management. Examples of each of these are described within the case study as noted below:

1. Parameters are noncritical, and therefore controls may be managed by a company's quality systems. This will be the most routine type of change.
2. Critical parameters are well-defined. Adjustment within licensed ranges may be made within firm's quality systems including confirmation of no adverse effect on CQAs.
3. Critical parameters are well-defined. Adjustment outside a licensed range is required to complete improvement. Confirmation of no adverse effect on CQAs and comparability can be shown, but must be managed through a regulatory reporting mechanism.

4. New knowledge regarding process parameters highlights potential for impact. New CPPs are defined. Confirmation of no adverse effect on CQAs and comparability can be shown, but must be managed through a regulatory reporting mechanism.

Among the most significant contributions/benefits of QbD is a decrease in the potential for items of the fourth type and more objective (less subjective) definition of the boundaries of each of the first three scenarios .

In cases where strong characterization tools are available, FDA and EMA have both facilitated the application of a specific type of supplement as a tool that may diminish the reporting requirements after a company has demonstrated a lack of adverse effect. This tool of a comparability protocol has a very specific set of conditions that are prescribed for it to be applicable to a change. While a comparability protocol potentially diminishes the reporting requirements after a company has demonstrated a lack of adverse effect, the comparability protocol itself must be approved as a prior approval supplement. Furthermore, it significantly reduces the flexibility of the company in responding to unexpected observations during execution of the protocol.

In this case study, we have selected one or two examples of situations where a comparability protocol is likely to have potential value. Refer to examples in the Purification and DP sections (and expand detail as appropriate in next paragraphs). In these sections, we outline some of the reasons why a comparability protocol may be useful or valuable in these instances as well as acknowledging any additional risks or costs incurred by choosing to use the comparability protocol. In the analysis, we point to how changes to one or more aspects of the case study may change the risk-reward balance of this analysis from the company (and potentially for the reviewing BOH).

a. A spectrum of risk has been articulated by health authorities, from examples cited as very acceptable: polysaccharide changes, cell bank location/process to examples highlighted as posing significant risk such as cross-linked conjugate. The examples in the case study are chosen to demonstrate where the additional data sets may reduce the perceived risk.
b. The range of application of animal models and/or clinical studies vs. physiochemical/analytical/process comparability is also demonstrated.

We also provide details of what a comparability protocol should include in these particular vaccine examples. And we look at how a company may have to react to unexpected data that may be generated during execution of the protocol and how a comparability protocol filed with an original marketing authorization may be maintained to ensure it remains relevant at various stages of the product life cycle.

8.5.2. Scope for Regulatory Flexibility and the Post-approval Change Management Plan

Once a design space has been established, movement within the approved design space can occur without further regulatory review; consequently, this is anticipated to reduce post-approval submissions.

The provision of the data and information for the design space and control strategy can be submitted either at the time of the initial application or during post-approval submissions.

Alongside this information, future post-approval changes can be presented in a "post-approval change management plan." In addition, ICH guidance introduces this concept in the *Q8/9/10-Implementation* paper *"Q8/9/10 Points to Consider,"* finalized in June 2011, where it is proposed that it can be incorporated into regulatory submissions as part of the manufacturing process description.

The purpose of the change management plan is to facilitate more effective and proactive management of future changes resulting from business or technical reasons, and the plan would be part of continuous improvement of the manufacturing and control processes. This enhanced process knowledge and prospective thought about appropriate analysis and data sets to support process changes will also accelerate handling of reactively driven post-approval changes that are the consequence of deviations, OOS, or other findings such as CAPAs.

It is anticipated that the level of regulatory oversight with an enhanced QbD filing will be inversely proportional to the demonstrated product and process knowledge and application of risk management. Thus, even for changes outside the design space that require regulatory oversight, a greater scope for a reduced reporting category is anticipated.

Based on ICH Q8, a change within an approved design space is not considered a change from a license perspective. However, all changes should be evaluated by a company's quality control system, which provides the mechanism to ensure that the manufacturing process is maintained within the boundaries described by the design space. This assessment would examine the risks associated with the particular move. Following the assessment, if the conclusion is that the proposed change is supported by the existing product and process knowledge, it can be concluded the change is within the design space. Thus, this enables the management of some CMC changes based on clearly defined and agreed-upon risk-based criteria without additional regulatory filing. However, if this condition is not met, then the standard regulatory application appropriate for the given change would have to be submitted.

The enhanced QbD approach brings increased process understanding, which reduces the risk that process changes will adversely impact product quality. We therefore anticipate that, once industry and health authorities have experience with and confidence in the application of QbD to vaccines, the regulatory application requirements for process steps filed under the QbD approach could be different from those for process steps filed under the traditional approach.

Additional avenues for potential regulatory flexibility are discussed in the following sections; please refer to examples found in Section 8.7 for the European Union and Section 8.10 for the United States.

8.6. Regulatory Framework for Enhanced/QbD Filings in the European Union

For products already licensed, the EU Variation Regulation 1234/2008 was revised in January 2009 and introduced the option to file a design space as variation application. The Classification Guideline refers to the introduction of a new design space or an extension of an approved design space for the active substance or finished product, items B.I.e.1 and B.II.g.1,

respectively. These changes are handled as Type II variations (the standard being a 60-day timetable).

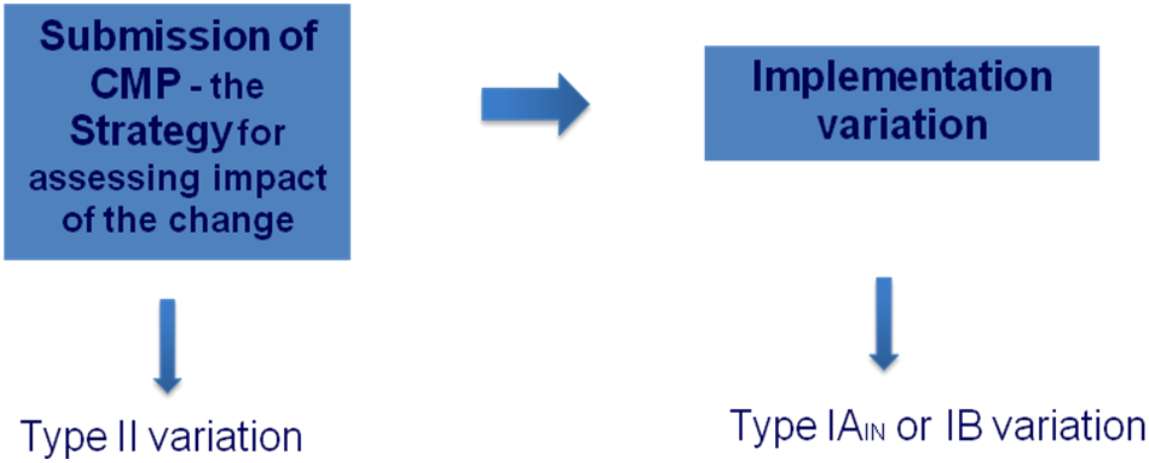
In addition to the introduction of design space, the concept of a Post-Approval Change Management Protocol (CMP) was introduced through the revised EU Variation Regulation 1234/2008 that went into effect in January 2010. The CMP concept provides a flexible mechanism to implement enhanced/QbD principles across the life cycle of a product and occurs in two steps (Figure 8-3). The CMP concept can also be included in the initial marketing authorization application and then follow the variation procedures for the implementation step.

The first step introduces the protocol to the license using a Type II variation as detailed in the Classification Guideline (**Introduction of a post-approval change management protocol** for the active substance and final product). In this step, the protocol presents a description of the proposed change; a risk assessment of the impact of the change on product quality, safety, and clinical performance; a description of the methods used to evaluate the effect of the change; the acceptance criteria for which the proposed change will be evaluated; and a commitment to update the protocol (if needed).

The protocol also includes how the changes will be reported to the regulatory authorities following approval of the protocol. This reporting is the second step or the implementation of the change.
The second step, or the implementation variation, can be managed either via a Type IA_{IN} or a Type IB variation procedure as detailed in the Classification Guideline. For a biological/immunological medicinal product, reporting under the current guidance is restricted to a Type IB.

Following the introduction of the CMP into the Classification Guideline, the EMA issued a Q&A document (EMA/CHMP/CVMP/QWP/586330/2010) providing information regarding the expectations in terms of content of the CMP. For example, for multiple changes a CMP can be used; however, in the submission a justification to demonstrate that the changes are interrelated is required. The Q&A document also details how a change should be implemented and reported and provides timelines for approval, etc.; and it describes the classification for a change to an already approved protocol for biologicals as a Type IB variation, B.I.e.z (active substance), and B.II.g.z (finished product), respectively.

Figure 8-3: Post-Approval Change Management Protocol EU Submission Procedure



8.7. Scope for Regulatory Flexibility in the European Union

The current and future regulatory environment for enhanced/QbD applications and variations introducing or changing design space and the CMP is expected to evolve over coming years. As manufacturers gain experience in the use of these regulatory paths and the authorities increase their assessment of these applications, regulations and guidance will develop or existing ones will be further amended.

The appropriate degree of regulatory oversight is based on the level of relevant scientific knowledge that will be provided in the registration application or variation and existing guidance. As more experience is gained, more flexibility can be introduced. Thus, the following sections explore how flexibility can be introduced to new applications and post-market product life cycle management by the application of the principles of the enhanced (QbD) approach and how the existing regulatory guidance could evolve in the future.

8.8. Reduction in End-Product Testing

The control strategy focuses on performing the appropriate testing at the appropriate point in the process and eliminating testing as appropriate. A further reduction in end-product release testing and/or implementation of skip testing could be achieved using the principles of QbD.

A traditional approach involves a discrete sample size that represents the minimal sampling expectations and will detect only major deviations in the manufacturing process. Use of an enhanced approach would make it possible to monitor relevant parameters that may involve assessing a CQA directly or indirectly using parameters associated with the CQA (e.g., temperature, pressure, pH, speed, time, etc.) Because the testing is during the manufacturing process (in-line, on-line, at-line), it does not represent discrete sampling; therefore, the data generated lends itself more to statistical analysis and trending of these parameters. This type of testing can be described under the umbrella of “real-time release testing” (refer to draft guideline on Real Time Release testing EMA/CHMP/QWP/811210/2009; Rev 1 was published in March 2010 in the European Union). Real-time release testing currently is unlikely to replace

end-product testing for a vaccine candidate; however, it can provide an opportunity for increased regulatory flexibility in the end-product testing. In addition, end-product testing will be required for other aspects of product quality such as in stability studies or OMCL release activities.

8.8.1. Flexibility in the Implementation of the CMP

Evolution in the existing legislation will facilitate the application of the CPM for biologics. The existing legislation on CMP provides a broad guidance of the applicability and use of the CMP. The CMP can be used to manage proactively and strategically the manufacturing and control changes during the life cycle of a product, and it could become a critical tool for life cycle management of CMC. It is therefore envisaged that CPM legislation will evolve in coming years to further facilitate the application of CMPs for biologics, including vaccines.

The following subsections explore how the existing CMP guidance could evolve in the future to be more flexible in the implementation of this concept for biologics.

8.8.1.1. Reporting category of the implementation variation of a CMP

Under current guidance, the reporting category for biologicals is a Type IB. As many biological changes have a default categorization of a Type II, the CMP provides a mechanism to downgrade the reporting category for these changes. However, in the guidance the category for the implementation variation does not distinguish between active substance and final product. It could be envisaged that certain changes, provided they are implemented as approved, will carry little or no risk regarding an impact on the quality, safety, or efficacy of the product. Thus, the possibility to report the implementation variation as a Type IA or Type IA_{IN} could potentially be supportable given the quality of the data package generated.

8.8.1.2. Cross-references within the dossier

A dossier can be structured well using smartly written text and cross-linking. This would lead to a reduction in post-approval changes. It is not related only to QbD but it is a general point to consider. There are specific opportunities available as companies begin to take advantage of filing a design space. Increased regulatory flexibility could be achieved by greater cross-reference within the dossier.

For example, methods for the determination of acceptance criteria for the CQAs or CPP could be cross-referred from the dossier section containing the description of the design space/CMP to the product dossier. When a minor method change is required, the dossier is updated via a regular variation, and the design space/CMP automatically reflects this change without additional regulatory action.

8.8.1.3. “Common/generic protocols” and combining work sharing and the CMP

The CMP protocol procedure is also interesting to manage in a strategic manner changes that are “common/generic” in nature. Changes that share common elements to demonstrating quality, safety, and efficacy might include secondary operations such as packaging or filling. The elements included in such a protocol would be equivalent, regardless of manufacturing site. Therefore, the use of common/generic protocols should be envisaged because this would greatly enhance the wider applicability of the CMP principle. The protocols are entirely

consistent with the facility and equipment knowledge and the types of data packages generated as part of enhanced programs.

In a similar way, it could be envisaged that a CMP could be written for a change that may affect multiple products of the same vaccine family. Thus, this type of CMP could be written and submitted in a work-sharing procedure for the vaccine family. Subsequently, for each product, individual secondary implementation variations providing the data could be submitted. An example might be a bulk manufacturing site of a drug substance that is present in the drug product of a number of vaccines in a vaccine family. This concept is similar to the US expanded change protocol (ECP), which takes a more holistic approach; it offers the use of a protocol providing the approach and acceptance criteria that can be applied to multiple manufacturing process changes or a process change across multiple related product types or manufacturing process platforms. Again, the facility and product knowledge generated should facilitate.

The current Q&A document (EMA/CHMP/CVMP/QWP/586330/2010), which provides information regarding the expectations in terms of content of the CMP, does not preclude the possibility of writing a protocol that could be used for a number of products. The CMP is an integral part of Module 3, and thus it is possible to write a protocol that becomes specific via cross-references within the dossier. However, this does infer that the vaccine family dossier structure needs to be sufficiently similar for the products to enable correct cross-referencing to occur. (Refer to Drug Product section).

8.9. Regulatory Framework for QbD Filings in the United States

- Limited FDA references exist to illustrate implementation of QbD principles into vaccine regulatory filings.
- Design space information may be incorporated into regulatory filings as part of an original license application or as a supplement to an approved license. In addition, design space information may be an important addition to a comparability protocol [i.e., 21 CFR 601.12(e) filing] by supporting prospectively defined acceptance criteria captured in the filing.
- The scope of regulatory flexibility will be defined by the ability of analytical methodologies to address two questions related to clinical significance of a change and robustness of the analytical methodology applied to assessing the change.

There are limited FDA references to the implementation of QbD principles beyond the adoption of principles contained in ICH documents Q8, Q9, and Q10. CDER issued a manual (dated Febr 8th, 2011) outlining and clarifying how CMC reviewers should apply the recommendations in the ICH Q8(R2), Q9, and Q10 guidances to new drugs approved under the FD&C Act; however, vaccines are regulated under a separate set of regulations and a different statutory authority. For new drugs regulated by CDER, reviewers are directed to ensure that applications contain at least the minimum information on pharmaceutical development described by ICH Q8(R2) as: “At a minimum, those aspects of drug substances, excipients, container-closure systems, and manufacturing processes that are critical to product quality should be determined and control strategies justified.”

The difference between vaccines and small molecule drugs in statutory authority and promulgated regulations in the United States adds a layer of complexity to the regulatory landscape. However, the concepts captured in the ICH guidance documents are consistent with the implementation of comparability protocols at CBER. This case study illustrates application of the principles of Q8, Q9, Q10, and Q11 to vaccine development and post-approval life cycle management through the enhanced process and project knowledge gained.

There are two means to incorporate a design space into a biologics license application (BLA):

- The incorporation of design space information into the original BLA or as part of a supplement to an approved BLA to support directions within the filed master batch record.
- The inclusion of design space to support acceptance criteria to be used under a regulatory comparability protocol (i.e., not to be confused with an assessment of product comparability performed by the license holder).

License application. Regarding incorporation of design space concepts into an original BLA, it is already fairly common for license applications to contain analogous types of data that provide a summary of product knowledge gained during the vaccine development process that supports operating parameters, specifications, and/or protocols. For example, stability protocols are often incorporated into the BLA to support extension of shelf life based on real-time commercial-scale stability experience; and these protocols prospectively define how change would be managed as additional data become available. The examples in this case study seek to illustrate additional means by which a more formalized QbD process can be used to enhance the control strategy and to establish a change management plan for review and approval by the FDA.

Comparability protocol. A comparability protocol (CP) is a well-defined, detailed, written plan for assessing the effect of specific CMC changes on the identity, strength, quality, purity, and potency of a specific drug product as they may relate to the safety and effectiveness of the product. A CP describes the changes that are covered under the protocol and the specific tests and validation studies and acceptable limits to be achieved to demonstrate the lack of adverse effect for specified types of changes on the safety or effectiveness of a product.

Upon approval of the CP, the FDA may determine that certain changes evaluated in accordance with the protocol may be reported at a reduced category. By providing an opportunity for FDA to review and approve the CP before it is used by the license holder to evaluate a change, FDA gains greater assurance that the change is being properly evaluated and, therefore, that there is less potential for the change to have an adverse effect on the safety or effectiveness of the product (*62 FR 39890*; 24 July 1997). Subsequent to implementation of the revised regulation, the FDA issued a number of guidance documents and conducted workshops to explore means to apply this regulatory approach to the reporting of changes in: manufacturing process; analytical procedures; manufacturing equipment; manufacturing facilities; container-closure systems; and process analytical technology (PAT).

In this spirit, this section seeks to extend the exploration of the CP approach as a means to apply the process knowledge and product understanding gained through application of the QbD approach to vaccine development and post-market product life cycle management.

License applicants and license holders are responsible for assessing, prior to distribution of a product, the effect of any post-approval CMC changes on the identity, strength, quality, purity, and potency of the product as they may relate to the safety or efficacy of the product. Such an assessment often includes data that demonstrate that the pre- and post-change products (i.e., the products manufactured prior to and subsequent to a manufacturing change) are comparable. Vaccine manufacturers must report pos- approval CMC changes to the FDA in one of the reporting categories described by the FDA. As part of its review and approval of a CP to evaluate the effects of a change if supported by the submission, the FDA may determine that a CMC change made under the CP will fall into a less restrictive reporting category. In many cases, using a CP will facilitate the subsequent implementation and reporting of CMC changes, which could result in moving a product into distribution sooner than if a protocol was not submitted.

8.9.1. Licensed/Marketed Products

The license for approved vaccines may be modified through the use of a supplement, which is filed under the Changes to Be Reported regulation, *21 CFR 601.12*. A new design space would be introduced into the license either as part of a supplement or a CP, which would provide the overall context for how the new design space information informs the ability of regulators to assess the means being used to evaluate impact of a change to product safety and effectiveness. The initial design space filing would generally be reviewed as a Prior Approval Supplement (PAS) as described under 21 CFR 601.12(e). A review action is taken within four months under PDUFA IV managed review process timelines.

For a CP filing, there is a second step in completing the reporting requirement for implementation of the change that requires a second submission. The follow-up submission is often submitted as a Change-Being-Effected-in-30-Days Supplement (CBE30); however, the agency has allowed increased regulatory flexibility and permitted the change to be reported at even lower categories [Change-Being-Effected Immediately (CBE) or Annual Report]. For changes to the manufacturing process, the design space data are incorporated into the license or license supplement as part of the justification of acceptance criteria to be applied to evaluation of the change.

Incorporation of design space data may be useful for other types of change that are amenable to use of a CP approach, such as changes to analytical procedures, manufacturing equipment, manufacturing facilities, and container-closure systems. For example, the understanding of CQAs gained from design space data collection may inform criticality of defined user needs and the most efficient means of assessing equipment capability in delivering these performance characteristics.

Other types of protocols that may be used during product life cycle management may benefit from design space information, including shelf life extension protocols and container-closure component interchangeability assessments. These protocols may also be submitted as supplements to approved vaccine license files and should include: a description of the proposed change; a description of how impact on product quality, safety, and clinical performance will be assessed; a description of the methods used to evaluate the effect of the change; and the acceptance criteria to be applied in evaluating the change. The protocol should also include how the changes will be subsequently reported to the FDA following approval of the protocol.

8.10. Options for Continuous Improvement in the United States

The options for continuous improvement will be defined by the ability of analytical methodologies to address two questions related to clinical significance of the change and robustness of the analytical methodology applied to assessing the change. To effectively implement design space in a vaccine license file, we will need to focus on providing information that not only evaluates the analytical result(s) within the context of the CQAs, but also provides a linkage back to the clinical relevance of the data. As nonclinical means of assessing immunological performance of a vaccine are validated and gain regulatory acceptance, we can hope to further advance our ability to address this question.

A second focus of potential reviewer questions can be expected around our level of confidence that the product is comparable if no change is observed in analytical results. The question here is whether the methodology is sensitive enough and how can we assure ourselves and the FDA that, in fact, there is not a significant impact on product safety or quality that has crept into the product after implementing change. For those changes that are amenable to definition of a design space, we can anticipate the ability to conduct dialog with regulatory health authorities to seek their advice on applicability.

8.10.1. Managing Repetitive Change

Because of the nature of Changes to Be Reported requirements in the areas of manufacturing facilities and equipment, CPs have also been used to decrease the regulatory reporting burden for repetitive and recurring changes. For example, qualification of a new working seed can be performed under a CP, which has undergone prior review and approval by the FDA to ensure that the regulatory authorities have confidence that the change will be assessed appropriately and that potential impact on product safety and effectiveness can be managed under the quality system.

In addition, more generic CP approaches have been used in instances where equipment- or facility system-related changes are being made and apply to multiple products. For example, replacement of terminal HEPA filtration casings throughout a large manufacturing facility can be a significant undertaking with potential to impact a variety of controlled manufacturing environments. It is possible to utilize a CP to define how systems and manufacturing environments will be assessed after a change and to achieve a lowered Changes to Be Reported category.

8.10.2. Reporting Category of the Implementation Supplement

Under current guidance, the FDA maintains a degree of flexibility in defining the Changes to Be Reported category for the supplement that provides results of a post-change assessment made under a CP. In general, these were handled as CBE30 submissions. However, in those instances where the FDA has sufficient confidence in the robustness of the comparability assessment, regulators have permitted subsequent reporting as CBE supplements or as part of the annual report. In the broader context, the precedents for enhanced downgrade to the reporting category are more limited with vaccines because of the more limited strength of the product

characterization capability and the more risk-averse nature of the patient population for vaccines.

8.10.3. Updating/Modifying the Content of the CP

Currently modification of the CP for vaccines would be a PAS filing. Updating the original protocol should be requested only when the original one becomes invalid because of substantial changes to the proposed test methods/acceptance criteria or new knowledge that becomes available.

8.10.4. Reduction in Lot Release Testing

Vaccines are subject to lot release testing on every lot of product intended for distribution to the US marketplace unless granted a waiver. The conditions for requesting a waiver from lot release testing include a demonstrated ability of the quality unit to release product lots over a period of time that meet specifications and confidence that release testing achieves a full assessment of all CQAs.

It may be possible to engage the FDA in a dialog to define the parameters that would be expected to request a waiver from lot release and to move a product to surveillance mode; however, because of the complexities of some vaccines (e.g., whole virus vaccine), the utility of this approach may be more readily acceptable for recombinant antigen vaccines with more well-characterized CQAs and a more robust strength of product characterization capability.

8.11. Future Challenges in QbD Implementation for Vaccines

As noted at the beginning of this section, the regulatory environment for incorporating design space into filings for vaccines is expected to evolve in coming years as regulators and vaccine companies gain more experience. As we look to the development of concepts in implementing QbD for small molecules, it is possible to identify some areas for further development of approaches for implementation that have not been discussed with regulatory health authorities for large molecules or vaccines. These include:

8.11.1. Secondary or Adaptive Acceptance Criteria in a CMP

In the development of a CMP, acceptance criteria for CQAs/CPP are required to build the control strategy. During manufacturing, it is possible that in testing of these criteria, a result may be at the limit of acceptance/failure. This could be handled as a deviation in the usual way and the CMP could be refiled, or more proactively it could be envisaged that secondary or adaptive criteria could be developed in advance.

Thus, using secondary or adaptive acceptance criteria, regulatory flexibility can be built into a CMP. The secondary acceptance criteria would be provided, along with details of the investigation and analysis that will be followed to determine acceptance and thus to justify the final conclusion that quality is maintained.

Following the triggering of the secondary acceptance, if it is assessed that this movement outside the design space is likely to re-occur, the design space should be reassessed and modified. The modification of the design space will then need to be submitted for regulatory review; please refer to Section 8.6.

8.11.1.1. Updating/modifying the content of the CMP

Currently modification of the CMP for biologicals is by default a Type IB variation under the EU legislation. Updating the original protocol should be requested only when the original one becomes invalid because of *substantial* changes to the proposed test methods/acceptance criteria or new knowledge that becomes available. However, minor noncritical deviations from the agreed protocol should be allowed via a Type IA and should not preclude submission of the minor deviation at the time of the implementation variation.

For instance, minor changes to the acceptance criteria can be justified in the variation submitted for the implementation of the change, to avoid having to delete the former CMP and submit a new type II variation with the updated CMP reflecting the adapted acceptance criteria. It could be envisaged in the future that minor changes could be notified or at the same time as the submission of the implementation variation as mentioned above in 1.1.3.3. Comparability protocols written in a more generic fashion.

The CMP protocol procedure is also interesting to manage in a strategic manner changes that are “common/generic” in nature. Changes that share elements common to demonstrating quality, safety, and efficacy might include secondary operations such as packaging or filling. The elements included in such a protocol would be equivalent, regardless of manufacturing site.

Therefore, the use of common/generic protocols should be envisaged because this would greatly enhance the wider applicability of the CMP principle. Also, they are entirely consistent with the facility and equipment knowledge and the types of data packages generated as part of enhanced programs.

9. Implementation Section

9.1. Executive Summary

This section covers the following key points:

- Multiple stakeholders (patients, manufactures, and regulators) benefit from the enhanced approach to vaccine process development. (See ICH Q8 and Q11 for concepts and definitions.)
- The key value of the enhanced approach is an improved ability to predict the value stream measures of safety and efficacy, availability, and cost effectiveness.
- A value stream approach can be used to successfully prioritize business and regulatory drivers, which support investment in the enhanced approach.
- Return-on-investment (ROI) analysis for the enhanced approach needs to be specific to the company, regulatory agency, and product because ROI factors drive the value stream and each situation may have unique considerations. In this case study, we provide an example framework that can be used to develop an individualized approach.

9.2. Implementation Section Overview

The objectives of this case study were to exemplify the utility of Quality by Design (QbD) tools for vaccine development, demonstrating that, in many cases, stakeholders can achieve superior value through implementation of the principles of the enhanced approach to process development (as defined in ICH Q8 and Q11). For this case study, determination of the costs and benefits of the enhanced approach for vaccine development was made using a value stream measure of improved efficiency. This measure was defined in terms of the organization's ability to predict:

- Safety and efficacy
- Product availability (robustness)
- Cost effectiveness

Superior value was achieved because the enhanced approach to vaccine process development provided an improvement in the organization's ability to predict metrics that directly impacted the three universal goals most vaccine stakeholders desire: safety and efficacy, product availability, and cost effectiveness. The value stream analysis demonstrated that implementation of the enhanced approach improved the efficiency in developing vaccines to meet patient needs, providing value over the life of the product to all stakeholders: patients, regulators, and manufacturers. Since all stakeholders receive value, the case for investment in the enhanced approach is justified.

The enhanced approach to process development offers great benefits but requires additional investment over more traditional process development methods. This additional investment is made primarily by the manufacturer during the development process when there is no

guarantee a product will even be launched. Value is returned to the manufacturer only if a product is launched and has a successful life cycle, thereby recouping the initial investment and generating profits for continued operation and additional investment.

Value stream analysis, focused on the universal goals all vaccine stakeholders desire (safety and efficacy, product availability, and cost effectiveness), can identify the value-generating levers supporting the business case for this additional investment. An analysis of this type provides analytical tools that can open a dialog and improve decision making.

For the purpose of this case study, the terms “traditional” and “enhanced” are used to differentiate two possible approaches. In a traditional approach, setpoints and operating ranges for process parameters are defined and the drug substance control strategy is typically based on demonstration of process reproducibility and testing to meet established acceptance criteria. In an enhanced approach, risk management and more extensive scientific knowledge are used to select process parameters and unit operations that impact critical quality attributes (CQAs) for evaluation in further studies; these studies establish design space and control strategies applicable over the life cycle of the drug substance.

As discussed in ICH Q8, for a drug product, a greater understanding of the drug substance and its manufacturing process can create the basis for more flexible regulatory approaches. The degree of regulatory flexibility is generally predicated on the level of relevant scientific knowledge provided in the application for marketing authorization (refer to ICH Q11). Traditional and enhanced approaches are not mutually exclusive. A company can use a traditional approach to drug substance/drug product development, an enhanced approach, or a combination of both in a hybrid filing.

The traditional approach refers to the methods manufacturers and regulators currently use in vaccine development. The traditional approach produces a safe and effective vaccine for the patient. However, the traditional approach may not fully investigate all the interactions in process inputs (e.g., parameters, raw materials) during development to identify those interactions impacting manufacturing. The traditional approach also may not always allow efficient technology transfer because it is less integrated, as well as less complete in identification of risks.

The enhanced approach explores the data from experiments at the lab scale through clinical material manufacturing scale to derive specifications for post-licensure manufacturing. It can be used for better processing and determination of when changes interact to affect the process and, ultimately, the resulting vaccine product. The enhanced approach allows a risk-based assessment that takes advantage of prior knowledge from earlier experiments. This permits effective and more information-based decisions and easier technology transfer. The enhanced approach also produces a safe, efficacious vaccine for patients, while allowing more flexibility for manufacturers and regulators by generating processes that are more robust and understood (refer to ICH Q11).

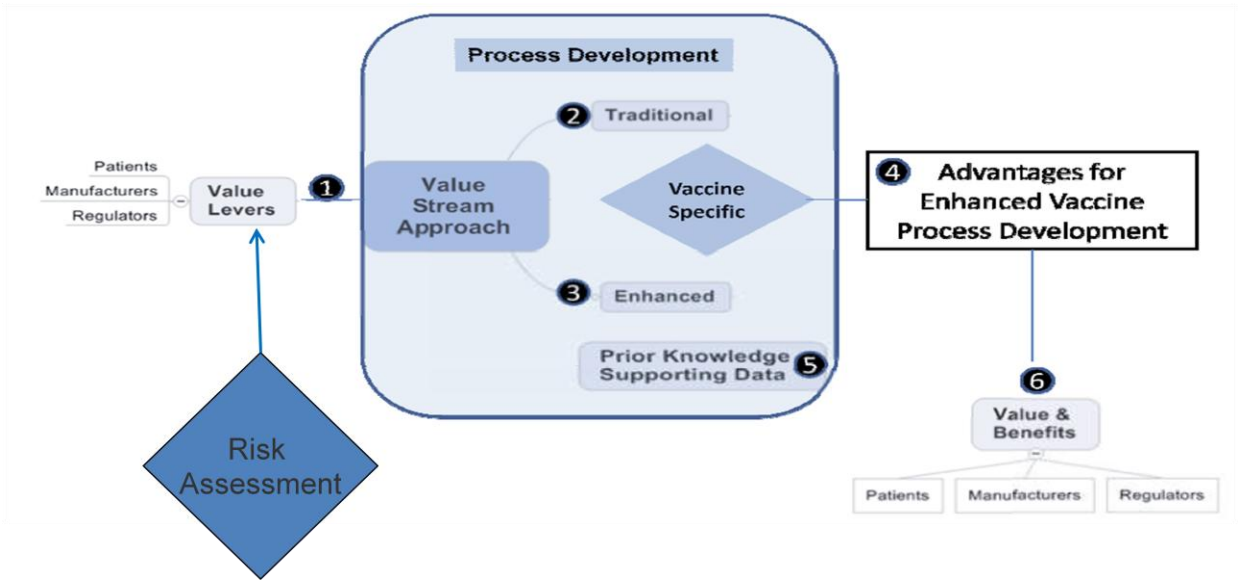
One impact of applying the enhanced vs. traditional approach to vaccine process development was to reduce overall investment during the product life cycle and improve the probability of predicting success before manufacturers and regulators have made substantial investments.

This approach is applicable to the decision process organizations undergo related to vaccine process development activities. A value stream approach as outlined provided the rationale and expected benefit in specific cases where an enhanced approach to vaccine process development generated superior value over traditional methods. Decisions to augment traditional methods by implementing the enhanced approach for vaccine development activities or to perform additional development studies for an existing manufacturing process need to be evaluated and made by each manufacturer or regulator on an individual basis.

Risk analysis outcomes are fed into a comparative analysis as outlined in the diagram below (Figure 9-1). The risk mitigation projects are then further refined through a value stream analysis of important business case levers. Comparison of traditional vs. enhanced process development identified those areas, specific to vaccines, where an enhanced approach provided value for the development of product and process knowledge while also reducing cost, resources, and development time over the product life cycle.

The six steps outlined in this case study provide a value stream tool to highlight possible advantages for specific areas of a vaccine manufacturing process if the decision to implement the enhanced approach to process development is made.

Figure 9-1: Value Stream Approach to Determining Implementation Costs and Benefits



The steps are as follows:

1. Identify all possible high-level business case value levers for manufacturers and regulators. Process and product risk areas, identified through a risk assessment tool or filter, generate high-risk items that can then be further prioritized through the value stream tool using these levers.
2. Qualitatively describe the traditional vaccine development process, and identify the disadvantage points and areas of opportunity based on previous risk analysis activities.
3. Qualitatively describe costs and benefits of using the enhanced approach in the areas that apply to vaccine process development.
4. Develop prioritization criteria, and select the business case levers where the enhanced approach is estimated to have potential beneficial impact.
5. Identify any prior knowledge or supporting data for the traditional or enhanced approach scenarios that is needed to support prioritized areas. Develop scoring ranges for implementation costs and benefits specific to vaccines.
6. Add detail supporting the scoring for the selected levers based on the key areas with greater estimated impact.

For this case study, examples from the process development chapters were selected. A few of the key business case levers/attributes were quantified in detail for each example. This exercise provides three examples of how to quantify and perform the six steps (Figure 9-1) for the determination of implementation costs and quantitative/qualitative benefits for the enhanced approach.

The benefits of implementing the enhanced approach must be large enough not only to cover implementation costs, but also to improve robustness and ultimately contribute to product availability, safety, and efficacy of the vaccine. For implementation of the enhanced approach, the key customers (patients, manufacturers, and regulators) must always be kept in mind.

9.3. Key Customers of the Enhanced Approach

The key customers of the enhanced approach are the patients, manufacturers, and regulators. The ultimate customer of enhanced approach efforts is the patient. The value to the patients is accrued by increasing the associated value for manufacturers and regulators to provide a robust supply of safe and efficacious vaccines within the time frame they are needed. The enhanced approach increases value to the patients by identifying the critical attributes directly relevant to patient needs. In many cases, the current state of vaccine technology may limit the availability of product and process knowledge obtainable through the enhanced approach. In these situations, the most effective solution might be the traditional approach. Moreover, medical providers are also customers because they can provide better care to their patients if they have an adequate supply of the appropriate vaccine. The payers of vaccines benefit by the enhanced approach through better availability and lower costs resulting from efficient and robust vaccine processes.

Vaccine manufacturers are customers of the enhanced approach in multiple categories. Senior managers are interested in fewer interruptions in supply, robust manufacturing processes, and flexibility in increasing their supplies. The enhanced approach benefits the chemistry,

manufacturing, and controls (CMC) process development customers since scale-up and technology transfer efforts can be more successful. A more complete CMC data package, developed using the enhanced approach, helps the regulatory groups compile high-quality submission documents. Site quality groups benefit from the enhanced approach with fewer nonconformances or regulatory actions. The additional product/process characterization associated with the enhanced approach also helps site quality groups expeditiously resolve manufacturing or testing issues that arise.

Regulators are key customers for the enhanced approach as well. They include the regulators in the review functions and in the inspectorate roles. Regulatory agencies, such as the FDA, EMA, and PDMA, can better assess the submissions due to the greater amount of process and product characterization information associated with the enhanced approach, as well as its focus on quality attributes.

9.4. Scope and Impact of the Enhanced Approach Implementation

The application of principles of the enhanced approach in the context of a new vaccine product candidate has the potential to impact and influence a vaccine's entire life cycle. To successfully apply the concepts as defined within ICH Q8, Q9, and Q10, some aspects of a manufacturer's pharmaceutical quality and associated systems will likely require "re-building/enhancement" to ensure application in a uniform and consistent manner. In that way, knowledge is conserved and the burden of repeating/verifying earlier work is streamlined or reduced. Execution of the enhanced approach to process development provides more knowledge of parameter design space. However, efforts to gain this knowledge are expected to increase compared with requirements of the traditional approach. In addition, start-up costs are associated with the enhanced approach, such as the cost of process and analytical equipment to execute design of experiment (DOE) development and associated cultural elements (e.g., the cost of training on such principles and retaining existing staff, development and maintenance of databases and knowledge bases, statistical services, and additional or contract staff for experiment execution and analysis).

Development costs likely increase based on enhanced development in comparison with traditional methods. Although there is an estimated cost increase, there are tangible gains, from both the manufacturer and regulatory perspectives, through knowledge management, information-based decisions, and operational flexibility linked to manufacturing processes. There is an expectation that the utilization of design space models results in gains for platform processes. These gains permit operational flexibility while maintaining a high degree of compliance through robust and reproducible operations. When platform process knowledge is supported by an enhanced process development approach, utilization of prior knowledge to support risk-based decisions is even more effective.

For an example illustrative vaccine, the estimated timeline for "break-even" ROI is about three years (Table 9-1). Within individual companies, the thrust is to integrate key concepts of the enhanced approach as fast as possible. This ultimately allows for timely market authorizations, such that the additional cost incurred with the enhanced approach models can be recovered even more quickly. There are also other tangible benefits linked to improved regulatory inspection performance. These benefits include shop-floor compliance drivers resulting from

deviation management, product release, and the ease and effectiveness of introducing changes based on the established design space. Other qualitative indices include, but are not limited to, employee satisfaction, morale, and retention, including maintaining or improving the respective company's reputation.

Table 9-1: Example Estimate for the Time to "Break-Even" ROI Analysis for Implementation of the Enhanced Approach

Item	Enhanced Approach Example	Traditional Approach Example
Cost of Product Development	$550 milliona	$500 million (1)
Time for Development Completionb	5 years	7 years (1)
Break-Even Point (Full Market Penetration)c	3 years	N/A

[a] Assumption: Introducing the enhanced approach principles to development and regulatory processes amounts to an increase in $50 million (excluding additional clinical studies) over the traditional approach and two years faster. Supplementary process development studies, required by the enhanced approach, are one of the factors for the increased cost of development in the enhanced vs. traditional approach.
[b] Development completion time for the enhanced approach is estimated to be less than that for the traditional approach because of better data continuity and documentation, reduced reliance on full-scale demonstration runs, and less redundancy of process development efforts.
c Assumption: Product sales for new vaccine candidate are $10 million at year one, $20 million at year two to a maximum of $50 million within three years of launch (1).
N/A - Not applicable because no additional costs were incurred based on the traditional approach.

Reference:
(1) Paragraph about waste is based on Better by Design, Sven Stegemann. World Pharmaceuticals Frontiers. 2010. Volume 1. pp 76 to 78

For the FDA's QbD pilot program for biologics, it was reported that "as of mid-2010 a total of five BLA and four post-approval supplements had been received." Within the same reference, it was noted that the FDA also extended its subscription period to its biologics QbD pilot program and the pilot results were not expected until 2015. (Quality by Design – Putting Theory into Practice, Siegfried Schmitt (Introduction, 2011)).

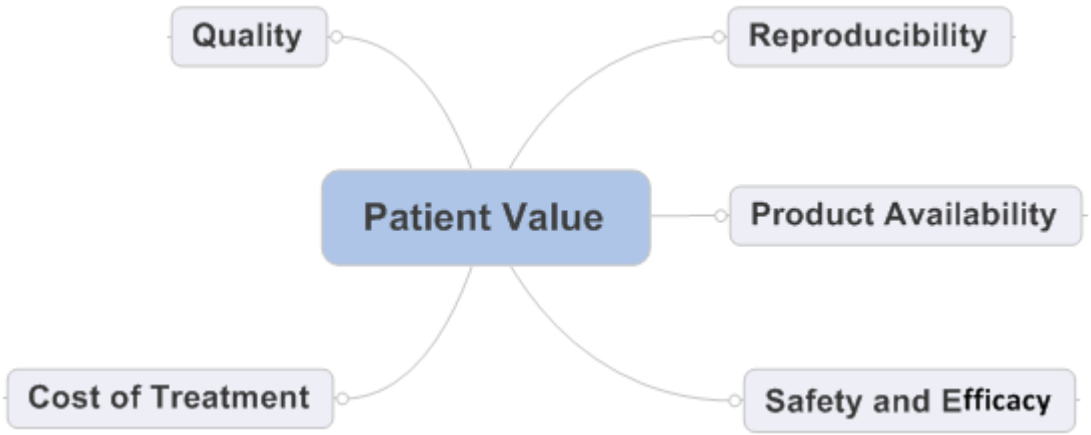
It is clear that the concept of QbD is still in its infancy. Although there are product candidates where this approach is being used, the full realization/gains are as yet unknown. The use of the enhanced approach for a new vaccine candidate has limitations from manufacturer as well as regulatory perspectives. Accordingly, if applied in its entirety, it requires a high degree of collaboration and upfront work from the sponsor (manufacturer) and the respective regulatory agencies. This concept requires an understanding of expectations and shared perspective from the manufacturer and the regulatory agencies, with the ultimate goal being the supply of a safe and efficacious product.

The enhanced approach may be beneficial for established unit operations (e.g., freeze drying, chromatography) that are directly scalable and where the concept of design space can be exploited for changes linked to established licensed processes. In this case, manufacturers and regulators alike are encouraged to partner and understand each other's expectations concerning the application of the enhanced approach in a regulated environment.

9.5. Business Case for Patient

The business case from the patient's perspective for the enhanced approach is shown by the mind map in Figure 9-2. The key levers identified for the patient are: reduced cost of treatment, availability of treatment supplies, reproducibility and consistency of the drug product, assurance that the product is safe and efficacious, and the highest consistent quality of the product. The improved patient value delivered through the enhanced approach may not be readily apparent to the individual consumer. In general, patients benefit directly from the value delivered to the regulators and manufacturers. Thus, no further work was done specifically on the patient business case.

Figure 9-2: Mind Map of Business Case Levers for Patient



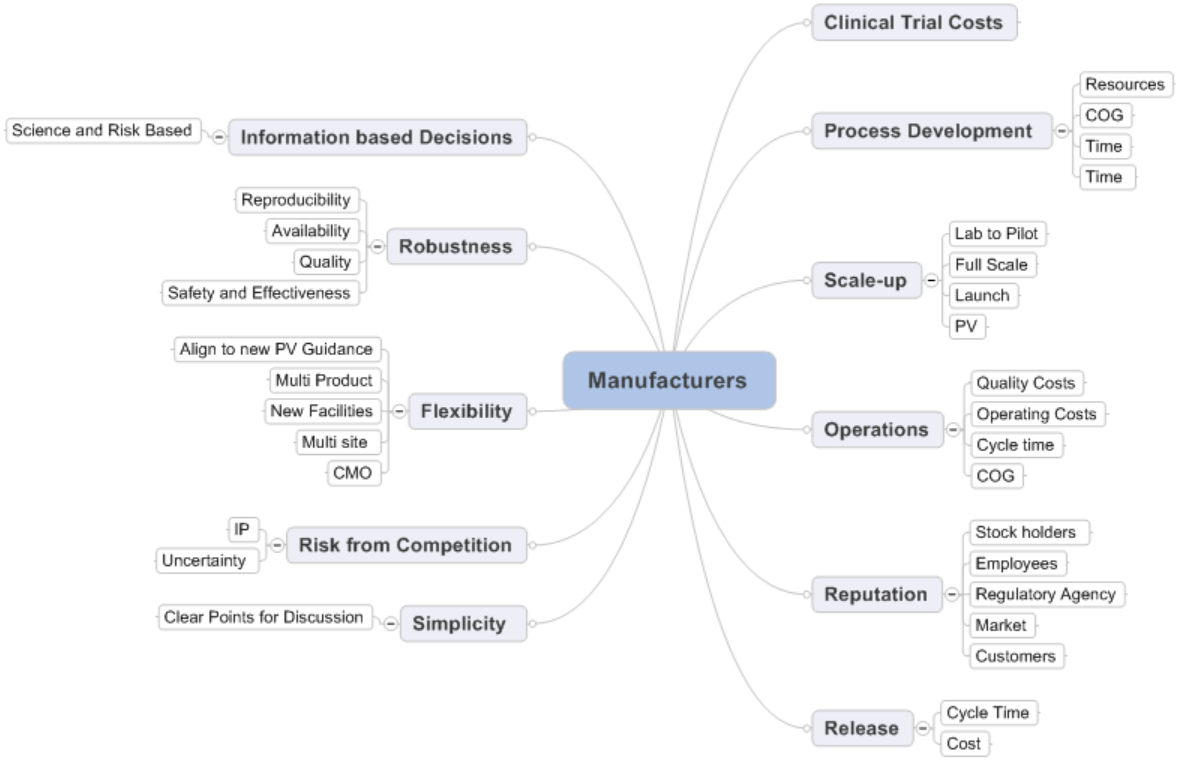
The enhanced approach could become a hardship for consumers if the additional workload substantially slows the development of new therapies or unreasonably limits regulatory approval of products already produced with the traditional approach.

9.6. Business Case for Manufacturer

The business case for applying the enhanced approach to vaccine development was constructed from the vaccine manufacturer's perspective. The thought process used was first to identify and prioritize the appropriate levers, then to determine the benefits and advantages of the enhanced approach for the levers specific to vaccine process development. Next, the implementation costs associated with the enhanced approach were evaluated. Comparing these costs along with the benefits, a vaccine-specific manufacturer's business case was constructed.

The levers impacting vaccine manufacturers were brainstormed based on the experience of the team members involved, and are depicted in a mind map (Figure 9-3). It is recommended that a company- and product-specific value stream brainstorming exercise be conducted in a cross-functional manner using risk management principles.

Figure 9-3: Mind Map of Business Case Levers for Manufacturers



Each of the 11 resulting lever categories was defined to assist in subsequent priority ranking (Table 9-2).

Table 9-2: Description of High-Level Levers for Manufacturers

High-Level Lever for Manufacturer	Definition
Release	• Release test selection (safety, efficacy, physicochemical characteristics, development), qualification during the course of development and validation, specification setting • Real-time release technology; batch release process simplification (electronic batch release process)
Flexibility	• Process definition achieved to maintain a high level of compliance/quality while being able to make changes within predetermined limits on the shop floor; streamline change control • Greater regulatory flexibility; ability to implement changes with

High-Level Lever for Manufacturer	Definition
	minimum regulatory burden and expedited time to approval
Clinical Trial Costs	• Ability to ensure clinical material is of the quality required to meet patient needs • Improved product CQA understanding in the clinic • Reduction of clinical bridging studies
Robustness	• Capability of the process to maintain acceptable ranges of quality and process attributes while operating within the predefined design space • Better assess manufacturability and achieve process/method reliability.
Operations	• Technical procedures driving production, release, and supply of product • Reduce or eliminate number of reworked batches, failures, atypical, OOSs, etc.
Process Development	• Ability to define the production methods, equipment, operating ranges, and specifications (process, product) that can be transferred into a manufacturing environment • Knowledge management - capture the associated know-how and know-why
Scale-up	• Ability to use appropriate scale-down models and comparability methodologies to develop successful large-scale procedures and operating ranges
Simplicity	• Similar to flexibility; greater process understanding and ability to relate inputs to resulting outputs
Reputation	• Capacity to speed up registration through strong partnership with agencies • Best-in-class supply chain (shorter cycle time, no recalls, etc.)
Risk from Competition	• Potential patent protection, ability to get to market (licensed) on time
Information-Based Decisions	• Data-driven decisions for process/analytical development, product release/resolution of atypical and overall scientifically sound decision-making processes

Prioritization ranking was accomplished, again based on the vaccine development and manufacturing experience of the team members involved, followed by team discussion, and documentation of the rationale behind the designated priority estimation (Table 9-3). During the prioritization, similar lever categories were merged to obtain seven remaining levers.

2347 **Table 9-3: Prioritized Drivers for Manufacturers and Associated Rationale**

Business Case Lever	Estimated Priority	Rationale
Robustness	High	Prevention of process drift and improved capability for CQAs ensure product availability.
Process Development (PD) Scale-up	High	Defined steps for PD ensure effective experiments are executed. Better process understanding and therefore simpler tech transfer/scale-up.
Flexibility/Simplicity	High	Improved facility utilization. Opportunities for process improvement/adaption, transfer, multi-products use, and comparability.
Information-Based Decisions	Medium	Management decisions based on process and product knowledge improve success rate.
Clinical Trial Costs	Medium	This is the most expensive part of development, so any opportunity to improve success rate has high return.
Release/Operations	Medium	Release costs are high as a result of nature of test, amount of testing, and timing for release. Enhanced approach with parametric release can allow simplification of release process. Enhanced approach application during development definitively simplifies operation on a daily basis (less nonconformances, less out-of-specifications, parametric release).
Reputation/Risk from Competition	Low	Companies embracing QbD may be able to demonstrate success and improved value.

2348
2349 The example prioritization criteria, although not formalized, were considered effective since
2350 there was a reasonable split among all three priority levels. The three high-priority levers were
2351 robustness, process development/scale-up, and flexibility/simplicity. The three medium-priority
2352 levers were information-based decisions, clinical trial costs, and release/operations. The sole
2353 low-priority lever was reputation/risk from competition. All levers were considered important,
2354 regardless of their ranked prioritization. Individual companies should complete this evaluation
2355 for each unique application.
2356
2357 The benefits of the enhanced approach were developed specifically for the high-priority levers
2358 only, and compared with drawbacks and pain points of traditional approaches (Table 9-4). Direct
2359 benefits largely were related to COGs and impacted high-priority levers such as robustness. Low
2360 COGs was particularly important to vaccine manufacturers to enable more global access to
2361 vaccines. Indirect benefits largely were intangible and impacted lower priority levers such as
2362 reputation. Intangible elements were particularly important to vaccine manufacturers because
2363 perceptions may reduce sales of vaccines, limiting illness prevention in target populations.
2364

Table 9-4: Comparison of Traditional and Enhanced Approaches for Vaccine Development for the Key Levers for Manufacturers

	Traditional	Enhanced
Robustness	• Interaction and impact of parameters not always explored	• Experiments and data from laboratory and nonclinical studies are used to derive specifications • Interactions are better understood • Less sensitivity to raw material and parameter input variations
Process Scale-up/ Development	• Manufacturing constraints not always integrated in the early development • Studies linked to development are process specific; transfer of data across multiple unit operations is rare • Work from laboratory experimental design is not always predictable; leads to resource and cycle time constraints	• Use of appropriate DOE and other statistical models allows appropriate key specifications linked to the target product/process to be derived; this also eventually offsets the upfront increase in cost of development • Better understanding of multivalent interactions (first order, second order, etc.) • Better use of PAT models
Flexibility/ Simplicity	• Limitations around changes and process improvements • Licensure-based changes lengthy in some cases • Limited risk assessments	• Potential changes can be made within the design space without need to extensive change control and regulatory oversight • Simplified comparability protocol or technical transfer

The costs of the enhanced approach for vaccines were the pre-investment — specifically, the effort and time involved implementing and performing enhanced approach activities. Most of the additional cost was associated with the following three tasks: establishment of a multivariate design space, adoption of advanced control strategies such as PAT, and performance of extensive analytical characterization. Since the extent of application of the enhanced approach for a vaccine was readily customizable based on previously identified risk areas, these costs were able to be readily managed to ensure a sufficient level of derived benefit.

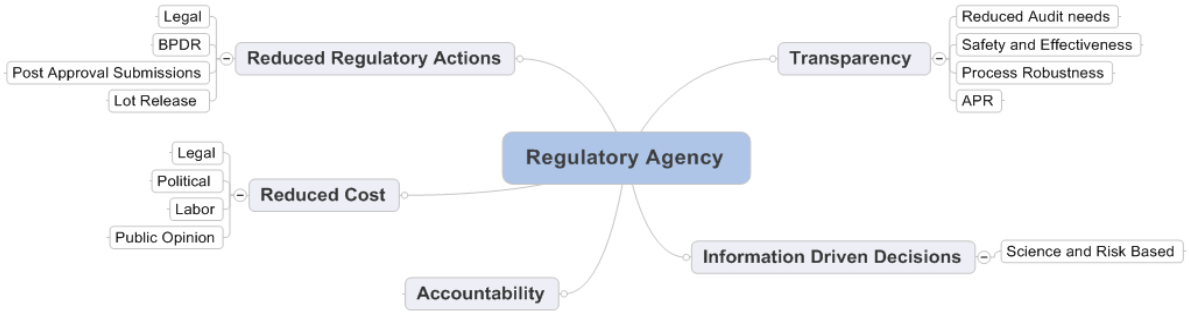
Costs and benefits were then compared to develop the business case. This comparison was considered in the general sense here, and more specifically in a later section. A few key principles were noteworthy: (1) Since enhanced approaches were an investment by development for manufacturing, the same part of the company did not always both spend the resources and reap the benefit. (2) Notable quality or supply interruption tended to limit vaccine sales more than expected based on the vaccine manufacturing costs associated with these events. (3) Most of the world has limited access to vaccines compared with the developed world. These three principles suggest that that application of enhanced approaches has been different for vaccines compared with other pharmaceutical products, and also likely different for specific vaccines.

9.7. Business Case for Regulator

The business case for applying the enhanced approach to vaccine development also was constructed from the vaccine regulator's perspective. The thought process used to identify and prioritize the appropriate regulatory levers was similar to those used for the manufacturer's business case. For the regulator's business case, the assessment of advantages of the enhanced approach includes a focus on scientific knowledge desired to maintain or improve the vaccine's safety and efficacy for the patient. A comparison of the costs together with the benefits to the regulator's business case was constructed.

The levers impacting regulators also were brainstormed based on the experiences of the team members from the working group and categorized using a mind map (Figure 9-4).

Figure 9-4: Mind Map of Business Case Levers for Regulators



Each of the five resulting lever categories was defined to assist in subsequent priority ranking (Table 9-5).

2407 **Table 9-5: Description of High-Level Levers for Regulators**

High-Level Lever for Regulator	Definition
Reduced Regulatory Action	• Interactions with regulatory agencies during development and post-licensure, including annual inspections and post-licensure amendments • Fewer inspections both PAI or general GMP • Classification of submissions from PAS to CBE-30 or CBE or to annual reportable • Reduced review time due to transparency of decision rationale and associated knowledge • Refocus resources to reduce oversight on lower-risk products/processes in favor of higher ones
Reduced Cost	• Lower costs resulting from "for-cause" inspections • Reduced cost associated with scheduled inspections and submissions/review for manufacturers and regulators • Enhanced approach filing could reduce filing review effort when submitting process changes within design space, etc.
Accountability	• Responsibility for decisions during development and care of the process post-licensure clarified for the regulators • Enhanced approach filing would help demonstrate that a reasonable level of product/process knowledge has been generated • Regulators assuring public that manufacturers met regulations for vaccine production. Enhanced approach filing provides regulators with knowledge they need to make their assessments.
Transparency	• Overt linkage of decisions made by manufacturers during development and post-licensure to prior knowledge or data for the current process • Encourages manufacturers to develop open and honest knowledge-driven relationship with regulators regarding inspections and submissions on manufacture of vaccine • Manufacturers notifying regulators if a problem or concern exists with vaccine production & distribution and the extent of its impact based on enhanced product/process understanding
Information-Driven Decisions	• Linking decisions to sound science based on available knowledge and understanding • Transparent justification of decisions with supporting data and risk-based rationale

2408

Prioritization ranking was accomplished based on informal interactions and discussion with regulators and manufacturing experiences of the team members involved, followed by team discussion and documentation of the rationale behind the designated priority (Table 9-6).

The regulator's business case for the enhanced approach offers some attractive advantages driven by the improved "ability to predict" from the knowledge developed from the enhanced over the traditional approach. Regulators can use the value stream approach presented to consider which applications might benefit from the additional investment in the enhanced approach. The traditional approach to process changes and product development often can be an effective path for managing product life cycle. However, some processes do not benefit as much as others from the additional knowledge provided by the enhanced approach to be robust, cost effective, efficacious, and safe. Considerations regarding the enhanced approach should be evaluated along with the expected value returned to regulators. Each project using the enhanced approach offers regulators, as well as manufacturers, unique opportunities and oversight challenges.

The approach outlined offers regulators a tool to prioritize important value stream goals relative to the specific situation under evaluation. In the case of a new or first-in-class vaccine, when prior knowledge is relatively low, regulators might highly value the improved transparency and clear information-driven decisions associated with the extensive process development of enhanced approach and thus be willing to invest additional resources to help guide manufacturers toward aligned expectations.

In the case where a manufacturer is entering a well-established market where there is a large body of prior knowledge, regulators may highly value the focus of the enhanced approach on risk-based knowledge gaps, ensuring robustness for this commodity product. Product entry into this market might offer competition and pricing benefits to patients, and the enhanced approach could minimize cost increases for regulatory agency oversight by ensuring process robustness. The enhanced approach offers additional knowledge that may offer manufacturers and regulators an improved ability to predict performance (and thus reliable resupply), but to be part of a sustainable business model, this approach must offer benefits over the traditional approach to both parties.

2443 **Table 9-6: Prioritized Levers for Regulators and Associated Rationale**

Business Case Lever	Estimated Priority	Rationale
Reduced Cost	High	• Fewer supply interruptions and associated oversight actions • Cost associated with reduced number of "for-cause" inspections and submissions reviews • Enhanced approach filing could reduce review times when submitting process changes within design space, etc.
Reduced Regulatory Action	High	• Effective and consistent interactions with manufacturers during development and post-licensure • Risk-focused approval and general GMP inspections • Fewer supplements by re-classification of some post-licensure submissions from approval supplements to annual reports
Information-Driven Decisions	High	• Linking decisions with scientific judgment based on available knowledge and understanding • Transparent justification of decisions with supporting data and risk-based rationale
Accountability	Medium	• Clear process decisions during development and planning for process verification post-licensure • Enhanced approach filing would help demonstrate that best effort for product/process knowledge has been generated • Enhanced approach filing would provide regulators with product and process knowledge they need to make assessments
Transparency (incorporated in information-driven decisions lever)	Low	• Linking decisions with scientific judgment based on available knowledge and understanding • Transparent justification of decisions with supporting data and risk-based rationale • Enhanced approach filing would provide regulators with product and process knowledge they need to make assessments

2444
2445 The prioritization criteria, although not formalized, were the same as those used for the
2446 manufacturer's drivers. It was considered effective for the regulator's drivers since there was a
2447 reasonable split among three priority levels. The three high-priority levers were: reduced cost,

reduced regulatory action, and information-driven decisions. The medium-priority lever was accountability. The low-priority lever was transparency. When the team revaluated these decisions, it was decided that Transparency was not a separate category, since it provided overlapping benefit within the information-driven decision lever. All levers were considered important, regardless of their ranked prioritization. The costs and benefits of the enhanced approach were developed specifically for the high-priority levers for manufacturers and for regulators. These were combined and compared with drawbacks and pain points of traditional approaches for vaccines (Table 9-7).

Table 9-7: Comparison of the Traditional and Enhanced Approaches for Vaccine Development for the Key Levers for Regulators

	Traditional	Enhanced
Operations	• Supply to market is sometimes uncertain because of slower resolution of manufacturing and testing issues • Product release and in-process controls based on battery of tests that are redundant in some cases, leading to increased cycle time and higher costs • Release based primarily on attribute testing	• Improvements can be made by reducing testing and utilization of key inputs linked to PAT models, cycle time, and oversight costs • Better (product/process) understanding of why certain procedures are being implemented • Improved process understanding and more well-characterized products leads to better evaluation of the impact of optimization and flexibility changes
Cost of Product Regulatory Oversight	• Periodic process redesign at development and commercial scales (analytical and clinical comparability), resulting in more complicated filings	• Potential improvements in product/process understanding, leading to fewer development iterations through licensure and straightforward development history • Process knowledge and design space provides clear guidance for determining quality impact of deviations from normal operating range • Human and physical resource savings required to assess manufacturer's provided information because of improved transparency

Information-Based Decisions	• Knowledge and technology transfer to manufacturing not always efficient because of fewer direct links with identified risks	• Decisions can be traced to supporting data and risk-based rationales for reviews throughout product life cycle • Improved transparency of experimental work, since development data is readily accessible for review

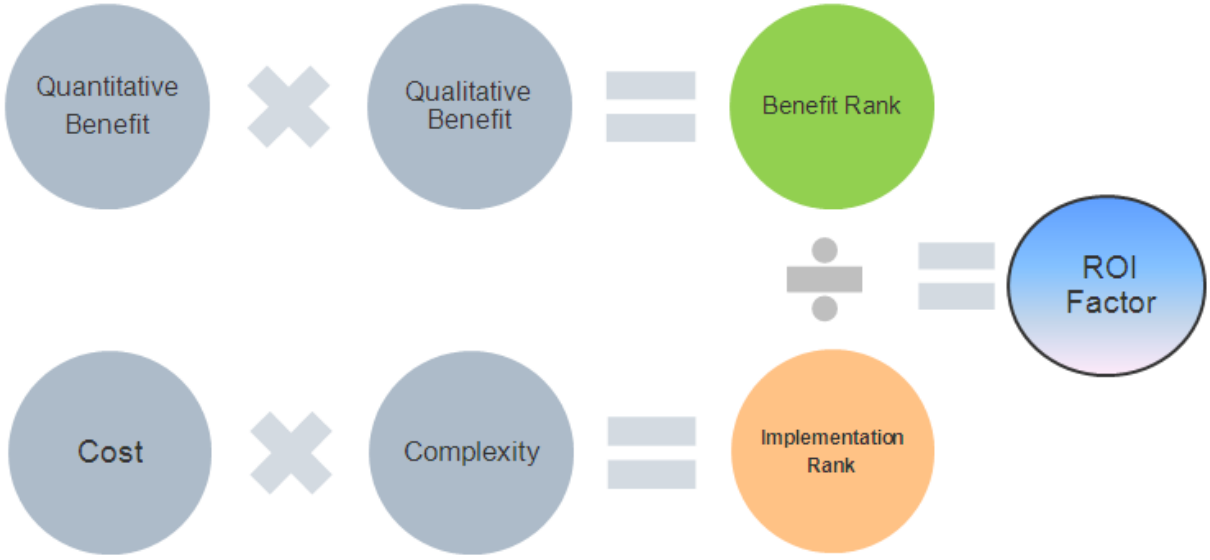
The benefits of the enhanced approach require a high degree of collaboration and exchange of information between the manufacturer and the regulator to attain the ultimate goal of providing a safe and efficacious vaccine product. A few of the benefits highlighted in the comparison of the traditional and enhanced approaches for both the manufacturers and regulators are: (1) better understanding of certain procedures being implemented for the product and process, (2) possible reduction in testing based on PAT models, (3) potential cost savings of human resources for assessment of information, and (4) potential to avoid repeats of earlier experimental work, since developmental data is more readily available. All of these benefits for the enhanced approach are obtained only by the manufacturers and regulators partnering and gaining an understanding from each other linked to the application of the enhanced approach in a regulated environment.

Costs of the enhanced approach increase if the manufacturers do not partner with the regulators and provide the initial pre-investment for implementation of the enhanced approach. The pre-investment cost for the enhanced approach consists of the time and effort for regulators to understand the implementation of enhanced approach activities in a regulated environment. These initial costs to regulators could relate to understanding: (1) the impact of a manufacturer's changes within the design space on the vaccine product, (2) changes in regulatory submission information from the manufacturer when initially implementing the enhanced approach, and (3) whether changes to improve processes or the product impact previous product/process characterization work conducted for that product. Partnering between the manufacturers and regulators for initial implementation of enhanced approach decreases the costs to both parties. The collaborative exchange of information outweighs start-up costs over time and results in an improved vaccine product, maintaining the safety and efficacy of the product as the ultimate goals.

9.8. Specific Business Cases for Implementation of the Enhanced Approach

A relative rating system was constructed to evaluate implementation costs and enhanced approach benefits.

Equation 9-1: Relative Return on Investment (ROI factor)



The relative return on investment (ROI factor) was defined as: ROI factor α [benefit rank/implementation rank], where the benefit rank was defined as: Benefit rank = [quantitative x qualitative], and the implementation rank was defined as: Implementation rank = [cost x complexity].

Quantitative benefits were easily calculated savings, such as lower OOS costs and fewer failed batches. Qualitative benefits were harder to quantify and included good will with patients and regulators.

For the enhanced approach, a ranking score that includes the relative quantitative benefit is multiplied by the relative qualitative benefit to obtain a benefit ranking. A score of 1 represents the least relative benefit rank, whereas a score of 25 represents highest relative benefit rank. Thereby, the relative benefit rank for the enhanced approach (vs. the traditional approach) can be evaluated for the degree of benefit.

2507 **Table 9-8: Benefit Rank Definition**

Increasing qualitative benefit					
5 (very high)	5	10	15	20	25
4 (high)	4	8	12	16	20
3 (same)	3	6	9	12	15
2 (low)	2	4	6	8	10
1 (very low)	1 (very low)	2 (low)	3 (same)	4 (high)	5 (very high)
	Increasing quantitative benefit				

Rank Score	Comments in terms of benefit ranking
15 to 25	Operations with high benefit – Flexible/favorable benefit ranking - High.
7 to 12	Operations with average benefit – Moderate benefit ranking - Med
1 to 6	Operations with less or negative benefits – Marginal benefit ranking - Low

2508
2509 Cost was defined as including cost of staff, equipment, and other materials for the process and
2510 associated analytical development, as well as production for that activity. It also included the
2511 time for the activity. For the examples in this case study, costs of additional clinical studies that
2512 might specifically be needed to support the enhanced approach were excluded, the base cost
2513 was the traditional cost, and the enhanced approach was believed to be able to lower as well as
2514 raise net costs, depending on the specific situation.
2515
2516 Complexity was defined according to whether the activity is new (not been done by any
2517 organizations to the best of our knowledge), unique (been tried by only a few companies, and
2518 only a few have had success), difficult (been tried by many companies and generally has had
2519 several challenging aspects), or simply semi-complex or noncomplex (routine).
2520
2521 For the enhanced approach, a ranking score that includes the relative level of complexity
2522 associated with the implementation is multiplied with the relative costs for implementation to
2523 obtain an implementation ranking. A score of 1 represents the least relative implementation
2524 rank, whereas a score of 25 represents highest relative implementation rank. Thereby, the
2525 relative implementation rank for the enhanced approach (vs. the traditional approach) can be
2526 evaluated for the ease of implementation.
2527

Table 9-9: Implementation Rank Definition

Increased complexity of implementation						
	5 (cutting edge)	5	10	15	20	25
	4 (unique)	4	8	12	16	20
	3 (difficult)	3	6	9	12	15
	2 (semi-complex)	2	4	6	8	10
	1 (noncomplex)	1	2	3	4	5
		1 (0.6 X base cost)	2 (0.8 X base cost)	3 (1 X base cost)	4 (1.25 X base cost)	5 (1.5 X base cost)
	Increasing Cost					

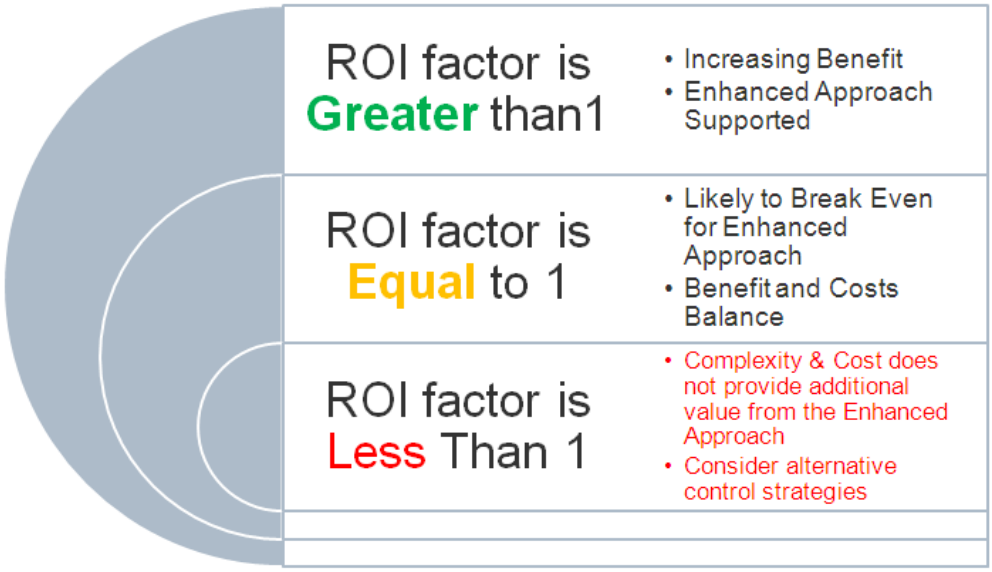
Rank Score	Comments in terms of implementation ranking
1 to 5	Operations with ease of implementation – Flexible/favorable ranking - High
6 to 12	Operations with average ease of implementation – Moderate ranking - Med
15 to 25	Operations with implementation linked to increased documentation practices – Marginal ranking - Low

Table 9-10: ROI Factor Definition

Benefit Rank						
	25	5	2.5	1.67	1.25	1
	20	2.5	2	1.33	1	0.8
	15	3	1.5	1	0.75	0.6
	10	2	1	0.67	0.5	0.4
	5	1	0.5	0.67	0.25	0.2
		5	10	15	20	25
	Implementation Rank					

Based on the above ranking system, relative ROI factors were obtained and interpreted according to the following framework:

Figure 9-5: Rank Score for Relative ROI



>1 ROI factors that were greater than 1 represented a benefit rank greater than the implementation rank and were more likely to produce gains.

=1 ROI factors that were about 1 represented a benefit rank about equal to the implementation rank and were considered to be "break even."

<1 ROI factors that were less than 1, represented a benefit rank that was less than the implementation rank and were less likely to produce gains (and may produce losses).

The ROI factor approach was next applied to establish specific business cases for three example steps or activities from the A-VAX case study. The examples selected were:

- Scale-up of a virus-like particle (VLP) conjugation time reduction by five hours, increasing manufacturing capacity of 24x7 operating plant by 20% for bottleneck process step
- Source change for enzyme for polysaccharide extraction to reduce cost by 5% by improving enzyme purity
- Site change for drug product lyophilization to increase industrial capacity

For each example, it was first decided whether it was appropriate to evaluate based on the aggregate activity or to divide the analysis into sub-activities (i.e., scale-up, tech transfer, validation, licensure) to evaluate the incremental ranking. If sub-activities were invoked, then the implementation investment was credited for subsequent activities, resulting in lower ROI factors. The individual, incremental ROI factors can then be averaged with appropriate weighting (not done here) or compared directly in a decision analysis.
Factors such as process development, technology transfer and scale-up, process validation, batch processing, and release of product were mapped to specific manufacturer and regulator

levers from Table 9-3 and Table 9-6. Relative rankings were based on quantitative/qualitative benefits and complexity/costs for implementation expected when the enhanced approach was applied for the example activity.

Scale-up of VLP conjugation time reduction example

The VLP conjugation process scale change example, when considered in four incremental activity stages, showed benefits of the enhanced approach in these stages. These steps reduced VLP conjugation time by five hours, increasing manufacturing capacity of 24x7 operating plant by 20% for bottleneck process step. The example clearly illustrated that every development activity showed benefit when using the enhanced approach. The ROI factor was higher than 1 in all cases (ROI factor = 1.2, 2.2, 1.8, 3.3) and generally increased for the activities positioned closer to commercial manufacturing. Although the implementation cost was higher for the early activities, even then there was a favorable ROI factor.

Table 9-10a: ROI Factor Rankings for Scale-Up of the VLP Conjugation Time Reduction

Key Levers (High-Priority Levers)		Step or Activity Example	Effort Level of Complexity	Implementation Cost	Implementation Rank[a]	Quantitative Benefit	Qualitative Benefit	Overall Benefit Rank	ROI Factor (Benefit/ Cost)
Business	Regulatory								
Process Dev't scale-up	Info-driven decisions	VLP conjugation-scale-up	3 Not much different	4 Added cost of dev't)	12	4 Improved scale-up success	4 More information available for the process	16	1.3 Despite higher costs, ROI factor still favorable
Process Dev't scale-up	Info-driven decisions	VLP Conjugation -tech transfer	3 Process remains the same	3 No additional investment	9	5 Additional dev't work supporting tech transfer	4 Helps achieve prerequisites for launch	20	2.2 ROI higher since leverage scale-up investments
Process Dev't scale-up	Info-driven decisions	VLP conjugation-process validation	3 Process remains the same	3 No additional investment	9	4 Fewer runs overall during PV than tech transfer	4 Helps achieve prerequisites for launch	16	1.8 ROI reduced since less benefit in PV of leveraging scale-up investment
Process Dev't scale-up	Info-driven decisions	VLP conjugation-licensed operation	3 Process remains the same	2 Lower costs of enabling licensed operation	6	5 Realize full benefit of investment (e.g., reduced losses, cycle time improvements)	4 Improved customer satisfaction	20	3.3

Enzyme source change example

This analysis also can be used to compare different change proposals to rank their expected ROI factors, helping to prioritize them. An example of the value stream analysis for three possible approaches for enzyme replacement source is shown below in Table 9-11B. The enhanced approach using small-scale DOE models provides the highest potential benefits (ROI factor = 6). This indicated that the value returned to stakeholders was higher than the traditional approach of full-scale process development and process validation (ROI factor = 0.8). Thus, there is clear advantage to implementing the enhanced approach for the enzyme replacement with a recombinant enzyme source.

An intermediate scenario was also explored because the enzyme replacement was a recombinant version of the enzyme rather than just an enzyme supplier change using a similar manufacturing process. The enhanced approach relies on application of product and process knowledge from the DOE used to determine the design space for the nonrecombinant enzyme at the small scale. Rather than checking the equivalence of the current and new enzymes at reference manufacturing-scale process conditions, the enhanced approach addresses whether the design spaces for the two enzymes overlap in the qualified scale-down model. The ability of small-scale models to predict manufacturing scale process performance with the recombinant enzyme is a critical consideration because the licensed design space was demonstrated with the nonrecombinant enzyme source.

Risk assessments should consider the potential for scale-up risk based on the small-scale model qualification and recombinant enzyme DOE studies. A compromise approach may be needed, where enhanced process development is performed at small scale but results are verified at full scale to confirm the recombinant enzyme design space. In this case, the value returned is much less (ROI factor = 1.3 vs. 6 without full-scale verification). This reduction was driven by the additional costs of a full-scale run and the lost opportunity for manufacturing runs while the facility is changed over for engineering or validation run activities. However, the scale-up uncertainty was mitigated and some value increase remained over the traditional approach, where traditional full-scale process validation was required because the ROI factor increased by 0.5 over the traditional approach ROI = 0.8.

When considering risk, manufacturers must balance their approach so that the project can be successfully delivered in an acceptable amount of time. Every project involves some risk and uncertainty that must be considered and mitigated by project teams. To provide value, teams cannot mitigate against all uncertainty, so teams must manage some level of residual risk for all projects. When considering the enhanced approach, teams must not only consider the risks they are mitigating, but also the additional value returned over more traditional methods.

In the enzyme replacement example, the team decided to execute the enhanced approach because the risk associated with enzyme replacement was relatively low and the step was well-understood and -documented through its established design space. Using this approach, an ROI factor as high as 6 was possible if the team was confident about its approach and could defend its rationale to regulators.

When risk is low, performing unnecessary full-scale activities causes the ROI factor to drop significantly because of the cost of full-scale runs and the lost opportunity for manufacturing

runs while the facility is changed over for engineering or validation run activities. In this case, the project team is doing much more work than needed for success. The incremental reduction in risk comes at a significant reduction in value returned (ROI factor = 1.3). The longer implementation timeline and lower ROI factor may force the company to consider abandoning the improvement altogether, unfortunately providing no value to stakeholders.

When used with risk management tools as outlined in ICH Q9, the value stream approach can help prioritize risk mitigation projects to ensure that implementation of the enhanced approach retains sustainable value.

Table 9-11B: Manufacturers' ROI Factor Rankings for Enzyme Source Change

Manufacturers' ROI Estimate:
Enzyme Source Change with Enhanced Approach and Process Monitoring Verification

Key Levers (high priority levers)		Step or Activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant benefit	Qualitative benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Process Development Scale-up	Reduced Resources and Time	Enzyme Source Change	2 Investment in small scale DOE in initial filing	1 No FS runs, but higher costs for DOE	**2**	4 Reduced RM cost	3 Improved purity and consistency	**12**	**6**
Complexity: Low for DOE small scale studies Imp Cost: Low for DOE studies in comparison with full scale Development runs but DOE costs still higher than traditional small scale experiments					Benefits: Faster realization of reduced raw material costs & improved purity/consistency over original enzyme source which may also improve process robustness				

Key Levers (high-priority levers)		Step or Activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant Benefit	Qualitative Benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Process Development Scale-up	Reduced Resources and Time	Enzyme Source Change	3 Added facility change over	3 FS run cost & lost facility time	**9**	4	3	**12**	**1.3**
Complexity: Increases for full scale verification because of added logistics for manufacturing facility scheduling and change-over. Imp Cost: Increases for full-scale verification because of full-scale run cost and lost facility time.					Benefits: No additional benefits of adding full scale engineering run to Enhanced approach but introduces additional costs and complexity				

Manufacturers' ROI Estimate: Traditional Approach with Full Scale PV / Commercial

Key Levers (high-priority levers)		Step or Activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant Benefit	Qualitative Benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Process Development Scale-up	Reduced Resources and Time	Enzyme Source Change	4 Scheduling of manufacturing facility, lack of small-scale data	4 FS PV runs costs and down time of manufacturing facility	**16**	4	3	**12**	**0.8**
Complexity: Higher for traditional approach since potential lack of adequate small scale model for trouble shooting and optimization would require development studies and PV at full scale. Costs: Significantly higher because licensed manufacturing facility must undergo change over for full-scale development and PV runs, resulting in lost manufacturing time and increased materials cost.					Benefit: Unchanged benefits but added complexity and cost of traditional method reduce ROI for implementation of improved enzyme source change.				

Table 9-12C: Regulator ROI Factor Rankings for Enzyme Source Change

This analysis also can be used by regulators to compare different change proposals and rank their expected ROI factors from the regulator's prospective. An example of the value stream analysis for the three possible approaches for enzyme replacement source is shown below in Table 9–11c. The enhanced approach using the qualified small-scale model to confirm the design space for the recombinant enzyme still provides the highest potential benefits from the regulator's prospective (ROI factor = 4). The regulator's cost in this case is lower than the manufacturer's ROI because regulators do not incur the costs associated with the process development and full-scale activities, the latter of which are avoided with the enhanced approach. The regulator's ROI factor still indicated that the value returned to stakeholders is higher than the traditional approach (ROI factor = 1.5). Thus, there appears to be clear

advantage to implementing the enhanced approach for the enzyme replacement with a recombinant enzyme source, from both the manufacturer's and regulator's view points. The expected ROI factors for the intermediate scenario from the regulator's view also were explored. Since the enzyme replacement was a recombinant version of the enzyme rather than just an enzyme supplier change, there may be potential for scale-up risk, because the filed qualified scale design space was demonstrated with the nonrecombinant enzyme source. An assessment of risks associated with small-scale model qualification only with the nonrecombinant enzyme in this case may suggest that a compromise approach might be needed, where the enhanced process development is performed at small scale but the result is verified at full scale. The small-scale model design, qualification, and correlation with full-scale operations are not covered in this case study, but this information should be considered when evaluating the scale-up risk.

If properly executed and documented, the enhanced approach provides clear rationale and supporting data to reinforce the decision to proceed with the enzyme change without full-scale run verification. In situations where the regulator's risk assessment indicates that the small-scale model data is not sufficient, then a discussion of the potential risks and ROI factors achieved for each of the proposed scenarios might support a compromise positions. For example, the enhanced process development could be performed at small scale, with an engineering run conducted for full-scale verification, but once success is demonstrated, then traditional process validation would not be executed. The value returned to regulators and manufacturers in this case is less (ROI factor = 2.7 and 1.3), but value is still returned to all stakeholders.

The value stream tool introduced in this case study provides process knowledge and implementation data that can improve the decision process when considering where to implement the enhanced approach. Manufacturers and regulators are encouraged to use formal value determination tools, such as this one, to ensure efficient and effective resource utilization. Each application should be customized for the manufacturer, the regulator, and the product.

Regulators' ROI Estimate: Enzyme Source Change with Enhanced Approach and Process Monitoring Verification

Key Levers (high priority levers)		Step or activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant benefit	Qualitative benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Process Development Scale-up	Reduced Review Resources and Time	Enzyme Source Change	2 DOE data reviews, trained resources	2 Focused review based on approved DS	**4**	4 Fewer investigations	4 Less supply interruptions	**16**	**4**

Enhanced Approach but with addition of a Full Scale Eng Run Verification

Key Levers (high priority levers)		Step or activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant benefit	Qualitative benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Process Development Scale-up	Reduced Resources and Time	Enzyme Source Change	2	3 Additional data from eng run to review	**6**	4	4	**16**	**2.7**

Traditional Approach with Full Scale PV / Commercial

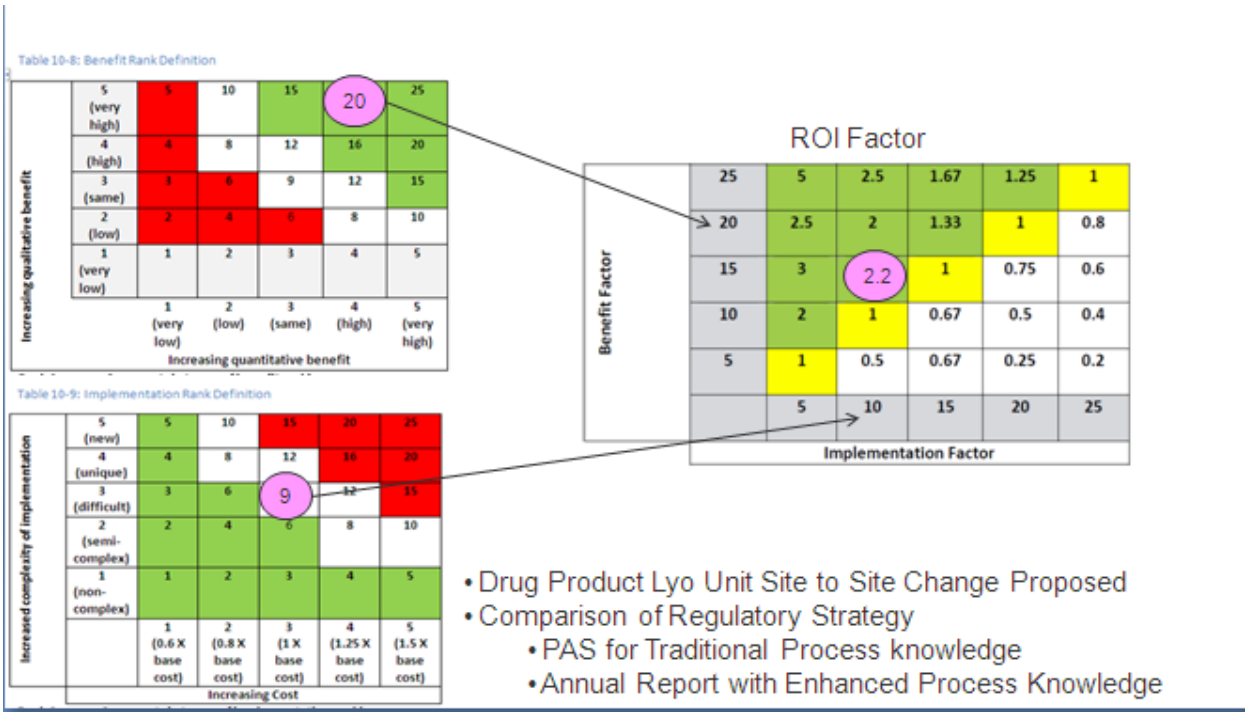
Key Levers (high priority levers)		Step or activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant benefit	Qualitative benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Process Development Scale-up	Reduced Resources and Time	Enzyme Source Change	2	3	**6**	3	3	**9**	**1.5**
					Benefits: Reduced owing to delay of enzyme change implementation owing to manufacturers' additional cost and complexity associated with Traditional Approach				

<u>Site change for drug product lyophilization</u>

The site change for drug product lyophilization to increase manufacturing capacity was considered in one activity stage. Breaking down the discrete items, such as facility, technology transfer, and comparability elements, was not pursued because site transfer to use additional capacity is a current industry practice. However, the cycle times associated with such transfers are lengthy and equivalency models are not equally nor consistently applied. With the utilization of the enhanced approach employing design space concepts linked to equipment and product comparability, such changes are expected to be facilitated and associated effort with cycle development and validation exercises significantly reduced. Based on the analysis and descriptors above, the ROI factors based on relative benefit and implementation costs scores yields are favorable.

Table 9-13c: ROI Factor Rankings for Site Change for Drug Lyophilization

Key Levers (high-priority levers)		Step or Activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant Benefit	Qualitative Benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Flexibility	Reduced regulatory action	Site change for DP	3 Process remains the same	3 Costs dominated by facility and equipment	**9**	5 Tangible gains from increased speed of transfer and approval	4 Meeting increased market demand reliably, patient supply	**20**	2.2 Validation then becomes more straight-forward



Overall, the main benefits of the enhanced approach for these three examples are: formalized assessment of risk, linkage of high-risk inputs to subsequent experiments for process understanding and/or subsequent control strategy, streamlined number of experiments through use of DOE, use of a scale-down model appropriate for manufacturing, and development of process models and quantification of variability to depict process understanding.

DOE experimental design was used in an integrated manner by linking studies to high-risk inputs and designing space studies with the goal of defining and understanding an appropriate design space. Consistent linkage also was made to proposed critical quality or key process attributes. Repeat of earlier work because of inefficient data and information (knowledge) management practices was minimized.

With an established design space that is relevant for manufacturing conditions, tech transfer is streamlined. Risk analysis is updated to generate prioritized experiments to fill identified gaps.

Using the enhanced approach, facility and equipment specifications as well as process batch records are developed faster.

Sufficient scale-down model studies resulted in scale-up success. Classical three-lot process qualification at the center point was replaced by single-lot confirmation at scale. Process understanding resulted in reasonable ranges for eventual manufacturing, translating into fewer atypicals. Process validation effort (new FDA guidance: stage 1, 2, and 3) is reduced since many documents generated through the enhanced approach can be directly applied to these deliverables.

Overall, the main implementation investments for the enhanced approach for these three examples fall into two categories:

- Equipment — The appropriate type and number of scale-down systems are needed to permit DOE and other types of experimentation in a timely manner. Although high throughput and/or miniature systems are not required, their use would assist in maximizing information obtained during available timeframes, if that were desirable. Analytical equipment is needed to match the process equipment to provide prompt assessment of product/process quality.
- Business processes — The enhanced approach is not about generating more information; rather, it is focused on generating the right type of information. Effective business processes need to be established to promote accurate assessment of risk, robust experimental design, leveraging of prior knowledge, etc. Process and analytical scientists need to be prepared to spend additional time discussing and planning their work in a cross-functional manner, then evaluating whether the results obtained generate the appropriate product/process understanding.

Of course, both of these categories require staffing. Whether it is more staffing or less staffing overall has been hard to ascertain. Many companies have staffing models where staffing estimates are incorporated. Many companies have time systems where staffing actual numbers are recorded. Few companies have been able to bridge the estimates to the actual within an accuracy of better than 10% to 20%. Thus, it can be difficult to evaluate changes in net staffing demand with the enhanced approach.

9.9. Business Case Customization Frameworks for Management Consideration

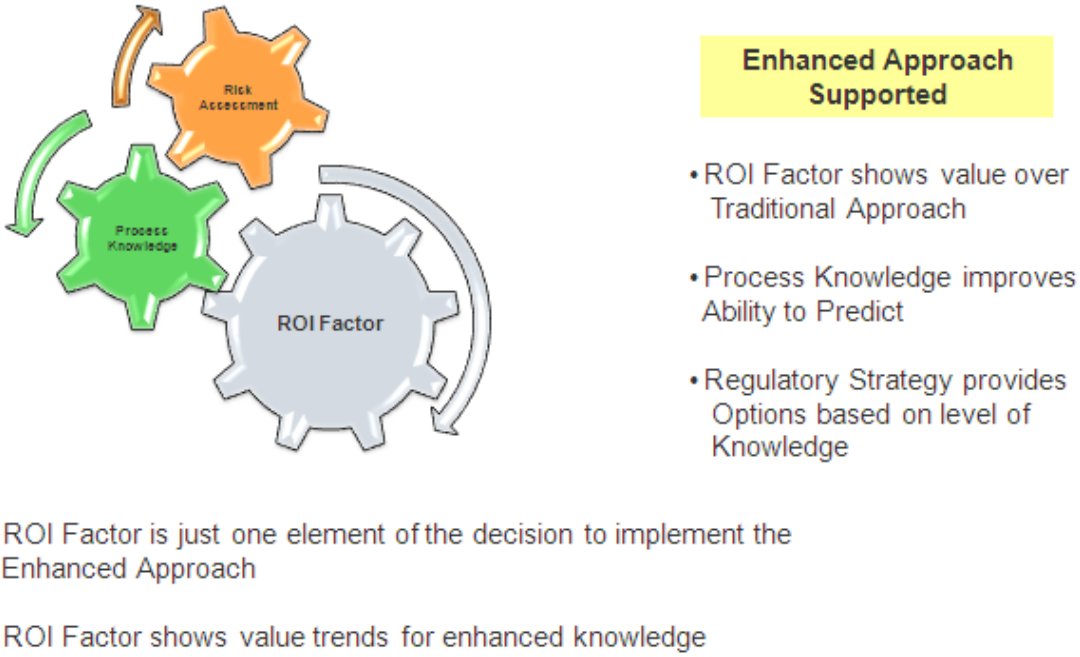
Companies need to figure out their specific implementation costs and benefits, and ROI factors. The total capitalized cost estimate of a new product is $802 million (year 2000 dollars) as published by DiMasi et al. (2003), with a base case out-of-pocket cost per approved new drug of $402 million. Furthermore, it is estimated for small molecules that nearly 25% of the classical (i.e., traditional) pharmaceutical industry expenses are incurred in product manufacturing, where waste and sampling/yield losses can be as high as 50% and that 5% to 15% of product loss occurs in later phases of operations (Better by Design, Sven Stegemann. World Pharmaceuticals Frontiers. 2010. Volume 1. pp 76 to 78). Similar values might be applicable for vaccine manufacturing. Accordingly, the cost incurred through product loss in manufacturing can add significantly to the cost of goods and present limitations to effective product turnaround.

Based on the numbers shown above, the cost to bring an entity to market is significant. However, after such investments, in some cases performance at industrial platforms shows a wide variance in write-offs as a result of product waste and loss. Although losses are not broken down by category, the limitations of traditional models may account for a significant portion of such losses. In the traditional model, batches are tested at several stages in the manufacturing process (i.e., raw materials, in-process material, and end product) against a number of parameters and quality attributes. Where a batch does not meet a required specification, it is typically discarded as out of specification, resulting in product loss and unavailability, which can lead to patient supply constraints.

Quality and performance are achieved primarily by imposed compliance with limited flexibility in the manufacturing process. Product specifications may be derived using test data from a limited number of development batches, which is not always based on a statistically significant sampling and can be a source of variability. Under this framework, process success is linked to the inherent variability of the process and the type of validation strategy executed and the limited development (design) characterization detailed in the license.

In contrast, where applied, the enhanced approach has the potential to offer a method that can improve overall manufacturing performance, reduce cost of goods, and assure compliance across the defined design space. It represents a scientific, risk-based approach to pharmaceutical process and product development with deliberate design considerations across the product development life cycle to final commercialization. (Refer to the key drivers linked to implementation and benefit ranking for the enhanced approach.)

Value Stream Decision to Go Beyond the Traditional Approach



The decision to supplement the traditional approach is complex, and implementation tools such as those introduced here should be developed to help support effective investment in the enhanced approach. The ROI factor is just one element in the decision to implement the

enhanced approach. A successful implementation strategy depends also on a rigorous demonstration of risk assessment rationale and process knowledge.

The enhanced approach may increase the upfront cost of development, but there will be ROI with better throughput for manufacturing operations, better efficiency, and more predictable controls via fewer deviations and reduced cost of goods (e.g., cycle time and reduced testing using PAT models). Application of the enhanced approach ensures predictability and the ability to consistently meet predefined product quality attributes by process control and understanding.

Furthermore, the enhanced approach promises to ultimately contribute to improving the safety of drugs compared with existing practices. With a product developed using the enhanced approach, there is continuous monitoring of critical parameters, and the ability to make changes to key process parameters based on feed-stream variability (e.g., raw material changes, equipment issues) is permissible based on data and scientific rationale. Also, control of operations is linked to technology-driven models where monitoring ensures the required product-critical attributes are achieved. It also provides efficiencies in investigations for out of specifications and allows for process simplifications.

An additional consideration is that the enhanced approach serves as the basis of a robust and detailed regulatory dossier. In that way, parameters and quality attributes that are linked to the clinical performance are understood. This linkage may allow for ease of implementation across sites when the necessary prerequisite elements are in place.

9.10. Key Implementation References

The price of innovation: new estimates, of drug development costs. Joseph A. DiMasi., Ronald W. Hansen, Henry G. Grabowski. Journal of Health Economics 22 (2003) 151–185

Better by Design, Sven Stegemann. World Pharmaceuticals Frontiers. 2010. Volume 1. pp 76 to 78. http://www.worldpharmaceuticals.net/editors_choice_march10.htm

10. Applying QbD to Live Vaccines (Upstream - LAIV)

10.1. Introduction for Viral-Based Vaccine Upstream

Vaccines based on viral components represent an important segment of the vaccines available on the market including influenza, poliovirus, and hepatitis A.

Because of their viral composition, these vaccines present process requirements that must be taken into account during their development to establish robust manufacturing. Such specificities include the biological complexity inherent in viruses, with an impact on the definition of suitable analytical tools for characterization, the use of several particular cell substrates susceptible to the virus to be produced (i.e., non-tumorigenic adherent cell lines such as vero cells), and the presence of certain process steps (e.g., production of viral seed stocks, viral infection and propagation steps during the production process).

These process constraints make the establishment of a process platform as for monoclonal antibodies' processes more challenging, with potentially less process history data and less prior knowledge to draw on in some cases.

Having these specificities in mind, the section of this case study dedicated to viral-based vaccines will illustrate how QbD methodology can be applied to their development. To illustrate this section, the proposed process is based on an adherence Madin Daby Canine Kidney (MDCK) cell line grown in static and dynamic conditions (microcarriers) using animal-free media formulations for the production of an influenza virus at the final bioreactor scale of 2,000 liters.

10.2. Executive Summary

The focus is put on specific process steps such as viral infection and the final cell growth in a bioreactor. It was decided not to address the question of tumorigenicity and adventitious agents in this case study because their control is complex and still relies on intensive testing of the different cell banks, viral stocks, raw materials, and process intermediate steps.

This section illustrates how to consider in parallel critical quality attributes (CQAs) and key process attributes (KPAs) during the development of a viral vaccine. A specific risk assessment methodology considering CQAs and KPAs is proposed.

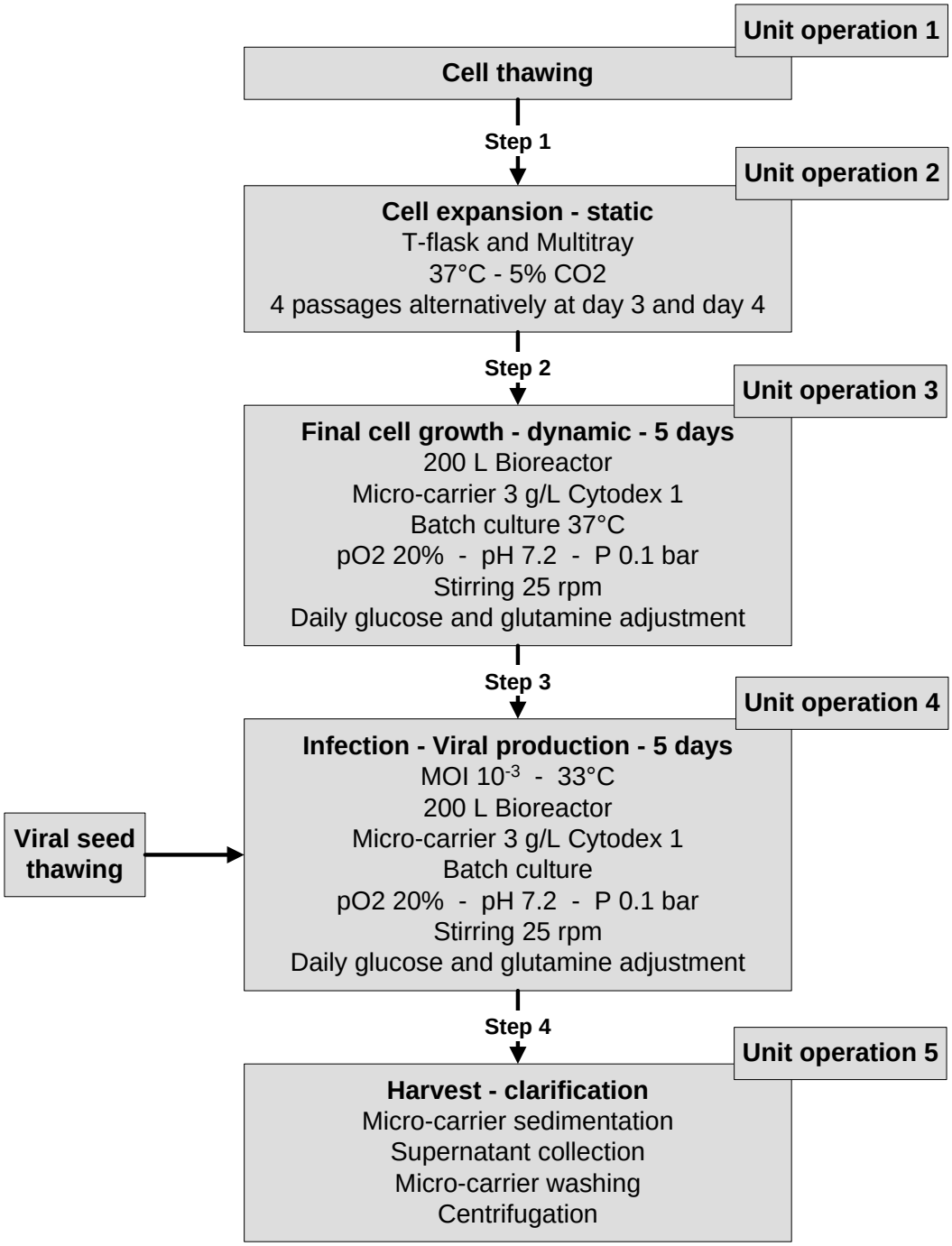
It is also important to carefully consider the variability of the analytical tools used during the development of such a vaccine. Some assays in the early stages of the product development might present variability too high to be suitable for DOE applications. The proposed strategy to define the design takes into account this analytical variability.

This section also illustrates the use of one-factor-at-a-time (OFAT)/univariate analysis for some of the process parameters, such as media stability evaluation.

A methodology is proposed to ensure the definition of an efficient way to scale up the bioreactor scale with the establishment of a scale-down bioreactor model taking into account the specificity of microcarrier-based cell culture (impact on mixing and shear stress).

10.3. Process Description (Phase II Process)

The production of an influenza virus on an adherent cell line has been chosen for the QbD analysis in this case study. A process flow diagram, as well as a brief description of the different process steps at the end of the phase II development, is presented in this chapter.



Cell culture

- Unit operation 1: **Cell thawing**
  The adherent cell line MDCK, stored in liquid nitrogen, is thawed at 37°C and seeded at 20.000 cells/cm² in T175 cm² followed by incubation at 37°C with 5% CO_2 during four days.
- Unit operation 2: **Cell expansion – static (multitray)**
  The production of the biomass necessary for the launch of the final bioreactor using an

animal-free proprietary medium is assured by four cell passages performed every three and four days respectively at 20.000 and 15.000 cells/cm². Cells are detached with a non-animal-origin enzyme and incubated at 37°C with 5% CO_2.

- Unit operation 3: **Final cell growth – dynamic culture in bioreactor**
  The production of the biomass required for the viral infection step in a stainless steel 200 L bioreactor is performed for five days. Cells are seeded at 150.000 cells/ml and grown on Cydotex 1 at 3g/L at 37°C. A daily glutamine (2 mM) and glucose (1 g/L) adjustment is performed. Bioreactor regulations are as follows:
- Regulation: pO2 20% - pH 7.2 - P 0.1 bar.
- Stirring: 25 rpm.
- Viral production
- Unit operation 4: **Infection and viral production**
  Infection is performed five days after the bioreactor seeding, when the cell density reaches at least $2.5x10^6$ cells/ml. Growth medium is replaced by a viral production medium, and temperature is decreased to 33°C. The wild type influenza virus is activated by addition of a serine protease at 100IU for 30 minutes and added at a multiplicity of infection (MOI) of 10^{-3}. Viral replication is allowed for five days with the same bioreactor regulations as for cell growth except for the temperature regulation, which is maintained at 33°C. Again a daily glucose and glutamine adjustment is performed as well as a daily addition of serine protease at 2IU/day for viral activation.
- Unit operation 5: **Harvest and clarification**
- This step is performed after five days of viral replication. The microcarriers are sedimented and the supernatant is harvested before clarification by centrifugation. The clarified harvest is then transferred for downstream purification.
- This phase II process will be the starting point for the different analyses described in sections 3, 4, and 5.

10.4. Unit Operations Selected

The unit operations selected for this case study will be identified by ranking their theoretical impact on chosen critical quality attributes (CQAs) and key process attributes (KPAs).

10.4.1. Identification of CQAs and KPAs

CQAs are output parameters linked to the quality of the product (safety and efficacy). Those considered for this case study are:

- Protein content: Total protein was chosen at this step of the process as a purity indicator that can be linked to host cell protein content.
- The virus integrity on crude harvest is assessed via the ratio hemagglutinin (HA) attached to the virus on total HA. The HA content is analyzed by SRD (single radial immunodiffusion), and the HA linked to the virus is measured after performance of an analytical sucrose density gradient.

Remark: Host cell protein and DNA are also critical at this stage. However, they are eliminated by the purification process steps. Purification of Phase I and II process was efficient enough to ensure a residual DNA and host cell protein content of the purified bulk significantly below the specifications. Therefore, the purification process capacity to ensure these impurities' removal will be checked after the Phase III process definition.

KPAs are output parameters linked to process consistency and business aspect (e.g., supply issue, production delay, cost impact). Those considered for this case study are:

- Antigenic titer (SRD): hemagglutinin (HA) content
- Cell density at the end of growth

10.4.2. Selection of the Unit Operations

The tool used for this selection is a cause-and-effect matrix. The theoretical impact of each unit operation (= input) on each above identified critical attribute (= output) will be scored according to the table below. The theoretical impact is estimated within the conditions usually encountered.

Rank/Weight	Input Process Step to CQA and KPA
10	Strong relationship known
7	Strong relationship is expected/likely
4	Not-so-strong relationship or not expected
1	Known to not have a relationship

It should be noted that an additional operation unit has been included (unit operation 2b: cell expansion – dynamic with a bead-to-bead transfer), as this additional step will be necessary to ensure the scale-up of the process.

This scoring will lead to a ranking of the different operation units as shown in the following table. The rankings reflect the link between the unit operations (input) and critical attributes (output).

	CQA		KPA		
	Total protein (HCP, DNA)	Virus integrity	Antigenic titer	Final cell density (end of growth)	Total
Unit operation 1: Cell thawing	1	1	1	7	10
Unit operation 2: Cell expansion - static (multitray)	1	1	5	7	14
Unit operation 2b: Cell expansion - dynamic (microcarriers, bioreactor)	5	1	5	7	18
Unit operation 3: Final cell growth - dynamic (microcarriers, bioreactor)	7	7	7	10	31
Unit operation 4: Infection - viral production (microcarriers, bioreactor)	10	10	10	na	30
Unit operation 5: Harvest and clarification	10	5	10	na	25

According to this analysis, three steps are identified as having more impact on the CQAs and KPAs. However, for illustration purposes, in this case study we will concentrate on two steps: final cell growth and infection and viral production.

10.5. Identification of Prior Knowledge (from Work Done Prior to the End of Phase 2 and from Other Processes)

All parameters linked to the cell expansion in the stationary phase and in the bioreactor, including the bead-to-bead transfer, were developed to support other products and can be considered as referring to a generic process. This process platform was implemented for this project with only minor adjustments.

Following are parameters that were developed during phase II and will not require further optimization:

- Composition of culture medium for cell growth and viral infection.
- Bioreactor seeding density: 150.000 cells/ml was selected during screening of this parameter based on antigenic titer and cell density at the end of cell growth.
- Cytodex concentration: Several concentrations were tested, and 3g/L was selected based on cell density at the end of cell growth and antigenic titer.
- Temperature during cell growth and infection: For cell growth, the range 36–38°C was screened and showed no impact on antigenic titer and cell density at cell growth end. During viral replication, the range 32–34°C was studied, showing no impact on antigenic titer.
- pO2 during cell growth and infection: Between 10% and 50%, the pO2 was shown to have no impact on growth and viral production.

- Pressure during cell growth and infection (has been taken from other cell culture processes using the same equipment)
- Cell infection duration: It was optimized to maximize yield; the impact on total protein and hence specific productivity was not investigated.
  - Seed thawing is independent of final scale and was defined for other flu processes.

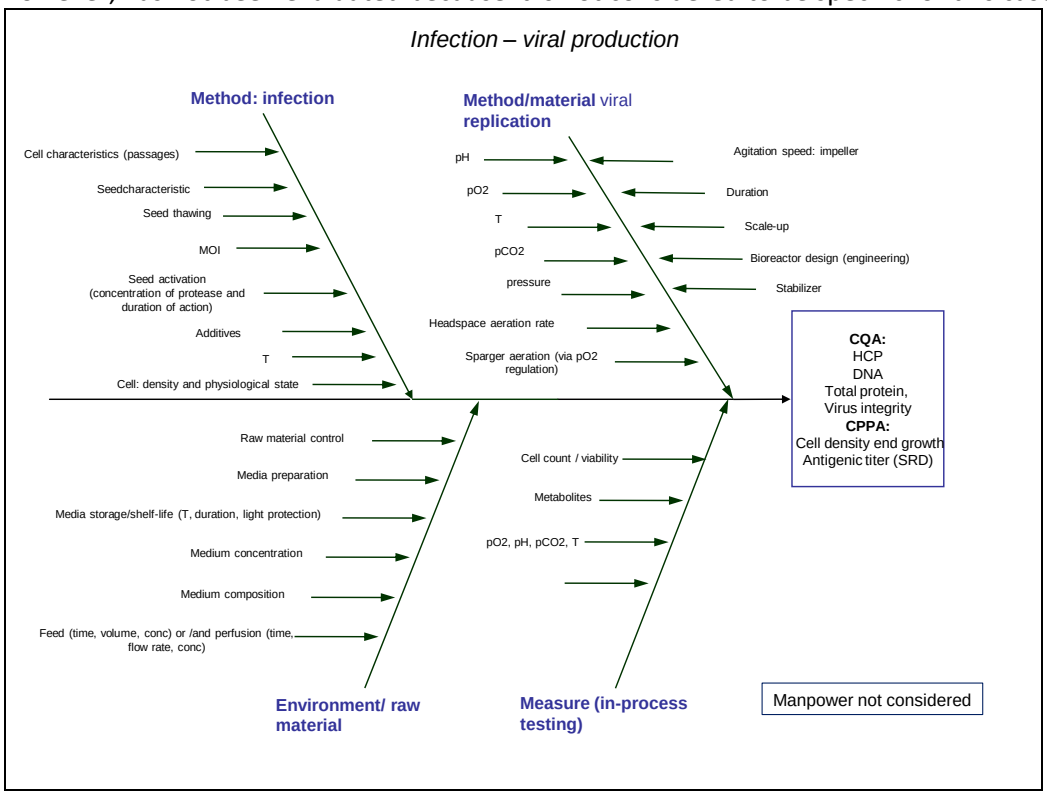
10.6. Process Risk Assessment at the End of Phase II

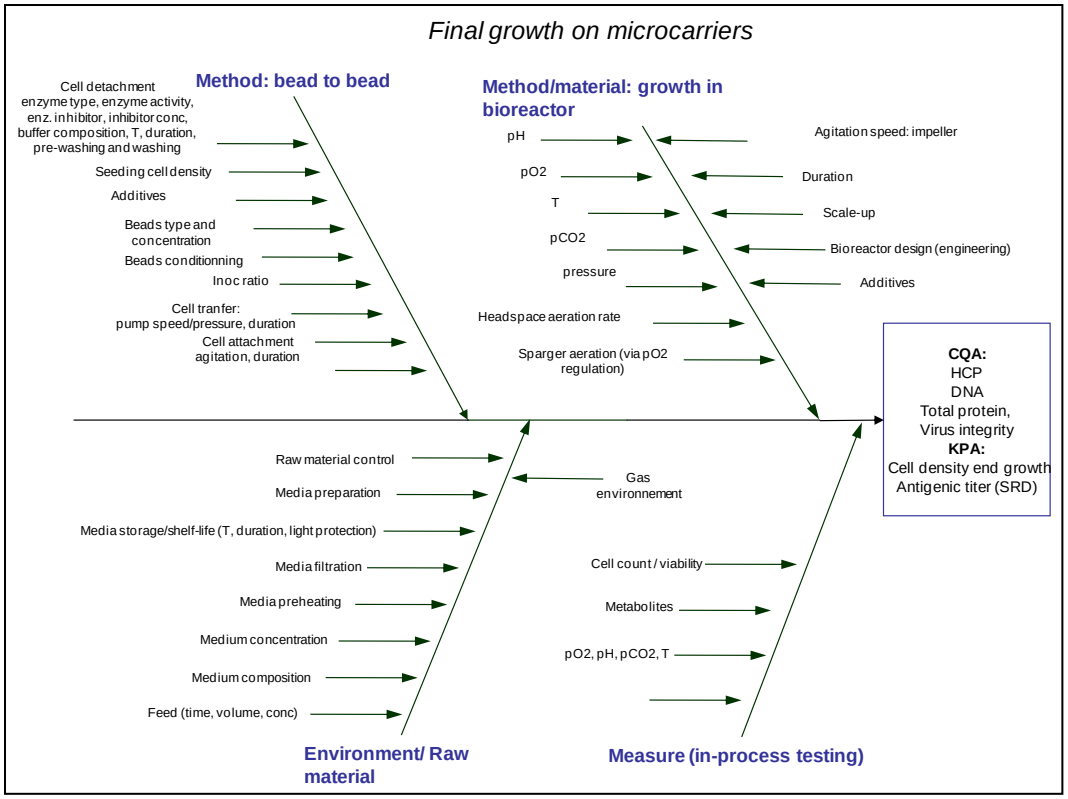
QbD is a continuous approach, and risk assessments will be performed all along the process. At the end of Phase II, the risks are based on the Phase II process and anticipation of the risks resulting from scale-up. The risk assessment will be repeated when the final-scale phase III process is developed.

10.6.1. Identification of High-Risk Process Parameters (Phase II 200 L Scale)

10.6.1.1. Lists of Parameters for Growth and Infection

First, all parameters of the final cell growth and infection/viral production steps having a potential impact on the CQAs and KPAs have been listed using the fishbone matrix. Manpower, however, has not been evaluated because it is not considered to be specific for this case study.





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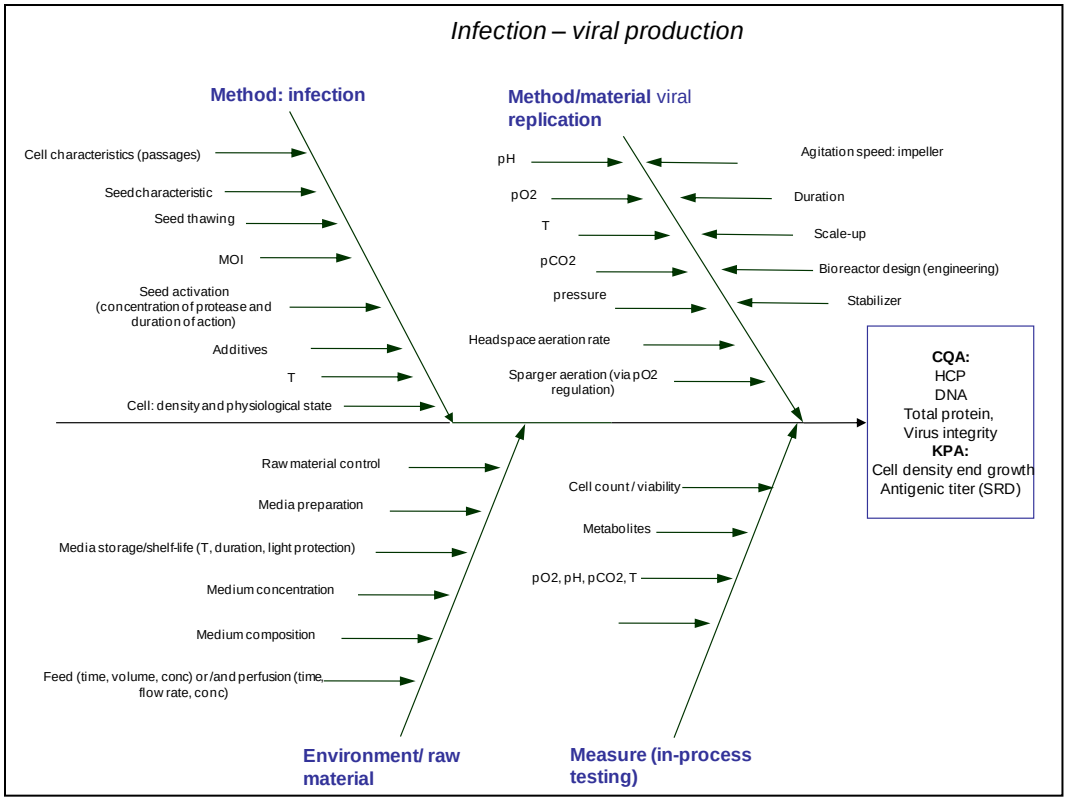

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10.6.1.2. Identification of Potential Critical Parameters

The tool chosen for this identification is called the FMEA approach (failure modes and effect analysis). The process parameters are ranked based on the RPN (Risk Priority Number). In this example of a process design phase, the knowledge is rated in the RPN to improve process understanding to assure a greater process robustness and manufacturability. The RPN is the product of four scores:

RPN = Severity * Knowledge * Occurrence * Detection

The severity factor itself is the product of the impact of a process parameter on the critical attributes and the criticality of the attributes. Higher scores for severity and knowledge were used than for occurrence and detection because:

- RPN places less emphasis on the occurrence, which may not always be scored reliably because of the limited number of data sets available at the end of phase II.
- Detection still largely relies on in-process and release testing.

Each step of the FMEA is described hereunder.

Step 1: Scoring of process outputs

Each CQA and KPA will be scored according to the following table.

Rank / Weight	Critical Quality Attribute (CQA)	Key Process Attribute (KPA)
10	Established or expected direct relationship to product quality (safety or efficacy)	
5	No knowledge on the impact product quality (safety or efficacy)	High supply issue or discontinuity, business loss
3		Significant production delay, high cost impact, rejection of product
1	No product quality (safety or efficacy) impact expected	No consistency impact expected

Step 2: Cause-and-effects matrix for severity calculation (S)

For the two unit operations selected (cell growth and infection/viral production), all process parameters are listed and scored according to their relationship with the CQAs and KPAs (see following table).

Rank / Weight	Input Process Parameter to CQA or KPA
10	Strong relationship known
5	Unknown relationship/ weak
1	Known to not have a relationship

Only negative and theoretical impacts should be considered (and not based on knowledge of the process). The impact should also be evaluated within the conditions usually encountered. After this scoring, a severity S factor will be calculated according to the formula blow:

$$S = \frac{\sum(\text{"CQA or KPA scoring"} \times \text{"process parameter/CQA or KPA relationship scoring"})}{\text{"number of CQA or KPA"}}$$

This severity score is finally normalized to obtain a final S score of 10, 7, 4, or 1.

Step 3: FMEA

In this step, the S' score of each process parameter will be modulated to manage and decrease the "potential" risk. Three different modulation levels exist:

Level 1: Knowledge scoring (L)

1	High	DOE/OFAT. Evaluations are "fit to purpose" (OFAT choice is justified). "Strong" bibliography or commercial retrospective data. = Existing Whole Design Space
5	Medium	Incomplete data/view (ex: Monovariate experiments performed for parameters needing a multivariate approach or retrospective commercial data) → OFAT for interdependent parameters = Incomplete Design Space
10	Low	Low Knowledge. Absence of data or few data, which do not allow conclusion. = No Design Space

Level 2: Occurrence scoring (O)

5	High	**No historical data**(? 30 batches) **Commercial/historical data: frequency (OOS, OOC, RD …)** X ? 3 %
3	Medium	**Commercial/historical data: frequency (OOS, OOC, RD …)** 1% ? X ? 3 %
1	Low	**Commercial/historical data: frequency (OOS, OOC, RD …)** X ? 1 %

OOS: out of specification
OOC: out of consistency
RD: deviation report

It has been decided to score all relationships with an occurrence of 1 so as not to create an artificial difference since the historical data available for all parameters is more or less equivalent.

Level 3: Detection scoring (D)

1	High	**Input= *relevant*control (alarm, device control, check on due time) + PAT, OR** **output = real-time detection, alarm and method of measurement variability:** X ? 10 %
3	Medium	**Input = *control with an appropriate measurement variability*, OR** **output = no real-time detection and method of measurement variability:** 10 ? X ? 20 %
5	Low	**Input= no control of theinput norcontrol on due time, OR** **output = method of measurement variability:** X > 20 %

Finally, a Risk Priority Number (RPN = S' x L x O x D) is calculated and will classify the input process parameters according to criticality.

All this information is gathered in the FMEA table, which is presented on the next page

OUTPUT PARAMETER LABEL		cell density end of growth	Antigenic titer	total protein	virus integrity					
LINK TO (CQA/CPPA)		KPA	KPA	CQA	CQA					
SCORE		5	5	5	10					
INPUT PARAMETER LABEL	FAILURE MODE					S"	L	D	O	RPN"
Growth										
pH	low negative impact outside range	10				10	10	1	1	100
daily glucose addition (concentration)		10				10	10	1	1	100
daily glutamine addition (concentration)		10				10	10	1	1	100
inoculation cell density	Lower: -> yield impact Upper: medium faster depleted	10				10	1	3	1	30
agitation speed (rpm)	mixing study done at 200L scale	10				10	1	1	1	10
incubation temperature	well defined for phase I-II process	10				10	1	1	1	10
air flow in head space (L/min)		1				1	5	1	1	5
media preheating T	Media preheated between 35 and 39°C	1				1	1	1	1	1
transfer time of preculture suspension	well controlled range no impact observed within range	1				1	1	1	1	1
final volume (L)	Weight measure	1				1	1	1	1	1
pO2 (%)	phase I/II showed no impact between 10-30 %. No issue to regulate at 20 % at 200 L.	1				1	1	1	1	1
culture duration		10				10	10	1	1	100
Infection										
cell density end of growth			5	5	1	4	5	3	1	60
pH			5	1	1	4	10	1	1	40
pO2 (%)	phase I/II showed no impact between 10-30 %. No issue to regulate at 20 % at 200 L.		1	1	1	1	1	1	1	1
seed activation by serine protease (concentration)	Scoring for lower failure mode		10	5	1	7	10	1	1	70
seed activation duration by serine protease			10	5	1	7	10	1	1	70
Multiplicity of Infection	Low: risk of virus degenerescence High: lower yield		10	5	5	10	5	1	1	50
daily serine protease addition (activity)	Scoring for lower failure mode		10	5	1	7	5	1	1	35
infection duration			5	1	5	7	5	1	1	35
daily glucose addition (concentration)			5	10	1	7	5	1	1	35
daily glutamine addition (concentration)			5	10	1	7	5	1	1	35
infection temperature			5	5	5	7	1	1	1	7
working seed thawing temperature	Thawing procedure defined		1	1	1	1	1	1	1	1
agitation speed (rpm)	mixing study done at 200L scale		5	5	1	4	1	1	1	4
air flow in headspace			1	1	1	1	1	1	1	1
working seed thawing duration	Thawing procedure defined		1	1	1	1	1	1	1	1

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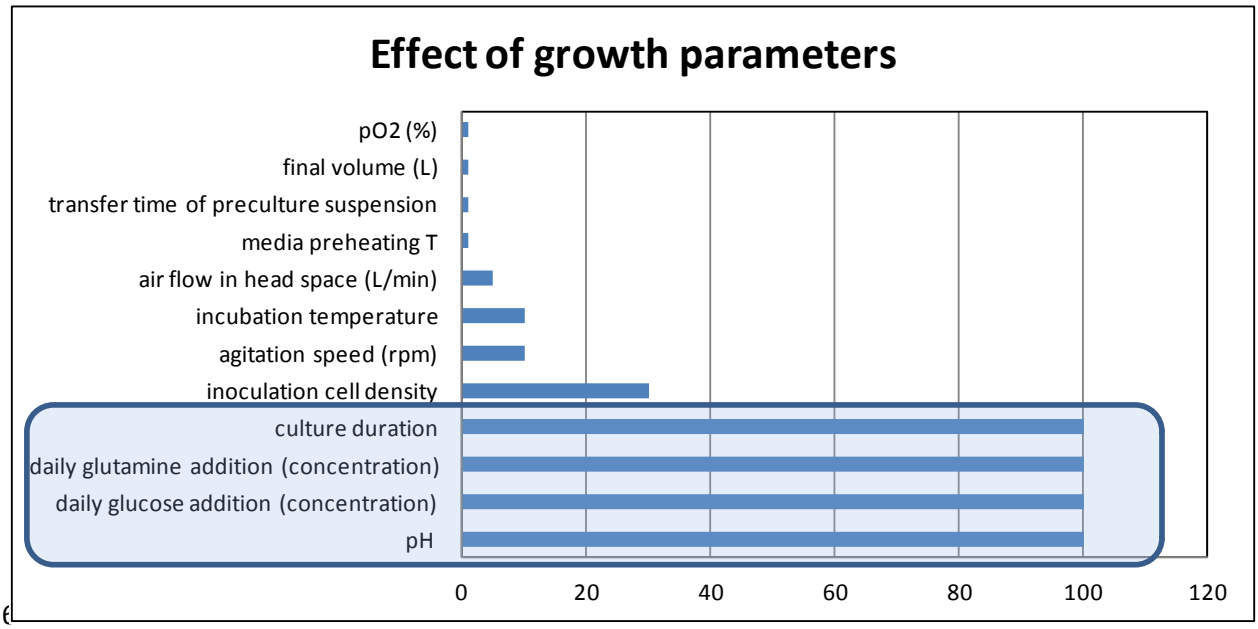
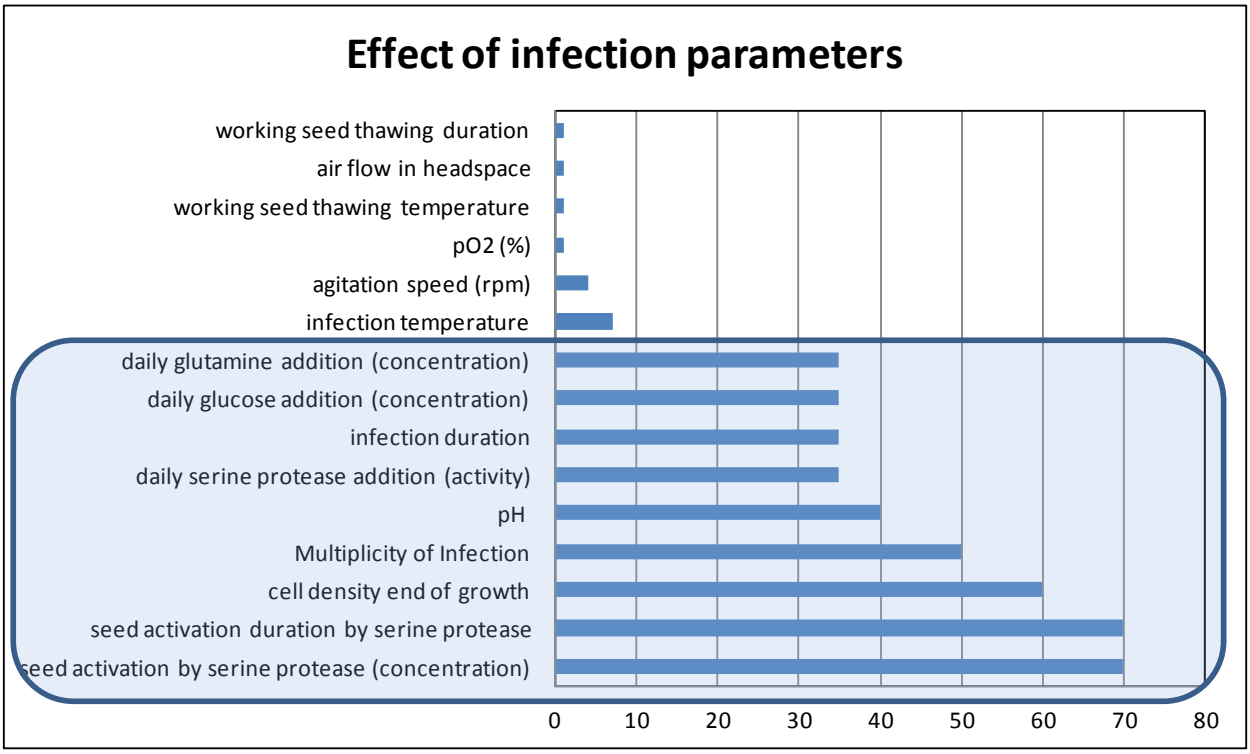
To select the critical parameters of each operation unit, all process parameters are ranked according to the RPN. The potential critical parameters are associated with those bars that are "exceptional signals" compared with the other bars considered as "noise signals." Bars associated with noise increase uniformly (like a staircase), while bars associated with a signal increase significantly in magnitude (like a wall). The critical parameters selected are highlighted in the blue boxes in the figures below.





Four growth parameters (duration, glutamine and glucose concentrations, pH) and nine parameters for infection (glutamine and glucose concentrations, infection duration, activation

by serine protease activity and duration, pH, MOI, cell density at end of growth, daily concentration of serine protease) are identified as high-risk process parameters from the FMEA analysis and will be further studied.

10.6.2. Identification of Phase III Scale-up Risks

The phase III development includes the final optimization of the process and the scale-up to the final process scale (2,000 L bioreactor).
The critical parameters identified in previous section are scale independent and will be studied at a scale-down model at 10L (section VII.B).
However, some additional parameters should also be studied to cover a successful scale-up of the process:

- Media preparation and filter size.
- Media stability.
- Implementation of a bead-to-bead cell passage required between the 200 L and the 2,000 L bioreactor (not described in this case study as it is part of prior knowledge).
- Addition of a final expansion step in a 2,000 L bioreactor (dynamic conditions) to reach the final biomass and infection at 2,000 L with the constraints linked to the scale-up.
- Scale-up of agitation: described in section VII.
- Addition of a shear protective additive (see section VIII).

10.7. Scale-up and Scale-down Models

An appropriate scale-up should assure that the process performances are similar at all scales. One specificity of adherent cell lines grown on microcarriers, in animal-free media, is their shear sensitivity. Therefore, the mixing is probably the biggest challenge for the scale-up to 2,000 L scale, justifying the rationale for its description in this section. An inadequate scale-up would impact the CQA and KPA (e.g., a higher shear at large scale could affect the cell density, the protein content in the harvest, and the antigenic titer).

Geometrical similarity is maintained for the design of the bioreactors from bench to pilot and manufacturing scales. That means that they all have the same shape, one being a uniform scaling (enlarging or shrinking) of the others (i.e., the ratio of all corresponding dimensions is equal).

10.7.1. Scale-up of Mixing

10.7.1.1. Maintain Microcarriers in Suspension

Microcarriers must stay in suspension during culture. The minimal speed required to maintain them in suspension follows the Corpstein law:

Equation 10-1: Minimal Agitation Speed to Suspend Microcarriers

$$N_{\min} = k.\left(\frac{\rho_s - \rho_l}{\rho_l}.v_{chute}\right)^{0.3}.f(d/D).(D/D0)^{-n} \; with: 0.5 < n < 1$$

Where:

Nmin = minimal agitation speed to suspend microcarriers [rps]

k = constant depending on the agitator type and the microcarriers' concentration

ρ = density (ρ_s for microcarrier, ρ_l for liquid) [kg/m³]

v_{chute} = microcarriers' settling speed [m/s]

d = impeller diameter [m]

D = vessel diameter [m]

f(d/D) = coefficient depending on the impeller type and diameter

D/D0 = ratio of vessel diameter at the two scales studied (scale factor)

A security factor of 10% is taken on the minimal required speed to take into account the accuracy of the Corpstein relation.

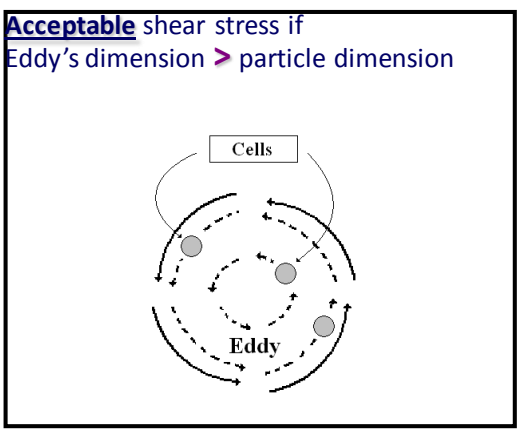
10.7.1.2. Liquid Homogeneity

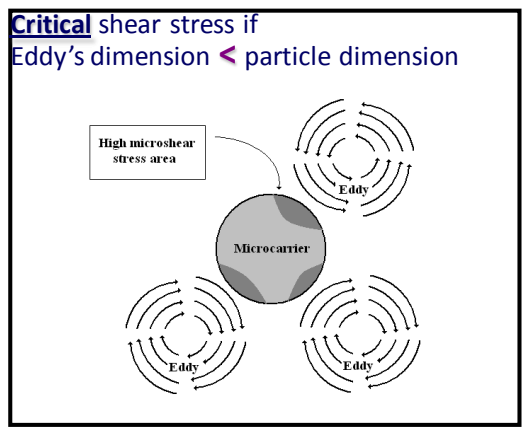
The mixing time is a good indicator of liquid homogeneity. For vessels in geometric similarity and in a turbulent hydrodynamic regime, the mixing time (tm) is maintained constant if the agitation speed (N) is conserved at all scales.

$$N\ tm = cst$$

10.7.1.3. Shear

The shear is much more detrimental for cells grown on microcarriers than for cells in suspension. Indeed, in turbulent flows, eddies are formed in the liquid. Larger eddies transfer their kinetic energy to smaller ones. These small eddies end up by dissipating their kinetic energy into heat. The cells are affected if their size (for cells in suspension) or the size of the microcarriers (for adherent cells) is of the same order of magnitude as the smallest eddies. The size of the smallest eddies depends on the specific volumetric power (P/V) input; high P/V leads to very small eddies and potentially more cell damage.





Suspension **cell culture**
- **➔ small particles (cells)**
- **➔ small microeddies acceptable**
- **➔ *high P/V* is acceptable**

Microcarrier **cell culture**
- **➔ big particles (microcarriers)**
- **➔ only big microeddies acceptable**
- **➔ *P/V becomes critical according to this theory***

Numeric exemple :

- Cell diameter ➔ ≈ 20 µm (=λ_1) = microeddies' critical dimension
- Microcarrier diameter ➔ ≈ 200 µm (=λ_2) = microeddies' critical dimension

$$\lambda = \left(\frac{\rho \cdot \upsilon^3}{P/V}\right)^{1/4} \Rightarrow \frac{\lambda_1}{\lambda_2} = \left(\frac{(P/V)_2}{(P/V)_1}\right)^{1/4} = 0.1 \Rightarrow \frac{(P/V)_1}{(P/V)_2} = 10\,000$$

$$P/V = N_P \cdot \rho \cdot N^3 \cdot d^5 \Rightarrow \frac{(P/V)_1}{(P/V)_2} = \left(\frac{N_1}{N_2}\right)^3 \Rightarrow \frac{N_1}{N_2} \approx 20$$

Where:

λ = particle diameter [m]

ρ and ν = fluid density and viscosity [kg/m³] and [m²/s]

P/V = volumetric power dissipated in the liquid [W/m³]

Np = power number (characteristic of the impeller, constant in turbulent regime)

N = agitation speed [rps]

d = impeller diameter [m]

=> In the same mixing configuration, cells in suspension can be agitated 20 times faster than cells on microcarriers without damage!

The scale-up criteria to reproduce the same eddy sizes at various scales would be to keep a constant volumetric power (P/V).

In turbulent flow, the volumetric power is calculated by:

$$P/V = N_P \cdot \rho \cdot N^3 \cdot d^5$$

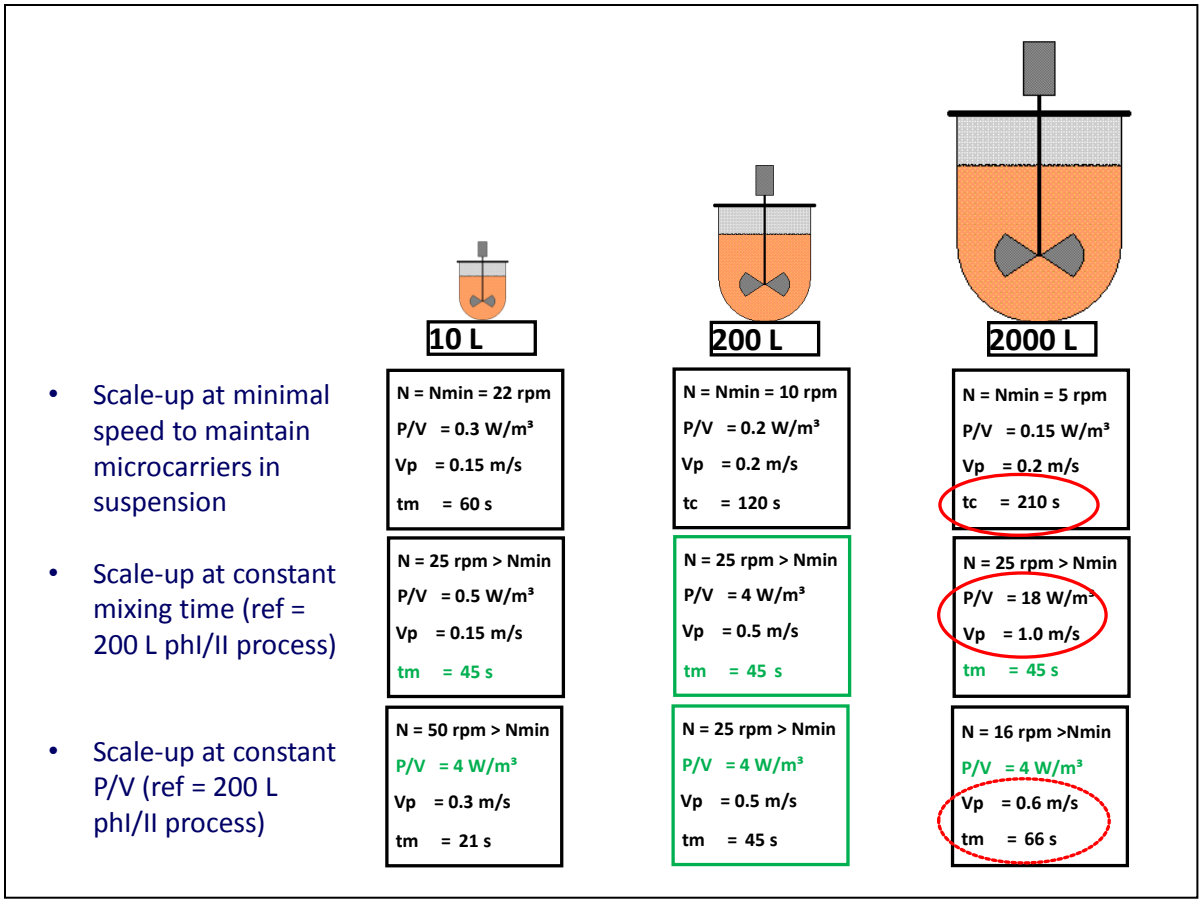
P/V is a good indicator of the mean shear. On the other hand, the maximal shear, produced at the edge of the impeller, can be correlated to the tip speed (vp = Π.d. N).

10.7.1.4. Mixing Scale-up Strategy

Three scale-up strategies are compared:

- Agitation fixed at each scale to maintain microcarriers in suspension
- Agitation calculated to achieve same mixing time at all scales
- Agitation kept at constant volumetric power

The starting point is the 200 L bioreactor operated at 25 rpm (phase I/II process).



The scale-up at the minimal speed to get microcarriers suspended is not optimal because the mixing time is increasing at large scale. Local nonhomogeneities — for example, during pH adjustment with base — could affect the cells.

The scale-up that keeps the mixing time constant (i.e., assuring the same liquid mixing efficiency) requires more power per volume at large scale. This can lead to cell damage and poor growth.

The preferred option is to perform the scaling-up at constant volumetric power. The starting point is the 200 L bioreactor. When the bioreactor is scaled up to 2,000 L, the agitation speed is fixed at 16 rpm; the tip speed (maximal shear) and the mixing time are only slightly increased.

10.7.2. Scale-down Models

Process optimization and process validation can be done to some extent at small scale. The lab bioreactors have a capacity of 10 L and are similar in geometry to the 200 L and 2,000 L bioreactors. The scale-up is performed at constant power per volume (4 W/m³). The corresponding speed at 10 L scale is 50 rpm. This is above the minimal speed required to maintain the microcarriers' suspension. There is no impact of the scale on the CQAs and KPAs as shown here.

Summary of process and quality attributes for small scale and 200 L scale for the phase II process:

	10 L scale	200 L scale
CQA		
Protein content (g/L)	1.37	1.33
Virus integrity (%)	84	80
KPA		
Antigenic titer (µg/ml)	91	97
Cell density end of growth (cells/ml)	$2.6\ 10^6$	$2.8\ 10^6$

The performances of the process at the 2,000 L manufacturing scale will be shown after the DOE section, on the Phase III optimized process.

10.8. Strategy for Phase III Process Optimization

10.8.1. OFAT Analysis

For some factors, an OFAT approach (one factor at a time) is appropriate for process understanding and/or optimization. The factors described here are investigated in univariate studies.

- Media preparation – filter resizing:
  - Increase of filtrated medium volume per filtration area for the scale-up from 200 L to 2,000 L to avoid an oversized area for the 2,000 L scale.
  - Tested on cell growth and viral production steps.
- Media stability – shelf life:
  - Powdered basal medium tested for two years to meet commercial constraints.
  - Rehydrated media tested for two months and validated for a one-month expiration that is in line with commercial-unit constraints.
  - Tested on cell growth and viral production steps.
- Addition of an additive to avoid cell damage at 2,000 L scale. The influence of the nontoxic additives on several physicochemical parameters such as k_la, foam, and bubble coalescence was studied. Six additives were screened for their physicochemical properties. Results are summarized in the following table:

Additives	Concentration [% w/w]	Toxicity	Surface tension [mN/m]	Bubble coalescence [%]	k_la impact [min^{-1}]	Foam
Water (reference)			73	10	0,06	Reference
Additive 1	0.05 0.1 0.15	OK TOX TOX	63 63 63	8 9 9	≈ + ++	More stable
Additive 2	0.05 0.1 0.15	LTOX LTOX TOX	62 62 62	0 0 13	- ++ ++	More stable
Additive 3	0.05 0.1 0.15	OK TOX TOX	63 62 62	0 5 20	≈ ≈ -	More stable
Additive 4	0.05 0.1 0.15	OK OK LTOX	68 68 68	18 21 17	+ ++ ++	Less stable
Additive 5: poor solubility	0.05 0.1 0.15	OK OK LTOX	/ / /	/ / /	- - - - - - - - -	Much more stable
Additive 6	0.05 0.1 0.15	OK OK OK	60 59 57	-30 0 14	- - - - - - - -	Much more stable

TOX=significant cytotoxic effect – LTOX = low cytotoxic effect

Additives 4 and 6 were further studied in culture at a concentration of 0.1%. Agitation and aeration were increased to highlight a potential shear-protecting action of these two additives during cell growth. The growth was monitored according to cell count and LDH assay in the supernatant. Additive 6 was confirmed as the best shear protector against agitation and aeration and thus selected for the 2,000 L process.

10.8.2. DOE Analysis

The high-risk process parameters selected in a risk assessment (FMEA; see Section VI.B) are investigated in a multivariate study. The growth phase and the virus production phase will be studied in the same DOE. The experiments are performed at 10 L scale; the scale-down models were qualified as representative of the final process scale (Section VII.B). Twelve parameters stand out in the risk assessment (the cell density at the end of the growth phase will be considered as an output of growth phase and not as a parameter).

The design of a model with 12 parameters would require overwhelming work; therefore, parameters are first tested in a screening study. The significant parameters are then further studied in a response surface design to establish the mathematical relationship between the process parameters and the critical attributes.

The process expectations are defined in terms of acceptable ranges of the critical attributes (CQAs and KPAs) detailed in the table below. The table also mentions the analytical variability of the tests.

	Target	Acceptable range	Analytical variability
Total protein	1.2 g/L	< 1.5 g/L	10%
Virus integrity	80%	> 70%	20%
Antigenic titer	100 µg/ml	> 80 µg/ml	10%
Cell density end of growth	3.0 10^6 cell/ml	2.5 10^6–3.5 10^6 cell/ml	20%

10.8.2.1. Screening for High-Risk Process Parameters

The Folded Plackett & Burman design is performed to select from the 12 high-risk process parameters those having a significant impact on the CQAs and KPAs. The factors are tested in a Minimum Run Equi-replicated Resolution IV Screening Design.

In this design, each factor is varied over only two levels. The resolution IV design allows estimation of main effects in a linear model while two-factor interactions will be aliased with other two-factor interactions. In this study, the testing of the 12 factors requires 26 experiments.

The 26 experiments are performed at 10 L scale. The investigated ranges of the parameters are fixed based on phase II process setpoints and knowledge built during phase II process development.

Parameters	Setpoint Ph II process	Range DOE
Growth		
. pH		6.8
. Daily glucose feeding		1–3
. Daily glutamine feeding	2	2–4
. Duration	5 days	4–6
nfection		
. pH		6.8
. Multiplicity of Infection	10^{-3}	10^{-3}
. Virus activation: activity of serine protease	100 IU/ml	50–200 I
. Virus activation: contact duration of serine protease	30 min	15–60

Time of harvest	5 days	3–6 days
. Daily addition of serine protease	2 IU/ml	1–10 IU/ml
Daily glucose feeding	1 g/l	1–3.0 g/l
1. Daily glutamine feeding	2 mM	2–4 mM

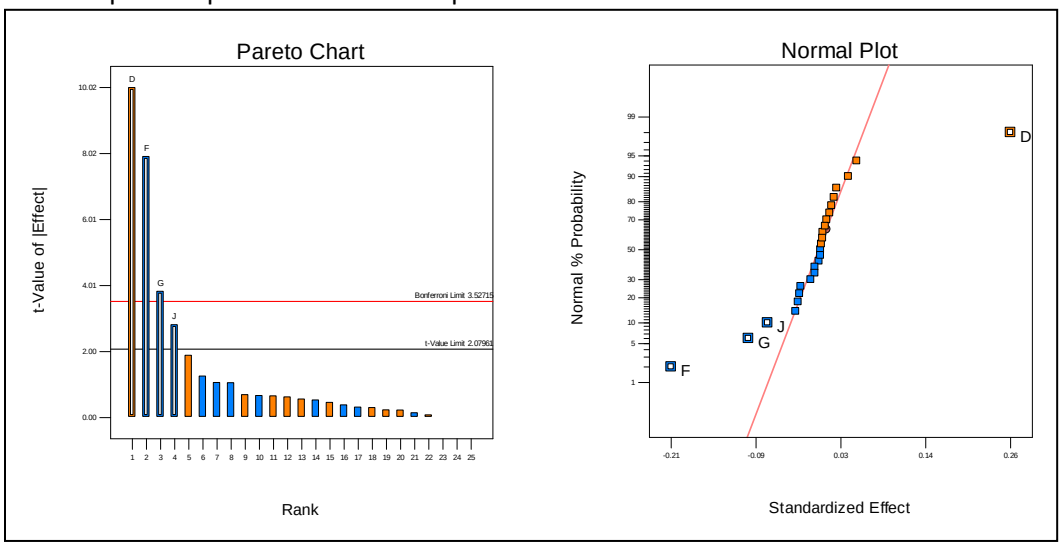
The results of the experiments were statistically analyzed. The first important finding is that out of the 12 potential high-risk process parameters, collectively five had a significant effect on the CQAs and KPAs: growth duration, multiplicity of infection, activity of the serine protease at the activation step, concentration of the daily addition of serine protease, and duration of infection. This is represented graphically in Pareto charts and normal plots.

In the Pareto chart, the effects and the interactions are ranked by decreasing amplitude of significance based on a Student t-test. The parameters above the black horizontal line are each significant at the 95% confidence level.

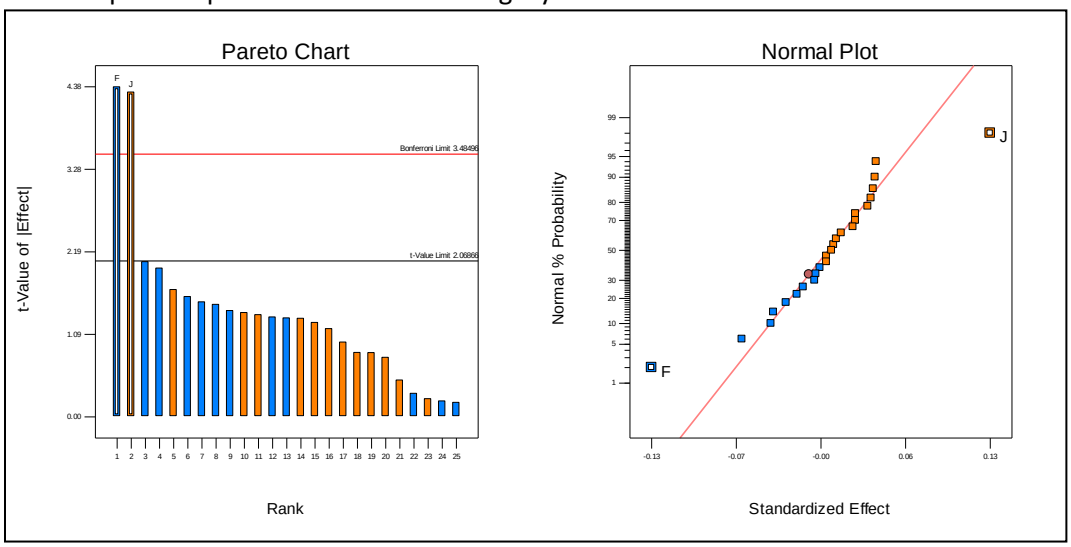
In the normal plot graph, the nonsignificant parameters should be distributed as noise and should be aligned in the Gaussian arithmetic scale. Significant effects are highlighted out of the line.

For both types of graph, the significant positive effects between a process parameter and a critical attribute are plotted in red, and the negative effects are plotted in blue.

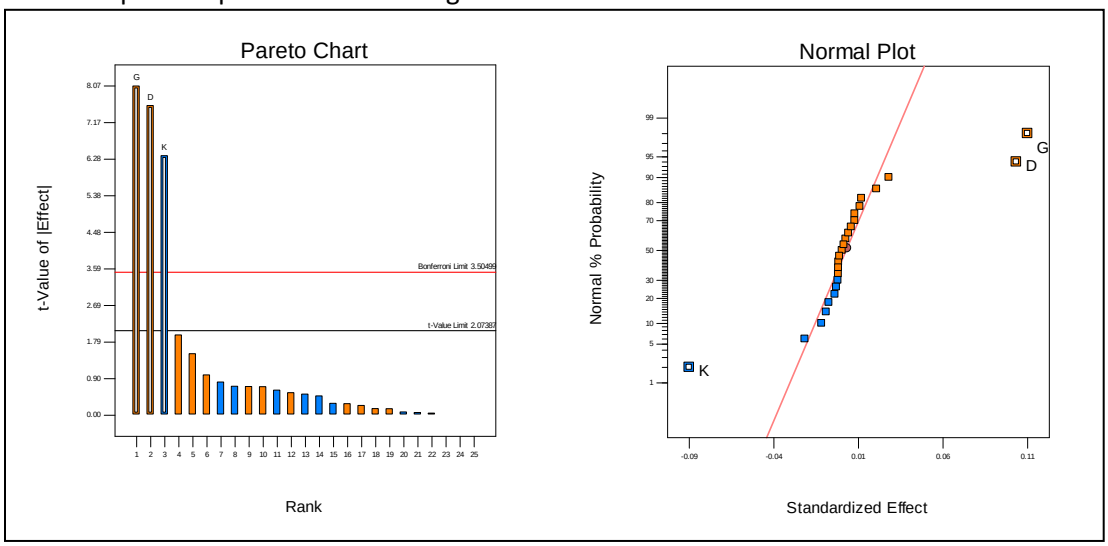
Effect of process parameters on total protein:



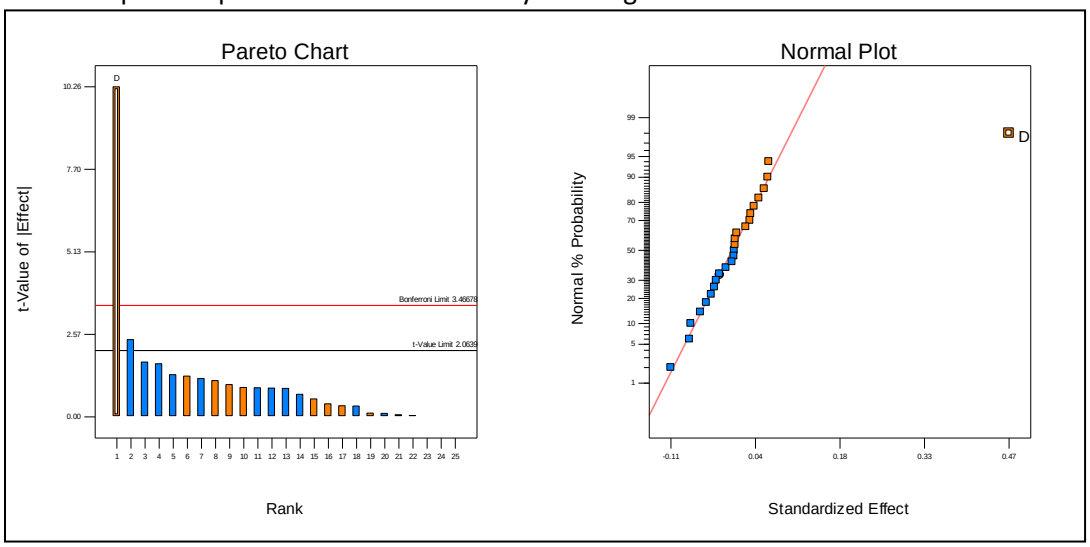
Effect of process parameters on virus integrity:



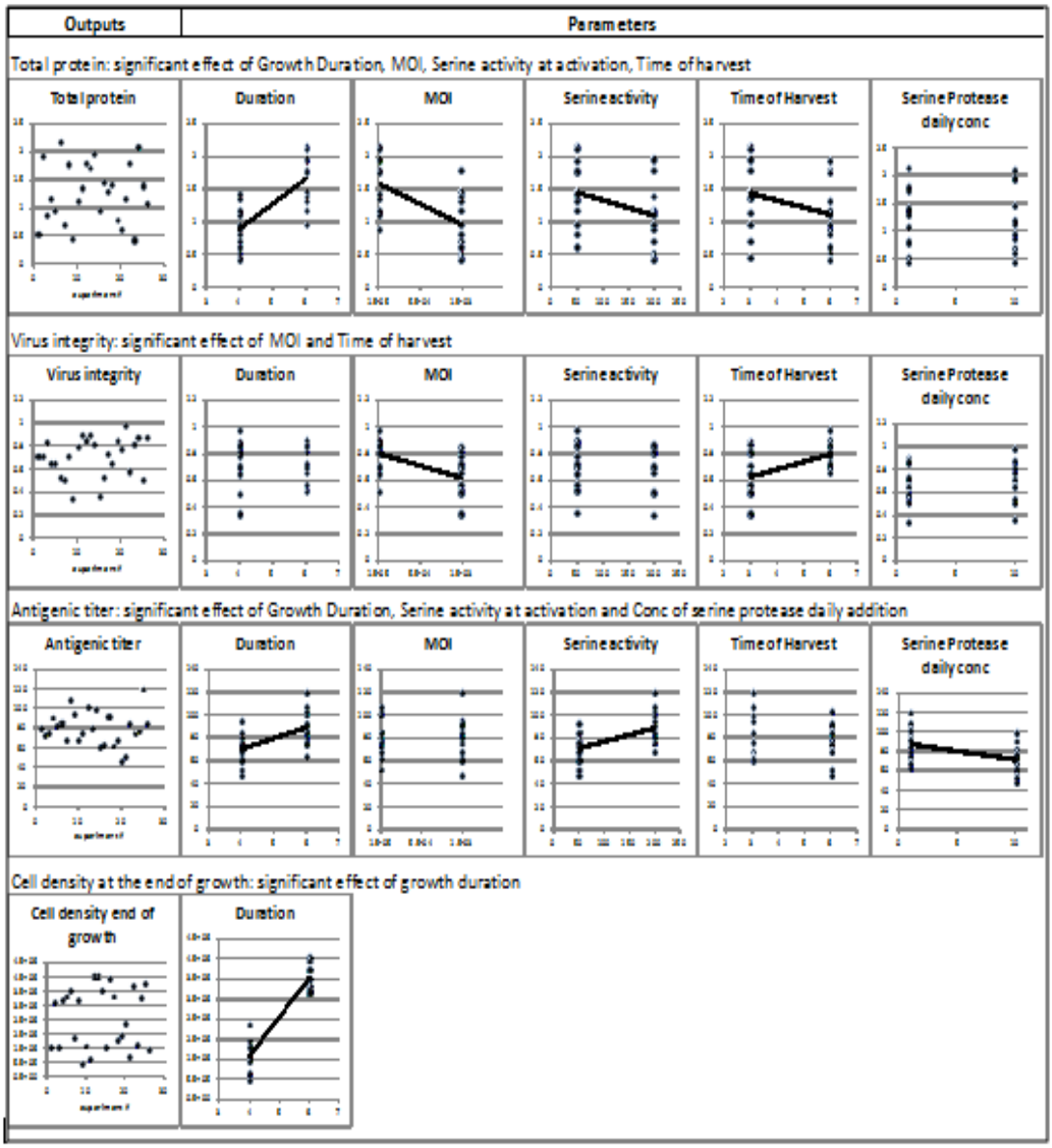
Effects of process parameters on antigenic titer



Effects of process parameters on cell density end of growth



The effects of the five significant process parameters are represented in the following graphs. For each critical attribute (total protein, virus integrity, antigenic titer, and cell density), a first graph shows the distribution of the values of the critical attributes for the 26 experiments. The data are then sorted by process parameters, each of which was tested in the DOE at two levels. If an effect is statistically significant, a linear trend links the process parameter and the critical attribute, showing the amplitude of the effect.



10.8.2.2. Response Surface for Process Optimization

The screening study identified five parameters having a significant effect on the CQAs and KPAs: growth duration, multiplicity of infection, activity of the serine protease at the activation step, concentration of the daily addition of serine protease, and duration of infection. The effects of those five process parameters are further studied using the Response Surface Methodology (RSM). With the RSM, the responses of interest are expressed as a second-order polynomial function of all the process parameters and their interactions; it will allow prediction of responses in the whole studied domain.

Five-factor, 29-run, face-centered central composite design is used, each factor being varied over three levels; it requires 29 experiments. The cultures are performed in 10 L bioreactors. The screening study has already shown the trends for the impact of the critical process

parameters on the critical attributes; consequently, the ranges of some parameters are adapted for the surface response DOE to achieve better performance. The upper limit of the multiplicity of infection was increased; the activity of the serine protease at viral activation was focused on high values, while the activity for the daily addition of serine protease was moved toward lower values.

Parameters	**Setpoint Ph II process**	**DOE range**
Growth		
Duration	5 days	4–6 days
Infection		
Multiplicity of Infection	10^{-3}	10^{-5}–10^{-2}
Virus activation: activity of serine protease	100 IU/ml	100–200 IU/ml
Time of harvest	5 days	3–6 days
Daily addition of serine protease	2 IU/ml	0.3–5 IU/ml

The results of the 29 experiments were statistically analyzed. The significant effects of the process parameters on the responses are shown by the p-value tables. A table is created for each response (i.e., critical attribute). The process parameters are listed in the lines and columns. The diagonal of the table represents the significance of the parameters on the selected response (first-order linear effect and second-order quadratic effect). The cells above the diagonal represent the significance of the interactions between two parameters on the response.

Significant effects are in red if the effect or synergy is positive (positive contribution to the output when variables are increasing) and in blue if the effect or synergy is negative (negative contribution to the output when variables are increasing). The threshold for a statistically significant effect is p-value <0.05.

Coefficients p-values from ANOVA (analysis of variance), for single effects (linear and quadratic), and two-way interactions.
Positive effect/synergy in the studied range
Negative effect/antagonism in the studied range

Total Protein

	Duration	MOI	Serine protease activity	Time of harvest	Conc. daily serine protease
Duration	lin. <0.001 Quad. 0.019	0.236	0.431	0.049	0.564
MOI		lin. <0.001 Quad. 0.09	0.101	0.164	0.194
Serine protease activity			lin. 0.004 Quad. 0.021	0.043	0.298
Time of harvest				lin. 0.035 Quad. 0.071	0.094
Conc. daily serine protease					lin. 0.034 Quad. 0.028

Virus integrity

	Duration	MOI	Serine protease activity	Time of harvest	Conc. daily serine protease
Duration	lin. 0.234 Quad. 0.453	0.462	0.241	0.497	0.378
MOI		lin. 0.01 Quad. 0.15	0.131	0.664	0.632
Serine protease activity			lin. 0.354 Quad. 0.575	0.369	0.564
Time of harvest				lin. 0.464 Quad. 0.069	0.697
Conc. daily serine protease					lin. 0.294 Quad. 0.642

Antigenic titer

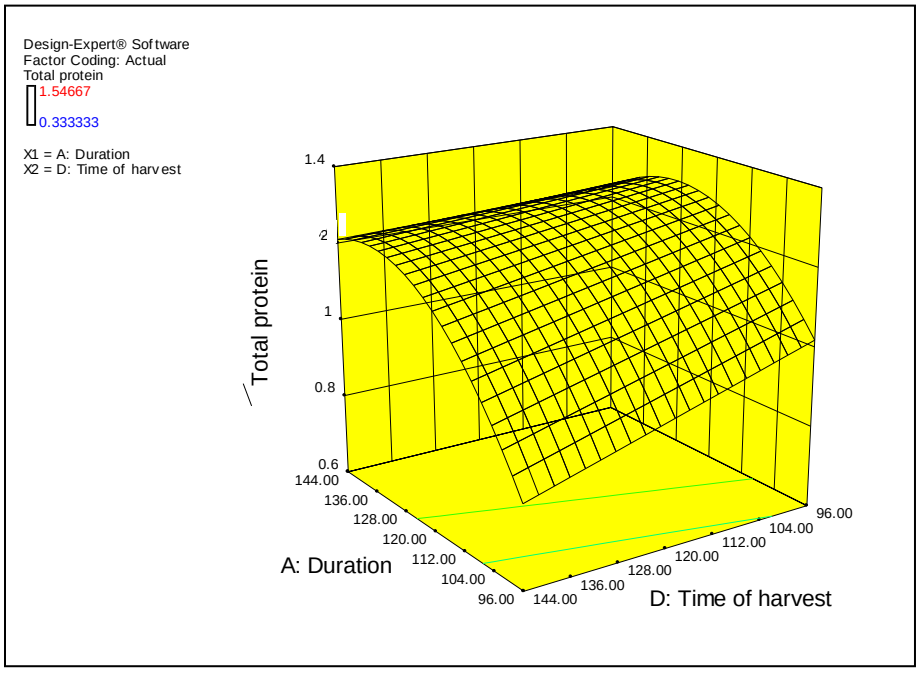
	Duration	MOI	Serine protease activity	Time of harvest	Conc. daily serine protease
Duration	lin. <0.001 Quad. <0.001	0.128	0.234	0.151	0.324
MOI		lin. <0.001 Quad. <0.001	0.043	0.037	0.049
Serine protease activity			lin. <0.001 Quad. 0.043	0.021	0.303
Time of harvest				lin. 0.621 Quad. 0.324	0.013
Conc. daily serine protease					lin. 0.033 Quad. 0.019

Cell density end of growth

	Duration	MOI	Serine protease activity	Time of harvest	Conc. daily serine protease
Duration	lin. 0.03 Quad. 0.07	NA	NA	NA	NA
MOI		NA	NA	NA	NA
Serine protease activity			NA	NA	NA
Time of harvest				NA	NA
Conc. daily serine protease					NA

3310

The relation between process parameters and critical attributes can be plotted in surface response graphs, as seen in the following figure example:



The response surfaces can be visualized for all critical attributes in function of two process parameters. Other examples of surface responses are shown in the design space section of this chapter. The phase III process setpoints were redefined based on the DOE results.

Parameters	**Setpoint Ph II process**	**Optimum setpoint Ph III**
Growth		
Duration	5 days	5 days
Infection		
Multiplicity of infection	10^{-3}	10^{-4}
Virus activation: activity of serine protease	100 IU/ml	200 IU/ml
Time of harvest	5 days	5 days
Daily addition of serine protease	2 IU/ml	1 IU/ml

10.8.3. Phase III Process Validation at Final Scale

The process optimizations were performed at 10 L scale. The DOE allowed the team to optimize the process and to predict the critical attributes at the reference conditions (Phase III process setpoints):

	Parameter	Phase III
Process	Duration (h)	120
	MOI (-)	10^{-4}
	Serine protease activity at activation (IU/ml)	200
	Time of harvest (h)	120
	Cc daily addition serine protease (IU/ml)	1
Responses	Total protein (g/L)	1.1
	Virus integrity (%)	88
	Antigenic titer (µg/ml)	104
	Cell density end of growth	3.2E+06

These optimizations were implemented at the 200 L and 2,000 L scales. No difference was observed within the scales.

Summary of process and quality attributes for the three scales for the phase III process:

	10 L scale	200 L scale	2,000 L scale
CQA			
Protein content (g/L)	1.13	1.17	1.22
Virus integrity (%)	90	82	84
KPA			
Antigenic titer (µg/ml)	103	96	107
Cell density end of growth (cells/ml)	$3.2\ 10^6$	$2.9\ 10^6$	$3.3\ 10^6$

10.8.4. Updated Process Description Based on Process Changes between End of Phase II and Final Process — Final Scale

Based on the OFAT and DOE optimizations and on the validation of those conditions at 2,000 L scale, the Phase III process is defined:

Parameter	Phase II (200 L)	Phase III (2,000 L)
Cell growth		
Cell passage	NA	**Bead-to-bead passage (200 L > 2,000 L)**
Cytodex concentration	3 g/L	3 g/L
Shear protective additive	-	**0.1%**
Seeding density	150.000 cells/ml	150.000 cells/ml
Temperature	37°C	37°C
pO_2	20%	20%
pH	7.2	7.2
Pressure	0.1 bar	0.1 bar
Stirring	25 rpm	**16 rpm**
Daily glucose adjustment	1 g/L	**2 g/L**
Daily glutamine adjustment	2 mM	**3 mM**
Growth duration	5 days	5 days
Viral infection		
Minimum cell density	$2.5\ 10^6$ cells/ml	**$3\ 10^6$ cells/ml**
Cytodex concentration	3 g/L	3 g/L
Temperature	33°C	33°C
pO_2	20%	20%
pH	7.2	7.2
pressure	0.1 bar	0.1 bar

Stirring	25 rpm	**16 rpm**
Daily glucose adjustment	1 g/L	**2 g/L**
Daily glutamine adjustment	2 mM	**3 mM**
MOI	10^{-3}	**10^{-4}**
Viral activation: serine protease activity	100 IU	**200 IU**
Viral activation: serine protease contact duration	30 min	30 min
Daily addition of serine protease: concentration	2 IU	**1 IU**
Viral replication duration	5 days	5 days

10.9. Design Space and Control Space

10.9.1. Critical Process Parameters

The design space must be determined in order to predict robust process conditions and demonstrate assurance of quality in the ICH definition of "design space": *"the multidimensional combination and interactions of input variables and process parameters (e.g. material attributes) that have been demonstrated to provide assurance of quality."*

The criticality of process parameters is reevaluated based on all the knowledge generated during phase III process development. The reevaluation uses a process risk assessment and takes into account the capability to control the process parameters.

In the following table, blank spaces indicate that parameters do not affect critical attributes in the ranges studied. Green and red denote parameters that affect critical attributes. Green indicates that capability of controlling the parameters is robust and effective. Red indicates that the range in which the parameters can vary before a CQA is potentially affected is close to the control capability.

Note that during Phase II process development, the optimal infection temperature was defined at 33°C, and this parameter did not come out of the risk assessment performed at the end of Phase II. Nevertheless, data available after Phase III process optimization suggested that the growth and the infection duration could interact with the infection temperature to affect antigenic titre.

Parameter	Quality attributes		Process attributes		Risk mitigation
	Total protein	Virus integrity	Cell density end growth	Antigenic titer	
Cell passage					Studied during Ph II development
Cytodex concentration					Studied during Ph II dvpt
Shear protective additive					OFAT study done during Ph III process optimization
Seeding density					Studied during Ph II dvpt
Temperature cell growth					Studied during Ph II dvpt
pO_2					Studied during Ph II dvpt
pH cell growth					DOE done during Ph III process optimization
Pressure					Studied during Ph II dvpt
Stirring					Scale-up based on Phase II dvpt
Daily glucose adjustment					DOE done during Ph III process optimization
Daily glutamine adjustment					DOE done during Ph III process optimization
Growth duration					**DOE done during Ph III process optimization. Potential interaction with induction T.**
Temperature infection			NA		**Studied during Ph II dvpt, range could be close to control capability. Potential interaction with process duration.**
pH infection			NA		DOE done during Ph III process optimization
MOI			NA		DOE done during Ph III process optimization. No interaction with other studied parameters.
Viral activation: serine protease activity			NA		**DOE done during Ph III process optimization. Ph III setpoint at the border of the studied range.**
Viral activation: serine protease contact duration			NA		DOE done during Ph III process optimization
Daily addition of serine			NA		**DOE done during Ph III process optimization.**

protease: concentration					**Range redefined around Ph III setpoint.**
Viral replication duration (time of harvest)			NA		**DOE done during Ph III process optimization. Potential interaction with induction T.**
Media preparation – filter size					OFAT study done during Ph III process optimization
Media stability – shelf life					OFAT study done during Ph III process optimization
Viral production media selection					Studied during Ph II dvpt

Since the multiplicity of infection has no interaction with other parameters (see DOE results during Phase III optimization), the limits of the design space for the MOI are fixed independently of the other process parameters. The design space for the MOI is fixed from 5 10^{-5} to 9 10^{-3}. Based on the Phase III optimization DOE results, within this MOI range, 95% of predicted future results will stay within specifications for CQAs and KPAs (Monte Carlo simulations).

Ideally the design space should be determined at the final step in a DOE combining all possible influent factors. But MOI has been studied in a pre-Phase III experiment and has shown a single effect pattern (not interacting with any other parameter). Its predicted impact (on pre-Phase III exp.) has been considered as an additive to all the further studied process parameters (post-Phase III exp.). It relies on a hypothesis (all possible interactions with MOI are negligible and its effect remains the same) that is considered reasonable from a theoretical point of view. This approach allows for making a profit from previously generated results, preserving them until final conclusions, without making experiments to retrieve the same information.

The other parameters will be studied in a DOE around the Phase III parameters' setpoints.

Parameter	Phase III process	DOE range
Duration (cell density end growth)	120 h	96–144 h
Temperature at infection	33°C	30–36°C
Virus activation condition (activity)	200 IU/ml	50–300 IU/ml
Daily addition of serine protease (activity)	1 IU/ml	0.3–3 IU/ml
Time of harvest	120 h	96 h to 144 h

	Target	Acceptable range	Analytical variability
Total protein	1.2 g/L	< 1.5 g/L	10%
Virus integrity	80%	> 70%	20%
Antigenic titer	100 µg/ml	> 80 µg/ml	10%
Cell density end of growth	3.0 10^6 cell/ml	2.5 10^6–3.5 10^6 cell/ml	20%

The responses conditioning the design space are the CQAs and KPAs.

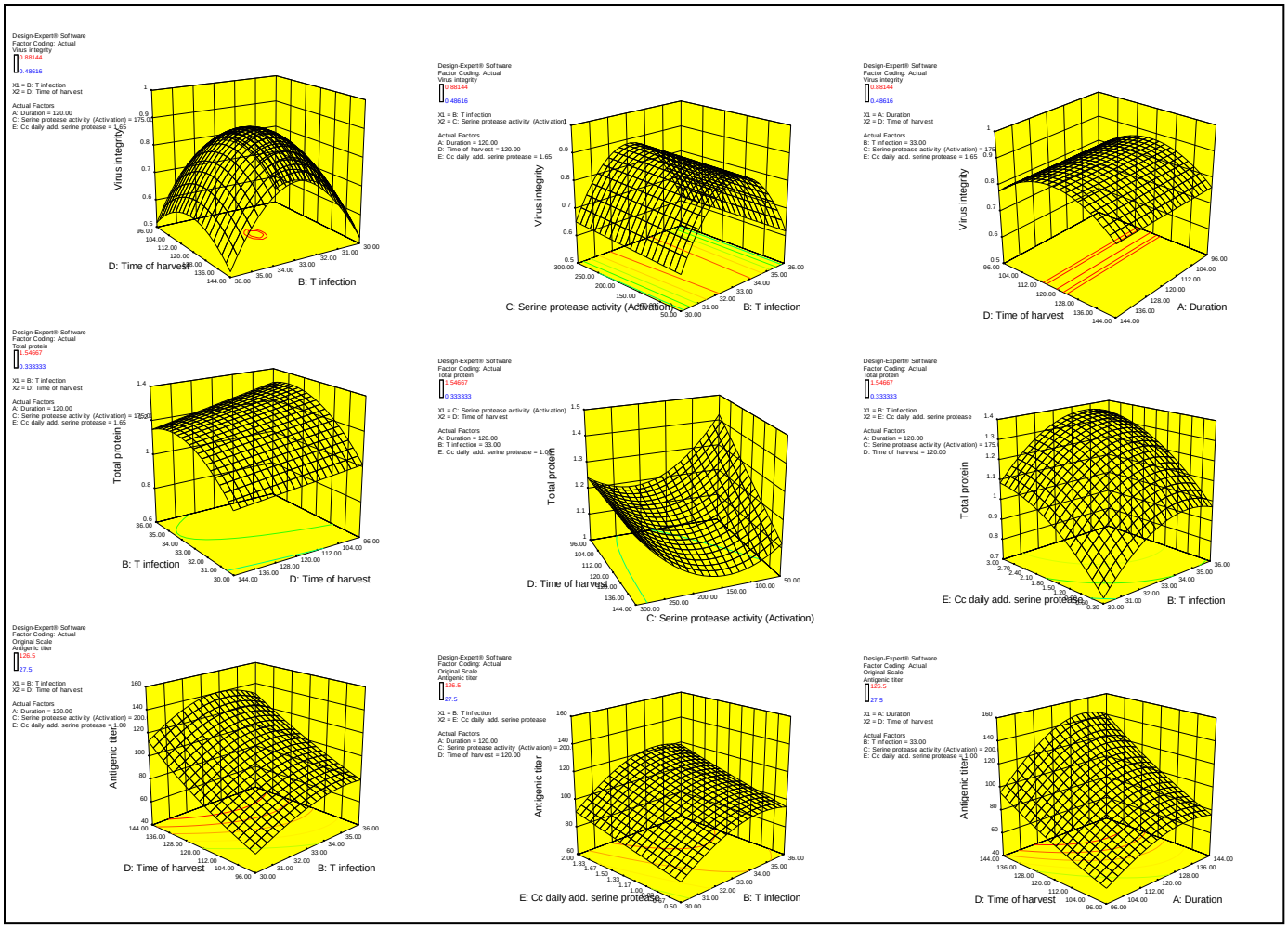
A faces-centered fractional central composite design is used; the responses of interest are expressed as a second-order polynomial function of all the process parameters and their interactions. It will allow prediction of responses in the whole studied domain. The faces-centered fractional central composite design requires 29 experiments with the five factors studied over three levels. The cultures will be performed in the 10 L bioreactors in representative conditions.

Four out of five parameters were studied by DOE during process optimization with different ranges for some parameters. Two options could be considered: (1) Enlarge the previous DOE to integrate the effect of infection temperature and the new ranges of some parameters, or (2) perform a new DOE. The second option was selected. Indeed, the cost saving with the first option was marginal (20 new experiments would be required in addition to the 29 performed during process optimization vs. 29 experiments for a new DOE), and the quality of the design would be poorer (two blocks of experiments with a long gap in time).

10.9.2. Prediction Model

For each response, a reduced polynomial model reproduces output variations using a selection of factor effects and interactions based on an analysis of variance (ANOVA). The response surfaces are graphical representations of those equations.

The effect of the five process parameters and their interactions on the four responses cannot be visualized altogether. Graphs in 3D illustrate the effects of two parameters on one critical attribute.



10.9.3. Optimal Process — Desirability Function

A desirability function is built to calculate the best process parameter region to get the optimal responses. The higher the desirability is, the better the objectives are fulfilled. More weight is given to the quality attributes (virus integrity and total protein). The objectives are:

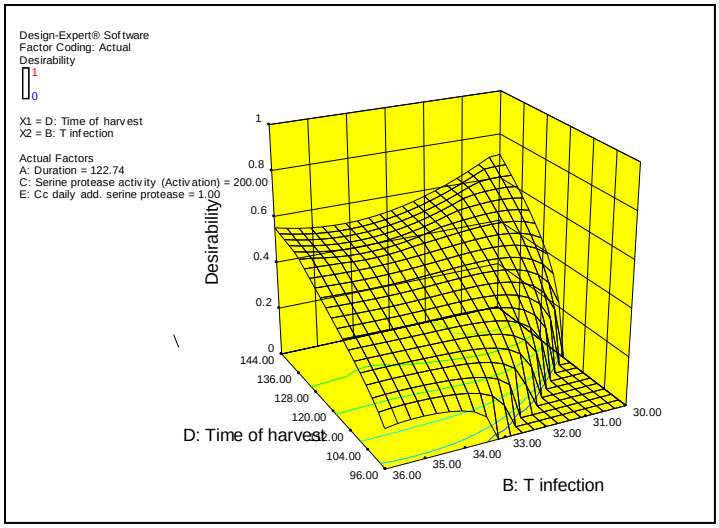
Maximize virus integrity (weight ****)

Minimize protein content (weight ****)

Maximize antigenic titre (weight **)

The final cell growth density is kept between 2.5 10^6 cells/ml and 3.5 10^6 cells/ml.

As an example, the desirability is represented here in function of the harvest time and the infection temperature.



For this particular graph, the desirability is very low for low infection temperature combined with short infection, with the other parameters being at the reference conditions. It is not an optimal area for the process. The highest value of the desirability function can be checked graphically taking the parameters two by two or numerically by maximizing the function. This method helps to find the optimal process conditions, or in our case to check whether the conditions fixed for phase III are close to the optimum.

10.9.4. Design Space

10.9.4.1. Approach

Numerous statistical approaches can be used to define the design space. The following ones have been applied and challenged:

An “average overlay plot approach” (as illustrated in ICH Q8)
For each input variable/process parameter, a prediction model is established. Then the design space (DS) is determined as the subset of the experimental domain where all quality attributes are predicted to be inside acceptance limits. Unfortunately with this approach, assurance of quality is not demonstrated because prediction models actually predict average values. In our case, at the thresholds of overlay plots, 50% of the predicted individual results are outside acceptance limits.

A “robustified average overlay plot approach”
This approach, very similar to the previous one, consists of adding confidence intervals (CIs) to overlay plots. DS will then be defined as the experimental domain subset where the lower/upper 95% CIs (depending if it is a minimal- or maximal-value criteria) of predicted quality attributes are inside acceptance limits. This method allows taking into account the prediction model quality, which is an improvement, but still not an assurance of quality for next process results.

A “tolerance intervals approach”
Instead of confidence intervals, coverage tolerance intervals are calculated around the predicted threshold for each response, taking into account experimental noise. DS being outside the

tolerance intervals, inside the DS β% of process results are predicted to be inside quality acceptance limits. Conceptually this approach is adequate to provide assurance of quality; however, when applying this approach on DOE data sets (by construction aiming to save experiments), calculated tolerance intervals were very wide, inducing the (almost) disappearance of the DS. This is probably due to the relatively small degrees of freedom leading to overly conservative limits with the calculation method used.

A "% of simulated failure approach"

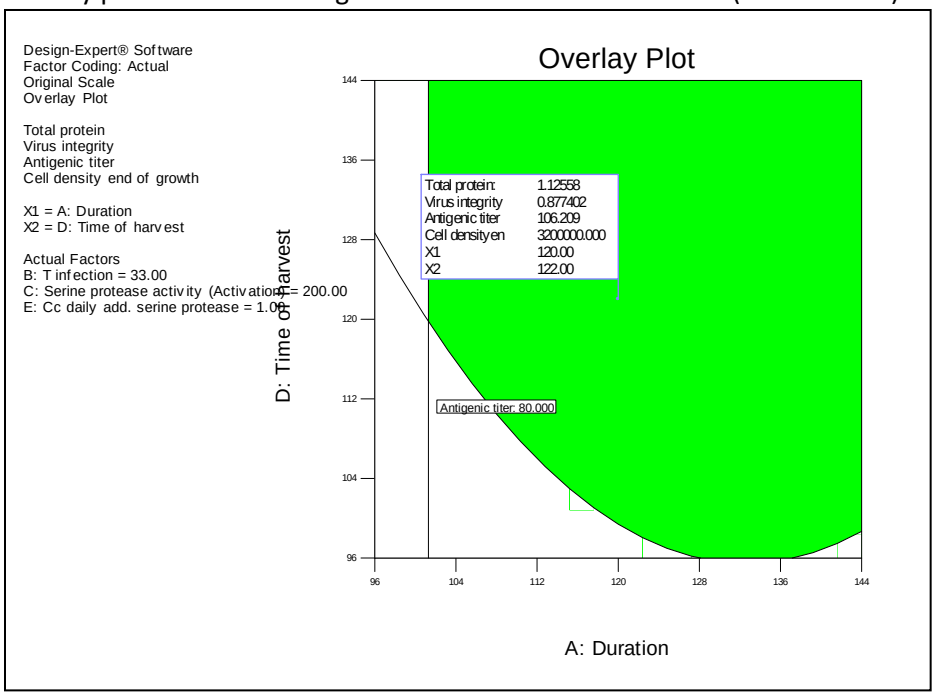
If mathematical/statistical calculations failed to determine the DS from a population point of view (all incoming process results), simulations allow doing so. The experimental domain is divided into cells; at each intersection (for each response), a huge number of simulations are made based on previously established prediction models (from DOE), adding random experimental noise (calculated from residuals). Then for all those locations the proportion of failed simulation can be calculated. Finally it is possible to determine a sub-domain with a defect rate below β%. Methodology is (partially) validated in our case by the fact that the 50% defect rate is exactly the same as the average overlay plot. Therefore, by decreasing the acceptable defect rate, we make the overlay plot approach more robust.

10.9.4.2. Overlay Plot

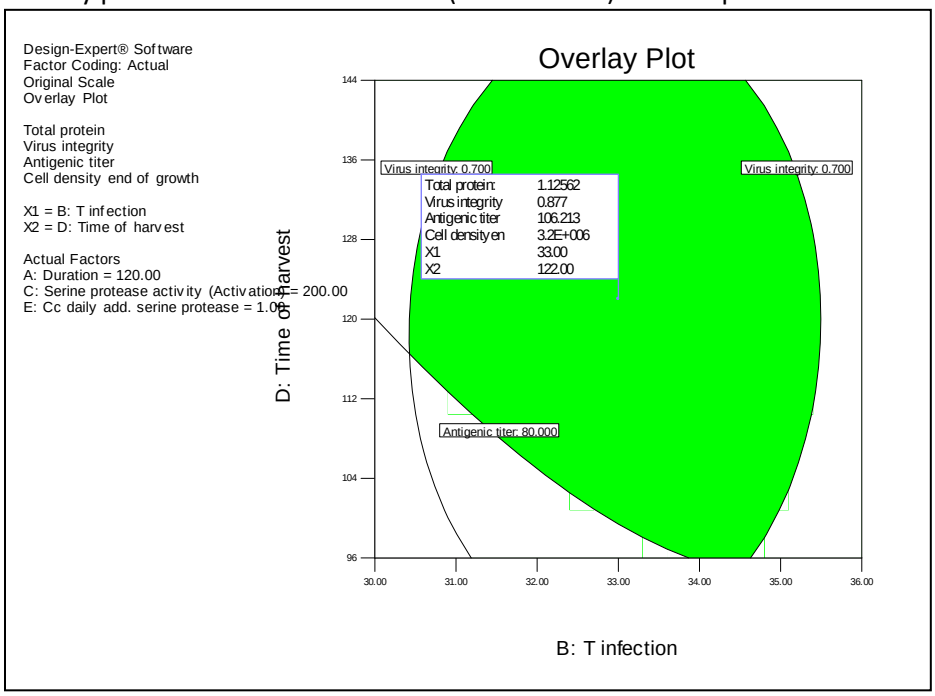
Overlay plots show the parameters' values for which the responses are within the specifications. At the limit of those regions, the responses, calculated by the polynomial relations with process parameters, are equal to the specifications (edge of failure). It can be represented by 2D graphs that look at two parameters at a time. For example, two overlay plots are shown here:

- Green: All specifications are met (in average).
- White: One or more responses are out of specification.

Overlay plot for duration of growth and duration of infection (harvest time):



Overlay plot for duration of infection (harvest time) and temperature of infection:



The design space could be extracted from the overlay plots, regions where the responses are within the specifications. In this way of defining a design space, the specifications are met in an average. This does not take into account the rate of failure because of the uncertainty of the model or the process variability or the analytical variability. For those reasons, another strategy will be adopted to determine the design space.

10.9.4.3. Design Space Determination

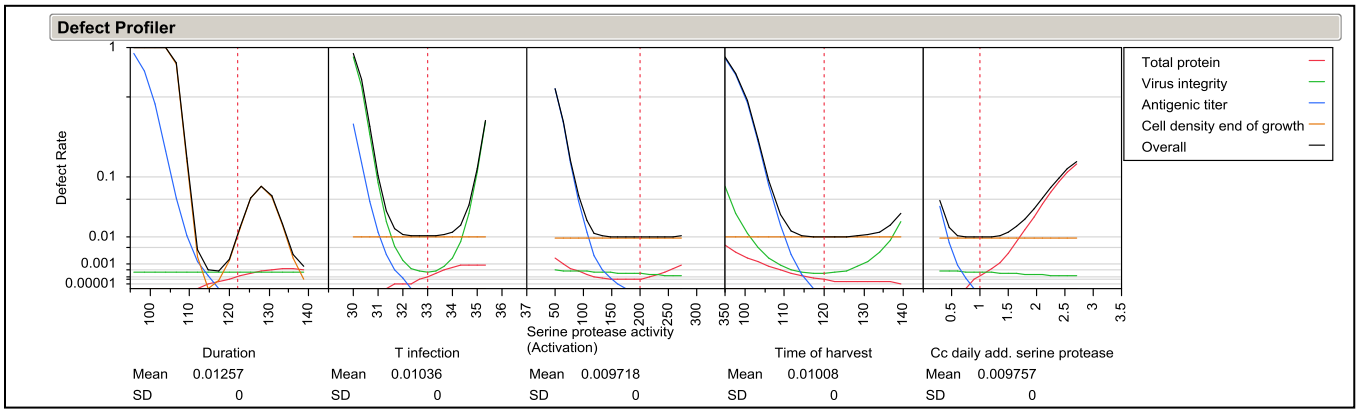
Rather than taking the limits where the mean responses of the process parameters yield to the specifications, it can be advantageous to integrate the variability of the responses (known from the DOE) to predict by simulations the regions where the specifications are met and the defect rates are acceptable. Those regions constitute the design space.

A failure (defect) is encountered when a batch falls out of the targets:

- Total protein < 1.5 g/L
- Virus integrity > 70%
- Antigenic titre> 80 µg/ml
- 2.5 10^6 ≤ Cell density end of growth≤ 3.5 10^6 cell/ml

The acceptable defect rate is fixed below 5% for the CQAs and below 15% for the KPAs.

The defect profiler tool helps to define the limits of the design space. The defect rate is represented in function of the process parameters. The reference conditions are shown by the red dashed vertical lines. The overall risk of failure, the black curve, is the combination of the failure for each response (illustrated by red curve for proteins, green curve for virus integrity, blue curve for antigenic titre and orange curve for cell density at the end of growth).



A parallelepiped design space is determined by an iterative algorithm, aiming to maximize process parameter ranges while keeping the defect rate below 5% for CQAs and 15% for KPAs in each point of the design space. Simulations are then performed in the proposed design space to quantify the defect rate.

			Design space		
	Parameter	**Ref.**	**Min.**	**Max.**	
Process	Duration	120	116	124	
	T infection	33	31.5	34.5	
	Serine protease activity (Activation)	200	190	250	
	Time of harvest	122	118	126	
	Cc daily add. serine protease	1	0.9	1.15	
	MOI	10^{-4}	$5\ 10^{-5}$	$9\ 10^{-3}$	
Defect rates	Total protein	0.01%	0.54%		
	Virus integrity	0.03%	1.91%		5% max.
	Antigenic titer	0.00%	14.56%		
	Cell density end of growth	0.96%	3.20%		15% max.
	All	0.99%	14.91%		

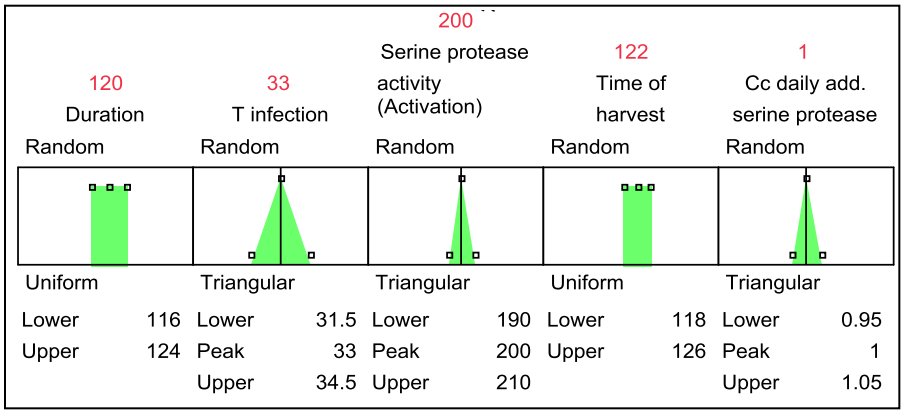
10.9.5. Control Space

Routine operations will be conducted within the boundaries of the control space. The control space is included in the design space. It is defined from process knowledge: control capability of process parameters, technical or equipment constraints, and flexibility of organization.

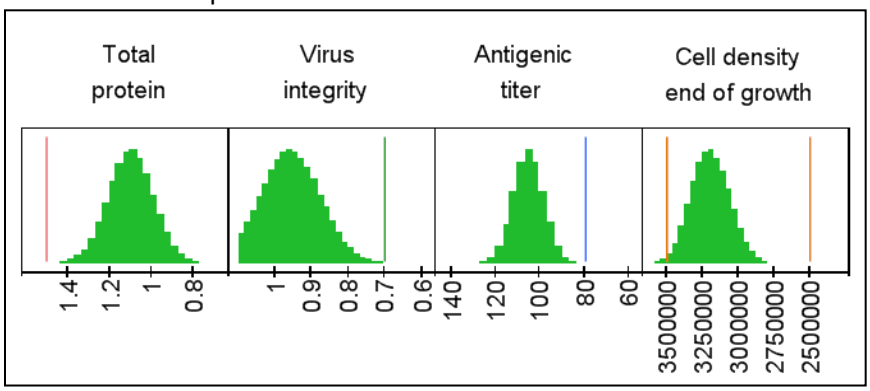
Parameter	Ref.	Design space	Proposed Range	Control space
Duration (h)	120	116-124	+/- 4	116-124
Multiplicity of infection (-)	10^{-4}	$5\ 10^{-5}$ - $9\ 10^{-3}$	+/- 0.5 LOG	$5\ 10^{-5}$ - $5\ 10^{-4}$
T infection (°C)	33	31.5-34.5	+/- 1.5	31.5-34.5
Serine protease activity at activation (IU/ml)	200	190-250	+/- 10	190-210
Time of harvest (h)	122	118-126	+/- 4	118-126
Cc daily addition serine protease (IU/ml)	1	0.9-1.15	+/- 0.05	0.95-1.05

As a confirmation, random error on routine process around reference conditions can be added to response variability in Monte Carlo simulations.

Distribution of random error on routine process:



The distribution of the results around the specification is illustrated below; the vertical lines are the limits of the specifications.

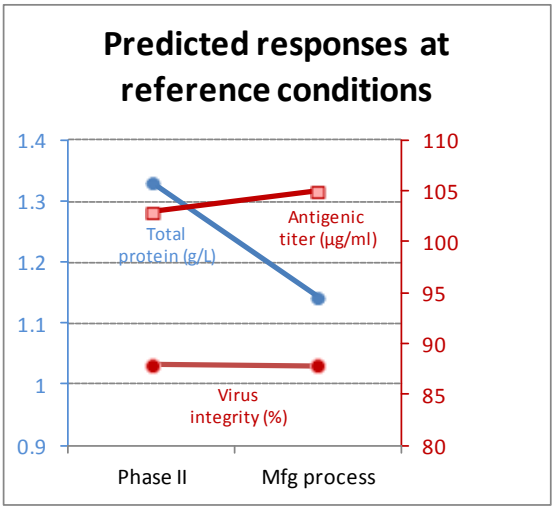


The predicted proportion of out-of-specification results is very low within the control space. The global defect rate is below 1% in the control space.

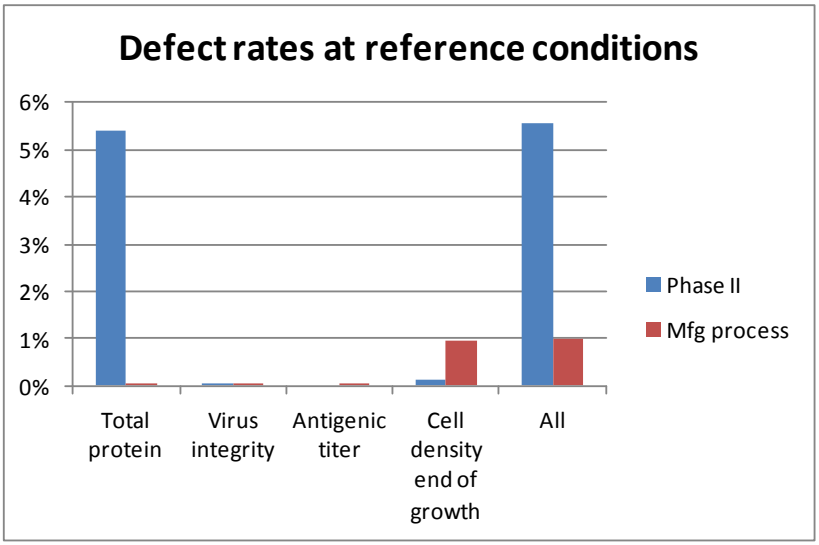
Defect	Rate
Total protein	0.01%
Virus integrity	0.11%
Antigenic titer	0.01%
Cell density end of growth	0.65%
All	0.78%

The simulations can also show the optimization between the Phase II and the manufacturing processes in terms of performance and robustness.

Process performances



Process robustness



10.9.6. Categorization of Process Parameters

The process parameters are categorized as noncritical process parameters, critical process parameters (CPPs), and well-controlled critical process parameters (WC-CPPs). The ICH defines a CPP as: *a process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality.* A WC-CPP is defined as: a CPP that *has a low risk of falling outside the design space.* The final assessment of CPPs and WC-CPPs is reevaluated based on the knowledge generated during design space definition. All critical parameters are WC-CPP.

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Parameter	Quality attributes		Process attributes		Risk mitigation
	Total protein	Virus integrity	Cell density	Antigenic titer	
Cell passage					Studied during Ph II development
Cytodex concentration					Studied during Ph II dvpt
Shear protective additive					OFAT study done during Ph III process optimization
Seeding density					Studied during Ph II dvpt
Temperature cell growth					Studied during Ph II dvpt
pO_2					Studied during Ph II dvpt
pH cell growth					DOE done during Ph III process optimization
Pressure					Studied during Ph II dvpt
Stirring					Scale-up based on Phase II dvpt
Daily glucose adjustment					DOE done during Ph III process optimization
Daily glutamine adjustment					DOE done during Ph III process optimization
Growth duration					DOE done during design space determination
Temperature infection			NA		DOE done during design space determination
pH infection			NA		DOE done during Ph III process optimization
MOI			NA		DOE done during Ph III process optimization
Viral activation: serine protease activity			NA		DOE done during design space determination
Viral activation: serine protease contact duration			NA		DOE done during Ph III process

					optimization
Daily addition of serine protease: concentration			NA		DOE done during design space determination
Viral replication duration (time of harvest)			NA		DOE done during design space determination
Media preparation – filter size					OFAT study done during Ph III process optimization
Media stability – shelf life					OFAT study done during Ph III process optimization
Viral production media selection					Studied during Ph II development

Noncritical process parameters are blank.

WC-CPP: Parameter impacts an attribute, but is well-controlled.

CPP: Parameter impacts an attribute, but the range is close to the control capability.