A-Mab: a Case Study in Bioprocess Development

CMC Biotech Working Group

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1 Introduction

1.1 Background and Acknowledgements

In August of 2008, company representatives from Abbott, Amgen, Eli Lilly & Company, Genentech, GlaxoSmithKline, MedImmune, and Pfizer were brought together to help advance the principles contained in ICH Q8(R2), Q9 and Q10, focusing on the principles of Quality by Design. The application of QbD to biotechnology products represents an important opportunity as the manufacturing and development of such products involves unique challenges with regard to both drug substance and drug product manufacturing due to the complexity of both the products and the biological manufacturing processes.

Through a series of inter-company and regulatory interactions, the group set out to create a case study that would stimulate discussion around how the core principles contained in these guidelines would be applied to product realisation programs, with a multitude of real world scenarios, as opposed to a singular approach. To that end, the CMC-Biotech Working Group set out to accomplish the following:

- Create a comprehensive biotechnology case study that would support teaching and learning for both Industry and Regulators
- Exemplify the more advanced principles and opportunities described in Q8(R2), Q9 and Q10 for both the active ingredient and the drug product
  - Demonstrate the concept of ‘prior knowledge’ and how it could be applied to demonstrate process understanding
  - Enable effective techniques for achieving continual improvement across the process development and commercial arenas
- Provoke and challenge current thinking in order to stimulate discussion and advance new concepts
- To examine the potential opportunities to enhance science and risk based regulatory approaches associated with these advanced concepts that would encourage greater implementation of the recent ICH guidelines across the industry.

The Facilitators would like to thank the efforts of each of these companies and their representatives for demonstrating their eagerness to create a document for public consumption and ultimately be used as the backbone for further discussion between industry and agencies across 2009-2010 and beyond.

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Amgen Team: Joseph Phillips (Lead), Lisa Ericson, Chulani Karunatilake, Bob Kuhn, and Anurag Rathore
Abbott Team: Ed Lundell (Lead), Hans-Juergen Krause, Christine Rinn, Michael Siedler, Sonja Simon, Carsten Weber, Brian Withers
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1**) Introduction** – Mark Schenerman
2) **CQA** – Mark Schenerman and Ron Taticek
3) **Control Strategy** – Mike DeFelippis
4) **Upstream** – Ilse Blumentals
5) **Downstream** – Ed Lundell and John Erickson
6**) Regulatory** – Leslie Bloom and Lynne Krummen
7**) Drug Product** – Joseph Phillips

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Finally, the CMC-BWG has requested that CASSS and ISPE place this document in the public domain. We thank these organizations for agreeing to host the case study and to continue with its use and development.

The Facilitator Team: John Berridge, Ken Seamon, and Sam Venugopal

1.2 Overall Case Study and Development Objectives

The objectives of this case study are to exemplify a QbD approach to product development.

An enhanced, **quality by design** approach to product development would additionally include the following elements:

- A systematic evaluation, understanding and refining of the formulation and manufacturing process, including;
    - Identifying, through e.g., prior knowledge, experimentation, and risk assessment, the material attributes and process parameters that can have an effect on product CQAs;
    - Determining the functional relationships that link material attributes and process parameters to product CQAs;
- Using the enhanced product and process understanding in combination with quality risk management to establish an appropriate control strategy which can, for example, include a proposal for a design space(s) and/or real-time release testing.

From ICH Q8(R2)

The overall approach for A-Mab product realization is shown in Figure 1.1 which illustrates a sequence of activities that starts with the design of the molecule and spans the development process ultimately resulting in the final process and control strategy used for commercial manufacturing.

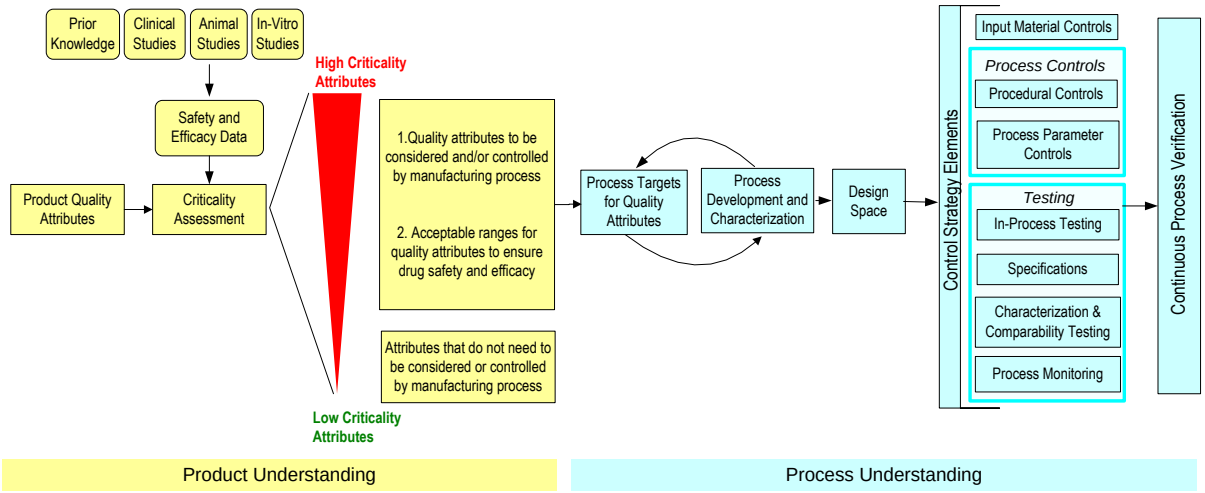

Figure 1.1 Overview of Product Realization Process

Having an effective and comprehensive methodology to identify all the relevant product quality characteristics that are linked to the desired clinical performance of the drug is a fundamental requirement and the cornerstone of a Quality by Design approach. The case study presents an example on how to link the Target Product Profile to the Critical Quality Attributes (CQAs) of the product based on product understanding.

Critical Quality Attribute

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: The suitability of either a drug substance or drug product for its intended use. This term includes such attributes as the identity, strength, and purity (from Q6A)

From ICH Q8(R2)

The challenges encountered in the identification of CQAs for large biological molecules are discussed. The proposed approach is exemplified through a series of examples, where risk assessments are used to rank potential quality attributes based on information derived not only from clinical exposure, but also from the fundamental understanding of the biology of the molecule and the disease, prior knowledge from similar class molecules, animal studies, and in-vitro experiments.

Risk

Risk is the product of the severity (consequences) and probability (likelihood it will go wrong).

What might go wrong (attribute)?

What are the consequences (severity)?

What is the likelihood it will go wrong (probability)? From ICH Q9

The outcome of this approach is not a binary classification of quality attributes into “Critical” and “Non-Critical”. Rather, the result is a “Continuum of Criticality” that more accurately reflects the complexity of structure-function relationships in large molecules and the reality that there is uncertainty around attribute classification. Based on this continuum, a set of quality attributes that must be monitored and controlled by the manufacturing process is identified. The assessment also provides a rationale for selecting the proposed target ranges for each quality attribute to ensure desired product quality.

These quality targets serve as the basis for process development activities and guide the selection of process steps, material attributes, equipment design and operation controls for the manufacturing process. Here, repeated risk assessments are performed throughout the development lifecycle to identify process parameters and material attributes that are most likely to impact drug substance and/or drug product CQAs (Figure 1.2).

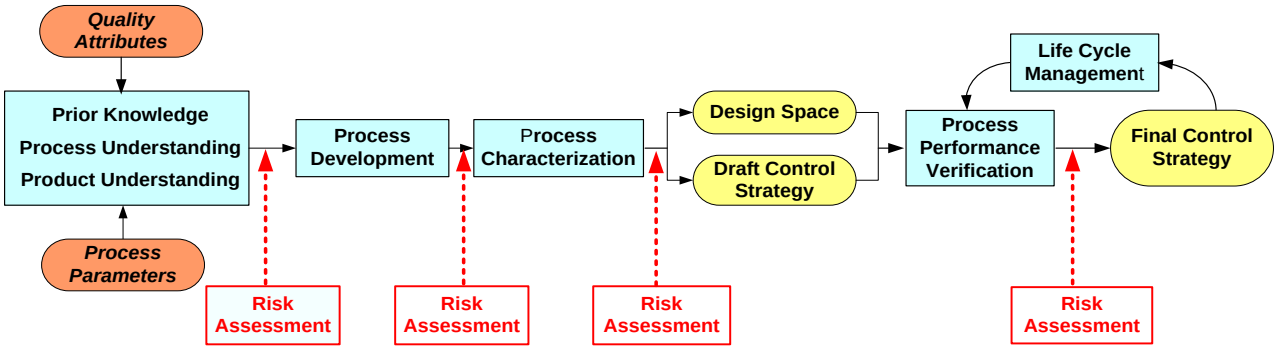

Figure 1.2 Risk Assessment Approach Used through A-Mab Development Lifecycle

The early risk assessments use prior knowledge and early development experience to identify parameters and attributes that must be considered for process characterization studies. A combination of multivariate (DOE) and univariate approaches are used to map process performance responses, identify parameter interactions, and define acceptable operating ranges. This cumulative process understanding serves as the basis for the late-phase risk assessments used to finalize selection of Critical Process Parameters (CPPs) that underpin the proposed design spaces and control strategy.

Critical Process Parameter

A process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure to process produces the desired quality.

In the case study, critical process parameters are sub-divided based on risk:

A **Well Controlled –Critical Process Parameter** (WC-CPP) has a low risk of falling outside the design space.

A **Critical Process Parameter** (CPP) has a high risk of falling outside the design space.

Here, the assessment of risk is based on a combination of factors that include equipment design considerations, process control capability and complexity, the size and reliability of the design space, ability to detect/measure a parameter deviation, etc.

The case study presents multiple examples on how design spaces can be defined. However, in all cases the design spaces represent the multivariate interactions of CPPs (or WC-CPPs) and critical quality attributes.

The overall control strategy is based on the design spaces of the unit operations and represents a science and risk based approach that provides a high degree of assurance that all product quality targets are met. For this, each quality attribute has an individual control strategy constructed based on a combination of control elements that include process and procedural controls as well as a rationale testing strategy. Thus, it is the sum of the individual control strategies that represent the overall process control strategy.

Product quality throughout the product life cycle is assured through a continued process verification approach.

1.3 Organization of Case Study

This case study is divided into sections that follow typical groups or sequences of activities that occur in the development of a monoclonal antibody. Within sections, key points and highlights are identified or summarized using blue text and boxes.

CHAPTER 2: CRITICAL QUALITY ATTRIBUTES

Product development begins with identification of the desired quality attributes of the antibody and its performance attributes using the target product profile. The molecule is designed to maximize the clinical safety and efficacy to achieve the desired profile. From the target product profile, an initial list of potential critical quality attributes was created and ordered, according to their criticality, using a novel spreadsheet tool which considers also the associated control tools. Novel assessment tools were utilized to assess the criticality of specific attributes which are described in detail. This provided an opportunity to demonstrate how to utilize knowledge from a number of sources including prior knowledge with similar molecules and experience from in-vitro, non-clinical, and clinical data for assessing the criticality of a quality attribute. For the purposes of this case study, four of the quality attributes are examined in detail and these are examined throughout the case study to define the design spaces and for developing specific control strategies.

CHAPTERS 3 AND 4: UPSTREAM AND DOWNSTREAM

A risk-based approach was used to evaluate each unit operation of the manufacturing process to identify process parameters and attributes that could pose risk to the quality of the product and process performance. The risk assessment tools are not described in detail as they are described with the ICH Q9 guideline and associated materials published by ICH. Prior knowledge gained through the use of platform processes and experience with other mAbs provided the initial basis for the risk assessment. Subsequent risk assessments incorporated the cumulative knowledge gained throughout the A-Mab development lifecycle.

As indicated in Figure 1.2, during A-Mab development, multiple rounds of risk assessments were conducted to guide process characterization and optimization studies. These studies were conducted using scale-down models that were demonstrated to be representative and predictive of full-scale manufacturing process performance. Results from the DoE studies provided an understanding of the relationships between input process parameters and output quality attributes. Additionally, clinical manufacturing experience added to the understanding of process performance and process control at various operational scales.

A detailed description of process parameter characterization for each unit operation is presented in the corresponding sections of the upstream and downstream processes. Only process parameters linked to product quality were used to define the limits of the design spaces.

CHAPTER 5: DRUG PRODUCT

Chapter 5 describes the formulation design, compounding, filtering and filling steps, again focusing on the limited set of critical quality attributes. A slightly different approach is used for the drug product. The extensive prior knowledge of formulation and manufacturing processes for monoclonals is such that it is possible to consider the product and its process to be essentially a platform process. Through risk assessments and targeted experimentation, it is shown that design space and proven acceptable ranges developed for other products can be re-used. The section also shows the use of dimensionless analysis to show scale independence.

In addition, an example of Fault Tree Analysis is included to demonstrate its utility in support of QbD principles.

Chapter 6: Control Strategy

The focus is on control of the critical and well controlled process parameters as these must be maintained within the limits of the design spaces to ensure product quality. For routine manufacturing, the process is operated within control spaces.

Control Space

Region within the Design Space that defines the operational limits (for process parameters and input variables) used in routine manufacturing. The control space can be a multidimensional space or a combination of univariate process ranges.

The control strategy utilizes a number of potential mechanisms for implementing and demonstrating control with specific detail for four important quality attributes: glycosylation, deamidation, host cell proteins, and aggregation. This provides an opportunity to demonstrate different types of control strategies for attributes that are different with regard to their criticality as well as process dependence.

Chapter 7: Regulatory Implications

Potential regulatory implications of the approaches described in the case study. ICH Q8(R2) describes opportunities for more flexible regulatory approaches, and ICH Q10 illustrates a number of potential opportunities to enhance science and risk based regulatory approaches. Based on the enhanced product and process understanding, opportunities and processes for lifecycle management are suggested.

Appendix 1: Glossary

You will see new and major terms and concepts highlighted throughout this case study in rectangular boxes. However, a more comprehensive glossary is included at the end of the case study.

What Next?

It's not essential to read through the sections in the order they are presented, but you will find that it helps because we have tried not to repeat information. We will also help you by indicating sections that contain lots of data or information for those with a specialist interest in a particular topic (e.g., dimensionless analysis, or engineering modeling). It is not essential to read all the detail in these sections if you are happy to accept the thesis being presented and its conclusions.

FINALLY

It is extremely important to recognize that what follows is not intended to be a mock regulatory submission.

A-Mab Case Study Objectives	
The case study does:	The case study does not:
Demonstrate implementation of the principles of Quality By Design	Present a prescriptive approach
Leverage the significant knowledge base of both commercial and investigative monoclonal antibodies	Follow a traditional approach
Show application to both drug substance and drug product	Deal with all possible unit operations,
Provide illustrative examples based on real data	Address all quality attributes or process parameters
Demonstrate a science and risk-based approach	Represent a 'mock' regulatory submission
Show there are many ways to implement QbD	Represent a standard

The authors hope you enjoy this case study and that it indeed stimulates the discussion, debate and learning that are intended.

2 Design of Molecule and Quality Attributes Assessment

A-Mab is a humanized IgG1 monoclonal antibody that was designed to maximize clinical performance and minimize potential impact from undesirable product quality attributes. It is intended as a treatment for non-Hodgkin's Lymphoma and its mechanism of action is B cell killing primarily through ADCC.

The case study illustrates how different risk assessment approaches (risk ranking, PHA or decision tree) and types of knowledge (prior or platform knowledge, laboratory data, nonclinical data and clinical data) may be used to assess the criticality of quality attributes. The risk ranking and PHA tools consider impact on efficacy, PK/PD, immunogenicity and safety in their assessments. Both tools do not consider process or manufacturing capability or detectability in their assessments and output a continuum of criticality. Three other similar commercial antibody products are considered the relevant prior or platform knowledge.

Rather than assess all quality attributes in this case study, a subset of QAs were chosen that span the continuum of criticality, vary in the impact on efficacy and safety and vary in the types of information used to assess criticality. The attributes assessed include aggregation, glycosylation, host cell protein, leached Protein A, methotrexate, oxidation, DNA, deamidation and C-terminal lysine. These attributes were also carried forward into the other sections of the case study as appropriate. The criticality assessments from the various tools were very similar. Some differences were observed but they did not change the overall assessment of which attributes were Critical.

The following attributes were assessed as Critical (high to very high criticality score): aggregation, glycosylation (galactose content, afucosylation, sialic acid content, high mannose content and non-glycosylated heavy chain) and HCP. The other attributes were assessed as very low to moderate in criticality: C-terminal Lysine, deamidation, DNA, oxidation, methotrexate and leached Protein A. Acceptable ranges for a subset of these QAs were established based on a combination of clinical experience, non-clinical studies, laboratory studies and prior knowledge. The acceptable ranges are used to establish the boundaries for the design spaces in the Upstream, Downstream and Drug Products sections of the case study.

Key Points

1. Different tools may be used to assess criticality of Quality Attributes.
2. Considering the effect on efficacy (through the most relevant biological activity assays), PK/PD, immunogenicity and safety is important for assessing the criticality of all QAs
3. Prior/platform knowledge, laboratory data, nonclinical data and clinical data are all important information sources for assessing the criticality of QAs.
4. A criticality continuum for QAs ensures that QAs are appropriate considered throughout the product lifecycle.

This section of the case study describes the Target Product Profile, the design strategy used for the development of the A-Mab molecule, historical ranges for quality attributes, and the ranking of the criticality of a subset of quality attributes for A-Mab. The quality attributes selected for ranking

encompass attributes across the criticality continuum and were chosen to illustrate the different types of information used in the criticality risk assessment.

2.1 Target Product Profile

Key aspects of a Target Product Profile important for the assessment of criticality of quality attributes are summarized below.

2.1.1 Clinical Aspects

Efficacy Claims

A-Mab is a humanized IgG1 antibody intended as a treatment for indolent non-Hodgkin's Lymphoma (NHL) in an adult population only. The mechanism of action for A-Mab is through binding to a tumor cell surface antigen, Lymph-1, and stimulating B cell killing. Although A-Mab was designed so that the B cell killing is primarily through ADCC activity, involvement of CDC activity cannot be completely ruled out. A-Mab is delivered by IV administration at a weekly dose of 10 mg/kg for 6 weeks. A completed treatment cycle is expected to result in 40% response in patients, as assessed by progression-free survival.

Safety Claims

The most common adverse event is expected to be infusion related and is limited to the duration of infusion. It is manageable with proper procedures. Severe events are expected to be rare. Toxic effects are not expected to impact neighboring cells and there is a very low level of renal or hepatic toxicity expected. There is a low level of HAHA (human anti-humanized antibody) response expected, but no evidence of neutralizing antibodies.

2.1.2 Drug Product Aspects

A-Mab is a sterile liquid formulation in a single-use vial at a concentration of 75 mg/mL to allow for dilution to approximately 25 mg/mL for patient dosing. Data will support a minimum shelf-life of two years at 5°C and 14 days at 25°C. The formulated Drug Substance is compatible with dilution in standard clinical diluents such as saline or D5W (5% dextrose), without use of any special devices. The formulation is colorless to slightly yellow and practically free of visible particles.

2.2 Molecule Design

2.2.1 Overview of Research Leading To Candidate Molecule

Lymph-1 (a surface antigen on CD20 B cells) has been shown to be expressed at high levels on the surface of B cells from NHL patients. CD20 cells in normal patients have no measurable levels of Lymph-1. Studies indicate a high level of selectivity to the tumor cells. An animal model for NHL has been developed in a SCID mouse system. When human lymphoma cells were transferred into the SCID mouse model, the lymphoma cells propagated and expressed high levels of Lymph-1. Based on these research studies, a panel of anti-Lymph-1 antibodies were developed using affinity optimization of the CDR to provide an IgG1 with maximal affinity for the Lymph-1 antigen. The top five candidate molecules were screened using a cytotoxicity assay to determine which molecule had the greatest ability to kill target B cells. The CDRs of the selected candidate molecule (4F7), named A-Mab, was further developed by transferring the CDR sequences onto a platform IgG1

framework and transfecting CHO cells to create the current CHO Master Cell Bank (and Working Cell Bank) using standard cloning and transfection procedures.

2.2.2 Design Features

A-Mab is a humanized monoclonal IgG1 κ light chain antibody produced by recombinant DNA technology. It is directed to an epitope on the surface of tumor cells. A-Mab was derived by in vitro affinity optimization of the complementarity determining regions (CDRs) of the heavy and light chains. The design strategy for A-Mab was based on creating a molecule that maximizes clinical performance (safety and efficacy) and minimizes potential impact on quality. The structure of the A-Mab was designed to mitigate risk from the following product attributes:

- Unpaired cysteine residues (reduced risk of undesirable disulfide bond formation)
- Potential deamidation sites in the CDRs (reduced risk of deamidation)
- O-linked glycosylation sites (reduced risk of heterogeneity and impact on bioactivity)
- N-linked glycosylation sites in the CDRs (reduced risk of heterogeneity and impact on bioactivity)
- Acid labile (DP) sequences (reduced risk of fragmentation)
- Oxidation sites in the CDR

2.2.3 Platform Knowledge

Platform knowledge is leveraged based on its relevance and applicability to the molecule under consideration. Table 2.1 summarizes the platform knowledge from other similar monoclonals, some of which may be applicable to A-Mab. Additional considerations include the nature of the target and the biological signaling associated with the target.

Table 2.1 Platform Knowledge Characteristics

Characteristic	X-Mab	X-Mab	Y-Mab	Z-Mab
CHO-derived?	Yes	Yes	Yes	Yes
Isotype	IgG1	IgG1	IgG1	IgG1
Indication	Oncology	Oncology	Inflammation	Oncology
Mechanism of Action (MOA)	ADCC-enhanced*	Primarily ADCC*	Binding Neutralizing	Primarily ADCC*
Humanized?	Yes	Human	Yes	Yes
Dosing	IV	IV	Sub-Q	IV

*CDC Activity cannot be ruled out as part of the Mechanism of Action.

2.3 Identification and Risk Assessment of Quality Attributes

2.3.1 Overview of a Science and Risk-based Approach

All quality attributes are assessed for criticality, which is defined in this case study as impact on safety and efficacy of the product. Examples are provided to illustrate how prior or platform knowledge, laboratory data, nonclinical data and clinical experience may be used to define the appropriate risk score (classification or ranking) for each quality attribute. Similarly, data are illustrated in the process sections (Section 3-5) to describe the capability of the process to deliver an attribute within this range of product knowledge.

A flowchart illustrating the overall approach to risk management related to quality attributes is presented in Figure 2.1.

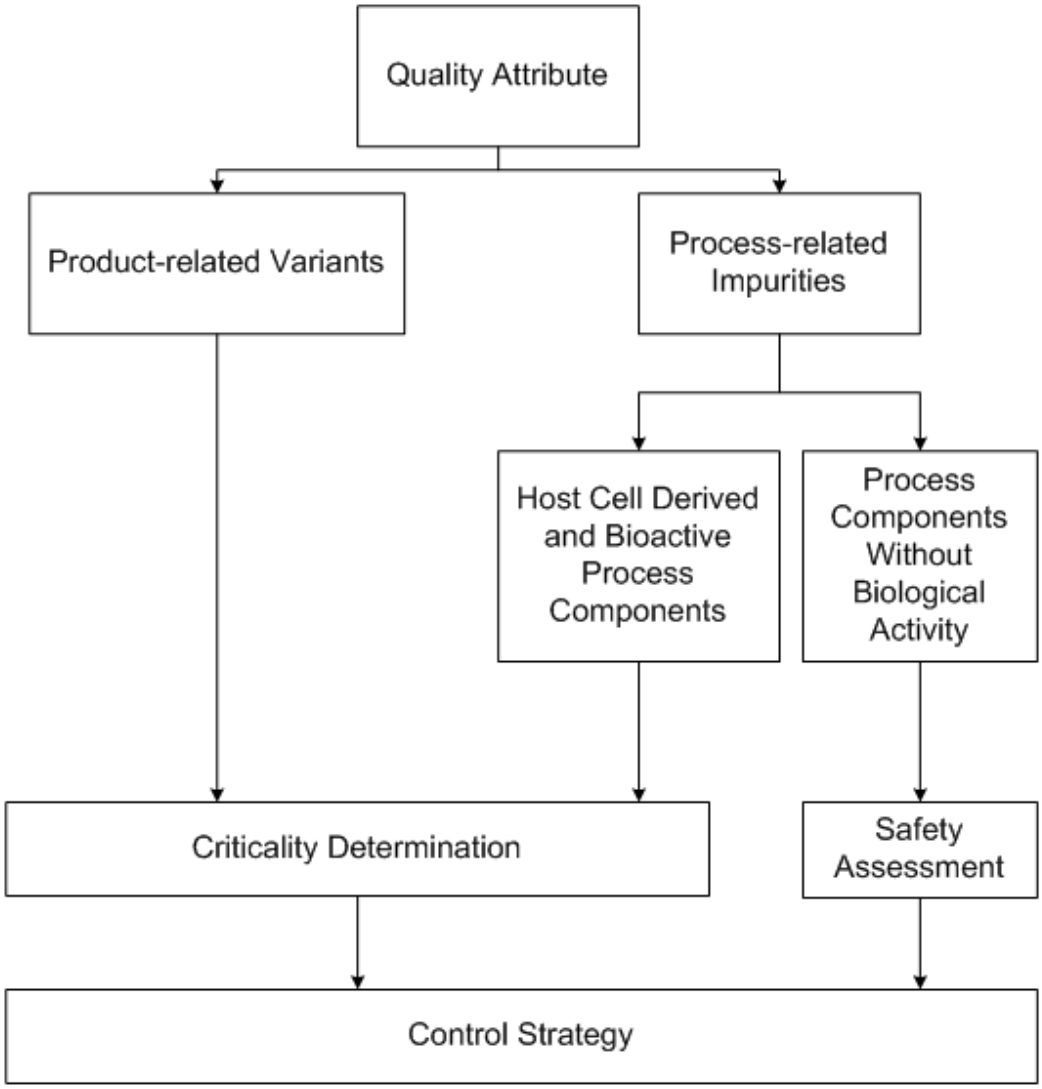

Figure 2.1 Quality Attribute Risk Management Approach

This case study considers three quality attributes (aggregation, galactose content and afucosylation) that have high criticality rankings, as well as others of medium to low criticality (host cell protein (HCP), leached Protein A, methotrexate, oxidation, DNA, deamidation, and C-terminal lysine). Based on prior knowledge for X-Mab, Y-Mab and Z-Mab, and the lack of data to suggest otherwise with A-Mab, it is assumed that process components without biological activity do not interact with the molecule and are therefore assessed on a safety basis only.

2.3.2 List of Quality Attributes

In order to evaluate quality attributes for criticality, it is first necessary to identify all the possible attributes for that product with consideration of molecular design. Molecular design aspects could include enrichment of an attribute (e.g., sialylation) or elimination of an attribute (e.g., fucosylation or Fc glycans). Table 2.2 lists typical quality attributes for a monoclonal antibody. When establishing the overall control strategy and judging its robustness, having a list of the relevant quality attributes, with the possible tests associated with each attribute, the purpose of each test, whether or not the method is stability indicating and to which ICH Q6B category(ies) it belongs is very useful.

A quality attribute listing tool (embedded Excel spreadsheet) is used to list and organize information about A-Mab quality attributes. The tool includes tests associated with each attribute, the purpose of each test, whether or not the method is stability-indicating and to which ICH Q6B category(ies) it belongs. An abbreviated example of such a spreadsheet is included here and summarizes the attributes covered in this case study:

<<Embedded Spreadsheet>>

Product Quality Attribute	Test	Purpose	Stability-indicating?	ICH Q6B Category
Aggregation	HPSEC	Detect product-related impurities (fragments, aggregates)		Identity, Purity, Stability
Aggregation	Gel electrophoresis	Detect product-related impurities (fragments, aggregates)		Identity, Purity, Stability
Aggregation	Analytical ultracentrifugation	Detect product-related impurities (fragments, aggregates)		Identity, Purity
Aggregation	HPSEC with MALLS	Detect product-related impurities (fragments, aggregates)		Identity, Purity
C-terminal lysine	Ion exchange chromatography	Detect charge isoforms		Identity, Purity, Stability
C-terminal lysine	Isoelectric focusing	Assess pattern of charge isoforms		Identity, Purity, Stability
C-terminal lysine	Peptide mapping with MS	Verify primary structure and identify posttranslational modifications		Identity, Purity, Stability
Deamidated isoforms	Ion exchange chromatography	Detect charge isoforms		Identity, Purity, Stability
Deamidated isoforms	Isoelectric focusing	Assess pattern of charge isoforms		Identity, Purity, Stability
Deamidated isoforms	Peptide mapping with MS	Verify primary structure and identify posttranslational modifications		Identity, Purity, Stability
Glycosylation	Monosaccharide composition analysis	Quantify monosaccharide composition		Identity
Glycosylation	Oligosaccharide profile	Assess pattern of oligosaccharide profile		Identity
Glycosylation	Sialic acid content	Measure sialic acid content		Identity
Glycosylation	Galactose content	Measure galactose content		Identity
Glycosylation	Fucose content	Measure fucose content		Identity
Oxidation	Peptide mapping with MS	Verify primary structure and identify posttranslational modifications		Identity, Purity,Impurity
Process-related Impurity	Methotrexate	Measure level of methotrexate		Impurity
Process-related Impurity	HCP	Measure host cell protein		Impurity
Process-related Impurity	Protein A	Measure residual protein A		Impurity
Process-related Impurity	DNA	Detect host cell DNA		Impurity

Quality attributes can have multiple tests associated with them. For example, the “deamidated isoforms” attribute can be associated with multiple tests such as ion exchange chromatography, isoelectric focusing, and peptide mapping. Similarly, aggregation can be associated with HPSEC, gel electrophoresis, analytical ultracentrifugation, and HPSEC with MALS detection. These tests may be used in various combinations for in-process controls, lot release, stability testing and characterization/comparability testing. The appropriate level of testing is ultimately based on a comprehensive control strategy.

Table 2.2 Typical Quality Attributes for a Monoclonal Antibody

Product Variants		Purity (including Process-related impurities)	
Aggregation	Fragmentation	Microbiological Purity	Selective agent
Conformation	Glycation	Viral Purity	Cell Culture Medium Components
C-Terminal Lysine	Glycosylation Oxidation	DNA	Purification Buffer Components
Deamidated Isoforms	Thioether link	HCP (Host Cell Protein)	
Disulfide Bonds		Protein A	

Drug Product Attributes	
Foreign Particles	pH
Clarity	Product Concentration
Color	Potency
Osmolality	Volume

2.4 Rationale for Selecting Quality Attributes for Case Study

Rather than evaluate all quality attributes of a monoclonal antibody, a subset of QAs was purposely selected for this case study that span the criticality continuum, and vary in their impact on safety and efficacy (higher vs lower criticality) and vary in the types of information that is used to assess criticality. The attributes selected include product-related and process-related quality attributes that can potentially have an impact on the safety and efficacy of the product and were selected in order to demonstrate how criticality can be assessed for both highly critical and less critical attributes, and to exemplify different control strategies based on process capabilities and impact of unit operations on attributes.

2.4.1 Quality Attribute Risk Assessment Tools

The basic principles of applying risk assessments to identify the criticality of quality attributes are well established (based on ICH Q9), however, the specific risk assessment tools may vary based on the type of quality attribute being assessed and the factors used to assess criticality (severity, occurrence, etc.). Three types of tools for assessing criticality of quality attributes are presented as examples: risk ranking (Tool #1), preliminary hazards analysis (PHA) (Tool #2) and a safety assessment decision tree for evaluating process-related impurities that do not have biological activity (Tool #3). Two examples of risk assessment tools are presented (Tool #1 and Tool #2) which are primarily designed for assessing the risk related to product variants and impurities typical of biotech drugs.

Tools #1 and #2 both consider criticality on the basis of impact to the patient, but leverage historical prior product knowledge/experience to differing degrees. In practice, knowledge from both sources

(prior or platform knowledge and product-specific knowledge) would be incorporated into the evaluation as warranted, but may shift as development proceeds. For this case study, these tools were developed independently, and as such, the relative risk scores are not expected to be identical. Importantly, Tool #1 and Tool #2 risk assessments do not take into account process and manufacturing capability (i.e., the likelihood that the attribute is present and, if present, well-controlled by the process) or detectability (i.e., whether a test exists that can detect the quality attribute and its sensitivity), and the outputs from both Tool #1 and Tool #2 are a continuum of criticality.

Tool #1 is a risk ranking tool. Risk ranking of complex systems usually involves evaluation of multiple factors for each risk. The approach taken with Tool #1 involved breaking down the risk into the multiple components required to capture the appropriate risk factors (i.e., the potential impact to safety and efficacy and the uncertainty around the information used to assess the potential impact) and developing a scoring matrix for each factor. The individual scores for each factor are then multiplied together to give a single risk score.

Tool #2 ranks the criticality of quality attributes using a PHA risk assessment approach based on severity (i.e., similar to impact in Tool #1) and likelihood (i.e., occurrence or probability of impacting safety and efficacy). The primary difference between Tools #1 and #2 is the use of uncertainty (in Tool #1) compared to likelihood (in Tool #2) in the second dimension of each tool.

The previously described tools are primarily useful in evaluating the criticality of product-related variants and impurities. Based on the outcome of the safety assessment, a rationale for not performing a clearance or impurity spiking study could be justified. For biologically active process components, the known clinically active dose or, when available, the NOAEL could be used for a safety assessment.

2.4.2 Quality Attribute Assessment Tool #1

Each quality attribute is evaluated for criticality using a risk ranking approach (per ICH Q9), which assesses the possible impact of each attribute on safety and efficacy. This ranking is determined by two factors: impact and the uncertainty (or certainty) of that impact.

Impact: The impact ranking of an attribute assesses either the known or potential consequences on safety and efficacy. The impact ranking considers the attribute's effect on:

1. efficacy, either through clinical experience or results using the most relevant potency assay(s),
2. pharmacokinetics/pharmacodynamics (PK/PD),
3. immunogenicity, and
4. safety.

The individual rankings for each impact category are provided in Table 2.3. The individual impact category with the highest ranking determines the overall impact ranking for an attribute.

Table 2.3 Impact Definition and Scale for Tool #1

Impact (Score)	Biological Activity or Efficacy[a]	PK/PD[a]	Immunogenicity	Safety
Very High (20)	Very significant change	Significant change on PK	ATA detected and confers limits on safety	Irreversible AEs
High (16)	Significant change	Moderate change with impact on PD	ATA detected and confers limits on efficacy	Reversible AEs
Moderate (12)	Moderate change	Moderate change with no impact on PD	ATA detected with in vivo effect that can be managed	Manageable AEs
Low (4)	Acceptable change	Acceptable change with no impact on PD	ATA detected with minimal in vivo effect	Minor, transient AEs
None (2)	No change	No impact on PK or PD	ATA not detected or ATA detected with no relevant in vivo effect	No AEs

AE = adverse event; ATA = anti-therapeutic antibody

[a]Quantitative criteria should be established for biological activity/efficacy and PK/PD. Significance of the change is assessed relative to assay variability.

Uncertainty: The uncertainty around the impact ranking is based on the relevance of the information used to assign the impact ranking (Table 2.4).

Table 2.4 Uncertainty Definition and Scale for Tool #1

Uncertainty (Score)	Description (Variants and Host Related Impurities)	Description (Process Raw Material)[a]
7 (Very High)	No information (new variant)	No information (new impurity)
5 (High)	Published external literature for variant in related molecule.	---
3 (Moderate)	Nonclinical or in vitro data with this molecule. Data (nonclinical, in vitro or clinical) from a similar class of molecule.	Component used in previous processes
2 (Low)	Variant has been present in material used in clinical trials.	---
1 (Very Low)	Impact of specific variant established in Clinical Studies with this molecule.	GRAS or studied in clinical trials

GRAS = generally regarded as safe

[a] Assesses the impact of a raw material as an impurity. Impact of the raw material on the product during manufacturing is assessed during process development.

Although this tool was originally developed for product variants and host-related impurities, a separate uncertainty scale was developed for process raw materials (e.g., insulin, glucose) to illustrate how a tool can be modified slightly to broaden its scope. The different scale was necessary for process raw materials because of the different type of information used to assess uncertainty of impact. Use of the process raw material uncertainty scale is applied to methotrexate later in the case study.

The impact and uncertainty scoring matrices were chosen to have different scales (2-20 for impact and 1-7 for uncertainty) to reflect the relative importance of the two factors, with impact outweighing uncertainty. The two values are multiplied to assign a risk score that determines an attribute's overall criticality.

Criticality (Risk Score) = Impact × Uncertainty

All quality attributes are assigned a degree of criticality (criticality continuum) based on their respective risk score. Risk scores range between a low of 2 to a high of 140.

This tool is applied throughout the product lifecycle starting pre-IND through licensure and post-approval. By performing this assessment at key points during process development, the development team will identify which attributes pose the highest risk and require mitigation. Mitigation will involve increasing the knowledge around the potential impact of that attribute through clinical, nonclinical and in vitro data, and/or through the control strategy employed. Over the product lifecycle, the criticality ranking (risk score) of the majority of quality attributes should decrease due to increased knowledge (lower uncertainty) at the same level of impact or due to a combination of less severe impact and increased knowledge.

2.4.3 QA Assessment Tool #2

In Tool #2, quality attributes are ranked for their criticality using a Preliminary Hazards Analysis (PHA) risk assessment approach based on two dimensions: Severity and Likelihood (probability). The severity takes into account risks associated with patient safety (toxicology, immunogenicity) and product efficacy (potency, pharmacokinetics/pharmacodynamics). Immunogenicity is a subset of the safety risk. The severity ranking of an attribute assesses the consequences, either known or potential, on safety and efficacy. It is based on product specific and general platform or prior knowledge (Table 2.5). Likelihood is defined as the probability that an adverse event impacts safety and/or efficacy due to a quality attribute being outside of established ranges based on current knowledge space. Knowledge space is based on clinical and non-clinical studies with this and similar molecules, and relevant literature information (Table 2.6). When limited clinical data is available for a particular quality attribute with respect to the likelihood of impacting safety and/or efficacy, a conservative score (≥ 5) is given.

A Risk Priority Number (RPN), which indicates the relative criticality of an attribute, is calculated by multiplying the Severity score and Likelihood score (see the equation below). The criticality of an attribute may decrease due to increased knowledge (typically reflected in a reduced Likelihood score) gained during the product lifecycle.

Criticality (Risk Priority Number [RPN]) = Severity × Likelihood

In Tool #2, a gradient approach is used to rank the criticality of product specific quality attributes, where, all quality attributes are assigned a degree of criticality (criticality continuum) based on their respective RPN ranking. RPN range is between a low of 1 to a high of 81.

Table 2.5 Severity Definition and Scale for Tool #2

Severity Score	Severity (Impact to Product Efficacy and Patient Safety)
9	Very high- death, microbiology related infections, hypersensitivity immune reaction
7	High- progression of cancer due to lower efficacy (potency, PK/PD) or serious immunogenicity response
5	Moderate- moderate immunogenicity or reduction in efficacy (potency, PK/PD)
3	Low- low immunogenicity potential or small reduction in efficacy (potency, PK/PD)
1	Very low- no measurable impact

Table 2.6 Likelihood Definition and Scale for Tool #2

Likelihood Score	Likelihood of Severity
9	Very high
7	High
5	Moderate
3	Low
1	Very low or never observed

2.4.4 Tool #3

Non-bioactive process components can be considered for their potential safety risk by evaluating an impurity safety factor (ISF). The ISF is the ratio of the impurity LD_{50} to the maximum amount of an impurity potentially present in the product dose:

$$\mathbf{ISF = LD_{50} \div Level\ in\ Product\ Dose}$$

where the LD_{50} is the dose of an impurity that results in lethality in 50% of animals tested, and the Level in Product Dose refers to the maximum amount of an impurity that could potentially be present and co-administered in a dose of product. Thus, the ISF is a normalized measure of the relationship between the level of an impurity resulting in a quantifiable toxic effect and the potential exposure of a patient to an impurity in the product. The higher the ISF, the greater the difference between the toxic effect and the potential product dose levels for an impurity, therefore, indicating a lower safety risk.

For the calculation of the ISF, the impurity Level in a Product Dose is determined based on worst-case assumptions. In the absence of an assay to detect an impurity, it is assumed that all of the impurity in the process co-purifies with the product, and no clearance is achieved by the purification process. Although this is a conservative assumption and unlikely to occur when orthogonal methods of separation are used in purification, it nevertheless allows calculation of the maximum potential content in the final product as a worst-case calculation. In the cases where a sufficiently sensitive

assay is available, the actual level of an impurity in the product is determined based on the assay quantitation.

LD_{50} values can be found in the literature for many process-related impurities. Therefore, the LD_{50} represents an established and quantitative indicator of acute toxicity that provides a useful comparator for assessing the risk posed by a process-related impurity. However, the LD_{50} is a relatively imprecise measure of toxicity, and LD_{50} values are generally orders of magnitude higher than the levels of process-related impurities. Another measure of toxicity, the NOAEL (no observed adverse effects level), represents the level of a compound shown to be safe in animal experiments. The NOAEL includes a longer term and more comprehensive assessment of organ-system safety compared to acute lethality by LD_{50} measures. Because the NOAEL is not readily available for most compounds, it cannot be routinely employed as a measure of safety. Comparison of LD_{50} and NOAEL information from the literature provides a link between safety and toxicity and can be useful for the assessment of risk. Literature searches have revealed examples of compounds for which both the NOAEL and the LD_{50} are reported, and these examples show that the NOAEL is generally one to two orders of magnitude below the LD_{50}. Based on this rationale, manufacturers can designate an ISF value that represents a conservative estimate of safety where values at or just above this threshold represent minimal risk. Alternatively, when available, the NOAEL can be used for safety risk assessment for process components.

The risk assessment strategy consists of a series of steps to evaluate an impurity in terms of its risk to product safety. This process is outlined in Figure 2.2 as a decision tree. Impurities can be eliminated from further consideration at any step where the safety risk is determined to be minimal.

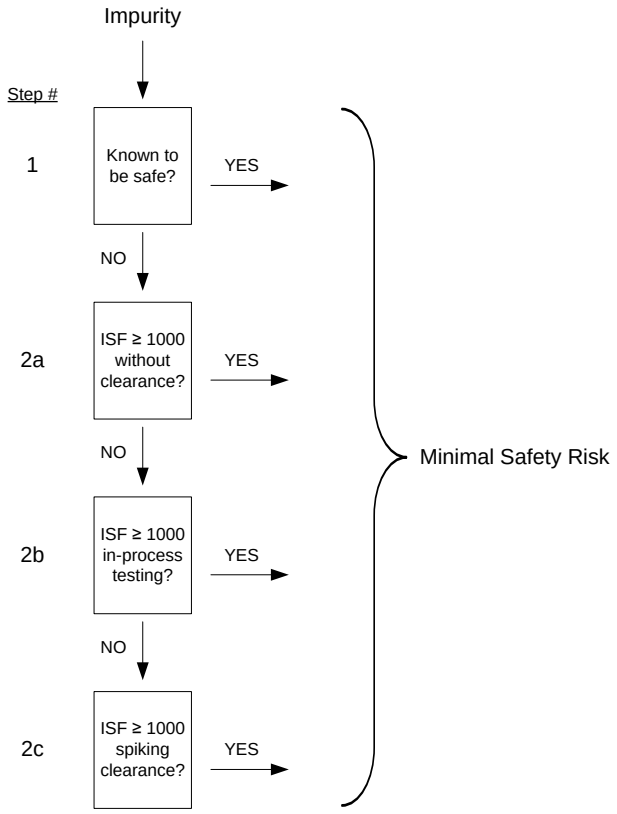

Figure 2.2 Representation of Process-related Impurities Safety Assessment Strategy

2.5 Examples of Quality Attribute Risk Assessment

Examples of quality attribute risk assessment are presented below. The examples provided below illustrate how the four types of information prior product knowledge (internal and external), laboratory data (in vitro data), nonclinical data and clinical data) are used to assess criticality. Note that in many cases, it may not be possible to gather all four types of information on a quality attribute.

2.5.1 Aggregation

Information used to assess the criticality of aggregation includes prior knowledge (both literature and platform knowledge), laboratory data and clinical data with A-Mab. The primary consideration for impact of aggregates is usually the potential for enhanced immunogenicity. In general, aggregated proteins have higher immunogenic potential (Rosenberg 2004; Rosenberg 2006; Hermeling et al, 2005). Based on literature for other similar antibodies, aggregates are also expected to affect binding to the Lymph-1 receptor, Fcγ receptors, and FcRn receptors compared to the monomer.

In *in vitro* studies, A-Mab aggregates (mostly dimer) have been purified and shown to have similar biological activity (both binding and functional activity) as monomer. Aggregates have been present in A-Mab clinical materials at a level of 1-3% and any ATAs (anti-therapeutic antibodies) that have been observed have been a part of the overall assessment of clinical safety and efficacy of the product. Table 2.7 describes the extent of prior knowledge, in-vitro studies, non-clinical studies, clinical experience and the claimed acceptable range associated with aggregation. Significant exposure to product with 5% aggregate was experienced in clinical trials with X-Mab with ATAs detected that had no effect on efficacy. X-Mab is similar to A-Mab in that they are both IgG1s, were used in oncology indications, their mechanisms of action are ADCC-related and both are administered by IV.

Table 2.7 Platform and Product Specific Experience with Aggregation

Prior Knowledge	In-vitro Studies	Non-clinical Studies	Clinical Experience	Claimed Acceptable Range
1-5% aggregate (at end of SL) in clinical studies and commercial production with X-Mab; minimal ATAs with no effect on efficacy; no SAE	Purified A-Mab dimer has similar biological activity to monomer	Animal models typically not relevant	1-3% aggregate	0-5%

SAE = serious adverse event; SL = shelf life

2.5.1.1 Tool #1

Because A-Mab aggregates have been purified and demonstrated to have no significant impact on potency, the score for biological activity/efficacy is 6 (2 for no impact and 3 for in vitro data for this molecule). Aggregates have the potential to have a moderate impact on PK based on literature data, so the score for PK/PD is 60 (12 for impact and 5 for literature data). Because aggregates have been present in A-Mab clinical lots and there were limited ATAs, the score for immunogenicity is 8 (4 for low impact and 2 for the uncertainty rank being based on A-Mab specific clinical trials). There have been a small number of SAEs during the A-Mab clinical trials, none of which could be directly

attributed to the level of aggregate. In addition, there is no known safety risk of aggregate independent of immunogenicity. Safety is scored as 8 (4 for low impact (acknowledging SAEs) and 2 for the basis that A-Mab aggregates have been in clinical trials). The highest score is 60 (for PK/PD), so aggregates are assigned a risk score of 60 and considered a high risk quality attribute.

2.5.1.2 Tool #2

Since there is specific immunogenicity data from A-Mab clinical trials, the immunogenicity impact is considered low for A-Mab (score of 3). An additional consideration is impact of aggregate on efficacy. Since A-Mab aggregate has been shown to have comparable potency, the efficacy impact is also taken into account, and considered low (score of 3). Since a relatively narrow range of aggregate levels have been tested in the clinic, the likelihood of A-Mab aggregate causing immunogenicity is considered high (score of 7). Considering that a wider range of aggregate levels have been used in the clinic for a similar antibody (X-Mab) and shown to be safe and efficacious, the likelihood score was reduced to a 5. Since aggregates have the potential to impact PK/PD based on literature data, the severity is considered moderate (score of 5). Since there is little known about this for A-Mab, the likelihood is scored at moderate (also a score of 5). The overall score (RPN) for aggregation is 25 (see Table 2.8) and is considered a moderate risk QA.

Table 2.8 Scoring Criticality of Aggregation using Risk Assessment Tools #1 and #2

Tool #1 (Impact x Uncertainty)				
Efficacy	**PK/PD**	**Immunogenicity**	**Safety**	**Risk Score**
2 × 3=6	12 × 5=60	4 × 2 = 8	2 x 2 = 4	60

Tool #2 (Severity x Likelihood)		
Severity	**Likelihood**	**Score (RPN)**
5	5	25

2.5.2 Glycosylation

A-Mab has been shown to be N-glycosylated at Asn residues in the constant region of each heavy chain. The oligosaccharide structure is of the complex biantennary type terminating in galactose. When both arms of the oligosaccharide chain terminate in galactose, the maximum moles galactose per mole heavy chain is two and the structure is referred to as G2. When one arm has terminal galactose, the structure is referred to as G1 and when there is no terminal galactose, the structure is referred to as G0. Criticality will be assessed separately for galactosylation (%G0, %G1 and %G2), sialylation, afucosylation, high mannose content and non-glycosylated heavy chain.

Table 2.9 describes the extent of prior knowledge, in-vitro studies, non-clinical studies, clinical experience and the claimed acceptable range associated with glycosylation.

Non-glycosylated mAbs are not ADCC competent (Tao 1989).

GALACTOSYLATION

ADCC requires binding by FcγIII receptor, which recognizes a determinant in the lower region of the Fc and is influenced by the Fc glycan (Jefferis 2005). Certain glycosylation variants can affect ADCC. For example, a-glycosylated IgG1 forms do not support ADCC, which is consistent with

the model where the lack of oligosaccharides modifies the Fc structure such that FcγRIII binding is abrogated.

The impact of the oligosaccharide moiety at Asn-A on both ADCC and CDC activities has been examined for A-Mab. A-Mab was treated with β-galactosidase to prepare variants that were completely G0 or treated with UDP-galactosyl transferase and UDP-galactose to convert all G0 and G1 structures to G2. Both the agalactosylated (G0) and fully galactosylated molecules (G2) had ADCC activity consistent with the control A-Mab. A statistically significant correlation between level of galactose and CDC activity was observed for A-Mab with CDC activity increasing with increasing galactose content. The fully agalactosylated material showed a 25% decrease in CDC activity over the control A-Mab, while the fully galactosylated material showed a 50% increase in CDC activity over the control. The observation with ADCC is consistent with literature studies with another IgG1 antibody that demonstrated that terminal galactose levels do not affect ADCC activity. Similarly, the observation with CDC is consistent with the literature (J. Hodoniczky et al, 2005).

The half-life of therapeutic IgGs are mediated through the neonatal Fc receptor, FcRn, pathway. Evidence suggests that Fc glycans do not influence interactions with FcRn and consequently are unlikely to impact the half-life or PK of the antibody (Jones et al, 2007).

Glycans produced by Chinese hamster ovary cells are found on endogenous human antibodies and therefore are not expected to impact immunogenicity or safety (Jefferis 2005). Although glycans containing galactose-α-1,3-galactose and N-glycoylneuraminic acid are potentially immunogenic (Jefferis 2005), these structures are not produced by Chinese hamster ovary cells. G0, G1, and G2 do not affect ADCC or proliferative activity and were therefore assigned a no impact.

AFUCOSYLATION

A non-clinical in vivo study suggested that ADCC is a key contributor to the efficacy of A-Mab against tumors. Clinical studies in adults with NHL indicated that patients treated with A-Mab had higher capability to mediate in vitro ADCC activity. Recent clinical evidence supports the role of ADCC in the in vivo effect of A-Mab at the level of the effector cell. In this study NHL patients were treated with A-Mab. Those patients with the FcγIIIa-158 V/V genotype, which confers higher ADCC of natural killer cells, had better response rates and progression free survival compared to FcγIIIa-158 V/F and FcγIIIa-158 F/F genotype.

Afucosylation of IgG1s correlates with ADCC (Shields 2002; Shinkawa 2003). Shields showed that fucose-deficient IgG1 had enhanced ADCC and improved binding to human FcγRIIIA. Shinkawa similarly demonstrated that an anti-human interleukin 5 receptor humanized IgG1 and an anti-CD20 chimeric IgG1 with low fucose had higher ADCC using purified human peripheral blood mononuclear cells (PBMCs) from healthy volunteers as effector cells. Afucosylated anti-HER2 antibody had significantly enhanced ADCC activity compared with the fucose-positive antibody using PBMCs from either normal donors or cancer patients (Suzuki 2007). A-Mab with 2-13% afucosylation was generated at small scale and tested in the ADCC assay. A linear correlation between afucosylation and ADCC activity was obtained with a range in ADCC activity of 70-130%. Taken together, the in vitro and in vivo data strongly suggest that ADCC is an important mechanism of action and that fucosylation can influence A-Mab efficacy.

SIALYLATION

Sialylation has also been shown to impact ADCC activity and inflammation. Higher sialylation resulted in lower ADCC activity and anti-inflammatory properties. A narrow range of sialylation (0-2%) on A-Mab has been tested in vitro and shown to have no detectable impact on binding to

FcγIIIa allotypes or ADCC activity, thus there is low risk that a much wider range of sialic acid than has actually been seen during the development history of the molecule (0-0.2%) would not be tolerable.

HIGH MANNOSE CONTENT

High mannose forms are afucosylated and are expected to significantly impact biological activity.

2.5.2.1 Tool #1

GALACTOSE CONTENT

Based on laboratory studies, the level of galactosylation (100% G0 and 100% G2) was shown to not affect ADCC activity for A-Mab, but did affect CDC activity significantly. Impact was assessed as high (score of 16) with an uncertainty of moderate (score of 3; in vitro data with this molecule). Literature data suggests that Fc glycans do not influence interactions with FcRn and consequently are unlikely to impact the PK of A-Mab. Based on this evidence, galactose content was assigned a no impact on PK (score of 2) and an uncertainty of 5 (published external literature for variant in related molecule). Glycans produced by Chinese hamster ovary cells are found on endogenous human antibodies and therefore are not expected to impact immunogenicity or safety. The impacts on immunogenicity and safety were assessed as none (score of 2) with an uncertainty score of 5 (published external literature for variant in related molecule). The overall risk score is 48 (based on efficacy) and is considered high risk.

Table 2.9 Platform and Product Specific Experience with Glycosylation

Attribute	Prior Knowledge	In-vitro Studies	Non-clinical Studies	Clinical Experience	Claimed Acceptable Range
Galactose Content	Clinical experience of 10-40% G0 for Y-Mab, another antibody with CDC activity as part of MOA; no negative impact on clinical outcome;	0-100% has statistical correlation with CDC activity with A-Mab	No animal studies	10-30%	10-40%
Afucosylation	1-11%; Clinical experience with X-Mab and Y-Mab; both X-Mab and Y-Mab have ADCC as part of MOA	A-Mab with 2-13% afucosylation tested in ADCC assay; linear correlation; 70-130%	Animal model available; modeled material (15%) shows no significant difference from 5%	5-10%; Phase II and Phase III	2-13%
High Mannose	Literature data show afucosylated forms impact ADCC	NA	NA	3-10%;	3-10%
Non-Glycosylated Heavy Chain	Literature data show that non-glycosylated forms impact ADCC	NA	NA	0-3%	0-3%
Sialic Acid	Literature data show sialylated forms can impact PK and ADCC	Level of 0-2% on A-Mab shows no statistical correlation to ADCC	NA	0-0.2%; Phase II and II	0-2%

Afucosylation

Since ADCC is thought to be the primary MOA for A-Mab and the extent of core fucosylation of A-Mab has been shown to inversely correlate with ADCC activity, the impact of afucosylation on efficacy has been assessed as very high (score of 20) with an uncertainty score of 3 (in vitro data with this molecule). PK, immunogenicity and safety are assessed the same as for galactose content. The overall risk score is 60 based on efficacy and is considered a very high risk.

Sialylation

Although a narrow range of sialylation on A-Mab had no detectable impact on binding to FcγIIIa allotypes or ADCC activity, sialylation variants were assessed as moderate impact (score of 12) since higher levels of sialylation can potentially reduce ADCC activity. The uncertainty score is 5 (published external literature for variant in related molecule). PK, immunogenicity and safety are assessed the same as for galactose content. The overall risk score is 60 (based on efficacy) and is considered a high risk.

High Mannose Content

Since high mannose structures are afucosylated, impact on efficacy was assigned the same as for afucosylation (impact score of 16; uncertainty score of 5). PK, immunogenicity and safety are assessed the same as for galactose content. The overall risk score is 80 (based on efficacy) and is considered a very high risk.

NON-GLYCOSYLATED HEAVY CHAIN

Since non-glycosylated forms do not support ADCC, their impact of efficacy was assigned the same as for afucosylation (impact score of 16; uncertainty score of 5) PK, immunogenicity and safety are assessed the same as for galactose content. The overall risk score is 80 (based on efficacy) and is considered a very high risk.

2.5.2.2 Tool #2

GALACTOSE CONTENT

The extent of terminal galactose (G0, G1, and G2) does not affect ADCC activity in A-Mab, but does affect CDC activity significantly. The impact of extent of terminal galactose on efficacy is then considered high for A-Mab (score of 7) based on CDC activity. Based on prior knowledge, likelihood of extent of galactose impacting efficacy is moderate (score of 5). The overall RPN score is 35 and is considered high risk.

AFUCOSYLATION

The extent of core fucosylation of IgG1s inversely correlates with ADCC activity (Shields 2002; Shinkawa 2003). The impact of extent of fucosylation on efficacy is considered high for A-Mab (score of 7) due to its dependence on ADCC. Based on prior knowledge, likelihood of extent of fucosylation impacting efficacy is moderate (score of 5). The overall RPN score is 35 and is considered high risk.

SIALYLATION

Since higher levels of sialylation can potentially reduce ADCC activity and due to the importance of ADCC to the mode of action of A-Mab, the impact of sialylation on efficacy is considered high for A-Mab (score of 7). However, based on prior knowledge of similar MAbs platform and/or published literature, there is a low likelihood that sialylation levels would be high enough to impact efficacy for A-Mab (likelihood score = 3). The overall RPN score is 21 and is considered moderate.

HIGH MANNOSE CONTENT

High mannose forms are a-fucosylated and expected to impact biological activity. The impact of high mannose on efficacy is considered high for A-Mab (score of 7) due to its dependence on ADCC. Based on prior knowledge, likelihood of high mannose impacting efficacy is moderate (score of 5). The overall RPN score is 35 and is considered high.

NON-GLYCOSYLATED HEAVY CHAIN

Non-glycosylated MAbs are not ADCC competent (Tao 1989). Therefore, the impact of non-glycosylated forms on efficacy is considered high for A-Mab (score of 7). Based on platform knowledge with similar MAbs, likelihood of non-glycosylated forms impacting efficacy is considered moderate (score of 5). The overall RPN score is 35 and is considered high.

Table 2.10 Scoring Criticality of Glycosylation using Risk Assessment Tools #1 and #2

Tool #1 (Impact × Uncertainty)					
Attribute	**Efficacy**	**PK/PD**	**Immunogenicity**	**Safety**	**Risk Score**
Galactose Content	16 × 3 = 48	2 × 5 = 10	2 × 5 = 10	2 × 5 = 10	48
Afucosylation	20 × 3 = 60	2 × 5 = 10	2 × 5 = 10	2 × 5 = 10	60
Sialylation	12 × 5 = 60	2 × 5 = 10	2 × 5 = 10	2 × 5 = 10	60
High mannose	16 × 5 = 80	2 × 5 = 10	2 × 5 = 10	2 × 5 = 10	80
Non-glycosylated heavy chain	16 × 5 = 80	2 × 5 = 10	2 × 5 = 10	2 × 5 = 10	80

Tool #2 (Severity x Likelihood)			
Attribute	**Severity**	**Likelihood**	RPN
Galactose Content	7	5	35
Afucosylation	7	5	35
Sialylation	7	3	21
High Mannose	7	5	35
Non-glycosylated Heavy Chain	7	5	35

2.5.3 Deamidation

Information used to assess the criticality of deamidation includes laboratory and nonclinical data with A-Mab. Deamidation at Asn or Gln residues is a common occurrence in human proteins (Huang et al., 2005; Lindner and Helliger, 2001) and recombinant monoclonal antibodies (Tsai et al., 1993). Asn-Gly sequences are present and conserved in the constant regions of IgGs, and these sites are known to undergo deamidation under physiological conditions.

The charged isoforms were characterized by fractionating A-Mab using ion-exchange chromatography (IEC). Peptide mapping of the fractions with on-line mass spectrometry (MS) demonstrated that the major deamidation sites of A-Mab are located in the Fc region. The primary deamidation site is Asn-A on the heavy chain as seen for other antibodies (Wang et al., 2005; Lyubarskaya et al., 2006). Other identified deamidation sites in A-Mab (Asn-B and Asn-C) were detected at lower levels. Because the deamidation sites are neither in the CDRs nor in a region of the Fc that affects Fc effector function, deamidation is unlikely to have an effect on the biological activity of the molecule. When tested, the deamidated isoforms exhibited similar antigen (Lymph-1) binding activity and biological activity compared to unfractionated A-Mab.

A-Mab was also incubated in human plasma at 37°C for up to 5 weeks. Peptide mapping was performed on all samples recovered and confirmed that the acidic isoforms were due to deamidation and identified the primary site of deamidation as Asn-A, which is located in the Fc region. Based on densitometry analysis of the native IEF gels, total deamidation of A-Mab ranged from 25% at the initial time point to 77% after 5 weeks of exposure to human plasma at 37°C. Deamidated A-Mab (up to 77% deamidation) exhibited antigen (Lymph-1) binding activity and was biologically active. For these assays the variability was greater than typically observed because the antibody isolated from the human serum was at very low concentrations. To overcome the limitations of the low

protein concentration in the plasma incubation study, a control study was conducted by incubating higher protein concentration A-Mab samples at the same conditions. Deamidation sites were confirmed to be the same as described above for the human plasma incubation study. In this study, binding activity was observed for deamidation levels of up to 79%. The results of the incubation studies support the conclusion that the deamidation occurs naturally in human plasma and does not impact A-Mab binding or biological activity.

A-Mab deamidated by incubation at pH 8.5 was evaluated in a PK study. Deamidated A-Mab (1 and 5 week incubation), as well as unmodified A-Mab, was administered to rats. A-Mab levels were measured in serum over time after IV dosing. The results showed no differences in the serum levels of A-Mab compared to deamidated A-Mab over time. These data indicate that deamidated A-Mab remains in serum at concentrations necessary for biological activity.

Although deamidation in the complementarity determining regions (CDRs) may affect antigen binding, no deamidation sites are present in the A-Mab CDRs. There are no known literature reports of immunogenicity in monoclonal antibodies linked to deamidation. The impact of deamidation on immunogenicity was evaluated in nonclinical and clinical studies. Cynomolgus monkeys were administered doses six-fold higher than the clinical dose at weekly intervals for six months and were thus exposed to far higher levels of deamidated product than would occur with the typical five monthly clinical doses. No immunogenicity or adverse reactions were observed.

Table 2.11 describes the extent of prior knowledge, in-vitro studies, non-clinical studies, clinical experience and the claimed acceptable range associated with deamidation.

Table 2.11 Platform and Product Specific Experience with Deamidation

Prior Knowledge	In-vitro Studies	Non-clinical Studies	Clinical Experience	Claimed Acceptable Range
Literature data reports that deamidation is a common occurrence	Stressed material (25-77%) tested in potency assay; no effect Serum studies showed rapid deamidation	Rat PK study showed no difference in serum levels between deamidated & non-deamidated A-Mab; No immunogenicity or AEs seen in cyno studies.	18-24%	No range claimed due to low criticality

2.5.3.1 Tool #1

Deamidation is unlikely to have an effect on the biological activity of A-Mab because the major deamidation sites are neither in the CDRs nor in a region of the Fc that affects Fc effector function. In addition, purified deamidated isoforms had similar biological activity as compared with unfractionated A-Mab. The score for biological activity is 6 (2 for no impact and 3 for laboratory data with this molecule). Deamidation is also expected to have no impact on PK based on the outcome of the rat PK study that showed no difference in serum levels over time between non-deamidated A-Mab and deamidated A-Mab. The score for PK for Tool #1 is 6 (2 for no impact and 3 for nonclinical data with this molecule). Immunogenicity is similarly scored a 6 (2 for no impact and 3 for nonclinical data with this molecule) based on the cyno study showing no immunogenicity at doses 6-fold higher than the clinical dose. Since deamidation occurs naturally under physiological conditions following dosing of A-Mab to patients and therefore the resulting charge isoforms were evaluated during clinical safety and efficacy trials, safety is scored a 4 (2 for no

impact and 2 for clinical studies). Using Tool #1, deamidation is scored overall as a 6 and is considered a very low risk quality attribute.

2.5.3.1 Tool #2

There were no immunogenicity or adverse reactions observed based on an animal nonclinical study. Deamidation occurs naturally under physiological conditions following dosing of A-Mab to patients. The impact of deamidation on efficacy is considered low for A-Mab (score of 3). Based on prior knowledge, the likelihood of deamidation impacting efficacy is low (score of 3). Using Tool #2, deamidation is given an RPN of 9 and is considered low risk.

Table 2.12 Scoring Criticality of Deamidation using Risk Assessment Tools #1 and #2

Tool #1 (Impact x Uncertainty)				
Efficacy	**PK/PD**	**Immunogenicity**	**Safety**	**Risk Score**
2 × 3 = 6	2 × 3 = 6	2 × 3 = 6	2 x 2 = 4	6

Tool #2 (Severity x Likelihood)		
Severity	**Likelihood**	**Score (RPN)**
3	3	9

2.5.4 Oxidation

The A-Mab amino acid residues most susceptible to tertiary-butyl hydroperoxide oxidation were determined to be heavy chain Met-250 and Met-420. Since those residues are not within the Fcγ receptor epitopes, oxidation of those residues is not expected to impact ADCC activity. This was confirmed by fully oxidizing those methionines in A-Mab by exposure to tertiary-butyl peroxide and showing that the oxidized material had comparable potency to the unoxidized control. The effect of A-Mab heavy chain Met-250 and Met-420 oxidation on PK can be inferred from studies that evaluated if those residues were involved in binding to human FcRn. Substitution of Met-250 with Ala had no effect on binding to FcRn and therefore is not expected to impact PK. Substitution of Met-420 with Leu did have a minor effect on FcRn binding (< 20% reduction) and therefore has the potential to impact PK, although it would be expected to be low. Although there is no A-Mab specific data related to oxidation and immunogenicity, oxidized A-Mab could lead to increased aggregation, thus increasing the potential for immunogenicity. Since oxidation has not been present in material used in the clinic, no A-Mab specific information about safety and oxidation is available. X-Mab did have a low level of oxidized methionines in its heavy chain in a number of lots used during clinical development. No difference in the level or type of adverse events was seen for those lots compared to others with no oxidation. X-Mab is similar to A-Mab in that they are both IgG1s, were used in oncology indications, their mechanisms of action are ADCC-related and both are administered by IV.

Table 2.13 describes the extent of prior knowledge, in-vitro studies, non-clinical studies, clinical experience and the claimed acceptable range associated with oxidation.

Table 2.13 Platform and Product Specific Experience with Oxidation

Prior Knowledge	In-vitro Studies	Non-clinical Studies	Clinical Experience	Claimed Acceptable Range
Some X-Mab lots had a low level of oxidation in heavy chain; no difference in adverse event type or frequency	Fully oxidized material tested in potency assay; no effect. Some effect on FcRn binding.	None	None	No range claimed due to low criticality

2.5.4.1 Tool #1

Oxidation at Met-250 and Met-420 is ranked as having a risk score of 6 for potency (2 for no impact and 3 for laboratory data). The effect on PK is ranked as a 12 (4 for low impact based on FcRn binding result for Met-420 and 3 for laboratory data). Since oxidized A-Mab could lead to aggregation, the score for the potential impact on immunogenicity is the same as for aggregation (4 for low impact and 2 for aggregates being present in A-Mab lots used in clinical trials; see Table 2.8). The impact on safety was assessed based on clinical data from X-Mab. The score is 6 (2 for no impact and 3 for data from a similar class of molecule). The overall risk score for oxidation is 12 and is considered a low risk.

2.5.4.2 Tool #2

Methionine oxidation in A-Mab by exposure to tertiary-butyl peroxide had no effect on potency. In addition, the oxidized residues are not within the Fcγ receptor epitopes and therefore are not expected to impact ADCC activity. However, oxidized A-Mab may lead to aggregation, thus increasing the immunogenicity potential. Therefore, the severity for oxidation in A-Mab was scored moderate (score of 5). A-Mab oxidation specific adverse events have not been observed in clinic, however, there is a moderate probability of increased immunogenicity due to oxidation (likelihood score of 5). The overall score for oxidation is 25 and represents moderate risk.

2.5.5 Host Cell Protein (HCP)

The information used to assess the criticality of Host Cell Protein (HCP) is prior knowledge with X-Mab. X-Mab is similar to A-Mab in that they are both IgG1s expressed from the same CHO cell host, were used in oncology indications, their mechanisms of action are ADCC-related and both are administered by IV. Because the Drug Substance manufacturing processes for A-Mab and X-Mab are very similar, and the same reagents are used for the detection of HCP, it is a reasonable assumption that both processes have a similar set of HCP.

Table 2.14 Scoring Criticality of Oxidation using Risk Assessment Tools #1 and #2

Tool #1 (Impact x Uncertainty)				
Potency	**PK/PD**	**Immunogenicity**	**Safety**	**Risk Score**
2 × 3 = 6	4 × 3 = 12	4 × 2 = 8	2 x 3 = 6	12

Tool #2 (Severity x Likelihood)		
Severity	**Likelihood**	**Score (RPN)**
5	5	25

In a dose escalation clinical trial (50 patients; Phase I) with X-Mab at the maximum dose of 30 mg/kg, one patient experienced a very mild allergic response. There were minor and transient adverse events in the Phase I trial. The material dosed in that Phase I trial contained 120 ng/mg HCP or a maximum of dose of HCP of 3600 ng/kg. In that trial, patients were exposed to 12-times the levels of HCP expected in the A-Mab process (i.e., a maximum dose of 200 ng/kg).

The X-Mab material with the high level of HCP did not show any difference in potency or FcRn binding compared to other X-Mab material containing no detectable HCP, therefore the impact on efficacy and PK are expected to be low.

Table 2.15 summarizes the extent of prior knowledge, in-vitro studies, non-clinical studies, clinical experience and the claimed acceptable range associated with host cell protein.

Table 2.15 Platform and Product Specific Experience with Host Cell Protein

Prior Knowledge	In-vitro Studies	Non-clinical Studies	Clinical Experience	Claimed Acceptable Range
Up to 3600 ng/kg in X-Mab Phase I trial (corresponds to 120 ng/mg HCP level)	NA	NA	5-20 ng/mg	0-100 ng/mg

2.5.5.1 Tool #1

All 4 categories were ranked based on data for X-Mab, so the uncertainty score is 3 (corresponding to data from a similar class of molecule). Potency and PK are scored as no impact (score of 2) because the X-Mab material containing a high level of HCP had similar potency and FcRn binding compared to material that did not contain a detectable level of HCP. Immunogenicity is ranked a moderate impact (in vivo effect was manageable; score of 12) with a risk score of 36. Safety is ranked as a low impact due to the minor and transient adverse events seen with X-Mab's Phase I clinical trial. The overall risk score for HCP is 36 and represents a moderate risk.

2.5.5.2 Tool #2 (HCP)

The primary concern for HCP is the potential for immunogenicity based on X-Mab. The severity score is moderate (5) to reflect the moderate level of immunogenicity seen with X-Mab. A low likelihood score (3) is assigned due to the broad clinical experience range seen with A-Mab. The overall score (RPN) is 15 and represents a moderate risk.

Table 2.16 Scoring Criticality of HCP using Risk Assessment Tools #1 and #2

Tool #1 (Impact x Uncertainty)				
Potency	**PK/PD**	**Immunogenicity**	**Safety**	**Risk Score**
2 × 3 = 6	2 × 3 = 6	12 × 3 = 36	4 x 3 = 12	36

Tool #2 (Severity x Likelihood)		
Severity	**Likelihood**	**Score (RPN)**
5	3	15

2.5.6 DNA

DNA is assessed for criticality based on literature and laboratory data. The theoretical risk associated with DNA is the potential for oncogene transfer. The World Health Organization (WHO) has recommended that DNA levels be consistently reduced to less than 10 ng DNA per dose for proteins intended for human therapeutics that are produced by continuous cell line such as CHO. The DNA limit recommended by the WHO has been widely adopted by the biotechnology industry.

DNA characterized from A-Mab and other MAbs produced by the same platform process (X-Mab, Y-Mab and Z-Mab) was determined to be less than 60 bp in size and, therefore represents a low risk of oncogene transfer. In vitro studies with Y-Mab spiked with its own purified DNA to a level consistent with the WHO limit showed no impact on potency or FcRn binding.

Table 2.17 describes the extent of prior knowledge, in-vitro studies, non-clinical studies, clinical experience and the claimed acceptable range associated with DNA.

Table 2.17 Platform and Product Specific Experience with DNA

Prior Knowledge	In-vitro Studies	Non-clinical Studies	Clinical Experience	Claimed Acceptable Range
Platform process (X-, Y-, Z-Mab) typically has DNA that are typically smaller than 60 bp; DNA spike studies with Y-Mab showed no impact on potency or FcRn binding	A-Mab: DNA size typically < 60 bp	NA	None as DNA is consistently cleared from the process	Less than 10^{-3} ng/dose

2.5.6.1 Tool #1

DNA is scored as having no impact (score of 2) for all 4 categories based on prior knowledge with a similar molecule (uncertainty score of 3). The efficacy and PK impact scores are based on the in vitro studies with Y-Mab, while the immunogenicity and safety scores are based on the fact that the DNA recovered across all 4 platform processes is typically smaller than 60 bp. The overall risk score is 6 and is considered a very low risk.

2.5.6.2 Tool #2

DNA was assigned a very low severity score (score of 1) based on no measurable impact on potency and PK, and no expected impact on immunogenicity or safety based on the size of DNA fragments observed. The likelihood score is very low (score of 1) because impact on safety, efficacy, immunogenicity or PK that has been attributed to DNA has never been observed with the platform process. The RPN score is 1 and is considered a very low risk.

Table 2.18 Scoring Criticality of DNA using Risk Assessment Tools #1 and #2

Tool #1 (Impact x Uncertainty)				
Efficacy	**PK**	**Immunogenicity**	**Safety**	**Risk Score**
2 × 3 = 6	2 × 3 = 6	2 × 3 = 6	2 x 3 = 6	6

Tool #2 (Severity x Likelihood)		
Severity	**Likelihood**	**Score (RPN)**
1	1	1

2.5.7 Leached Protein A

The criticality of Protein A is assessed based on literature and prior knowledge, and nonclinical studies. Protein A is a cell wall protein deriving from Staphylococcus aureus, which exhibits unique binding properties for a variety of mammalian IgGs. Protein A interacts primarily with the Fc domains of IgG molecules, although there is some binding to Fab regions for certain isotypes. Protein A may have immunogenic (Gomez et al., 2004) and mitogenic effects (Kraft and Reid, 1985).

Protein A immunoadsorption is approved by the FDA to treat idiopathic thrombocytopenic purpura (ITP) and rheumatoid arthritis (RA). Silica-Immobilized Protein A (PROSORBA, Fresenius HemoCare, Inc) is a single use therapeutic medical device approved for the extracorporeal irnmunoadsorption of IgG and circulating immune complexes, containing 200 mg of Protein A. Plasma depleted of IgG is returned to the patient during a two hour period of plasmapherisis and the therapeutic regimen calls for weekly treatments for 12 weeks. Because of this medical use, the human health implications of potential Protein A have been extensively studied. No adverse events were associated with Protein A leachate for PROSORBA. Adverse events, with differing opinions about their level of significance, are attributed to activation of complement by the immobilization to circulating immune complexes and IgG on the column, and possibly activation of T cells.

Previous studies in cynomolgus monkeys showed that doses of Protein A at 1 mg/kg over a period of four weeks were well tolerated. Two male monkeys per dose group were tested at 0.16, 0.4 and 1.0 mg/kg, daily. Histopathology showed no treatment-related changes in any animals receiving Protein A at any dose level.

Table 2.19 summarizes the extent of prior knowledge, in-vitro studies, non-clinical studies, clinical experience and the claimed acceptable range associated with leached protein A.

Table 2.19 Platform and Product Specific Experience with Leached Protein A

Prior Knowledge	In-vitro Studies	Non-clinical Studies	Clinical Experience	Claimed Acceptable Range
Protein A is used in approved therapy (PROSORBA)	None	Primate studies showed doses up to 1 mg/kg well tolerated	None as protein A is always cleared from the process	No range claimed due to low to moderate criticality

2.5.7.1 Tool #1

If Protein A was present with A-Mab, it would be expected to bind the Fc domain of A-Mab and impact both efficacy and PK. The impacts were both assessed as moderate (score of 12) since not all leached protein A is intact (Carter-Franklin et al., 2007). Both efficacy and PK were also scored with very low uncertainties (score of 1; studied in clinical trials on the Process Raw Material scale). Since there is literature data that suggests Protein A could be immunogenic, immunogenicity was scored as high impact (score of 16; significant change) and very low uncertainty (score of 1; studied in clinical trials on the Process Raw Material scale). Both the PROSORBA and cynomolgus monkey data indicate that no adverse events (independent of immunogenicity) are likely due to Protein A. Safety was assessed no impact (score is 2) based on clinical/nonclinical data with the molecule (uncertainty score of 3). The overall risk score is 16 (based on immunogenicity) and is considered a low to moderate risk.

2.5.7.2 Tool #2

Based on the potential immunogenicity of Protein A, a moderate severity (score of 5) is assigned. Low likelihood score (score of 3) is assigned based on prior knowledge and primate tolerance data. The RPN score of 15 is considered a moderate risk.

Table 2.20 Scoring Criticality of Leached Protein A using Risk Assessment Tools #1 and #2

Tool #1 (Impact x Uncertainty)				
Efficacy	**PK/PD**	**Immunogenicity**	**Safety**	**Risk Score**
12 × 1 = 12	12 × 1 = 12	16 × 1 = 16	2 x 3 = 6	16

Tool #2 (Severity x Likelihood)		
Severity	**Likelihood**	**Score (RPN)**
5	3	15

2.5.8 Methotrexate

The criticality of methotrexate (MTX) is assessed based on knowledge from clinical exposure to MTX in other applications. MTX is assessed using Tool #1 and Tool #3.

Methotrexate is a cytotoxic chemical that acts by inhibiting dihydrofolate reductase (DHFR), and also by directly inhibiting the folate-dependent enzymes of *de novo* purine and thymidylate

synthesis. It is used in the early seed cultures to maintain selective pressure. It is not used in production cultures.

Medically, MTX is indicated for the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis. MTX has the potential for serious toxicity if used in high doses, resulting in a "Black Box Warning" in its label for bone marrow, liver, lung and kidney toxicities. However, these toxicities are related to dose and frequency, and most adverse effects are reversible if detected early enough. MTX is an FDA-approved drug for the treatment of certain neoplastic diseases, severe psoriasis and adult rheumatoid arthritis.

Table 2.21 describes the extent of prior knowledge, in-vitro studies, non-clinical studies, clinical experience and the claimed acceptable range associated with methotrexate.

Table 2.21 Platform and Product Specific Experience with MTX

Prior Knowledge	In-vitro Studies	Non-clinical Studies	Clinical Experience	Claimed Acceptable Range
MTX is used in approved therapies	None	None	None as MTX is always cleared from the process	No range claimed due to low criticality

2.5.8.1 Tool #1

With no expected impact on potency, PK and immunogenicity based on human clinical trials with MTX, MTX is ranked as having no impact (score of 2) with an uncertainty of 1 (studied in clinical trials; Process Raw Material) for those 3 categories. Because of the extensive list of adverse events that are reversible, impact based on safety was ranked high (16, reversible AEs) with an uncertainty of very low (1, studied in clinical trials; Process Raw Material). The overall risk score is 16 (based on safety) and is considered a low to moderate risk.

Table 2.22 Scoring Criticality of Methotrexate using Risk Assessment Tools #1

Tool #1 (Impact x Uncertainty)				
Potency	**PK/PD**	**Immunogenicity**	**Safety**	**Risk Score**
2 ×1 = 2	2 × 1 = 2	2 × 1 = 2	16 x 1 = 16	16

2.5.8.2 Tool #3

The risk associated with methotrexate was assessed using the impurity safety factor (ISF) method described as Tool #3 (see Table 2.23). The results of the assessment are shown in the table below. As the ISF was determined to be greater than 1000, it was concluded to be a minimal safety risk. Because methotrexate is used clinically, an alternative approach would be to use the known clinically active dose or the NOAEL for the safety assessment.

2.5.9 C-terminal Lysine Truncation

The criticality of C-terminal lysine truncation was assessed based on prior knowledge and laboratory studies with A-Mab.

C-terminal lysine is a common post-translational modification in humanized monoclonal antibodies (Harris, et al, 1995). The effect of C-terminal lysine variability on bioavailability has been evaluated for another type of recombinant protein, lenercept [an immunoadhesin comprising the Fc domain of human IgG1 and two TNF binding domains derived from the TNF receptor TNFR1], the cleavage of C-terminal lysine varied from 50% to 89%, however, this variation had no impact on PK profiles (Keck, et al, 2007).

It has previously been shown that a similar monoclonal (X-Mab) produced using two different cell culture processes had significantly different levels of C-terminal lysine processing (see Table 2.24). In addition, the pattern for X-Mab Process II is very similar to the pattern for A-Mab. Table 2.25 shows PK data from human serum following a 3 mg/kg IV dose of representative lots of X-Mab Process I and Process II. The data show no significant difference in PK. Together, the C-terminal lysine distribution and PK data demonstrate that C-terminal lysine truncation does not affect the bioavailability of other similar MAbs.

Table 2.23 Scoring Criticality of Methotrexate using Risk Assessment Tools #3

Methotrexate Safety Factor Calculation Cell culture A-Mab titer: 4.1 mg/mL Dose (A-Mab/body weight): 10 mg/Kg Route of administration: Intravenous					
Component	**Concentration (mg MTX/L CM)**	**Impurity/A-Mab (mg MTX/mg A-Mab)**	**Dose [TME] (mg MTX/Kg)**	**LD_{50} (mg/Kg)**	**ISF (LD_{50}/TME)**
Methotrexate	0.018177	4.43×10^{-6}	0.0000443	6	135,000

MTX = methotrexate
CM = conditioned medium
TME = theoretical maximum exposure

LD_{50} = median lethal dose, LD_{50} of 6 is for intraperitoneal administration in rat
Note: as a reference 1 mg/kg or 1 mg/L = 1 ppm

Because of the identical amino acid sequence of the Fc portions of A-Mab and X-Mab, and the nearly identical C-terminal lysine distributions between X-Mab Process II and A-Mab, C-terminal lysine truncation is not expected to affect the bioavailability of A-Mab. No differences in adverse events or immunogenicity were seen between clinical trials that used X-Mab material from either Process I or Process II.

Table 2.24 C-terminal Lysine Distribution Pattern

Molecule	% 0-Lys	% 1-Lys	% 2-Lys
X-Mab (Process I)	52.4 ± 3.4	25.6 ± 0.9	22.0 ± 2.7
X-Mab (Process II)	84.9 ± 1.5	13.8 ± 1.2	1.3 ± 0.5
A-Mab	87.4 ± 2.3	11.2 ± 1.8	1.4 ± 0.8

Table 2.25 Trough Concentrations and Half-life of the 3 mg/kg IV Dose of Representative Lots of Process I and Process II X-Mab

Molecule	Process	C_{trough} (µg/ml)	Half-life ($t_{1/2}$) (days)	Clinical Study
X-Mab	Process I	8.8 ± 1.78	20.1 ± 3.28	001
	Process II	9.7 ± 1.78	19.8 ± 3.38	002

A-Mab C-terminal lysine variants were purified to produce material containing predominantly the 0-Lys variant, 1-Lys variant and 2-Lys variant. All 3 preparations had no measureable difference in biological activity compared to the A-Mab Reference Standard that contained all lysine variants. This demonstrated that C-terminal lysine truncation does not have an effect on the biological activity of A-Mab.

To investigate the effect of serum incubation on C-terminal lysine heterogeneity, a time course study was performed where A-Mab was incubated in human serum for 0, 1, 6, 24, and 72 hours at 37°C. A-Mab was isolated and analyzed at each time point and the levels of the predominant lysine-containing species were determined. The results show that, when A-Mab is incubated in human serum, the 1-Lys variant is converted to the 0-Lys form within 6 hours, presumably by endogenous serum carboxypeptidases. Because this conversion occurs rapidly, and the IV half-life of A-Mab is much longer than 6 hours, the 0-Lys form would be expected to be the predominant circulating form of A-Mab in the serum. An additional analysis was performed using A-Mab isolated from a clinical sample (clinical study 001, Day 3). When the isolated A-Mab was analyzed for the presence of C-terminal lysine variants, all of the isolated A-Mab was in the 0-Lys form. This further supports the findings from the serum incubation study that suggests that the predominant form of circulating A-Mab is the 0-Lys form.

The assessment that C-terminal lysine truncation is a Quality Attribute with low criticality applies to intravenous administration. Other products delivered via other routes of administration would need to be assessed independently.

Table 2.26 summarizes the extent of prior knowledge, in-vitro studies, non-clinical studies, clinical experience and the claimed acceptable range associated with C-terminal lysine truncation.

Table 2.26 Platform and Product Specific Experience with C-Terminal Lysine Truncation

Prior Knowledge	In-vitro Studies	Non-clinical Studies	Clinical Experience	Claimed Acceptable Range
X-Mab Clinical data with two different versions of X-Mab show no difference in PK.	A-Mab C-Terminal Lysine variants are equally potent.	None	A-Mab containing C-terminal Lysine variants used in the clinic. Serum samples show predominant species is 0-Lys.	No range claimed due to low criticality

2.5.9.1 Tool #1

The impact of C-terminal lysine truncation on efficacy is ranked as none (score of 2) due to the laboratory studies with purified C-terminal lysine variants showing no difference in potency. The uncertainty for efficacy would be scored a 3 (in vitro data with this molecule). The impact on PK is similarly scored as none (score of 2) due to the clinical results from X-Mab. The uncertainty for PK would also be scored a 3 (clinical data from a similar class of molecule). Since it is likely that the predominant form of A-Mab circulating in the body is the 0-Lys form, there is likely no effect of C-terminal lysine truncation on immunogenicity and safety. Based on that fact and the clinical results from X-Mab, both immunogenicity and safety were given an impact rating of none (score of 2) with an uncertainty ranking of low (score of 3; clinical data from a similar class of molecule). The overall risk score is 6 (based on all 4 categories) and is considered a very low risk.

2.5.9.2 Tool #2

C-terminal lysine truncation does not have a significant affect on the biological activity or bioavailability of A-Mab. In addition, C-terminal lysine processing is observed frequently in plasma derived antibodies. Therefore, the impact of C-terminal lysine truncation on efficacy is considered very low for A-Mab (score of 1). There is a very low likelihood of C-terminal lysine truncation impacting efficacy (score of 1) and is considered a very low risk.

Table 2.27 Scoring Criticality of C-Terminal Lysine using Risk Assessment Tools #1 and #2

Tool #1 (Impact x Uncertainty)				
Potency	**PK/PD**	**Immunogenicity**	**Safety**	**Risk Score**
2 × 3 = 6	2 × 3 = 6	2 × 3 = 6	2 x 3 = 6	6

Tool #2 (Severity x Likelihood)		
Severity	**Likelihood**	**Score (RPN)**
1	1	1

2.6 Quality Attribute Risk Assessment Summary

A summary of the attribute risk assessments illustrated in this case study is shown in Table 2.28. The table lists the attributes and the risk rankings for Tools 1, 2, and 3. Although both Tools #1 and #2 do not categorize attributes specifically as Critical or Non-Critical, a level of criticality has been assigned to all of the attributes in Table 2.28. The levels are very low (VL), low (L), moderate (M), high (H) and very high (VH). The attributes that are of high and very high criticality have been called "Critical". All other attributes are referred to as either being of very low, low or moderate criticality.

The two risk assessment tools are not expected to give identical scoring because of the different ranking and numerical scoring that each tool is based upon. In general, the relative scores are very similar between the two tools. For many of the attributes, the score for Tool #1 is approximately twice that of Tool #2 consistent with the scoring range for Tool #1 being approximately 1.7 times that of Tool #2. There are a few differences between the results for each tool (e.g., aggregation and sialic acid being scored high risk with Tool #1 and medium risk with Tool #2; oxidation and leached protein A being scored low with Tool #1 and medium with Tool #2). These differences can be primarily attributed to the difference between how uncertainty and likelihood are scored. Tool #2 considers the likelihood score associated with use of platform data to be higher relative to how it is ranked in Tool #1. In addition, the likelihood scale for Tool #2 is somewhat more subjective than the uncertainty scale for Tool #1.

Scoring could change significantly as a product moves through its lifecycle and more knowledge is gained about the product (changing the impact assessment and reducing the uncertainty). Using Tool #1, if ADCC was not thought to be part of the MOA for A-Mab in early development, afucosylation would have been scored a 10 based on PK, immunogenicity or safety (2 for no impact and 5 for literature data; see Table 2.28). As more data and information is obtained through development identifying and confirming that ADCC is part of the MOA for A-Mab, the afucosylation risk score would eventually change to 60 (see Table 2.28). Scoring using Tool #2 would be similar.

Table 2.28 Summary of Quality Attribute Risk Assessments

	Tool #1			Tool #2			Tool #3
Product Quality Attribute	**Impact**	**Uncertainty**	**Risk Score**	**Severity**	**Likelihood**	**RPN**	**ISF**
Aggregation*	12	5	60 (H)	5	5	25 (M)	ND
C-terminal lysine	2	2	4 (VL)	1	1	1 (VL)	ND
Deamidated isoforms	2	2	4 (VL)	3	3	9 (L)	ND
Galactose Content*	16	3	48 (H)	7	5	35 (H)	ND
Afucosylation*	20	3	60 (H)	7	5	35 (H)	ND
Sialic Acid Content*	12	5	60 (H)	7	3	21 (M)	ND
High Mannose Content*	16	5	80 (VH)	7	5	35 (H)	ND
Non-Glycosylated Heavy Chain*	16	5	80 (VH)	7	5	35 (H)	ND
Oxidation	4	3	12 (L)	5	5	25 (M)	ND
DNA	2	3	6 (VL)	1	1	1 (VL)	ND
Methotrexate	16	1	16 (L)	ND	ND	ND	268000
HCP*	12	3	36 (M-H)	5	3	15 (M)	ND
Protein A	16	1	16 (L)	5	3	15 (M)	ND

ISF = impurity safety factor; ND = not determined; RPN = Risk Priority Number; VH = very high; H=high; M=moderate; L=low; VL=very low.
*Considered Critical Quality Attributes.

2.7 Attribute Ranges

Table 2.29 summarizes the range of experience for select quality attributes considered in this case study and the corresponding claimed acceptable range for each attribute. These attributes were selected to illustrate the principles of QbD. Although, the risk ranking did not identify deamidation as a significant risk, it is included here because it is used as a measure of consistency in the process characterization studies.

Table 2.29 Basis for Acceptable Ranges for the Quality Attributes Discussed in the Case Study

Attribute	Prior Knowledge	In-vitro Studies	Non-clinical Studies	Clinical Experience	Claimed Acceptable Range	Rationale for Claimed Acceptable Range
Afucosylation	1-11%; Clinical experience with X-Mab and Y-Mab; both X-Mab and Y-Mab have ADCC as part of MOA	A-Mab with 2-13% afucosylation tested in ADCC assay; linear correlation; 70-130%	Animal model available; modeled material (15%) shows no significant difference from 5%	5-10%; Phase II and Phase III	2-13%	2-13% afucosylation correlates with 70-130% ADCC activity. Lower end covered by prior knowledge; upper end covered by modeled material in animal model.
Aggregation	1-5% aggregate (at end of SL) in clinical studies and commercial production with X-Mab; minimal ATAs with no effect on efficacy; no SAE	Purified A-Mab dimer has similar biological activity to monomer	Animal models typically not relevant	1-3% aggregate	0-5%	5% upper range claimed based on prior clinical experience with X-Mab.
Deamidated isoforms	Literature data reports that deamidation is a common occurrence	Stressed material (25-77%) tested in potency assay; no effect; Serum studies showed rapid deamidation	No animal studies	18-24%	None claimed; measure of consistency	NA
Galactose Content	Clinical experience of 10-40% G0 for Y-Mab, another antibody with CDC activity as part of MOA; no negative impact on clinical outcome;	0-100% has statistical correlation with CDC activity with A-Mab	No animal studies	10-30%	10-40%	Range is based on a combination of prior knowledge (Y-Mab experience) and clinical experience.
HCP	Up to 3600 ng/kg in X-Mab Phase I trial (corresponds to 120 ng/mg HCP level)	NA	NA	5-20 ng/mg	0-100 ng/mg	100 ng/mg upper limit claimed based on prior clinical experience with X-Mab.
Sialic Acid	Literature data show sialylated forms can impact PK and ADCC	Level of 0-2% on A-Mab shows no statistical correlation to ADCC	NA	0-0.2%; Phase II and II	0-2%	In vitro studies with A-Mab.
High Mannose	Literature data show afucosylated forms impact ADCC	NA	NA	3-10%;	3-10%	Clinical Experience with A-Mab.
Non-Glycosylated Heavy Chain	Literature data show that non-glycosylated forms impact ADCC	NA	NA	0-3%	0-3%	Clinical Experience with A-Mab.

SAE = serious adverse event; SL = shelf life

2.8 Testing Plan as a Part of Control Strategy

A testing plan is a part of the overall control strategy (see Section 6) that takes into account the assessment of quality attribute criticality and the process' ability to control the quality attribute. Testing can include routine monitoring, characterization testing, in process testing, stability testing, or raw material testing. All product quality attributes are evaluated to determine the appropriate testing required as part of the product testing plan. Not all high risk CQAs automatically map to testing and all low risk CQAs are not automatically excluded from testing. For example, high risk QAs with low to moderate process capability would typically require in-process control or specification testing, while high risk QAs with high to very high process capability would not typically require testing. In the latter case, validation testing on the Qualification campaign lots would be sufficient to demonstrate control and testing would not be required. QAs with very low or low criticality and moderate to high process capability would likely not require testing.

The information gathered during the QA risk assessment is useful for justifying specifications and rationalizing the selection of various control mechanisms, such as raw material control, in-process testing, release testing, and stability testing, as well as, comparability testing requirements for post-approval changes. The latter case may include testing the high risk QA with high process capability (e.g., HCP) and some of the QAs with low criticality and moderate to high process capability (e.g., DNA, methotrexate and leached Protein A). The decision to test these additional QAs would be based on the post-approval change being made and whether or not the QA could potentially be impacted by that change.

The acceptable ranges for these quality attributes are important in that they set the acceptable range or boundary for process parameters included in the design space.

2.9 References

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3 Upstream Manufacturing Process Development

The upstream process for A-Mab represents a well established platform with extensive process performance history. The seed expansion steps are solely based on the existing platform process and no further process optimization was done for A-Mab. By contrast, the production bioreactor was further optimized to meet commercial demands.

The upstream process development approach leverages prior knowledge from other antibodies to guide process development and characterization studies. Multivariate models are created to describe the interactions between process parameters and quality attributes. A Bayesian statistical approach is used to define the limits of the production bioreactor design space to provide a high degree of assurance that the product quality attribute limits are met. The design space is based on scale-independent process parameters and thus is applicable to all scales of operation.

The concept of an Engineering Design Space is presented for the production bioreactor. This concept is defined as the multidimensional combination of bioreactor design characteristics and engineering parameters that provide assurance that the production bioreactor performance will be robust and consistent and will meet product quality targets. Characterization of bioreactor design, operation parameters, control capabilities, product quality and cell culture process performance provide the basis for scientific understanding of the impact of scale and equipment design that underpins the Engineering Design Space.

A life-cycle approach to process validation is described. This begins with process development activities, and carries through process characterization to a continuous process verification approach for commercial manufacturing, which is based on multivariate statistical analysis to provide assurance of product quality throughout the product life cycle.

Key Points from Upstream Section

1. Platform process and prior knowledge obviate need to optimize seed expansion
2. Design space established for production bioreactor
    - Scale independent
    - Supported by Bayesian statistical model
    - Supported by concept of Engineering Design Space.
3. Lifecycle approach to process validation incorporating continuous process verification

3.1 Upstream Manufacturing Process Development

This section summarizes the approaches used to develop the upstream manufacturing process for A-Mab using the principles of Quality-by-Design (QbD). The examples provided in this case study show how the knowledge gained through prior experience with similar monoclonals and process development studies and manufacturing experience with A-Mab provides a scientific understanding to support the establishment of the design space and the Control Strategy.

The upstream development sections include exemplification of the following QbD principles:

1) Use of prior knowledge and A-Mab development data to support categorization of the seed expansion steps as non-critical because they do not impact product quality.
2) Use of prior platform knowledge, risk assessments and DOE approaches to define the commercial manufacturing process for the production bioreactor step.
3) Examples of risk assessments and DOE approaches to link process parameters to product Quality Attributes. Description of how this information is used to create a multivariate model to define design space.
4) Rationale for control strategy based on design space and risk assessment results
5) Demonstration of how the design space is applicable to multiple operational scales and bioreactor configurations. This includes the use of multivariate analysis models to justify the use of scale-down models for the production bioreactor and a detailed engineering analysis to describe the design space in terms of scale-independent parameters.
6) Description of a lifecycle approach to validation which includes continuous process verification through statistical Multivariate Analysis to demonstrate that the process is in a state of control and delivers product quality attributes as predicted by the design space.
7) Examples of anticipated post-launch process movement within the Design space as part of the product life-cycle management.

Table 3.1 provides a summary of how the QbD approaches exemplified in this case study contrast with “traditional” process development and validation approaches. Here, we recognize that traditional approaches can span the gamut from using One-Factor-At-a-Time (OFAT) experiments to full DOEs, and that many larger and well established biotechnology companies have been using aspects of QbD principles for many years. However, it is important to highlight that it is the holistic application of such principles that provide the enhanced QbD approach that this case study embodies – i.e. it is the sum of approaches outlined in this table that provide the scientific and risk based approach for process and product understanding and that serve as the basis for the proposed design space, control strategy and continuous process verification.

Table 3.1 QbD Compared to "Traditional" Approach for Upstream Development

Quality by Design Approaches Exemplified in the A-Mab Upstream Process	"Traditional" Upstream Process Development Approaches
Thorough process understanding is based on prior knowledge and product specific experience.	Process understanding is limited to product-specific empirical information
Establish predictive relationships between process parameters and product quality attributes using statistically designed experiments. Acceptable operating conditions expressed in terms of a design space	Some experiments conducted using single-variable approaches, potentially overlooking parameter interactions. Acceptable operating ranges expressed as univariate Proven Acceptable Ranges
Systematic process development based on risk management tools.	Process development based on established industry precedents.
Rational approach to establishing a control strategy supported by thorough process/product understanding. Control strategy focuses on critical control points and control of critical process parameters.	Control Strategy based on prior experience and precedent. Product quality controlled primarily by end-product testing
Design space applicable to multiple operational scales. Predictability and robustness of process performance at multiple scales is ensured by defining an engineering design space	Process performance at multiple scales is demonstrated through empirical experience and end-product testing.
Lifecycle approach to process validation which includes continuous process verification to demonstrate that process remains in state of control. Continual improvement enabled Use of multivariate approaches for process verification.	Process validation based on limited and defined number of full-scale batches. Primary focus on corrective action. Process performance generally monitored using single variable approaches

3.2 Upstream Process Overview

The upstream commercial manufacturing process for A-Mab comprises 4 steps. A summary of the upstream process is provided below and presented in graphical form in Figure 3.1.

- Step 1. Seed culture expansion in disposable vessels
- Step 2. Seed culture expansion in fixed stirred tank bioreactors
- Step 3. Production Bioreactor
- Step 4. Harvest by centrifugation and depth filtration.

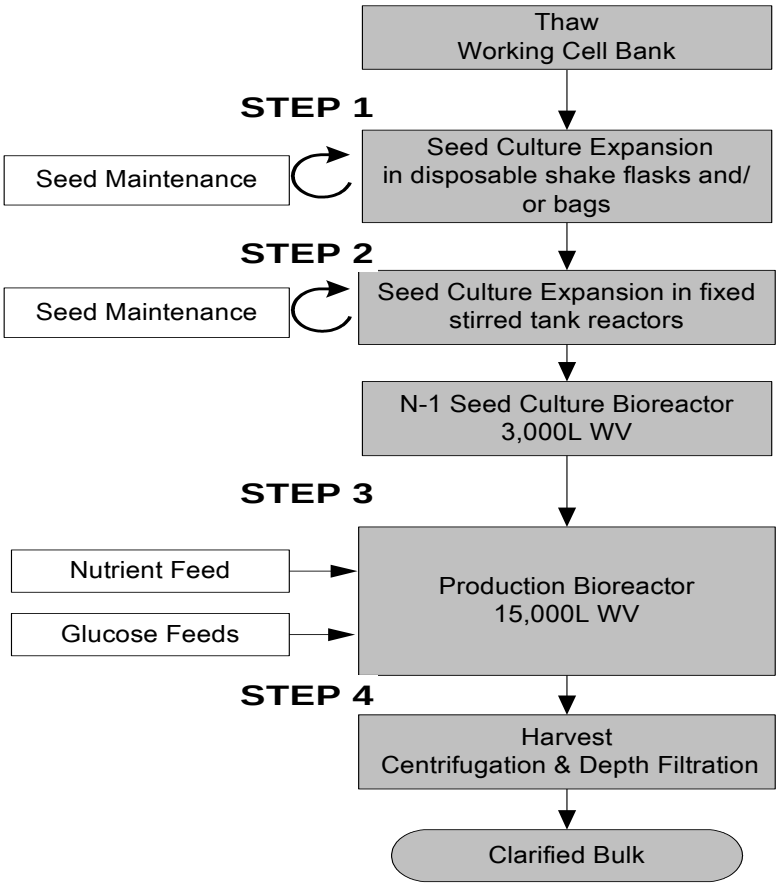

Figure 3.1 Upstream Process Flow Diagram

The A-Mab cell culture process uses a proprietary, chemically defined, basal medium formulation. The medium is essentially protein free as recombinant human insulin (1 mg/mL) is the only protein component that is added. The growth medium also contains 1 g/L Pluronic and 50 nM methotrexate, which is added up to the N-2 seed bioreactor. The N-1 and production bioreactor steps do not contain the methotrexate.

In the seed expansion steps (Steps 1 and 2) one container of Working Cell Bank (WCB) is expanded to a volume of culture that contains enough cells to meet the target initial cell density of the production bioreactor (Step 3). For this, the seed cultures are expanded through multiple passages by increasing the volume and/or number of disposable culture vessels in Step 1 and by increasing the bioreactor volumes in Step 2. To provide flexibility in the manufacturing schedule, the seed cultures can be maintained for additional culture passages or used to generate additional inoculum trains.

The production bioreactor (Step 3) is inoculated to achieve a range of initial Viable Cell Concentration (iVCC) and cultivated at controlled conditions for temperature, pH, and dissolved oxygen (DO). A bolus addition of nutrient feed, NF-1, is added at a defined time post-inoculation and multiple discrete glucose feeds are used to maintain the glucose concentration at $\geq$ 1.0 g/L. Antifoam C solution is added as required for foam control up to a maximum of 100 PPM. Viable cell concentration (VCC), culture viability and residual glucose concentration are monitored periodically starting at the day of inoculation.

Cultures are clarified by a primary continuous centrifugation step that uses a disk-stack centrifuge to remove the bulk of suspended cells and cell debris. A secondary clarification step is performed to remove remnant solids and smaller debris using a depth filtration system. The resulting clarified bulk is held under controlled conditions up to a maximum allowed time prior to further processing.

3.3 Batch History

Two processes have been used to manufacture A-Mab. Process 1 was used to manufacture A-Mab for Phase 1 and 2 clinical studies, and to generate Reference Standard RS-PR1. Process 1 represents a well established platform with extensive process performance history and thus provided a high level of assurance that the desired quality attributes of A-Mab would be met without requiring extensive process development.

To accommodate expected commercial demand, the process was further optimized to increase product titers. The resulting process (Process 2) was used to manufacture Phase 3 supplies for pivotal clinical study at the 5,000 L scale and to generate Reference Standard RS-PR2. Process 2 was subsequently transferred and scaled-up to 15,000 L to support commercial launch, as well as to establish the commercial reference standard (RS-MF1). The A-Mab batch history and upstream process changes are summarized in Table 3.2.

Table 3.2 A-Mab Batch History with Upstream Process Changes

Process	Scale	Number of Batches	Disposition
Process 1 Steps 1 to 4: Platform Process	N-1 Seed: 100 L Prod BioRx: 500 L	2	Supply pre-clinical studies
Process 1	N-1 Seed: 200 L Prod BioRx: 1,000 L	3	Supply clinical and pre-clinical studies and provide product/process understanding. Generate Reference Std RS-PR1
Process 2 Steps 1 and 4 : Platform Process Step 2: Platform Process up to N-2 Optimized platform for N-1 Step 3: Optimized Platform	N-1 Seed: 1,000 L Prod BioRx: 5,000 L	5	Supply pivotal clinical studies and confirm end-to-end process performance. Generate Reference Std RS-PR2
Process 2	N-1 Seed: 3,000 L Prod BioRx: 15,000 L	2	Build commercial launch supplies. Confirm design space and Control Strategy at commercial scale Generate Reference Std RS-MF1

Modifications made to Process 1 to develop the commercial manufacturing Process 2 and the rationale for the changes is described in the process development sections below. Assessment of the impact of these changes on the quality of the product is also included. Biochemical comparability of A-Mab drug substance was established through extensive characterization of product derived from the 1000 L, 5000 L and 15,000 L scales (data not included in case study).

3.4 Process Understanding

The upstream process leverages extensive prior knowledge gained from development of previously licensed antibodies (X-Mab, Y-Mab, and Z-Mab). The process understanding derived from these mAbs is applicable to A-Mab because they utilize the same process platform which includes the parental CHO host cell line, expression system, and cell culture process. Specific use of prior knowledge is discussed in detail under each process step.

For the purposes of this case study, only a subset of quality attributes was considered in the analysis of drug substance and drug product development; these include aggregate, galactosylation, a-fucosylation, deamidation, and HCP. In a real-life case scenario, the examples and approaches described here would include all relevant product quality and material attributes.

The following section describes the prior knowledge, development history, summary of process characterization, equipment engineering and risk analysis used to support the definition of design space and control strategy.

An initial risk assessment was conducted using the extensive prior knowledge for the A-Mab upstream platform process. This assessment identified the production bioreactor as the only upstream process step that posed a significant risk to product quality. The other process steps (seed expansion and harvest) had a low risk of impact to product quality. Also, all steps had a high risk of impacting process performance and consistency as identified through the relationship with Key Process Attributes. The results from this initial risk assessment (Table 3.3) were used to guide the process development and characterization studies.

> *Note: For the purposes of simplicity, the risk assessments presented in the upstream section of the case study do not include raw material and medium composition considerations. In a real-life scenario, upstream process risk analysis would require a thorough understanding of the impact of medium and raw material variability on process performance and product quality.*

Table 3.3 Initial Risk Assessment

Process Step		Risk of Impact to Product Quality Attributes	Risk of Impact to Key Process Attributes
1	Seed Culture expansion in disposable shake flasks and/or bags	Low	***High***
2	Seed Culture expansion in bioreactors	Low	***High***
3	Production bioreactor	***High***	***High***
4	Harvest: centrifugation and depth filtration	Low	***High***

The following sections describe the approaches used to identify parameters linked to product quality and process performance that serve as the basis for defining the design space for the upstream process. The classification of process parameters used in this section is based on the decision logic presented in the control strategy section.

3.4.1 Step 1: Seed Expansion in Disposable Culture Vessels

Risk analysis based on cumulative process understanding gained from prior knowledge and process characterization studies show that the A-Mab seed expansion steps from vial thaw through N-1 seed bioreactor do not impact product quality. Therefore the seed expansion steps are not included in the design space of the upstream process.

If the reader is not interested in studying the data and rationale that support the above statement, the reader can skip this section and go to Step 3 (Production Bioreactor).

3.4.1.1 Development History

The seed expansion process for A-Mab corresponds to a well established platform process. Process understanding has been derived from previous development and clinical experiences with other mAbs. X-Mab and two other similar products (Y-Mab and Z-Mab) have been cultured in spinner or shake flasks, cell-bag bioreactors and fully-instrumented bioreactors for the toxicity, Phase I, Phase II and Phase III/commercial processes. No significant difference has been seen in process performance, as measured by cell specific growth rate and % viability at the end of the culture (Table 3.4). Moreover, results show that process performance has been consistent and robust demonstrating that all three options may be used to culture cells in the seed expansion stage. Note that Y-Mab and Z-Mab bracket the growth rate of X-Mab, supporting the robustness of these 3 options for cell expansion. Data for A-Mab corresponds to manufacturing experience of clinical supplies; results are in alignment with previous mAb experience.

Table 3.4 Prior Process Experience for Seed Culture Steps

Product	Performance Parameter	Seed Culture Expansion Platform		
		Shake Flasks	Wave Bag Bioreactor	Fixed Bioreactor
X- Mab	Specific Growth Rate	0.55 ± 0.10 $days^{-1}$	0.60 ± 0.08 $days^{-1}$	0.62 ± 0.07 $days^{-1}$
	% Viability at End of Culture	92 ± 7	90 ± 9	95 ± 5
Y- Mab	Specific Growth Rate	0.40 ± 0.12 $days^{-1}$	0.38 ± 0.10 $days^{-1}$	0.45 ± 0.09 $days^{-1}$
	% Viability at End of Culture	90 ± 9	92 ± 7	94 ± 5
Z- Mab	Specific Growth Rate	0.65 ± 0.15 $days^{-1}$	0.62 ± 0.13 $days^{-1}$	0.69± 0.11$days^{-1}$
	% Viability at End of Culture	88 ± 10	90 ± 7	93 ± 6
A- Mab	Specific Growth Rate	(Shake flasks only) 0.60 ± 0.10 $days^{-1}$	0.59 ± 0.09 $days^{-1}$	0.62± 0.11$days^{-1}$
	% Viability at End of Culture	95 ± 3	92 ± 4	94 ±3

The risk assessment results (Table 3.5) show that the seed culture steps present a low risk to product quality based on the following considerations:

- A negligible amount of product is accumulated during seed expansion steps.
- Extensive historical experience with X-Mab, Y-Mab, and Z-Mab has demonstrated that seed culture process performance using various configurations of culture vessels does not impact product quality.

This risk assessment assumes that the seed expansion process is operated following well established and successful process control strategies to ensure that seed culture performance is robust and reproducible. Bach record procedures, SOPs, process descriptions and process controls ensure that the seed expansion steps are monitored and operated within established limits. This would include limits for parameters and attributes such as inoculation seeding density, culture duration, viability, pH, temperature and CO2.

It is also important to note that, as stated in the previous section, this risk analysis has been simplified by not including medium and raw material considerations. It could be assumed that such sources of variability have been identified and that the appropriate raw material control strategies are in place based on platform process knowledge and prior experience with other mAbs. If such knowledge and controls are not available, the risk assessments would be used to guide a comprehensive evaluation of the impact of medium and raw material variability on process performance and product quality. The results of such studies would then serve as a basis to establish appropriate testing and control strategies to ensure that raw materials and media meet their respective quality acceptance criteria.

Table 3.5 Risk Assessment Results

Seed Culture Steps	Product Accumulation	Risk of Impact to Product Quality
Seed expansion in spinner or shake flasks	Negligible	Low
Seed expansion in wave bag bioreactor	Negligible	Low
Seed expansion in fixed bioreactor	Negligible	Low

3.4.2 Step 2: Seed Expansion in Fixed Stirred Tank Bioreactors

3.4.2.1 Development History

Similar to culture expansion in disposable vessels, the A-Mab seed expansion in fixed-tank bioreactors uses a well established platform process where processing understanding is derived from extensive prior knowledge with other mAbs. This prior information has demonstrated that the cell culture expansion steps are robust and reproducible in different scale of operations and bioreactor configurations. The clinical experience with A-Mab has also shown consistent performance of the seed bioreactor steps (data not shown). Based on process understanding, no further process development studies were deemed necessary for A-Mab seed culture expansion up to the N-2 step. However, since experience with other mAbs has shown that the N-1 seed bioreactor can potentially affect product quality, process characterization and seed-to-production bioreactor linkage studies were conducted for this last seed expansion step.

Some changes to the N-1 bioreactor stage were implemented throughout the course of A-Mab development, to address the increased scale of operation, and are outlined in Table 3.6 below. Table 3.7 summarizes the range of data from the clinical batches for operational parameters and process attributes.

Table 3.6 Operational Parameters in N-1 Seed Bioreactor

Parameter	Process 1	Process 2	Rationale for Change
Scale	200 L	1000 L (Phase 3 lots) 3000 L (commercial lots)	Increased scale of operation
Temperature Set-Point	37°C	37°C	No change
Dissolved Oxygen Set-Point	30%	27%	Changed setpoint to account for liquid head in order to ensure same oxygen concentration
pH Set-point	7.0	7.0	No change
Culture Duration	2 to 4 days	3 to 5 days	To allow for the increased seed density required in production bioreactor
Basal Medium Concentration	1.0 X	1.2 X	To allow for the increased seed density required in production bioreactor

Table 3.7 N-1 Seed Bioreactor Process Performance Ranges in Clinical Batches

Parameter	Process 1	Process 2
Seed density	$2.4\text{-}5.0 \times 10^5$ vc/mL	$2.0\text{-}3.9 \times 10^5$
pH	6.8-7.2	6.9-7.2
Dissolved Oxygen	20-70%	25-40%
Split Ratio	3.8-5.1	3.0-4.1
Temperature	36.8-37.1°C	36.9-37.1°C
Passage cell density	$2.7\text{-}4.3 \times 10^6$ vc/mL	$3.9\text{-}6.0 \times 10^6$ vc/mL
Viability at Passage	88-97%	90-99%
Maximum medium storage	72 hours (25°C)	15 hours (37°C)

3.4.2.2 Process Characterization

A comprehensive DOE study was carried out to gain better understanding of the A-Mab N-1 seed bioreactor performance and its impact on the production bioreactor stage performance and the quality of product expressed. A full-factorial DOE was executed in 2L bioreactors, to characterize the impact of bioreactor pH, DO and Temp, on peak VCC, Viability at passage and duration of culture in the N-1 stage to reach the passage criteria. The cultures from this study were subsequently passaged into the production bioreactor stage also performed in a 2L scaled-down bioreactor. The production bioreactor stage was operated at the set-point conditions. The harvest samples from the production bioreactor were tested for product quality. Table 3.8 below summarizes the results of the study, by reporting the p values of the statistical analysis (t-test) of the data.

Table 3.8 DOE Study results for N-1 Bioreactor

N-1 Bioreactor Process Parameters	P-Values							
	N- 1 Bioreactor Performance			Production Bioreactor Performance	Production Bioreactor Product Quality			
Variables (Levels)	Peak VCC	Viab.	Culture Duration	Harvest Titer	a-Fucos.	Galactos.	HCP	Aggregate
pH (6.8, 7.0, 7.2)	0.03	0.24	0.04	0.001	0.27	0.53	0.63	0.64
Dissolved oxygen (10, 40, 70 %)	0.31	0.25	0.19	0.35	0.77	0.73	0.31	0.49
Temperature (36, 37, 38°C)	0.02	0.05	0.03	0.005	0.43	0.22	0.23	0.60
pH × Dissolved Oxygen	0.04	0.78	0.65	0.37	0.17	0.78	0.59	0.85
pH × Temperature	0.32	0.26	0.32	0.02	0.98	0.36	0.80	0.36
Dissolved Oxygen × Temperature	0.42	0.86	0.74	0.37	0.80	0.38	0.61	0.26

Based on the results from the characterization study summarized above, none of the operating parameters for the N-1 seed bioreactors had an impact on product quality in the Production bioreactor step. Also, N-1 bioreactor pH and temperature were designated key process parameters (KPP) due to their impact on process attributes; peak VCC, viability and culture duration.

In conclusion, the cumulative process understanding gained from prior knowledge, results from process characterization studies and risk analysis show that the A-Mab seed expansion steps from vial thaw through N-1 seed bioreactor do not impact product quality and thus do not need to be included in the definition of the design space.

3.4.3 Step 3: Production Bioreactor

3.4.3.1 Development History

Clinical and preclinical manufacturing of A-Mab for toxicity, Phase 1, and Phase 2 studies used Process 1. This corresponds to a well established platform that was first used for the commercial manufacturing of X-Mab and subsequently used to manufacture supplies for Phase 1 and 2 clinical studies for licensed products Y-Mab and Z-Mab. This platform is also currently used to support other multiple mAb products in various phases of development. Platform Process 1 conditions are summarized in Table 3.11.

A summary of the process knowledge gained through development and manufacturing experience for Process 1 is summarized in Table 3.9 (for the purposes of the case study, only selected quality attributes are discussed). This process knowledge is based on development and manufacturing experience with other mAbs (X-Mab, Y-Mab, and Z-Mab) as well as A-Mab process performance in toxicity and Phase 1 and 2 manufacturing campaigns. This cumulative knowledge served as the basis for process optimization studies leading to the development of Process 2 for commercial manufacturing of A-Mab.

As described in the seed expansion sections, for the purposes of simplicity raw material and medium variability considerations are not included in this case study. It has been assumed that such sources of variability have been identified and that the appropriate raw material control strategies are in place based on platform process knowledge and prior experience with other mAbs. If such knowledge and controls are not available, the risk assessments should include a comprehensive evaluation of the impact of medium and raw material variability on process performance and product quality.

Table 3.9 Summary of Prior Knowledge of Platform Process

Parameter	Summary of Knowledge
Initial Cell Density	Impacts peak VCC, integral of VCC, final titer, and culture duration. Also affects timing for nutrient bolus addition, glucose feeding regime, overall glucose and base consumption. Does not impact growth rate, specific productivity, specific glucose consumption or specific lactate production. **Quality Impact and Risk:** Does not impact product quality and hence is considered low risk.
Temperature, pH	Impact peak VCC, integral of VCC, final titer, culture duration, growth rate, specific productivity, specific glucose consumption and specific lactate production. Also impact timing for nutrient bolus addition, glucose feeding regime, overall glucose consumption and base consumption. The optimal temperature/pH and the extent of temperature/pH effects are cell-line dependent. The target temperature and pH for the platform process have been shown to be acceptable for all tested cell lines. **Quality Impact and Risk:** Temperature and pH can affect glycosylation (afucosylation and galactosylation levels), charge heterogeneity, host cell protein levels, and aggregate formation, and hence is considered high risk.
Dissolved Oxygen	Does not impact product quality of process performance within a wide range. Must be maintained above a minimum DO concentration to ensure that product quality and process performance are not affected. **Quality Impact and Risk:** Has been observed to occasionally impact product quality and hence is considered medium risk based on process control and monitoring capabilities.
pCO_2	Does not affect process performance and product quality within a relatively wide range. If pCO_2 exceeds acceptable range it can affect process performance: peak VCC, integral of VCC, final titer, culture duration, growth rate, specific productivity, specific glucose consumption and specific lactate production. Also can impact product quality; the effects are cell-line specific. **Quality Impact and Risk:** Has been observed to occasionally impact product quality and hence is considered medium risk.
Mixing and gassing strategy	Acceptable process conditions have been established at various operation scales and bioreactor configurations based on engineering characterization of the production. **Quality Impact and Risk:** Has been observed to occasionally impact product quality and hence is considered medium risk.
Feeding Strategy	Feed concentration, volume and timing do not impact product quality within a wide range of operations. The feeding strategy can affect process performance: peak VCC, integral of VCC, final titer, culture duration, growth rate, specific productivity, specific glucose consumption and specific lactate production. Platform process conditions might not be optimal for all cell lines, but have been demonstrated to result in consistent and robust process performance. **Quality Impact and Risk:** Does not impact product quality and hence is considered low risk.
Culture Duration	Extended culture duration can impact product quality. Cultures are harvested within an acceptable duration based on culture viability, product quality and product titer considerations; this can be cell line dependent. **Quality Impact and Risk:** Has been observed to impact product quality and hence is considered high risk. Culture duration also impact levels of host cell protein and DNA in the clarified culture broth.

OPTIMIZATION OF PROCESS 1

In order to meet anticipated commercial demand, Process 1 was further optimized to increase product titers while ensuring no significant impact on product quality. Parameters for optimization studies were chosen based on prior process knowledge (Table 3.9). A DOE approach was taken to optimize process conditions for pH, temperature, iVCC, and pCO_2; two DOE studies were performed using a fractional factorial design. Dissolved oxygen and pCO_2 levels were not varied. The composition of the basal medium and nutrient feeds were also adjusted based on individual nutrient consumption data (not shown). The parameters and ranges used in these studies are summarized in Table 3.10.

Table 3.10 Parameters and Ranges for DOE Process Optimization Studies

Parameter	DOE Range		
	Low	Middle	High
Temperature (°C)	34	35.5	37
pH	6.75	6.90	7.05
Medium concentration (X)	0.75	1.0	1.5
Nutrient feed volume (% of WV)	6	10	14
iVCC (MM/mL)	0.5	1.0	1.5
Culture duration (days)	13	15	17

Results from these DOE studies were used to define optimized process conditions for pH, temperature, iVCC, culture duration, medium concentration and feeding strategy. The optimized process resulted in a higher integral of the viable cell concentration, longer culture duration and thus higher product titers. Results from the optimized process also demonstrated that there were no significant differences in product quality attributes compared to Process 1. The cumulative knowledge gained through these process development studies was used to define Process 2. A summary of process conditions and results for Process 2 is presented in Table 3.11 alongside results from A-Mab manufacturing experience with Process 1.

Table 3.11 Summary of A-Mab Process Parameters, Performance, and Product Quality for Process 1 and Process 2

Process Parameter	Process 1	Process 2
Initial Cell Density (MM/mL)	0.7	1.0
Temperature (°C)	36.0	35.0
pH	6.9	6.85
DO (% sat) Target	50	50
CO_2 (mmHg) Target Range	40-100	40-100
Medium Concentration (X)	1.0	1.2
Feed 1 Volume (% of WV)	6	12

Table 3.11 Summary of A-Mab Process Parameters, Performance, and Product Quality for Process 1 and Process 2

Glucose Feed Addition time and Volume	As needed	As needed
Culture Duration (days)	13-15	16-17
Quality Attributes and Process Performance Attributes		
Titer	1.8-2.1 g/L	4.1-5.0 g/L
Viability at Harvest	50-80%	40-70%
Turbidity at Harvest (NTU)	5-25	10-25
Aggregate	1.2-1.4 %	1.4-1.6 %
aFucosylation	5.1-8.2%	6.3-9.6%
Galactosylation	G1: 17.5-19.7% G2: 8.5-10.2% % Galac: 35.4-38.9%	G1: 12.2-14.2 % G2: 5.8-7.3% % Galac: 24.7-27.7%
ADCC	88-108%	85-113%
CDC	93- 115%	90-108%
Deamidation	Consistent with Ref Standard	Consistent with Ref Standard
Host Cell Protein	$3\text{-}5 \times 10^5$ ppm	$4\text{-}8 \times 10^5$ ppm
DNA	$0.8\text{-}1.4 \times 10^3$ ppm	$1.2\text{-}2.2 \times 10^3$ ppm

3.4.3.2 Process Characterization

Figure 3.2 is a pictorial representation of the body of data that served as the starting point for the design of the characterization studies. A summary of the process knowledge gained from the process optimization and development studies is summarized in Table 3.12. This cumulative knowledge served as the basis for the risk assessments and process characterization studies leading to the definition of the design space and control definition for the production bioreactor step.

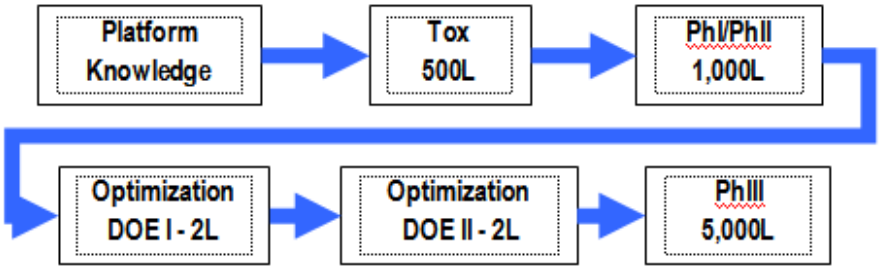

Figure 3.2 Body of Data available as starting point for process characterization studies

Table 3.12 Summary of Knowledge from Process Optimization Experiments

Parameters	Summary of Knowledge from Process Optimization Experiments
Initial cell density (iVCC)	Higher initial iVCC was required to maximize integral of viable cell concentration and product titer in the production bioreactor. There was no effect on product quality within the ranges of the optimization studies, thus iVCC was considered as low risk
Temperature	Lower temperature resulted in higher specific productivity, longer culture duration and higher product titers. The lower temperature also resulted in slightly higher levels of a-fucosylation and slightly lower galactosylation. There was no significant effect observed on aggregation, host cell protein or DNA concentrations. Based on impact on product quality, temperature was considered as high risk.
pH	Lower culture pH increased specific productivity, culture duration and product titers. Lower pH also resulted in slightly increased levels of a-fucosylation. The effect on galactosylation was relatively minor and temperature dependent; at the lower temperature target for Process 2 galactosylation levels were slightly lower compared to platform process conditions. There was no significant effect observed on aggregation, host cell protein and DNA. Based on impact on product quality, pH was considered as high risk.
Basal medium concentration	A higher basal medium concentration was required to maximize integral of viable cell concentration and product titer in the production bioreactor. Concentration of medium components (e.g., amino acids, vitamins, trace elements) was adjusted based on nutrient consumption rates. There was no effect on product quality within the ranges of these studies. Medium concentration was considered as low risk for product quality but critical for optimizing product titers.
Nutrient feed volume	A higher feed volume was required to maximize integral of viable cell concentration and product titer in the production bioreactor. There was no effect on product quality within the ranges of these studies. The higher feed volume requirements reflect the increased nutrient consumption associated with higher iVCC and culture densities. Nutrient feed volume was considered as medium risk for product quality but critical for optimizing product titers.
Culture duration	Culture Duration had an impact on titer, and product quality. Longer culture times resulted in higher titers and lower a-fucosylation levels. Also, prolonged culture durations resulted in lower final culture viabilities and thus higher HCP and DNA levels. Culture duration was considered as high risk for product quality.
Linkage to downstream process	Worst case scenario culture conditions for high DNA, HCP and aggregate levels were established to provide material to the downstream development group for clearance studies. Results showed worst case at the following bioreactor conditions: High pH, high Temp, high iVCC, and late harvest. These conditions resulted in a rapid decline of viability at the end of the culture process and thus yielded higher levels of HCP and DNA. The highest levels of HCP and aggregate tested were 1.3×10^6 ppm and 3.1% respectively.

Initial Risk Assessment for Production Bioreactor Step

An initial risk assessment was completed for the production bioreactor and the N-1 seed culture steps with the purpose of identifying equipment design, control parameters, processing conditions and starting materials that pose a significant risk to the quality attributes of the product. All these parameters were analyzed using the Ishikawa or fishbone diagram shown in Figure 3.3. The results of this risk assessment are presented in Table 3.13, except for those related to scale effects, which are discussed in Section 3.9.4.

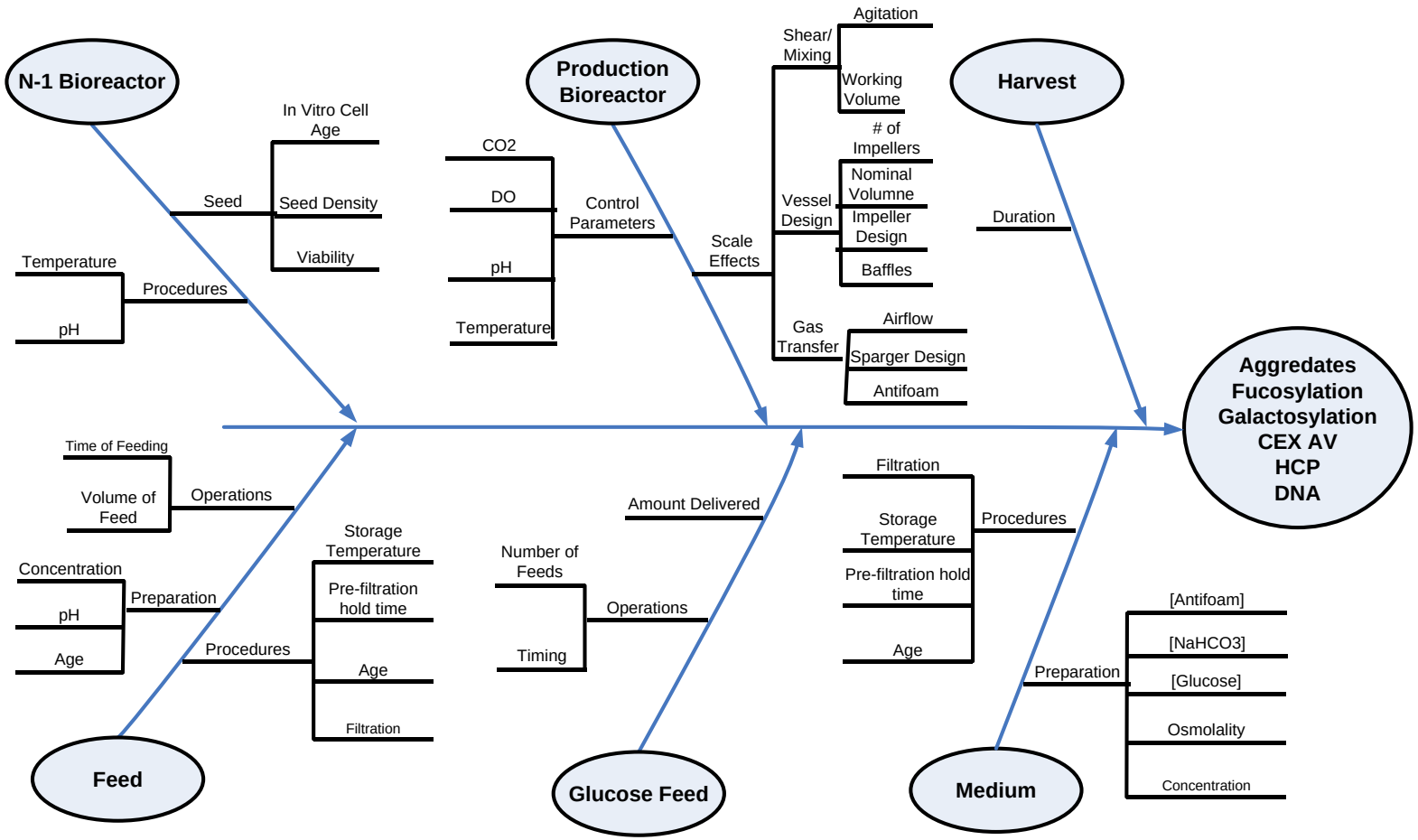

Figure 3.3 Ishikawa Diagram Indicating the Process Parameters Analyzed in the Risk Assessment of the Production and the N-1 Bioreactors

The risk ranking in Table 3.13 evaluated process parameters in Figure 3.3 with respect to their potential to affect either one of the process attributes - product yield or viability and turbidity at harvest - or selected CQAs - soluble aggregates, afucosylation, galactosylation, deamidation, HCP or DNA. Green denotes parameters that can significantly affect a process attribute; yellow and red denote parameters that can potentially affect a CQA. Yellow indicates that capability of controlling the parameters is robust and effective. For example, the nutrient and components concentrations in the feeds and medium are tightly controlled through the formulation and therefore pose a low risk to CQAs. Red indicates that the range in which the parameters can vary before a CQA is potentially affected is close to the control capability, e.g., pH.

Table 3.13 also summarizes the activities that were undertaken to mitigate the identified risks:

- **DOE**: Multivariate studies to establish relationships between parameters and CQAs
- **DOE Indirect**: Parameters were indirectly varied during DOE studies. For example, glucose was fed as needed to maintain cell viability, which resulted in different feed amounts at different time points, leading to different concentration profiles.
- **Linkage Studies**: Seed-to-production bioreactor studies
- **EOPC**: End of Production Cell studies to establish limit of in-vitro cell age
- **Medium hold studies**: Studies performed to justify medium and feed hold times.
- **Not required**: Indicates that no special risk mitigation was performed. These parameters were controlled and recorded and data was retrospectively analyzed for correlations.

Table 3.13 Results of the Risk Analysis Performed in the Production and N-1 Bioreactors

Process Parameter in Production Bioreactor	Quality Attributes						Process Attributes			Risk Mitigation
	Aggregate	aFucosylation	Galactosylation	Deamidation	HCP	DNA	Product Yield	Viability at Harvest	Turbidity at harvest	
Inoculum Viable Cell Concentr										DOE
Inoculum Viability										Linkage Studies
Inoculum In Vitro Cell Age										EOPC Study
N-1 Bioreactor pH										Linkage Studies
N-1 Bioreactor Temperature										Linkage Studies
Osmolality										DOE
Antifoam Concentration										Not Required
Nutrient Concentration in medium										DOE
Medium storage temperature										Medium Hold Studies
Medium hold time before filtration										Medium Hold Studies
Medium Filtration										Medium Hold Studies
Medium Age										Medium Hold Studies
Timing of Feed addition										Not Required
Volume of Feed addition										DOE
Component Concentration in Feed										DOE
Timing of glucose feed addition										DOE-Indirect
Amount of Glucose fed										DOE-Indirect
Dissolved Oxygen										DOE
Dissolved Carbon Dioxide										DOE
Temperature										DOE
pH										DOE
Culture Duration (days)										DOE
Remnant Glucose Concentration										DOE-Indirect

Green denotes parameters that can affect a process attribute.
Yellow and red denote parameters that can affect CQAs. Yellow 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.
Blank indicates that parameter does not affect attribute.

3.5 Definition of Design Space for Production Bioreactor Step

The design space was defined based on process characterization studies conducted using a qualified scale-down model of the production bioreactor (See section "Qualification of scale-down model for production bioreactor").

Initial Screening Study

An Initial screening study using a fractional factorial experimental design indicates that temperature, CO_2, pH, osmolality and culture duration have statistically significant impact on quality attributes to merit further investigation.

Process characterization was based on multi-factorial experiments (DOE) that included process parameters ranked either high (red) or medium (yellow) in the above risk analysis. The parameters and ranges used in the DOE studies are given in Table 3.14. The parameters were tested in an initial screening study, a resolution IV fractional factorial experimental design augmented with four center points. This type of experimental design is not able to resolve all the interactions between parameters and it would have to be augmented on the subset of parameters shown to impact CQAs. The center-point conditions align with the target process conditions. The effect of culture duration was assessed by assaying samples at days 15, 17 and 19 of each culture. These samples were assayed for afucosylation and galactosylation using CE-LIF, soluble aggregates using aSEC, HCP using ELISA, DNA using qPCR and the acidic variants using aCEX. This last technique is used as an indicator for deamidation.

Table 3.14 Parameters and Ranges Tested in the Design Space Definition Study

Process Parameter	Low	Middle	High
Temperature (°C)	34	35	36
DO (%)	30	50	70
CO_2 (mm Hg)	40	100	160
pH (% sat)	6.6	6.85	7.1
Medium concentration (X)	0.8	1.2	1.6
Osmolality (mOsm)	360	400	440
Feed 1 volume (% of WV)	9	12	15
iCC (MM/mL)	0.7	1	1.3
Culture duration (days)	15	17	19

Figure 3.4 contains a matrix of plots indicating each of the effects found in the DOE. Results clearly indicate that pH, CO_2, temperature, osmolality and culture duration exert the largest influence on the levels of the CQAs. Table 3.15 summarizes the process parameters found to significantly affect CQAs. Arrows pointing up, (↑), indicate that the parameter causes an increase in the level of the CQA. Similarly, arrows pointing down, (↓), indicate a decrease in the level of the CQA. Effects that were not detected are identified by ND. Grey arrows indicate the effect was detected statistically but is too small to have an appreciable effect on the quality of the material produced. For example, it is seen that medium concentration had a statistically significant effect on aFucosylation ($p = 0.001$). However, by reviewing Figure 3.4 it is seen that its effect was very shallow. In this case, changing the medium concentration from 0.8 to 1.6 X only changed the aFucosylation levels by 0.3 %.

Table 3.15 Effects of Parameters Tested in Multifactorial Experiment on the CQAs Defined in the Production Bioreactor. Statistical significance is indicated by p-values

	aFucosylation	Galactosylation	HCP	Sol Aggregates	Acidic Variants	DNA
Temperature	↓ p<0.0001	↑ p<0.0001	ND p=0.37	ND p=0.37	↑ p=0.0005	ND p=0.42
DO	ND p=0.25	ND p=0.37	ND p=0.85	ND p=0.25	↓ p=0.03	ND p=0.34
CO2	↓ p<0.0001	↓ p=0.003	ND p=0.22	ND p=0.25	↓ p=0.002	ND p=0.85
pH	↓ p<0.0001	↓ p<0.0001	ND p=0.15	↑ p<0.0001	↓ p=0.006	ND p=0.24
Medium Conc	↑ p=0.001	ND p=0.13	ND p=0.77	ND p=0.62	ND p=0.74	ND p=0.76
Osmolality	↓ p=0.003	↓ p=0.001	ND p=0.54	ND p=0.45	↓ p=0.0003	ND p=0.82
Feed 1 Vol	ND p=0.75	↓ p=0.05	ND p=0.25	ND p=0.64	ND p=0.42	ND p=0.65
iVCC	ND p=0.62	↓ p=0.02	ND p=0.37	↑ p=0.008	↑ p=0.05	ND p=0.27
Culture Duration	↓ p<0.0001	ND p=0.65	↑ p=0.003	↑ p<0.0001	ND p=0.32	↑ p=0.0001

Arrows pointing up, (↑), indicate that the parameter causes an increase in the level of the CQA.
Arrows pointing down, (↓), indicate that the parameter causes a decrease in the level of the CQA.
Grey arrows indicate the effect was detectable but very small. Effects that were not detected are identified by ND

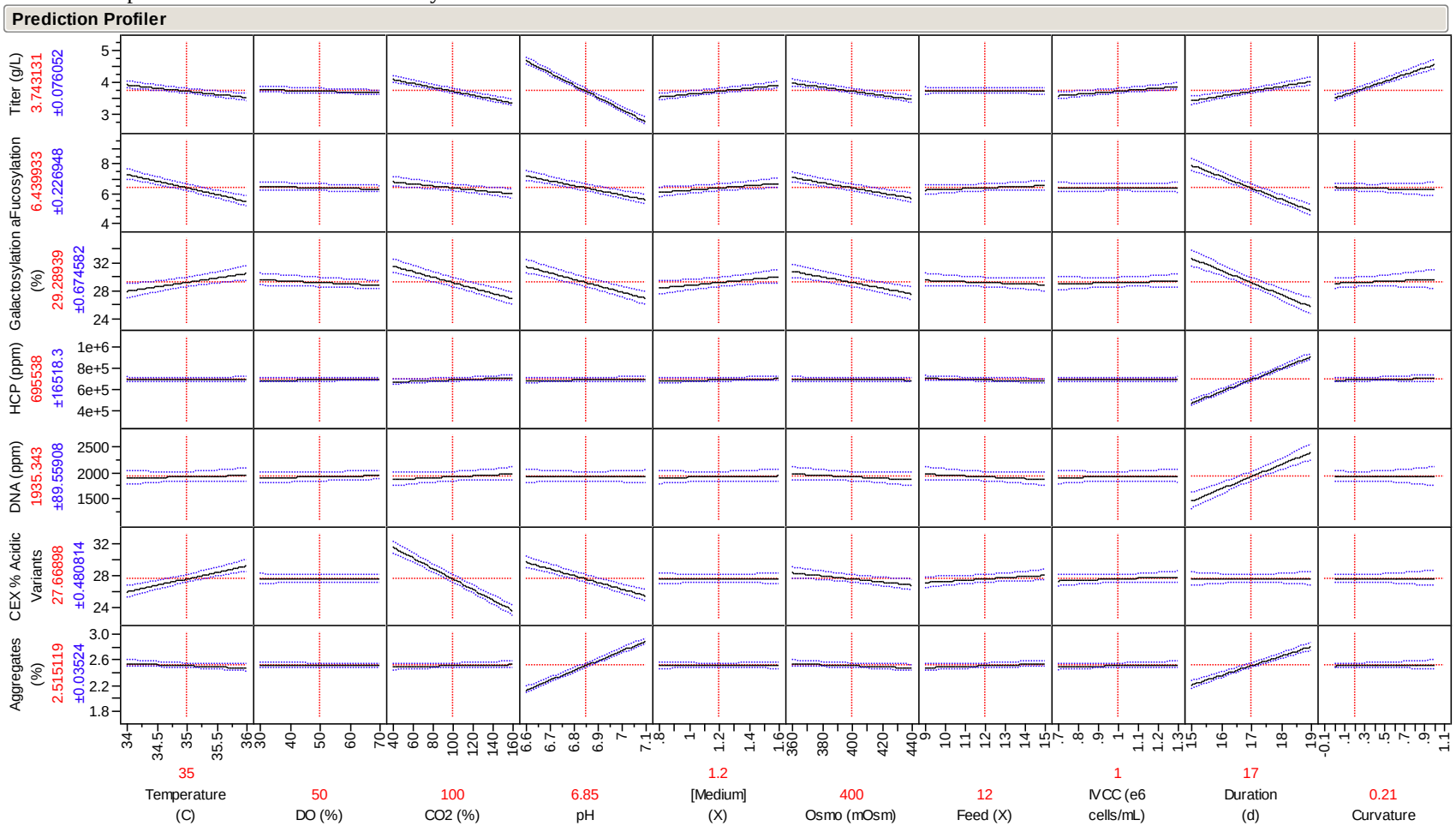

Figure 3.4 Results from Multifactorial DOE in Production Bioreactor: Initial Screening Studies

The results (Figure 3.4) indicate that temperature, CO_2, pH, osmolality and culture duration had statistically significant effects that were strong enough to merit further investigation. This was accomplished by augmenting the screening design to enable the estimation of a full response surface model containing all main effects, two-way interactions and quadratic effects for these four parameters.

Follow-up DOE Study to Develop Multivariate Surface Response Model

An augmented DOE study was used to identify parameter interactions and develop a full response surface model for the production bioreactor

The augmentation consisted on eight additional runs to form a full factorial in the four parameters of interest and another eight axial points. The factorial runs enabled the estimation of all interactions among Temperature, CO_2, pH, Osmolality and Culture Duration, and the estimation of the quadratic effects to assess curvature in the responses. The ranges for all additional runs were the same as in Table 3.14, hence the additional runs effectively augmented the fractional factorial to a Central Composite Design in these four parameters. Four additional center points were also included.

The results from the combined data set are given in Table 3.16, which provides all significant parameter estimates for response surface models for the CQAs, i.e., Aggregates, Acidic Variants, Galactosylation, afucosylation, HCP and DNA. P-values are also included to assess statistical significance. The parameter estimates provide a measure of how much a given response, e.g., aFucosylation, changes as a function of an input parameter, or combination in the case of interactions. Table 3.16 provides all estimates for main effects, two-way interactions and second order terms, which combined form the coefficients of the response surface model. This model is suitable for predicting mean levels of the CQAs over the ranges of the process parameters included.

Table 3.16 Parameter Estimates from Second Order Polynomial Models Fitted To CQAs

	aFucos (%)		Galact (%)		HCP (ppm)		DNA (ppm)		CEX AV (%)		Aggr (%)	
Term	Scl. Est	p-value	Scl. Est	p-value	Scl. Est	p-value	Scl. Est	p-value	Scl. Est	p-value	Scl. Est	p-value
Intercept	6.59		30.45		6.9E+05		1.9E+03		28.0		2.5	
Temp (C)(34,36)	-0.62	<.0001	1.67	<.0001					1.6	<.0001		
CO2 (mmHg)(40,160)	-0.53	<.0001	-2.42	<.0001					-4.0	<.0001		
pH(6.6,7.1)	-0.99	<.0001	-2.43	<.0001					-2.1	<.0001	0.4	<.0001
Osmo (mOsm)(360,440)	-0.75	<.0001	-1.37	<.0001					-1.0	<.0001		
Temp*CO2												
Temp*pH	-0.57	<.0001	1.88	<.0001					1.1	<.0001		
Temp*Osmo			2.04	<.0001								
CO*pH	-0.84	<.0001	1.96	<.0001					-0.4	0.0365		
CO2*Osmo			0.65	0.0270								
pH*Osmo												
Temp*Temp (C)												
CO2*CO2												
pH*pH	-1.09	0.0003										
Osmo*Osmo												
Duration (d)(15,19)	-1.36	<.0001	-3.76	<.0001	2.0E+05	<.0001	4.7E+02	<.0001			0.3	<.0001
Temp*Duration	0.32	0.0180										
CO2*Duration												
pH*Duration	1.35	<.0001	2.96	<.0001							-0.3	<.0001
Osmo*Duration	0.71	<.0001										

Estimates are scaled based on the ranges tested in the DOEs, so that they measure change in the response value by half-range. These estimates represent the coefficients of the response surface that

models changes in the CQAs as a function of the level of the process parameters. Only effects that are significant at $p < 0.05$ level are shown.

The design space for the production bioreactor was defined using the response surface models in Table 3.16 and the levels of the CQAs indicated in Table 3.17. The design space is the multivariate combination of process parameters that provides high assurance that all CQAs will be within the limits in this table.

The limits for afucosylation and galactosylation represent the process targets for these quality attributes which are based on safety and efficacy data (CQA section). By contrast, the upper limit for soluble aggregates is based on the demonstrated capability of the purification process to clear these impurities. The limits for acidic variants are derived from acceptable changes in the level of deamidation based on past clinical and pre-clinical experience with A-Mab.

Table 3.17 Levels of CQAs Used To Define the Production Bioreactor Design Space

CQA	Lower Limit	Higher Limit
Afucosylation (%)	2	13
Galactosylation (%)	10	40
Acidic Variants (%)*	20	40
Soluble Aggregates (%)	0	3

Acidic variants are not considered CQAs per the criticality assessment. They were included in the model to ensure process consistency.

Note, that the multivariate model was developed using afucosylation and galactosylation levels rather than ADCC and CDC activity results. The rationale for this approach is based on the following premises:

- The potential effects of process conditions on bioactivity, based on CDC and ADCC bioassays, were studied by assaying samples with extreme levels of galactosylation and afucosylation. Results showed that there is a direct correlation between afucosylation levels and ADCC activity (see CQA section). CDC activity was not correlated to galactosylation levels.
- Analytical measurements for afucosylation and galactosylation are less variable than the cell based assays and thus provide more certainty in the models.

The design space for this unit operation is fairly complex due to the interactions and non-linear behavior found in the DOE studies. First, a graphical depiction of the intersection between the response surface models in Table 3.16 and the limits in Table 3.17 is given in Figure 3.5. The shaded regions in these plots indicate the regions where the mean levels of the CQAs will exceed the acceptable limits or specifications. Notice that the limits on acidic variants and soluble aggregates are not exceeded within the ranges tested in the DOEs.

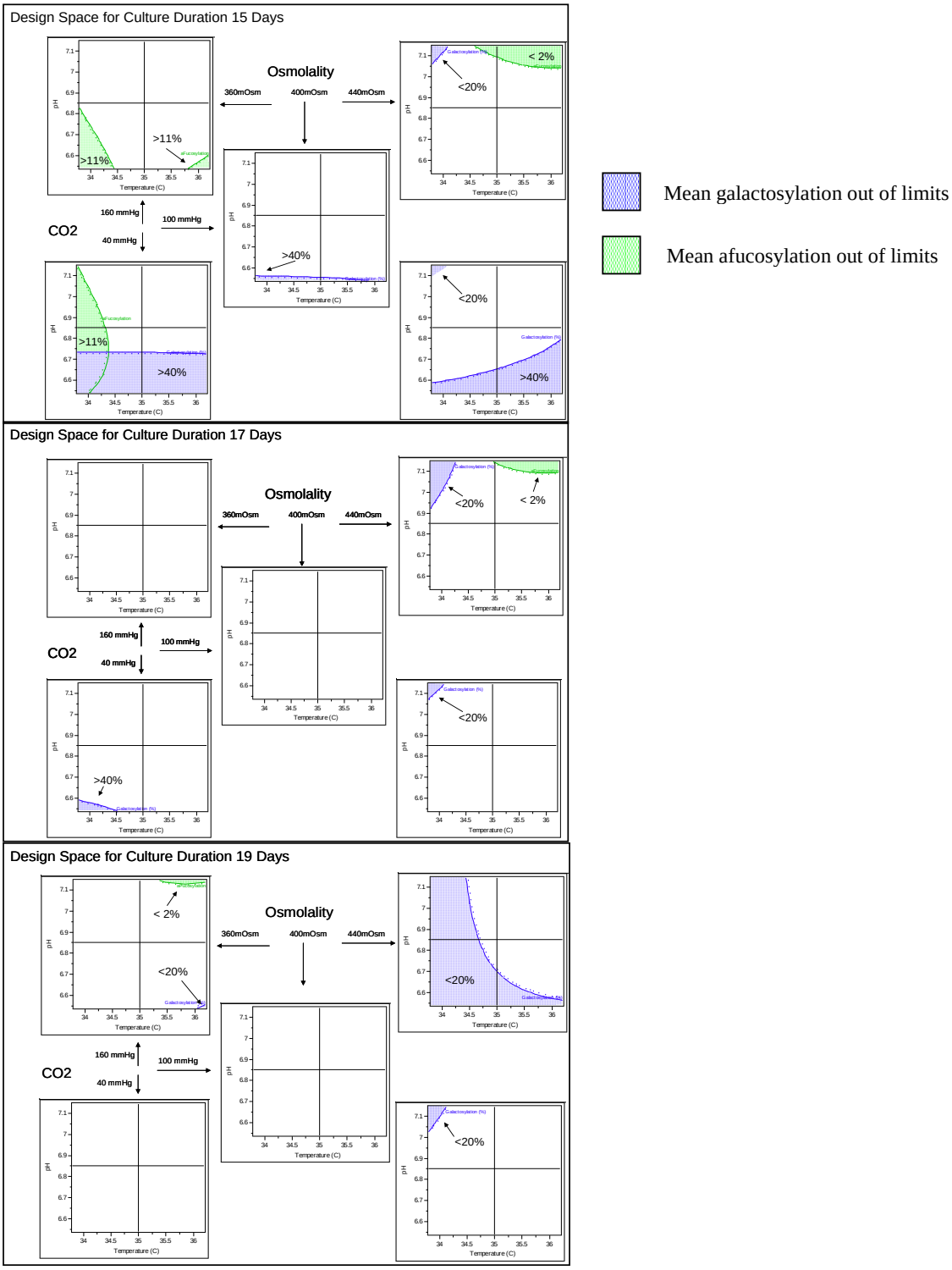

Figure 3.5 Graphical Representation of the Multivariate Studies for the Production Bioreactor

To establish the limits of the proposed design space a Bayesian Statistical approach was used. This approach provides a high level of assurance that all critical product quality attributes will meet their acceptance criteria and therefore satisfies the requirement to demonstrate assurance of quality in the ICH definition of design space: "The multidimensional combination and interactions of input variables (e.g. material attributes) and process parameters that have been demonstrated to provide assurance of quality".

Since the response surface models used to create Figure 3.5 represent mean levels, the reliability of the process at the edges of the shaded regions in would be roughly 50% if the variability is symmetrical around the mean values. In order to determine the multivariate combination of process parameters that results in a highly reliable process, i.e., one that produces acceptable Drug Substance with a high level of confidence, a Predictive Bayesian Reliability approach (Peterson, 2008, Stockdale, 2008, Peterson J. J., 2004, Peterson J. J., 2009, Stockdale, 2009) was used. The results are given in Figure 3.6. Notice that in this case the contours in the plot represent the probability levels that all the quality attributes included in the model will be within the acceptable limits defined in Table 3.17. In this case study the design space is defined as the multidimensional subset of process conditions that result in a reliability >99% of satisfying these limits simultaneously. This is represented by the dark-red region in Figure 3.6. Results show that culture duration of 17 days provides the highest reliability profile for all combination of parameters.

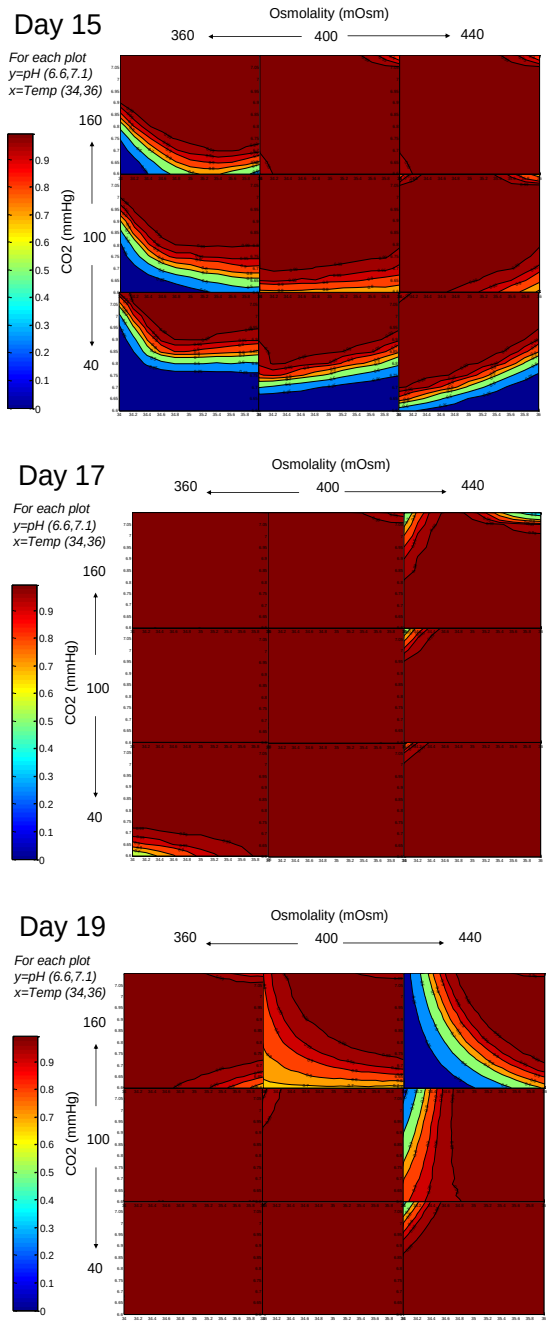

Figure 3.6 Design Space for the Production Bioreactor Based on the Overall Reliability of the Process

Regions in dark-red possess > 99% reliability to satisfy the CQA limits in Table 3.17. Each panel represents a different harvest day or culture duration. Within each panel there are 9 contour plots of all 3 × 3 combinations of osmolality at 360, 400, and 440 mOsm and CO_2 at 40, 100, and 160 mmHg. Each plot depicts contours of the reliability of the process to satisfy all limits in Table 3.5-4. as a function of temperature and pH.

3.5.1 Step 4: Harvest

Detailed risk analysis and process development/optimization information for the harvest step were not included due to document length considerations. Also, for simplicity, it has been assumed that the harvest step has no impact on product quality. This might not be true in a real life scenario, where it would be necessary to consider and evaluate sources of variability and potential impact to process performance and product quality.

3.6 Upstream Process Risk Assessment and Control Strategy

A final set of risk assessments were conducted after the completion of the process characterization studies to define the control strategy for the commercial upstream manufacturing process for A-Mab. Information from two (2) commercial scale batches was used to verify process performance (see Lifecycle Approach to Validation section) and demonstrate the control strategy at full commercial scale. Figure 3.7 is a pictorial representation of the body of data that served as the basis for the final risk assessments that underpins the proposed control strategy.

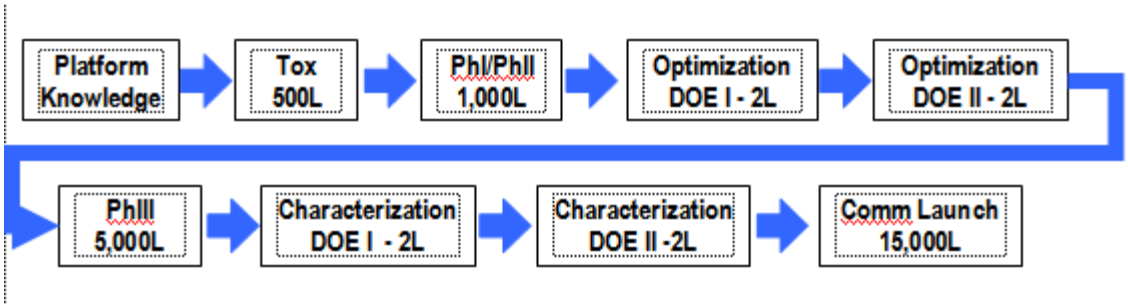

Figure 3.7 Body of Data used for Final Risk Assessment to define Control Strategy

An overall risk assessment for the upstream process identified the unit operations that had an impact on product quality; results are summarized in Table 3.18. The seed expansion steps (Steps 1 and 2) present a low risk to product quality because there is essentially no product accumulation during these steps and prior knowledge indicates that seed culture performance up to the N-2 bioreactor has no effect on product quality. The risk analysis also showed that process conditions in the N-1 seed bioreactor and harvest had an impact on key process attributes (e.g., product titer/yield) but did not impact product quality attributes. The production bioreactor step was identified as the only upstream process step that impacted A-Mab product quality.

Based on the results from the overall risk assessment and potential impact to product quality, the Production Bioreactor was the only step included in defining the limits of the design space for the upstream process.

Table 3.18 Upstream Process Risk Assessment: Impact of Upstream Process Steps on Quality Attributes and Process Performance

Product Quality Attribute	Seed Culture Expansion in Shake Flasks and/or Bags	Seed Culture Expansion in Bioreactors	Production Bioreactor	Harvest: Centrifugation and Depth Filtration
Aggregate	NO	NO	Form	NO
Deamidated Isoforms	NO	NO	Form	NO
aFucosylation	NO	NO	Form	NO
CHO HCP	NO	NO	Form	NO
DNA	NO	NO	Form	NO

Yellow indicates that operation includes a WC-CPP that impacts process attribute. "Form" indicates that CQA is generated in the step. "NO" indicates that step has no impact on attribute

Process Performance	Seed Culture Expansion in Shake Flasks and/or Bags	Seed Culture Expansion in Bioreactors	Production Bioreactor	Harvest: Centrifugation and Depth Filtration
Product Titer/Yield	NO	Yes	Yes	Yes
Culture Viability	Yes	Yes	Yes	NA
Viable Cell Concentration	Yes	Yes	Yes	NA
Cycle Time	Yes	Yes	Yes	Yes

"Yes " indicates that step impacts performance. Green indicates that operation includes a KPP.
"NO" indicates that step has no impact on process performance. NA = Not Applicable

3.6.1 Categorization of Process Parameters

To identify process parameters linked to product quality and process performance, input process parameters were categorized using the decision logic outlined in the Control Strategy section. Classification of parameters was based on a risk assessment approach that considered potential impact to product quality and process performance, the likelihood of a parameter to exceed acceptable limits, and the ability to detect and/or correct a failure if it occurred. A failure is when a parameter exceeds its acceptable limits. For quality-linked parameters (CPPs and WC-CPPs) the acceptable limits are defined by the design space.

Critical Process Parameter (CPP) and Well-Controlled Critical Process Parameter (WC-CPP)

Both, CPPs and WC-CPPs, are process parameters whose variability have 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 has a low risk of falling outside the design space.

A CPP has a high risk of falling outside the design space.

Here, the assessment of risk is based on a combination of factors that include equipment design considerations, process control capability and complexity, the size and reliability of the design space, ability to detect/measure a parameter deviation, etc.

Results from the risk assessment showed that there are no quality-linked parameters (CPPs or WC-CPPs) in Steps 1, 2, and 4. Results also showed that there are no Critical Process Parameters (CPPs) in Step 3 since all parameters are well controlled within their acceptable limits and have demonstrated robust process operation. Thus, all quality-linked process parameters for Step 3 were classified as WC-CPPs. The rationale for classification of quality-linked process parameters is summarized in Table 3.19 and the summary of the risk assessment for the production bioreactor is presented in Table 3.20.

The risk assessment also identified parameters that are linked to process performance consistency and robustness; these are classified as KPPs based on the decision logic outlined in the Control Strategy section. These parameters do not impact product quality but are important to ensure successful and reliable commercial manufacturing operations.

Table 3.19 Risk Assessment results that support classification of Quality-Linked Process Parameters in the Production Bioreactor Step

Process Parameter	Impacted Quality Attribute(s)	Likelihood of Parameter Exceeding Design Space	Ability to Detect and/or Control Parameter Failure	Classification based on Risk Assessment
Temperature	aFucosylation Galactosylation Deamidation	Low	Good	WC-CPP
pH	aFucosylation Galactosylation Deamidation Aggregate	Low	Good	WC-CPP
Dissolved CO_2 Concentration	aFucosylation Galactosylation Deamidation	Low	Medium	WC-CPP
Culture Duration	aFucosylation Deamidation Aggregate Host Cell Protein Residual DNA	Low	Good	WC-CPP
Osmolality	aFucosylation Galactosylation Deamidation	Low	Medium	WC-CPP

Note: Process Parameter classification algorithm is described in Control Strategy Section

Table 3.20 Final Risk Assessment Results for Process Parameters in the Production Bioreactor

Process Parameter in Production Bioreactor	Quality Attributes						Process Attributes			Risk Mitigation
	Aggregate	aFucosylation	Galactosylation	Deamidation	HCP	DNA	Product Yield	Viability at Harvest	Turbidity at harvest	
Inoculum Viable Cell Concen.										DOE
Inoculum Viability										Linkage Studies
Inoculum In Vitro Cell Age										EOPC Study
N-1 Bioreactor pH										Linkage Studies
N-1 Bioreactor Temperature										Linkage Studies
Osmolality										DOE
Antifoam Concentration										Not Required
Nutrient Concentration in medium										DOE
Medium storage temperature										Medium Hold Studies
Medium hold time before filtration										Medium Hold Studies
Medium Filtration										Medium Hold Studies
Medium Age										Medium Hold Studies
Timing of Feed addition										Not Required
Volume of Feed addition										DOE
Component Conc. in Feed										DOE
Timing of glucose feed addition										DOE-Indirect
Amount of Glucose fed										DOE-Indirect
Dissolved Oxygen										DOE
Dissolved Carbon Dioxide										DOE
Temperature										DOE
pH										DOE
Culture Duration (days)										DOE

CPP = Parameter impacts a Quality Attribute - Must be controlled tightly, limited robustness
WC-CPP = Parameter impacts a Quality Attribute - Well controlled, robust operation
KPP = Parameter impacts Process Attribute
Non-KPP = Parameter does not impact a QA or PA

3.7 Summary of Design Space

The limits of the design space for the upstream process are defined by process parameters (WC-CPPs) in the production bioreactor that impact CQAs. Since none of the other upstream process steps (seed expansion and harvest) had an impact on CQAs, these are not included in the design space.

The design space for the production bioreactor shown in Figure 3.8 is highly complex and, by definition, multidimensional. Furthermore, since it is based on the process reliability it does not necessarily follow the response surface models shown in Figure 3.6. In order to unequivocally define the limits of the design space, the following equations were developed that closely follow the limit of the design space as shown in Figure 3.8. There is one equation for the limit of each CQA that constrains the design space. In this case study, both the low and high limits for galactosylation

and afucosylation were exceeded when the process was operated within the tested ranges thus defining edges of failure and imposing limits within the multidimensional cubic form of the knowledge space. Therefore, combinations of pH, temperature, CO_2, osmolality, and culture duration define the limits of the design space according to the sum of the following inequalities:

Inequality 1: For aFuc < 13%

$$aFuc < 13\% \, if:$$
$$14.7 + 0.62CD + 0.84CO2 + 0.51Osm + 2.83pH + 1.42T - 1.27CD \times CO2 - 1.82CD \times Osm$$
$$- 4.36CD \times pH - 2.26CD \times T - 0.31CO2 \times Osm + 3.11CO2 \times pH + 0.04CO2 \times T - 1.44Osm \times pH$$
$$- 0.81Osm \times T + 3.00pH \times T - 2.09CD^2 - 0.15CO2^2 - 1.66Osm^2 + 3.26pH^2 - 2.73T^2 \geq 4.60$$

Inequality 2: For aFuc > 2%

$$aFuc > 2\% \, if:$$
$$15.29 + 0.35CD - 1.53CO2 - 0.41Osm - 0.28pH - 1.90T - 0.57CD \times CO2 + 2.80CD \times Osm + 0.18CD \times pH$$
$$+ 0.81CD \times T - 1.05CO2 \times Osm - 1.89CO2 \times pH - 0.41CO2 \times T - 0.73Osm \times pH - 0.22Osm \times T - 2.91pH \times T$$
$$+ 0.31CD^2 - 0.51CO2^2 - 0.42Osm^2 - 5.32pH^2 + 1.70T^2 \geq 4.60$$

Inequality 3: For Gal < 40%

$$Gal < 40\% \, if:$$
$$13.2 + 2.31CD + 1.81CO2 + 1.86Osm + 2.82pH - 1.63T - 1.44CD \times CO2 - 0.26CD \times Osm$$
$$- 4.12CD \times pH + 0.76CD \times T - 0.12CO2 \times Osm - 2.80CO2 \times pH + 0.50CO2 \times T - 0.22Osm \times pH$$
$$- 2.20Osm \times T - 2.27pH \times T - 2.49CD^2 - 0.43CO2^2 - 0.32Osm^2 - 0.33pH^2 + 0.06T^2 \geq 4.60$$

Inequality 4: Gal > 10%

$$Gal > 10\% \, if:$$
$$11.08 - 3.06CD - 2.87CO2 - 1.61Osm - 2.27pH + 1.21T - 0.34CD \times CO2 - 0.63CD \times Osm + 3.65CD \times pH$$
$$+ 1.79CD \times T - 0.19CO2 \times Osm + 2.61CO2 \times pH + 0.11CO2 \times T - 0.13Osm \times pH + 2.35Osm \times T$$
$$+ 3.16pH \times T - 0.23CD^2 + 0.41CO2^2 + 0.33Osm^2 - 0.20pH^2 - 0.16T^2 \geq 4.60$$

and process parameters are bounded within the following limits:

- $6.6 \leq pH_u \leq 7.1$,
- $34 \leq T_u \leq 36$ (C),
- $40 \leq CO2_u \leq 160$ (mmHg),
- $360 \leq Osmo_u \leq 440$ (mOsm),
- $15 \leq CD_u \leq 19$ (days)

where, pH_u, T_u, $CO2_u$, Osm_u, and CD_u refer to pH, temperature, dissolved CO_2, osmolality and culture duration. For the models in inequalities 1 to 4, these process parameters were normalized according to the following Equations:

$$pH = \frac{pH_u - 6.85}{0.25}, \quad T = T_u - 35, \quad CO2 = \frac{CO2_u - 100}{60},$$

$$Osm = \frac{Osm_u - 400}{40}, \quad CD = \frac{CD_u - 17}{2}$$

TRANSLATING DESIGN SPACE INTO MANUFACTURING OPERATIONS

To consider how these types of equations would be used in commercial manufacturing it is important to remember that routine operations will be conducted within the boundaries of the control space which lies within the design space.

Control Space

Region within the design space that defines the operational limits (for process parameters and input variables) used in routine manufacturing. The control space can be a multidimensional space or a combination of univariate process ranges.

As such, the manufacturing instructions and process controls would be based on acceptable minimum and maximum ranges that define the control space and ensure that the process is operated well within the design space.

In the case where a parameter or attribute exceeds the limits of the control space, these inequalities need to be evaluated to verify that the design space limits have not been exceeded. This verification can be performed in several ways, including automated real-time evaluation or off-line analysis. For the automated real-time evaluation, the inequalities would be programmed into a computer and evaluated using real-time process data continuously throughout the batch. Such an automation system could also include various alarm levels to indicate if the process has exceeded the control space and/or the design space.

For off-line analysis, the equations can be evaluated using a spreadsheet-based template such as the one exemplified in Figure 3.8. In this spreadsheet, the four inequalities that define the limits of the design space are evaluated using process data for pH, Temp, CO_2, osmolality and culture duration and results compared with acceptance limits. In this example, if the calculated results are within the acceptance limits of the design space, the spreadsheet displays the word “TRUE”. In the case that the design space is exceeded, the spreadsheet would display the word “FALSE”. This is a simple yet effective way to demonstrate that the process was operated within the design space.

Finally, in the case that the control space is moved within the design space, these equations would be checked to ensure that all process parameter interactions will result in product that meets all quality target criteria.

	Test Condition	Design Space Boundaries Low	Design Space Boundaries High
Duration	17	15	19
CO2	100	40	160
Osmo	400	360	440
pH	6.85	6.6	7.1
Temp	35	34	36

Design Space Equations	Holds?
Fuc < 11%	TRUE
Fuc > 2%	TRUE
Gal < 40%	TRUE
Gal > 20%	TRUE

	Equations Fuc <11%	Fuc > 2%	Gal < 40%	Gal > 20%
Intercept	14.70	15.29	13.15	11.08
Cult Dur (days)(15,19)	0.62	0.35	2.31	-3.06
CO2 (mmHg)(40,160)	0.84	-1.53	1.81	-2.87
Osmo (mOsm)(360,440)	1.54	-0.41	1.86	-1.61
pH(6.6,7.1)	2.83	-0.28	2.82	-2.27
Temp (C)(34,36)	1.42	-1.90	-1.63	1.21
Cult Dur (days)*CO2 (mmHg)	-1.27	-0.57	-1.44	-0.34
Cult Dur (days)*Osmo (mOsm)	-1.82	2.80	-0.26	-0.63
Cult Dur (days)*pH	-4.36	0.18	-4.12	3.65
Cult Dur (days)*Temp (C)	-2.26	0.81	0.76	1.79
CO2 (mmHg)*Osmo (mOsm)	-0.31	-1.05	-0.12	-0.19
CO2 (mmHg)*pH	3.11	-1.89	-2.80	2.61
CO2 (mmHg)*Temp (C)	0.04	-0.41	0.50	0.11
Osmo (mOsm)*pH	-1.44	-0.73	-0.22	-0.13
Osmo (mOsm)*Temp (C)	-0.81	-0.22	-2.20	2.35
pH*Temp (C)	3.00	-2.91	-2.27	3.16
Cult Dur (days)*Cult Dur (days)	-2.09	0.31	-2.49	-0.23
CO2 (mmHg)*CO2 (mmHg)	-0.15	-0.51	-0.43	0.41
Osmo (mOsm)*Osmo (mOsm)	-1.66	-0.42	-0.32	0.33
pH*pH	3.26	-5.32	-0.33	-0.20
Temp (C)*Temp (C)	-2.73	1.70	0.06	-0.16

Figure 3.8 Spreadsheet-based Tool for Evaluation of Design Space

3.8 Control Strategy for Upstream Process

In this case study, the design space and control strategy for the upstream process have been limited to include process parameters that are linked to a sub-set of product quality attributes. In a real case scenario, the control strategy would be based on all relevant product quality attributes and would also include considerations for raw material and medium variability quality testing.

The proposed control strategy for the upstream process has a dual purpose:

1. Ensure that the process delivers a product that meets its specifications.
2. Ensure that the commercial manufacturing process is consistent and robust.

Product quality is ensured by operating the process within the limits of the design space- i.e. all quality-linked process parameters (CPPs and WC-CPPs) must meet the inequalities defined by the equations in the design space section. On the other hand, process consistency is ensured by

controlling key process parameters (KPPs) within established limits, and by monitoring relevant process attributes.

A summary of the control strategy for the commercial A-Mab upstream process is presented in Figure 3.9. Here, quality linked process parameters must be controlled within the design space and in-process quality attributes (i.e. microbial and viral safety) must be within specified limits to ensure drug safety and efficacy. Although key process parameters and key process attributes have been shown not to impact product quality, they are included in the control strategy because their monitoring and control ensures that the process is operated in a consistent and predictable manner. The control of key process parameters and attributes also ensures that commercial success criteria such as cycle time and yield are met.

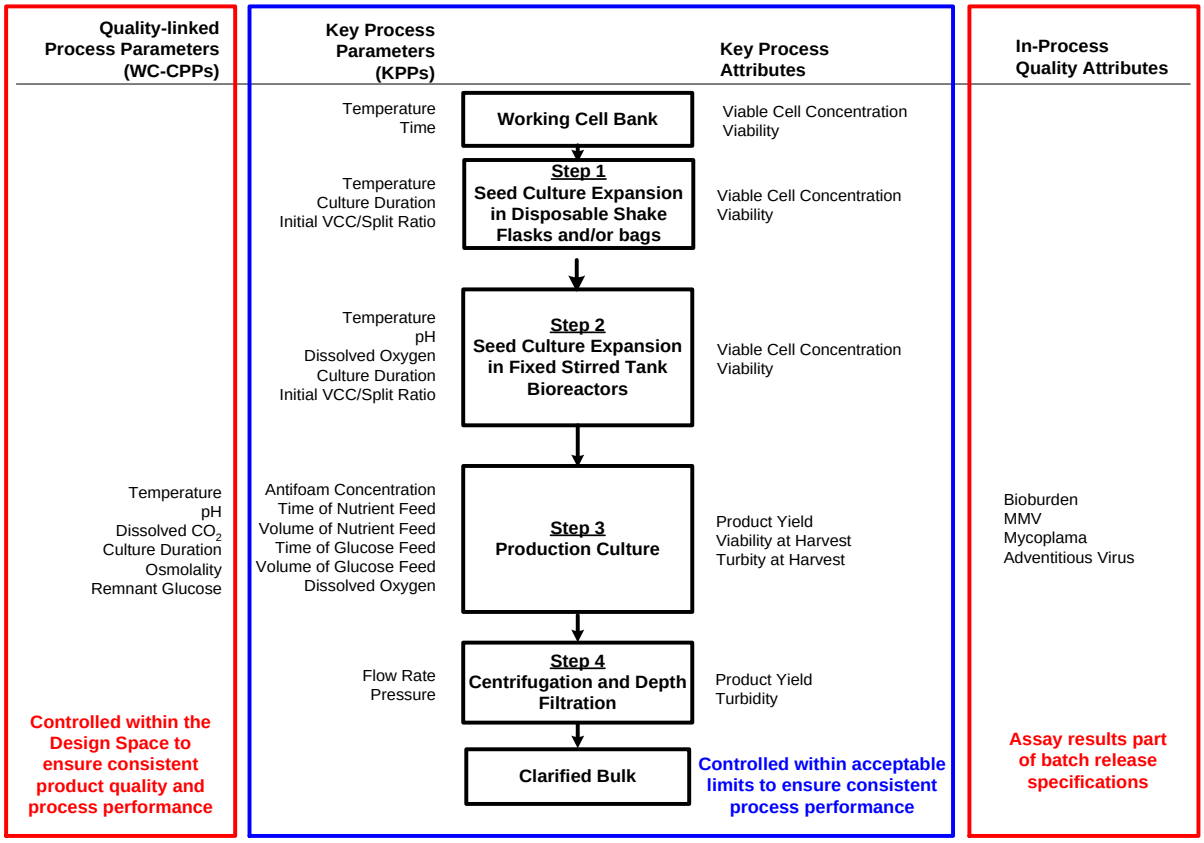

Figure 3.9 Overview of Control Strategy for Upstream Manufacturing Process

3.9 Applicability of Design Space to Multiple Operational Scales and Bioreactor Configurations: Engineering Design Space

The limits of the design space for the A-Mab production bioreactor step were largely based on data derived using a 2-L scale-down model. The demonstration that the design space is applicable to multiple operational scales includes three parts:

- Demonstration that the 2-L scale-down model is representative and predictive of large-scale manufacturing performance
- Demonstration that design space applies to various scales of operation
- Definition and creation of Engineering Design Space

3.9.1 Qualification of Scale-down Model for Production Bioreactor

In order to demonstrate the applicability of the scale-down model to predict large scale production bioreactor performance, manufacturing data from a previous product (X-Mab in this case) was used. Since significant amount of both small scale (2 L) and production scale (15,000 L) are available for X-Mab, which uses the same cell host, expression system and a similar cell culture process, comparison of performance between these scales, serves as a sound approach to qualify this model system for A-Mab.

The scaled-down model for the production bioreactor has a similar design and capabilities to the full-scale production vessels. Both are stirred tank bioreactors with equivalent design characteristics (e.g., mixing, aeration, mass transfer) and process control capabilities (e.g., pH, dissolved oxygen, temperature, nutrient addition, etc). For the qualification studies, scale-independent variables (pH, temperature, iVCC, DO, culture duration, etc) in the scale-down bioreactors were operated at the proposed target process values of commercial operations. For scale-dependent parameters (agitation, gas flow rates, pressure, volume, pCO_2, etc), operating conditions at small scale were established to match process performance at full-scale.

In order to assess the comparability of bioreactor performance and product quality between the 2 L and 15K L scales, a Principal Component Analysis (PCA) model was developed. PCA transforms a large number of possibly correlated variables into a smaller number of uncorrelated variables called principal components which are formed with different loadings of the original variables. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability. The strength of this approach can be viewed as revealing the internal structure of the data in a way which best explains the variance in the data. Thus, this multivariate approach represents a powerful means to assess if the correlation structure between key performance attributes and quality attributes in the scale down model data is comparable to results from full-scale bioreactors.

The resulting model is more sensitive than commonly used univariate comparisons, e.g., t-tests, because it can detect observations that don’t fit the predicted response patterns while resulting in fewer false-positive signals.

The model was built based on data from X-Mab commercial batches for which extensive 15K L experience is available (N=40). Thirteen (13) variables were included in the analysis: Peak VCD, Final Viability, culture pH, Glucose, Lactate, Peak Lactate, Titer, Final IVC, and product quality attributes P5, P6, P7, P5/P6, and P5/P7 (galactosylation, afucosylation and acidic variants). These variables were chosen based on their significance for process performance and product quality as established through process characterization studies and represented by the design space model.

The PCA model results showed that the first five principal components explained 99.4% of the variability in the data set, thus capturing all the significant trends and correlations in the data set. ($R^2X = 0.994$). Cross-validation of the model indicated that it could predict 96.9% of variation in future observations ($Q^2(cum)=0.969$). A 95% confidence ellipsoid for the five principal components was constructed based on the 15K scale data set (Figure 3.10), then data from the 8 2 L runs was used to predict the spatial coordinates of these batches in the multivariate space formed with the large scale batches. As shown in Figure 3.10, the small scale batches reside well within the multivariate confidence ellipsoid of the large scale batches indicating that both data sets have comparable trends and possess similar correlation structures.

As explained earlier, process similarity provides justification for using the qualification of the X-Mab scale-down model for A-Mab. However as part of continuous process monitoring in commercial operations, a PCA model will be developed for A-Mab once sufficient full scale data becomes available. In the interim, PCA was performed using A-Mab process and product quality data from 2 L small scale batches. In this analysis (not shown) the confidence ellipsoid was built using the small scale results (N=20) and the predictions for 5K (N=5, Phase 3) and 15K (N=2, full-scale) were shown to fit within this ellipsoid.

The successful outcome of this multivariate analysis, lends credibility to both the applicability of the 2 L bioreactors as an acceptable scale-down model for 15,000 production bioreactor as well as a validation strategy that relies more on the continuous process verification rather than a minimum number of “validation batches” typically practised.

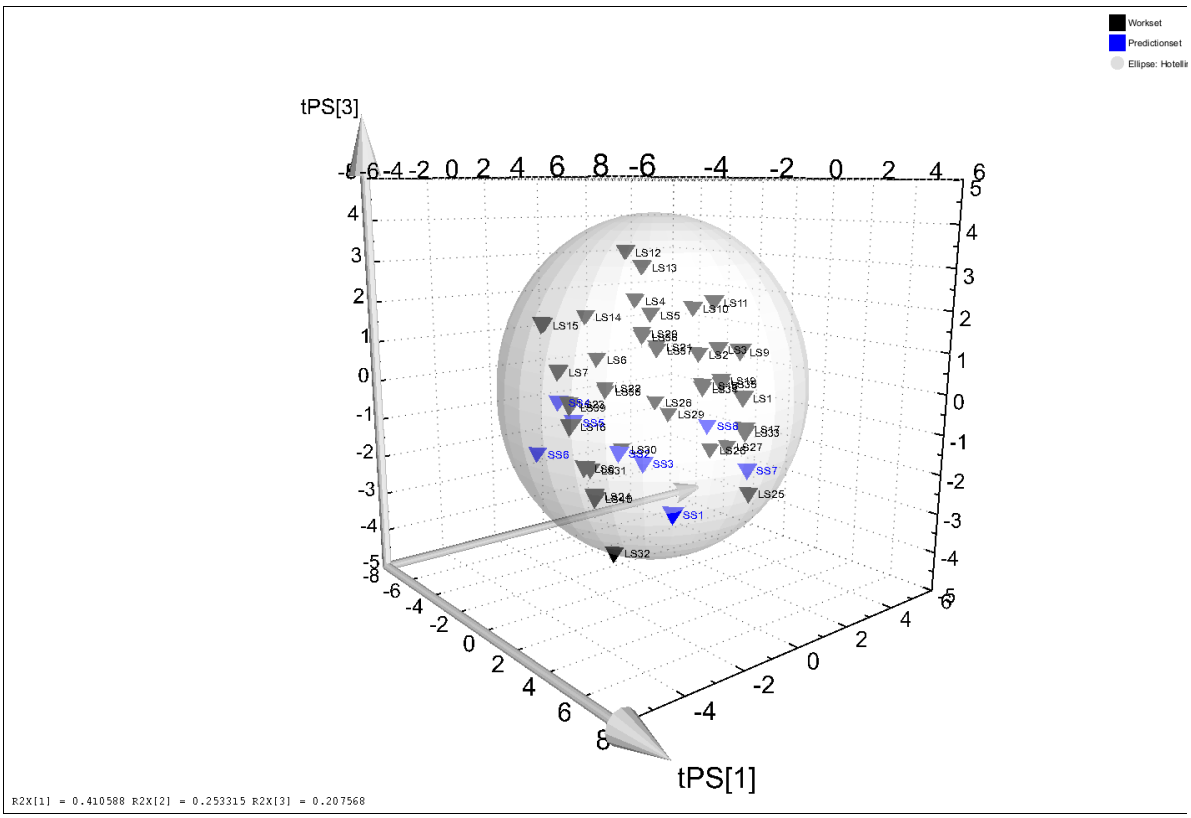

Figure 3.10 Scale Comparison for Large-scale and Small scale X-Mab Runs. Comparison is done using PCA

The sphere is a Hotelling's 95% confidence ellipsoid built from the large scale data (black markers). Plots show how all the small scale runs (blue markers) fall within the ellipsoid, showing that scales produce comparable process performance and product quality results. (▼) Black triangles correspond to 15K liter scale data, (▼) Blue triangles correspond to 2L scale data)

3.9.2 Design Space Applicability to Multiple Operational Scales

The design space described in Section 3.7 is based on the quality-linked process parameters summarized in Table 3.21. All these parameters are considered scale-independent variables and thus apply to all operational scales. However, there are other scale-dependent parameters that must be considered for successful and consistent process performance when operating at various scales. Characterization of the relationships between bioreactor design, operation parameters, control capabilities, product quality and process performance provides the basis for a scientific understanding of the impact of scale.

The intrinsic goal of a successful scale-up is to ensure that the cells in suspension consistently and reliably experience the same micro-environment that was used to establish the design space at small scale. The biological response of the culture to process conditions (e.g., pH or temperature) and process actions such as nutrient feed or base addition must be predictable across scales. While it is impossible to exactly replicate every parameter across scales due to inherent physical constraints, it is possible to create a bioreactor environment that ensures that the scale-independent design space established in Section 3.7 is applicable to bioreactors of different design and scale.

Using previous platform mAb and A-Mab process experience we have demonstrated that by identifying the appropriate design and operational conditions, product quality, product yields, and culture performance can be reliably translated between 2 L and 15,000 L scales. The combination of bioreactor design considerations and process engineering parameters define an engineering design space for the A-Mab cell culture process.

This section describes how prior knowledge, bioreactor engineering characterization and scale-up criteria were used to identify scale-dependent parameters to be included as part of the design space description.

3.9.3 Prior Knowledge

The equipment and control features of a bioreactor are designed and tested to assure axenic conditions and an environment conducive for optimal and reproducible cell growth and expression of the desired recombinant protein. Significant prior knowledge on fermentor design and operation exists from a long history of large-scale fermentations (many in excess of 100,000 liters) including processes to produce antibiotics, related secondary metabolites, and more recently active peptides. In addition, since the approval of the first mammalian cell culture based processes, significant experience has been gained using CHO and other suspension cell culture systems for commercial manufacturing of biopharmaceutical products. This extensive prior knowledge encompasses published literature and practical experience and provides a scientific understanding of the relationships between the physical and chemical environment in the bioreactor, and the physiological response of the cells being cultivated. Thus, this vast experience with reactor design and operation at large scale fermentations serves as significant and relevant prior art for the scale-up of the A-Mab production bioreactors.

3.9.4 Scale-up Criteria

The micro-environment experienced by the cells is what determines the biological performance of the culture. The challenge for scale-up is that this micro-environment cannot be explained by the partial view provided by average bulk parameters such as mixing time and volumetric mass transfer. While these parameters are very valuable to describe bioreactor performance, on their own they do not provide sufficient information to predict possible non-homogeneity in the culture environment. In addition, due the complexity of interactions between multiple parameters, the successful scale-up of cell culture processes cannot be based on a simple model.

The scale-up strategy used for A-Mab is based on a combination of approaches that include engineering design characterization and empirical methods that have a proven track record of successful scale-up in aerated stirred-tank bioreactors.

This section describes the scale-up approaches used for A-Mab and the rationale for selecting bioreactor design characteristics and engineering parameters that can be used to define an Engineering Design Space.

The scale-up considerations used for A-Mab include the following:

- Bioreactor Design
- Mixing regime: Specific energy dissipation rates and mixing time
- Oxygen and CO_2 mass transfer: superficial gas velocity, k_La, gas hold-up volume, pCO_2 stripping

It is important to note that during scale-up the selected parameters do not need to remain constant, but rather be within an acceptable combination of ranges that ensures adequate mixing and mass transfer.

3.9.5 Bioreactor Design

Process experience has demonstrated that a wide range of bioreactor scales and designs have been successfully used to produce A-Mab; a summary of the bioreactor design characteristics that have been used for A-Mab manufacturing is presented in Table 3.21. Although a detailed analysis of the bioreactor design considerations is outside the scope of this document, a high level summary is presented below.

The maximum productivity and quality achievable depend primarily on bulk mixing, oxygen mass transfer and hydrodynamic conditions, which are governed by bioreactor design, impeller type, and process operation. A summary of the primary and secondary reactor design features that must be evaluated to assess the capability of a bioreactor to support a high-density cell culture process, such as the one for A-Mab is shown in Table 3.21. This matrix was derived based on published literature, bioreactor engineering industry best practices, and extensive prior experience with cell culture operations at multiple scales. Primary design parameters are known to have a direct impact on bioreactor performance capability, while secondary parameters have a lesser impact.

Aspect Ratio (height to diameter ratio): Aspect ratio similarity can be maintained from bench to pilot scales but it is not practical to apply to large scale commercial bioreactors. The aspect ratio typically used in smaller bioreactors is 1.0-1.5 but is increased to 2-3 in large bioreactors due to power input considerations.

Impellers and agitation: As the height of the vessel increases, the number of required impellers is also typically increased. From a design perspective, good mixing at large scale is ensured by using multiple impellers of 0.4 to 0.5 vessel diameter with proper clearance between them. Although the specific impeller design can vary (hydrofoil, “elephant ear”, etc) the general requirement consists of high-flow, low power number impellers (e.g., wide-blade axial flow) that provide good liquid blending and good air dispersion. These types of impellers can be combined with radial flow Rushton turbines. The agitation rate required depends on the power number of the impeller.

Sparger Element Design and Location: Prior experience has shown that spargers (sintered stone, etc) and drilled pipes can be successfully used. The drilled pipes produce larger bubbles and thus a

lower volumetric mass transfer (k_La) while the sparge stone produces very small bubbles and a much higher k_La. At smaller scales, successful DO and pCO_2 control strategies have employed either type of sparger elements, at larger scale drilled pipes alone or in combination with sintered sparger are recommended.

Addition port design and location: For pH and nutrients, addition of concentrated feeds or base must be mixed rapidly to prevent localized regions of high concentrations. Rapid dispersion is obtained by ensuring that the addition ports are located in a well mixed region, preferably sub-surface and within the impeller mixing zone.

Table 3.21 Final Risk Assessment Results for Process Parameters in the Production Bioreactor

	Bioreactor Performance Indicators													
Design Parameters	Hydrodynamic Shear	Power per Volume	Mixing Time	Culture Heterogeneity	Gas Transfer (Kla)	Superficial Gas Velocity	Gas Holdup Volume	CO2 stripping time	Bioburden Control	pH	DO	pCO_2	Temperature	Osmolarity
Biorx. Aspect Ratio	P	S	P	P	P	S					P		P	
Baffles			P	P	S									
Impeller Design/size	P	P	P	P	P	S								
Number of Impellers	P	P	P	P	P	S								
Agitation Rate	P	P	P	P	P	S	S	S		S	S	S	S	S
Gas composition, flow rates, control)				P	P	P	P	P			P	P		
Sparger Design & Location	S	P		P	P	P	P	P		S	P	P		
Location of addition ports/tubes				P						S				
Feed Addition Rates				P						S			S	S
Vessel Pressure									P	S	S	S		
Probe Locations				S						P	P	S	P	S
DO Control Loop						S				S	P	S		S
pH Control Loop						S				P	S	S		S
Temp. Control Loop										S	S	S	P	
Foam Control						P	P	S						

P = Primary design consideration expected to impact bioreactor capability. Impact assessment based on prior knowledge, engineering fundamentals, and/or modeling studies (e.g., Computational Fluid Dynamics).
S= Secondary design indirectly impacts bioreactor capability - based on prior knowledge and engineering standard design.

3.9.6 Mixing Regime: Specific energy dissipation rates and mixing time

Mixing is usually uniform in small scale bioreactors resulting in homogeneous microenvironments for the cells. As scale increases, circulating times increase and the microenvironment experienced by the cells becomes a function of bulk flow, mixing and turbulence. The interactions between these parameters are complex and difficult to predict. In most cases, non-homogeneous distribution of nutrients, pH, DO, and pCO_2 are responsible for differences in performance between scales of operation.

Two parameters have been chosen to describe bioreactor performance: specific energy dissipation rate (also referred as power per volume, P/V) and mixing time (Tm). Hydrodynamic shear was not included based on prior process experience and recent published literature that indicate that shear effects are not significant when mammalian cells, including CHO cell lines, are cultivated under standard bioreactor process conditions.

P/V was chosen because it directly impacts mixing and mass transfer. The average P/V provides a measure of the total energy input in the bioreactor while the maximum local P/V is found in the impeller zone and provides a measure of the highest specific energy dissipation rates experienced in the culture. P/V is a function of impeller design, the agitation rate, and working volume of the bioreactor, as shown in **Equation 1**. For the calculation of the maximum local P/V, the impeller sweep volume is used.

Equation 1: $$P/V = \frac{P_0 \rho N^3 D^5}{V}$$

where: P=Power (W), Po = Power number, impeller dependent (--), ρ = density of the liquid (kg/m3), N = agitation speed (s-1), Di= impeller diameter (m), and V= Volume of liquid in bioreactor.

Mixing time provides useful information in terms of the time it takes to achieve a certain degree of homogeneity. Although mixing time is an average bulk measurement and thus cannot describe the possibility for non-homogeneity, it provides valuable information on the performance of the bioreactor. Mixing time is proportional to the mean circulation time, which provides a measure of the time interval during which a cell circulates through the bioreactor and hence possibly encounters different microenvironments.

Both, P/V and mixing time are proportional to the agitation speed; higher agitation rates provide better mixing and gas dispersion. Empirical experience has demonstrated that cell culture processes can withstand much higher agitation rates than those erroneously assumed based on a historical perception of sensitivity of mammalian cells to hydrodynamic shear stress. The upper limit of agitation speed with potential for shear damage at high power inputs (~10^5 kW/m^3) is several orders of magnitude higher than the maximum local P/V used in the A-Mab cell culture process (~5×10^{-2} kW/m^3) (Ma, 2002).

3.9.7 Oxygen and CO2 Mass Transfer: Superficial Gas Velocity, kLa, Gas Hold-up Volume, pCO2 Stripping

Adequate oxygen supply is one of the most important considerations for bioreactor design and operations. Due to its low solubility in water, oxygen must be supplied continuously from the gas phase and proper mixing is required to prevent localized depletion. Insufficient mixing and

inadequate mass transfer will result in DO gradients. Similarly, pCO_2 removal is critical because high levels of dissolved carbon dioxide impact product quality and yield.

The ability of the bioreactor to deliver oxygen is defined by the oxygen transfer rate (OTR) relationship shown in **Equation 2**.

Equation 2 $$OTR = \frac{d[O_2]}{dt} = k_L a\left([O_2]^*_{gas} - [O_2]\right)$$

Where, K_La is the volumetric mass transfer coefficient, $[O_2]$ is the dissolved oxygen concentration in the liquid phase, $[O_2]^*_{gas}$ is the equilibrium oxygen concentration in the liquid. The maximum oxygen uptake rate (OUR_{max}) of the culture is related to the maximum viable cell concentration (VCC_{max}) and the maximum specific oxygen uptake rate($Q_{O2\ max}$), as indicated by **Equation 3**.

Equation 3 $OUR_{max} = (Q_{O2\ max}).\ VCC_{max}$

For A-Mab the maximum OUR was estimated to be 1.5 mmol/L-hr at 15×10^6 cells/mL. The oxygen transfer requirements for a bioreactor are calculated based the oxygen consumption of the culture. To ensure that there are no oxygen limitations, the OTR must exceed the maximum oxygen consumption of the culture. The required OTR is achieved by a combination of providing an appropriate volumetric mass transfer rate (k_La) and adjusting the concentration of oxygen ($[O_2]^*_{gas}$). The K_La is a function of P/V and superficial gas velocity as described in **Equation 4**. The application of this equation for the 15,000 L bioreactor used for the production of A-Mab is shown in Figure 3.11.

Equation 4 $K_La = k\,(P/V)^{\alpha}\,(v_s)^{\beta}$

Where, P/V= energy dissipation rate, v_s = superficial gas velocity, k, α and β = constants that depend on bioreactor system configuration and medium composition.

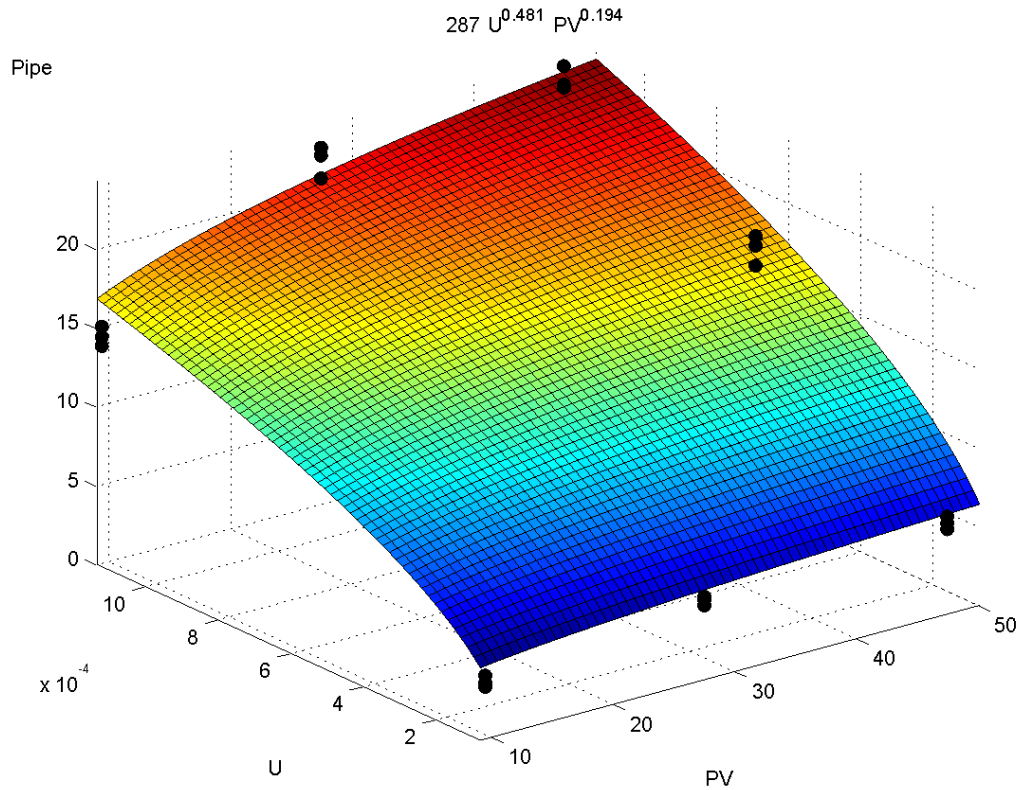

Figure 3.11 Plot of kLa as a Function of Superficial Gas Velocity and Power per Unit Volume for an Open Pipe Design in a 15,000 L Reactor

Black dots represent experimental data and color surface model predictions
U = superficial gas velocity (10^4 m/sec); P/V = power per unit volume in W/m^3 ; $k_La = hr^{-1}$

The characterization of this relationship allows estimation of the OTR capabilities, prediction of required gas flow rates and accumulation of pCO_2 in any bioreactor. It is important to note that in the k_La equation (**Equation 4**), the impeller type, tip speeds and diameter are expressed in the P/V term. Thus one can change impeller types as equipment is scaled up and be certain to meet the required k_La. If the proposed production bioreactors are geometrically similar to existing pilot/commercial scale equipment, similar k_La performance may be expected at the same superficial gas velocity and power per unit volume.

Choosing appropriate gas flow rates and concentrations must ensure that the dissolved oxygen in the bioreactor is maintained within acceptable levels while carbon dioxide is effectively stripped from the bioreactor. Prior experience has shown that a wide range of gas flow rates can be successfully used in the cell culture processes. This is consistent with extensive literature publications that have demonstrated that cell damage associated with aeration is due to bubble-bursting at the gas-liquid interface when the bubbles exit the liquid surface and can be minimized by the addition of surfactants, like Pluronic F68.

The volumetric flow rate (VVM) is related to the superficial gas velocity (Vs) by the following relationship: VVM = [60/H .Vs], where H is the liquid height. Thus, if Vs is kept constant, the

VVM will decrease. If VVM is kept constant, Vs will be higher and foaming can occur. For A-Mab scale-up, VVM and Vs were adjusted at each scale to meet OTR and CO_2 stripping requirements.

Accumulation of pCO_2 depends on a combination of parameters; contributions include bicarbonate concentration in the basal medium, CO_2 generated from cell metabolism, CO_2 stripping and CO_2 addition for pH control. Bicarbonate concentration is fixed by media batch procedures while CO_2 generated by cell metabolism is largely a function of pH and temperature, which are maintained constant across scales. Carbon dioxide stripping is controlled by selecting an appropriate volumetric air flow rate that ensures proper mass transfer from the liquid into the gas phase. Prior experience has shown that carbon dioxide levels are influenced more by the volumetric gas flow rate and the gas residence time than by the agitation speed in the bioreactor. Carbon dioxide stripping rates can be easily measured experimentally to demonstrate that bioreactor operation conditions have been properly selected.

It is important to note that pCO_2 control is also linked to pH and base consumption. Increasing airflow rate strips carbon dioxide and thus reduces pH and potentially leads to an overall reduction of caustic addition. In turn, the reduced amount of caustic can lead to lower pCO_2 at the end of the culture when lactate levels typically decrease.

The successful pCO_2 stripping strategy must be combined with the DO control strategy. There are multiple combinations of DO and pCO_2 control strategies that have been successfully implemented in large scale bioreactors. These include the use of separate sparger elements for pCO_2 and oxygen, combinations of perforated pipes and sintered sparger and positioning of multiple sparger elements within the impeller mixing zones. For the commercial manufacturing of A-Mab, the control strategy conditions in the 15,000 L bioreactor will ensure that the pCO_2 will be maintained between 40 and 100 mmHg by using a constant air flow rate through two drilled pipe sparger elements. The dissolved oxygen will be maintained by adjusting the oxygen gas flow into the gas supply. The control strategy has been successfully demonstrated and an illustration of the results is presented in Figure 3.12.

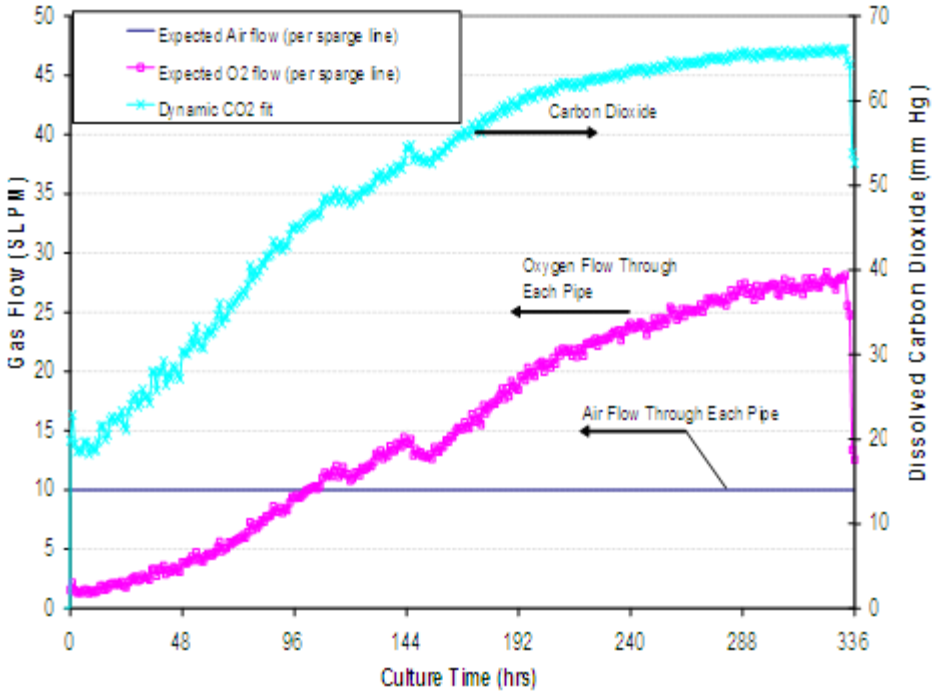

Figure 3.12 Gas Flow Rates and Carbon Dioxide Accumulation in A-Mab Process at 15,000 L Scale

3.9.8 Engineering Design Space

Analogous to the design space defined by scale-independent parameters, the engineering design space is a multidimensional combination of bioreactor design characteristics and engineering parameters that provide assurance that the production bioreactor performance will be robust and consistent and will meet product quality targets. In other words, the engineering design space supports the entire A-Mab design space.

The sections above have summarized the rationale for identifying an engineering design space. The summary of the reactor design and engineering parameters data included in the A-Mab engineering design space is shown in Table 3.22. The A-Mab process has been successfully run in bioreactors from 2 L to 15,000 L working volumes and various design configurations.

The primary design parameters described earlier (aspect ratio, impeller design and number, baffles, addition port location, and sparger design) were verified during A-Mab process scale-up from 500 L to 5000 L and 15,000 L bioreactors to ensure that the bioreactors could support the A-Mab process. Similarly, process engineering parameters (P/V, superficial gas velocity, k_La, mixing time, gas hold-up volume and CO_2 stripping times) were measured and confirmed to meet A-Mab process requirements. Information from 2 L scale-down characterization studies (using Process 2) is also presented to provide additional support to the notion that wide ranges of engineering parameters can

be used and have demonstrated to be acceptable, both in terms of process performance and product quality.

Table 3.22 Summary of Bioreactor Design and Engineering Characterization Data for Various Scales of Operation for A-Mab

	2L Standard Scale-Down Model	2L Process Characterization Studies	500L	1,000L	5,000L	15,000L	25,000L (Future)
Bioreactor Design Characteristics							
Nominal Volume (L)	3	3	600	1250	6200	19000	31500
Working Volume (L)	2	1.5 – 2.25	500	1000	5000	15000	25000
Aspect Ratio (H:D)	1.5	1.0 – 1.5	1.1	1.5	1.4	3.0	3.0
Impeller Design	Marine	Rushton or Marine	A320	A320 & Rushton	Pitched Blade Axial flow	A320	A320
Number of Impellers	1	1	1	2	2	2	2
Baffles	4	4	4	4	4	4	4
RPM	200	150-400	65	75	50	45	40
VVMs	0.1	0.03-0.2	0.16	0.08	0.1	0.1	0.1
Sparger Design	Drilled pipe	Sintered Sparger, open pipe, drilled pipe	2 lines: open pipe & sparge stone	2 lines: open pipe & sparge stone	2 drilled pipes	2 drilled pipes	2 drilled pipes
Bioreactor Engineering Parameters							
Average P/V (W/m^3)	2.8	2.8 -30.2	13.6	28.2	27.4	26.0	25.6
Max local P/V (W/m^3)	5.95	5.95-47.6	25	60	54	52	52
Vs ($x10^{-3}$ m/s)	1.6	0.8 – 4.0	6.0	5.0	8.0	15.0	17.0
KLa (hr^{-1})	10	8-17	8	10	10	20	20
Mixing Time (s)	20	13- 45	48.3	43	62	81	91
CO_2 stripping time	1	1 - 5	1.5	1.7	4.0	2.1	2.3
Gas hold-up volume (L)	ND	ND	5.4	10.5	87.5	350	662

Results (Table 3.23) show that process performance was comparable across all the scales and that product quality was within the predicted design space presented in 3.7.

Of particular interest is the process performance of the 5K bioreactor; where the average titer was approximately 15% lower than in the 15K commercial scale. The lower titer is a consequence of a lower Integral of Viable Cell Concentration (IVC) that is associated with the higher pCO_2 accumulation at the 5K scale. These results are aligned with the multivariate model predictions based on DOE studies that show higher pCO_2 levels lead to lower IVCs and thus lower titers. These results are also in line with the engineering characterization of the bioreactors that show that the 5K design has a somewhat slower pCO_2 stripping rate.

These results demonstrate the design space defined using scale-down data accurately predicts performance at various operational scales.

Table 3.23 Summary of Process Performance and Product Quality for Various Scales of Operation for A-Mab

	2 L Scale-down Model Process 2	2 L Process Characterization Process 2	500 L Process 1	1,000 L Process 1	5,000 L Process 2	15,000 L Process 2
Process Performance Attributes						
Max VCC (cells/mL)	25×10^6	$18\text{-}30 \times 10^6$	5.2×10^6	4.9×10^6	24×10^6	27×10^6
IVC ($\times 10^6$ cells/mL.day)	120	110-140	49.4	51.9	101.3	125
Final Viability (%)	60	45-55	70	75	50	51
Culture Duration (days)	17	15-19	14	14	17	17
Titer (g/L)	4.9	3.2-5.4	1.95	2.1	4.0	5.0
Specific productivity (pg/cell.day)	39.0	38-43	39.5	40.5	39.5	40
Specific Oxygen Uptake rate (mmol/cell.hr)	1.0	0.8-1.2	1.0	1.0	1.0	1.0
Max pCO_2 (mmHg)	60	50-70	65	70	120	65
Quality Attributes						
Aggregate (%)	1.2	1.4-1.6	1.3	1.25	1.6	1.4
aFucosylation (%)	6.7	6.3-9.6	6.5	5.1	7.5	7.4
Galactosylation (%)	26.2	23.5-25.6	35.7	37.8	26.8	25.9
Deamidation	CRS	CRS	CRS	CRS	CRS	CRS
Charge Heterogeneity	CRS	CRS	CRS	CRS	CRS	CRS
ADCC (%)	98	83-107	99.4	89.5	105	110
CDC (%)	101	92-105	112	98	94	98
HCP (ppm)	2.8×10^5	$3.2\text{-}4.1 \times 10^5$	4.3×10^5	2.7×10^5	5×10^5	2.5×10^5
DNA (ppm)	1.7×10^3	$0.8\text{-}1.7 \times 10^3$	1.2×10^3	0.9×10^3	1.5×10^3	0.9×10^3

CRS = comparable to reference standard
Bioreactors operated at standard (set-point) process conditions.
All results are averages, unless a range is a more appropriate metric.

3.10 Lifecycle Approach to Validation

Process validation encompassed the cumulative data and information from early process development through to commercial scale production and continuous process verification to provide a scientific understanding and assurance that the process will consistently deliver product with acceptable quality attributes.

The model used for the validation of A-Mab process is presented in Figure 3.13.

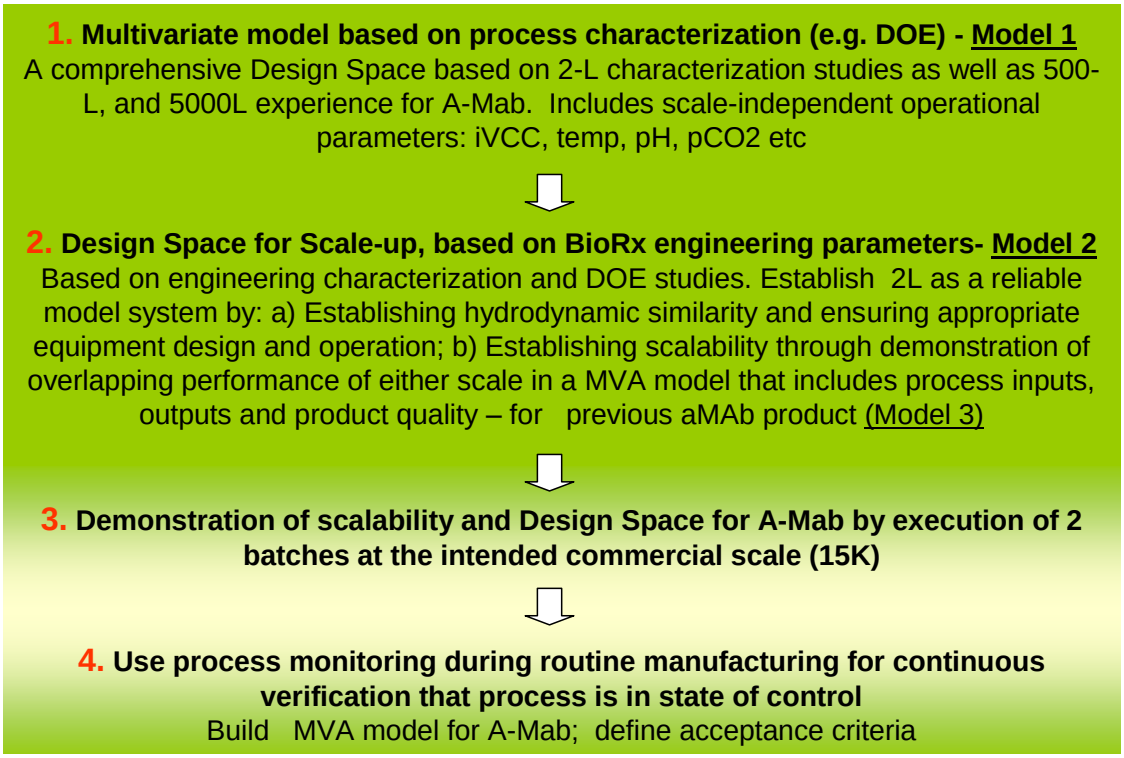

Figure 3.13 Lifecycle Approach to Process Validation

DOE and characterization studies were used to understand the impact of variation of process parameters on product quality. This knowledge is expressed in 2 multivariate models: 1) a model based on scale-independent parameters that serves as the basis for the design space and 2) a model based on the interactions between engineering and bioreactor design considerations at different scales that serves as the basis for an engineering design space. Data at the 2 L scale down model and 5K clinical manufacturing scale provided assurance that the commercial process is robust and consistently delivers product with the right quality. Sources of variability (e.g., raw materials) are controlled through the control strategy which was established based on the cumulative process understanding and demonstrated at the 5K scale.

The proposal for process qualification at 15K scale is a departure from the traditional 3-batch process validation approach. The only element pertinent to the A-Mab PQ is the demonstration that process performance and product quality at the 15K scale are within the predicted design space.

Performance qualification (PQ) will be considered separately from the design of the facility and qualification of the equipment and utilities. The qualification of the equipment, utilities, operation, etc, is considered to have been demonstrated by the successful and extensive commercial manufacturing of X-Mab, Y-Mab, and Z-Mab that demonstrate adherence to cGMPs and suitability of the facility to support the A-Mab process. Similarly, the PQ components related to training of personal, control of batch procedures and materials are also considered proven based on the experience with these other mAbs.

Data from 2 batches at the 15K scale were used to demonstrate the validity of the design space at the intended commercial manufacturing scale. Results showed that product quality and process performance were within the desired acceptance criteria (Table 3.23).

The rationale for using 2 batches includes the following considerations:

1. Confirm Design Space:

- Confirm that process performance at 15K was within model predictions for scale-independent design space (pH, temp, etc)
- Confirm engineering design space for A-Mab by including data from 15K scale of operations

2. Product Characterization & Comparability

- Complete comparability analysis for product made at 1000L, 5000L and 15,000L scales

3. Comply with GMP requirement for Process Qualification:

- The 2 batches are the start of the continuous process verification process and part of the lifecycle approach to validation

However, it is recognized that such a reduced number of batches cannot adequately capture the expected process variability at commercial manufacturing scale. To provide continued assurance that the process remains in a state of control throughout the life of commercial manufacturing, we will create a multivariate statistical partial least squares model (PLS) as part of continued process verification.

PLS is more powerful than standard univariate Statistical Process Control (SPC) approaches in that it ensures that the internal correlations among the different variables are also considered. For example if at any given time the titer is lower than expected for the measured viable cell concentration, the PCA model will be able to detect this as a potential out of norm signal even if both parameters are within their respective univariate ranges. Thus, a PLS model can be used to create a “fingerprint” of the process that detects a larger number of potential shifts, trends and excursions that would not be detected by univariate monitoring tools.

Such a model has already been created and successfully used for Z-Mab. For continuous monitoring of manufacturing batches, time (treated as a Y variable) was modeled as a function of offline (daily VCD, viability, glucose, lactate, titer, etc.) and on-line and at-line data (pH, temp, DO, sparge rates, pressure, reactor weight, etc.) using PLS (Projection to Latent Structures or Partial Least Square) analysis of historical batches. In the model, data from 40 batches (data acquisition frequency = every 20 minutes, number of variables = 26) was used and the PLS model resulted in 4 principal components. These components could cumulatively explain 72.6% and 96.1% of variation in X and Y data, respectively ($R^2(X) = 0.726$, $R^2(Y) = 0.961$). Also, the model had a high predictive power

(Q^2(cum) = 0.961). The control chart of first principal component (t1), its allowable range (mean ± 3 stdev), and the predicted trajectory of an ongoing batch is shown in Figure 3.14.

This multivariate batch modeling technique maps the dynamic nature of the batch process characterizing the design space and identifying process boundaries. Real-time monitoring assures consistent manufacturing and provides early trend detection.

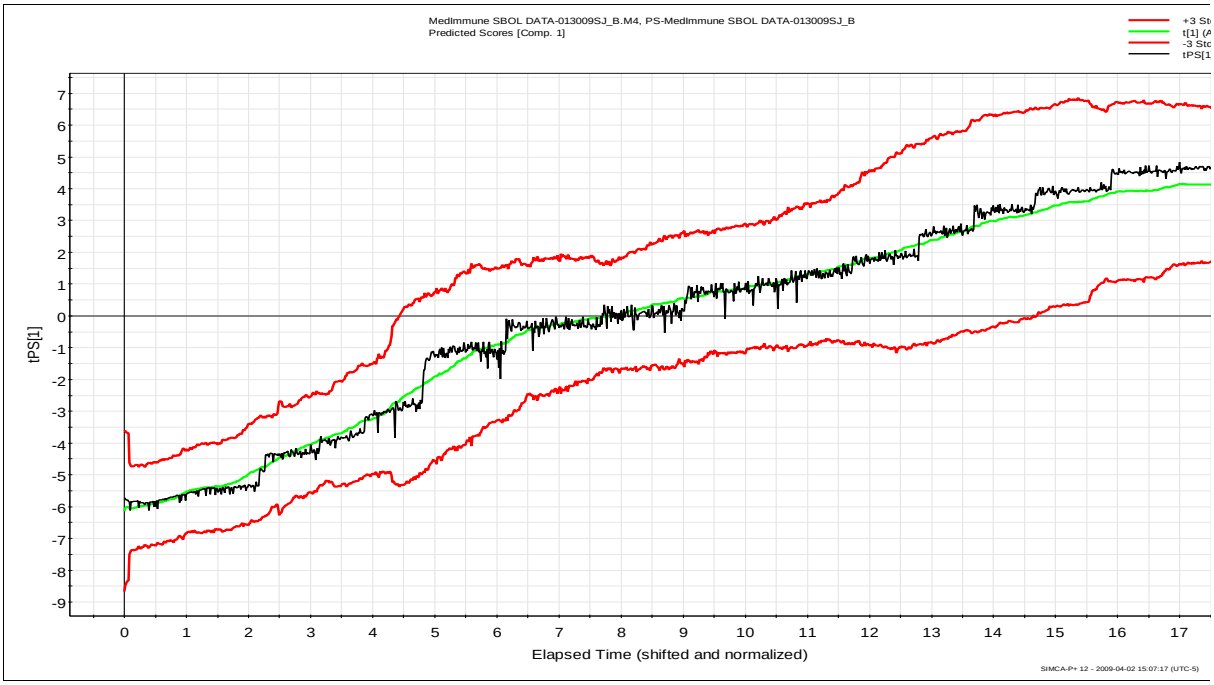

Figure 3.14 Example of a PLS Model for Z-Mab Batch Monitoring

For those parameters that are not built into this PLS model, additional monitoring such as univariate SPC charts, and other routine process monitoring will be carried out. Because of its utility as a process monitoring tool, the PLS model will also have alert and action limits; and when the process result exceeds the action limit a deviation will be initiated.

3.11 Anticipated Post-launch Process Movement within the Design Space

To supply expected commercial demand, it is anticipated that A-Mab manufacturing process will be scaled-up further to 25,000 L scale. The process will be the same as the 15,000 L, except for the scale of operations. For the purposes of this case study, it is assumed that the 25K plant has an extensive and proven commercial manufacturing record of cGMP compliance and monoclonal antibody production.

Based on bioreactor design and engineering parameter characterization (Table 3.22), the 25K bioreactors are within the engineering design space and this provides a very high degree of assurance that operation at this scale will result in comparable process performance and expected product quality. Specifically, vessel, impeller, and sparger configurations meet design requirements and engineering parameter characterization (P/V, mixing time, Vs, k_La, CO_2 stripping time and gas-hold-up volume) is within the proven combination of ranges for successful A-Mab process

operation. Thus, the scale-up to the 25K bioreactor is considered a movement within the engineering design space.

For a change to a different bioreactor (e.g., different impeller design, geometry, etc) an assessment would be conducted to determine if the bioreactor characteristics fall within the engineering design space. If they do not then equipment modifications and/or changes in operational parameters would be considered to bring the bioreactor operation within the approved engineering design space.

3.12 Bibliography

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4 A-Mab Downstream Process Description and Characterization

4.1 Summary

The downstream process for A-Mab represents a well established platform with extensive process performance history. It has been used for the production of commercially licensed antibodies and the supply of multiple clinical studies. The large body of knowledge derived from this experience has demonstrated that the downstream process is robust and consistently produces Drug Substance of acceptable yield and quality. This extensive process experience therefore reduced the amount of process optimization studies required for the A-Mab downstream process.

The QbD approaches exemplified in this section leverage this extensive prior knowledge and are augmented with A-Mab specific data, where necessary, to provide input to risk assessments, DOEs and process characterization studies that provide a science-based approach to process understanding, definition of design space and establishment of the control strategy.

Each unit operation leverages this prior knowledge to guide process characterization studies and support a design space proposal. A multivariate model for HCP clearance was developed that, in this case includes Protein A, Cation exchange, and Anion exchange chromatography steps.

Also presented is a comprehensive approach to viral clearance that leverages the extensive prior knowledge and justifies a modular approach for all 3 viral clearance steps: low-pH inactivation, anion exchange (AEX) chromatography and small-virus retentive filtration. This prior knowledge also justifies that no additional virus spiking studies are necessary for the A-Mab low-pH treatment step.

Key Points from Downstream Section

1. Platform process and prior knowledge obviate need to conduct optimization studies.
2. Linkage of process performance of 3 chromatography steps: Protein A, Cation Exchange, and Anion Exchange to provide greater understanding of the inter-step dependence of unit operations
    - Exemplified through HCP clearance
3. Viral clearance claims based on modular approach. Prior knowledge leveraged to define design space for A-Mab.

Specifically, this section includes discussion of the following QbD approaches:

- Through risk assessments, use of the extensive prior knowledge that is available for the downstream process to identify parameters that could impact product quality and process performance for each process step. This information is used to guide the design of multivariate and univariate process characterization studies for A-Mab.
- The use of scale-down models for process characterization studies to define design space.
- Development of a linkage model linking for all three purifications steps (Protein A, AEX and CEX) to gain a more complete understanding of the purification process and to define the overall design space for the downstream process.

- Leveraging prior knowledge and A-Mab results to justify a modular approach to viral clearance. Defining parameters for the process design space based on viral clearance considerations.
- Leveraging the extensive process characterization data with other mAbs to support elimination of AEX and CEX resin re-use studies for A-Mab.
- Science and risk-based approaches taken to justify two potential post-launch process changes: 1. Change of protein A resin and, 2. Change from a resin to a membrane format in the AEX step.
- Exemplification of the use of a risk assessment tool to assess clearance of cell culture impurities.

4.2 Downstream Process Overview

The downstream process captures A-Mab from the clarified harvest and purifies the antibody by a combination of chromatography unit operations. Also included in the process are two orthogonal steps dedicated to virus inactivation and removal. The antibody is formulated through a diafiltration and ultrafiltration step to a composition and concentration suitable for drug product manufacturing. The formulated product is 0.2 µm filtered, filled into the appropriate containers and stored frozen.

The downstream manufacturing process for A-Mab comprises 7 steps which are presented in the flow diagram Figure 4.1. The purpose of each step and the scope of information included in the case study are summarized in Table 4.1. Detailed step descriptions and process performance analyses are presented in the sections that describe each step.

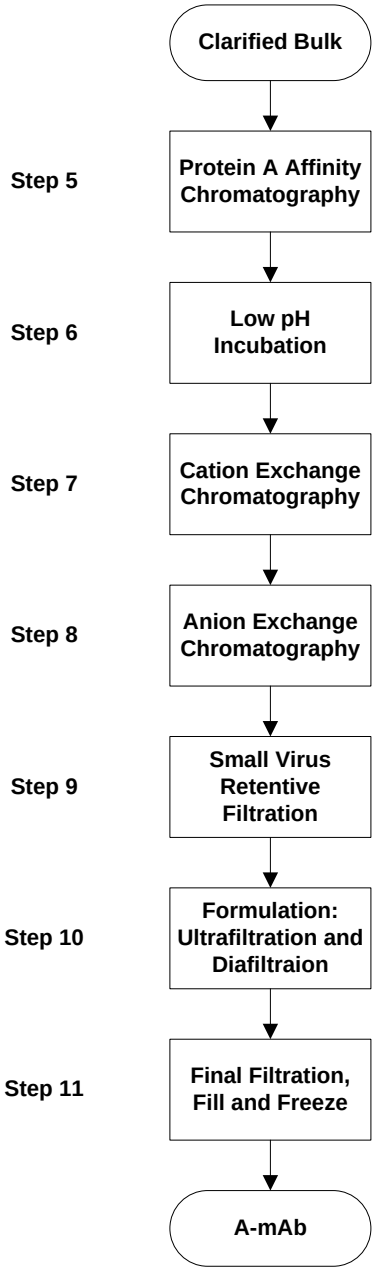

Figure 4.1 Downstream Process Flow Diagram

Table 4.1 Overview of Downstream Process Steps

Downstream Step	Purpose of Step	Scope included in Case Study
Protein A Affinity Chromatography	▪ Capture of monoclonal antibody from the clarified harvest liquid. ▪ Removal of process related impurities: HCP, DNA and small molecules.	▪ Risk assessment to define DOE process characterization studies. ▪ Linkage of Protein A chromatography step to the CEX and AEX chromatography steps ▪ Column lifetime studies. ▪ Protocol to introduce a new Protein A resin into the process
Low pH Viral Inactivation	▪ Inactivate enveloped viruses that are potentially present in therapeutic protein products derived from mammalian cell culture	▪ Worst case condition studies for low pH hold times to demonstrate A-Mab stability. ▪ Modular viral clearance approach that leverages data from several antibodies to characterize viral inactivation process and define design space.
Cation Exchange Chromatography	▪ Reduce aggregate to acceptable levels for drug substance. ▪ Reduce HCP to acceptable levels for subsequent processing by AEX chromatography.	▪ Establish a predictive capability model based on DOE data and risk assessments. ▪ Establish model based on linkage between Protein A, Cation exchange and anion chromatography steps
Anion Exchange Chromatography	▪ Remove HCP, DNA, Protein A and endotoxins to levels that meet drug substance acceptance criteria. ▪ Virus removal	▪ Modular viral clearance approach that leverages prior knowledge. ▪ Use of prior experience and A-Mab data to define a model based on linkage to Cation exchange step. ▪ Replacement of the anion exchange resin with a membrane format
Small Virus Retention Filtration	▪ Removal of small parvoviruses such as minute virus of mice (MVM) and larger viruses such as murine leukemia virus (MuLV) potentially present in product derived from mammalian cell culture.	▪ Leverage prior knowledge to demonstrate that step does not impact product quality ▪ Discuss separate modular claims based on small and large virus removal
Ultra-Filtration/Diafiltration	▪ Formulation and concentration of mAb to drug substance specifications (e.g. 75 g A-Mab/L)	▪ UF/DF process step not included in case study. ▪ Formulation studies are presented in Drug Product section
Final Filtration, Filling and Freezing	▪ Sterilize filtration and dispensing for Drug Substance storage.	▪ Not included in case study.

4.3 Process Understanding based on Prior Knowledge

Utilising the extensive prior knowledge, an initial risk assessment was conducted to identify which downstream process steps potentially impact product quality. The resulting matrix (Table 4.2) provided guidance on which unit operations to evaluate for process characterization studies.

Table 4.2 Quality Attributes Potentially Affected by the A-Mab Downstream Unit Operations

Quality Attributes	Risk of Impact to Product Quality Attribute						
	Protein A Chromatography	Low pH Treatment	Cation Exchange Chromatography	Anion Exchange Chromatography	Small Virus Retentive Filtration	Ultrafiltration and Diafiltration	Final Filtration and Bottling
Identity							
Protein Content						✓	
ADCC							
Aggregate	✓	✓	✓	✓	✓	✓	
Color							
Clarity							
Oligosaccharide Profile							
Charge Variants	✓	✓	✓	✓			
pH						✓	
Osmolality						✓	
Residual HCP	✓	✓	✓	✓			
Residual Protein A	✓	✓	✓	✓			
Residual DNA	✓	✓	✓	✓			
Residual Methotrexate	✓		✓	✓		✓	
Bioburden							✓
Endotoxin			✓	✓			
Viral Safety		✓		✓	✓		

For the purposes of this case study, only a subset of quality attributes is considered:

- Aggregate,
- Galactosylation,
- A-fucosylation,
- Deamidation,
- HCP

By contrast, the extensive prior knowledge has demonstrated that the distribution of glycosylation variants (e.g. galactosylation and fucosylation) is minimally impacted by downstream processing and is mainly influenced by the upstream process conditions. Based on this assessment, glycosylation variants were not included in the testing for characterization studies of the downstream process steps.

Viral clearance and process residuals (e.g. protein A, methotrexate) were also included in the downstream process discussion. In an actual study, the examples and approaches described here would include all relevant product quality and material attributes.

The lack of clearance or modification of glycosylation variants through the downstream platform process is consistent with the binding mechanisms of the respective chromatography steps. Protein A, when operated under platform conditions, does not separate glycosylation variants of monoclonal antibodies. The charge-based separation steps, cation exchange and anion exchange chromatography, also do not discriminate between different glycosylation variants, except for sialylated structures. However, sialylation variants are only present at very low levels in A-Mab and thus are not considered critical to product quality.

4.4 Prior Knowledge for Viral Clearance

Extensive prior knowledge exists for viral clearance steps. Full process characterization studies have been conducted for the low pH treatment, anion exchange chromatography and small virus retention filtration steps. These studies were conducted with three licensed IgG1 monoclonal antibodies (X-Mab, Y-Mab and Z-Mab) with similar physicochemical properties that include isoelectric point of the mAb (pI), acidic variant distribution, and glycosylation variant profiles. These studies included using scale down models for the platform process (similar steps, same sequence, run under similar conditions) and a modular process approach (similar steps, different sequence, run under similar conditions).

Consistent with the FDA Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (1997), the modular clearance study demonstrated virus removal or inactivation in individual steps during the purification process. Here, each module in the purification scheme was studied independently of the other modules.

Four model viruses were selected to provide a range of virus characteristics representative of the diversity of potential adventitious agents, Xenotropic Murine leukemia Virus (XMuLV), Minute Virus of Mice (MVM), Simian Virus 40 (SV40) and Pseudorabies Virus (PRV). However for purpose of brevity, only data for MVM and XMuLV are provided in the case study. XMuLV was selected as a relevant model for the retrovirus-like particles that are expressed endogenously in the Chinese hamster ovary (CHO) cell line. MVM, a small, chemically resistant virus, was chosen as a

challenge for removal by small virus retentive filters. Properties of the selected viruses are shown in Table 4.3.

Table 4.3 Properties of Model Viruses

Virus	Family	Envelope	Genome	Size (nm)	Shape
XMuLV	Retroviridae	Yes	ssRNA	80-130	Spherical
MVM	Parvoviridae	No	ssDNA	18-24	Icosahedral

4.5 Batch History

The downstream platform process did not require any significant changes to accommodate the increased productivity of the cell culture process or facility changes made through the development life cycle. The only changes made to the downstream process represent scale increases to match the upstream process scales. The A-Mab batch history is summarized in the upstream process section.

4.6 Downstream Process Characterization

The following sections describe the approaches used to identify parameters linked to product quality and process performance that serve as the basis for defining the design space for each process step. The classification of process parameters used in this section is based on the decision logic presented in the Control Strategy Section.

NOTE– The following terms are used in the downstream section. The definitions are repeated here to aid the reader.

1. Critical Process Parameter (CPP) and Well-Controlled Critical Process Parameter (WC-CPP). Both, CPPs and WC-CPPs, are process parameters whose variability have 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 has a low risk of falling outside the design space.

A CPP has a high risk of falling outside the design space.

Here, the assessment of risk is based on a combination of factors that include equipment design considerations, process control capability and complexity, the size and reliability of the design space, ability to detect/measure a parameter deviation, etc.

2. Key Process Parameter. An adjustable parameter (variable) of the process that, when maintained within a narrow range, ensures operational reliability. A key process parameter does not affect critical product quality attributes.

4.6.1 Step 5: Protein A Chromatography

The Protein A step is linked to the performance of the CEX and AEX chromatography steps. The information used to build the design space is based on prior knowledge with other monoclonal antibodies and A-Mab data. The design space is described in the form of a multivariate model in Section 4.7 "Linkage of Unit Operations".

The Protein A chromatography step is linked to the upstream process and Cation exchange steps as follows:

Table 4.2. Protein A Affinity Chromatography Step Linkages

Input from Clarified Harvest	Output to Low pH Inactivation
Protein concentration ≤ 5 g/L	Protein concentration ~ 20 g/L
pH 6.9	pH > 4.0
Aggregate < 3.1%	Aggregate < 3.1%
Acidic variants ~ 10%	Acidic variants ~ 10%
HCP ~ 900,000 – 1,300,000 ppm	~ 7,200 ppm but may range from 3,000 to 12,000 ppm

4.6.1.1 Step Description

The Protein A step is the first chromatographic unit operation in the purification process. This step uses an immobilized Protein A resin which binds the mAb from the harvested cell culture fluid (clarified harvest). The affinity capture is an inherently robust processing step, with a rich platform performance history that supports the proposed design space. Process impurities such as HCP, DNA, and small molecules are removed in the flow through or wash. A low pH buffer elutes the mAb and sets up the subsequent low pH inactivation step. While viral clearance can be demonstrated for Protein A chromatography steps, there are no claims made for this step.

The Protein A column is packed to a bed height of 10-30 cm. The column is purged of storage buffer, equilibrated and the clarified harvest is loaded. After loading to 10-50 g/L, the column is washed with equilibration buffer. The mAb is eluted from the column with a low pH elution buffer. The start of collection is based on the absorbance at 280 nm, and is ended by the absorbance at 280 nm or based on specified column volumes. The column is then regenerated and re-equilibrated prior to starting the next load cycle (several cycles are used to process a single harvest, and the individual elution pools are combined after the final cycle). Upon completion of the processing of the entire harvest, the column is washed with and stored in storage buffer until the next use.

4.6.1.2 Scale-down Model

A scale-down laboratory system was qualified as a model of the manufacturing-scale process. The model was designed based on well-established principles of chromatography scaling, maintaining the same bed height, linear flow velocities, load, wash and elution volumes (normalized to column volumes), and column efficiency based on plate count and peak asymmetry. The model qualification used triplicate runs of the lab-scale system, with statistical comparisons of the mean values of the performance parameters for lab, pilot- and manufacturing-scale, product yield, peak volume, impurity removal (e.g. HCP, DNA, and insulin), and levels of leached Protein A. In all cases, there were no statistically significant differences in column efficiency or performance

parameters between scales (data not shown) and therefore, the scale-down model accurately represents the full-scale system and is suitable for use in process characterization studies.

4.6.1.3 Risk Assessment Used To Plan Process Characterization Studies

This section provides an example of a risk assessment tool used to identify which parameters need to be included in the design of process characterization studies, which include DOEs and univariate approaches.

A risk assessment approach was used to categorize all Protein A process parameters into three groups: i) parameters warranting multivariate evaluation, ii) secondary parameters whose ranges could be supported by univariate studies, and iii) parameters which did not require new studies, but instead would employ ranges based on knowledge space or modular claims established from prior knowledge.

Also, because different cell lines and corresponding upstream culture feed- streams may have unique characteristics, it was not possible to apply data from resin re-use studies from other mAbs to A-Mab. Therefore, independent studies with A-Mab were conducted to support resin re-use and process pool hold times (data not shown).

The risk assessment approach used risk ranking to classify process variables based on their potential impact to CQAs, process performance and possible interaction with other parameters. Each parameter was assigned two rankings: one based on the potential impact to CQAs (main effect) and the other based on the potential of interactions with other parameters. The rankings for impact to CQAs were weighted more severely than the impact to lower criticality QAs or process attributes (Table 4.4). If no data or rationale were available to make an assessment, the parameter was ranked at the highest level.

Table 4.4 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

** Note: The impact assessment is considered for variation of a parameter within the proposed design space range*

Main and interaction effects were multiplied to calculate the overall "Severity Score" which served as the basis for identifying the minimum level of experimental complexity required for characterization studies (See Table 4.5).

Table 4.5 Severity Score Calculation

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

Three experimental design strategies were considered for characterization studies: multivariate studies, univariate studies, or no further study needed (Table 4.6).

Table 4.6 Severity Classification

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

For "Severity Score" values of 8 or 16, further assessments were required to determine if a parameter was considered for univariate or multivariate studies. In the absence of such rationale, these parameters were assigned to multivariate studies. In this risk assessment approach, parameters with low scores can always be included in multivariate studies to provide additional process understanding.

Platform process development and process characterization knowledge from other mAbs, manufacturing history, and scientific knowledge were used to rank each process variable in the initial risk assessment, and set the ranges for evaluation. The Protein A risk ranking results are summarized in Table 4.7.

Table 4.7 Risk Ranking for Protein A Chromatography Step

Phase	Parameter	Main Effect (CQA)[a]	Main Effect (PA)[b]	Highest Main Effect Score	Interaction (CQA)[a]	Interaction (PA)[b]	Highest Interaction Score	Severity (MxI)
All phases	Column Bed Height (cm)	1	1	1	4	2	4	**4**
Load (HCCF)	Flow Rate (CV/hr)	4	2	4	2	2	2	**8**
Load (HCCF)	Operating Temperature (oC)	4	1	4	4	1	4	**16**
Load (HCCF)	Protein Load (g/L)	4	4	4	4	4	4	**16**
Load (HCCF)	Load Concentration (g/L)	1	1	1	1	1	1	**1**
Equil & Wash	Buffer pH	1	1	1	1	1	1	**1**
Equil & Wash	Buffer Molarity (mM Tris)	1	1	1	4	1	4	**4**
Equil & Wash	Buffer Molarity (mM NaCl)	1	1	1	4	1	4	**4**
Equil & Wash	Buffer Molarity (mM EDTA)	1	1	1	1	1	1	**1**
Equil & Wash	Flow Rate (CV/hr)	4	2	4	4	1	4	**16**
Equil & Wash	Operating Temperature (oC)	1	1	1	1	1	1	**1**
Equil & Wash	Volume (phase duration)	1	1	1	4	1	4	**4**
Elution	Buffer Molarity/pH (mM Acetic acid)	4	1	4	4	1	4	**16**
Elution	Flow Rate (CV/hr)	1	2	2	1	1	1	**2**
Elution	Operating Temperature (oC)	1	1	1	1	1	1	**1**
Elution	Start Pool Collection (OD)	1	1	1	1	1	1	**1**
Elution	End Pool Collection (CV)	1	1	1	8	1	8	**8**

4.6.1.4 Multivariate DOE Studies

Based on this risk assessment (Table 4.7), five variables were identified for the multivariate studies: protein load, flow rate, temperature, elution buffer pH, and end of collection based on column volumes. A randomized 19-run study was conducted with six-factor, 16 run Resolution IV fractional factorial design which included a link to the cell culture process. Two culture harvests, one early and one late harvest were used to get feed stocks with extremes of low and high viability and titers, since these parameters could impact the Protein A performance. The three center points consisted of equal volume mixture of the two extreme feed stocks.

Table 4.8 lists the multivariate parameters and test ranges, their potential interactions, and rationale for inclusion in the study.

Table 4.8 Process Parameters in Multivariate Study A

Parameter	Testing Range	Severity Rating	Potential Interactions	Scientific Rationale
Protein load	10-50g/L	16	• Bed height • Load flow rate • Temperature • Elution pH • End collection	Moderate interactions expected with high flow rate decreasing yield. There is potential for slightly higher pool impurities at high protein load. No impact is expected at lower protein loading.
Flow rate	100-300 cm/hr	8	• Bed height • Protein load • Temperature	Expect to have moderate interaction with high protein load causing a decrease in yield. At low flow rate there is an unknown impurity impact and possibly some interaction of operating temperature.
Temperature (entire step)	15-30°C	16	• Load flow rate • Protein load	Expect higher HCP at and lower leached Protein A at low operating temperature. It is possible to have moderate interaction with load flow rate and protein load leading to higher pool impurities.
Elution buffer pH	pH 3.2-3.9	16	• Protein load • End collection parameter	There are potential interactions with load and end collection parameter resulting in low pool pH or high pool pH, as well as increasing HCP at low pH.
End collection	2.0-3.2 CV	8	• Elution buffer pH • Protein load	Platform experience has shown no effect on process or product quality. Potential interactions with elution pH and load resulting in low pool pH or high pool pH.
Cell culture clarified harvest	Two feedstocks (A and B) from early and late harvests having low and high titer/ and viability	Linking variable	• Protein load • Elution buffer pH	There is a potential for product titer and/or viability levels to influence Protein A step performance and product pool purity.

The process performance outputs included quality attributes (HCP, acidic variants and aggregate levels) as well as performance indicators (product yield, product concentration in the product pool, and turbidity/filterability).

The multivariate study revealed that the following parameters impacted process performance and product quality:

- The elution pH and protein loading had a significant impact on HCP. (Figure 4.2). Based on the impact on this CQA and because both parameters are well controlled in the process, they were classified as “well controlled –critical process parameters (WC-CPPs).
- Protein load, flow rate, and end collection (CV) had a significant impact on product yield. The process model included two main effects as well as a non-linear interaction term. Based on these results, flow rate and end collection (CV) were classified as Key Process Parameters (KPPs).

- Results also showed that none of the process parameters had a significant effect on aggregate or acidic variants (deamidation). While small differences in these product quality attributes was seen in Protein A pools of the multivariate runs, no significant statistical correlation was established. Based on these results, all other process parameters were classified as General Process Parameters (GPPs)
- The combination of protein loading and pool collection criteria resulted in product concentration in the pool that ranged from 2 to 20 g/L, well below the upper limit of protein concentration tested in the low pH viral inactivation step (35 g/L).

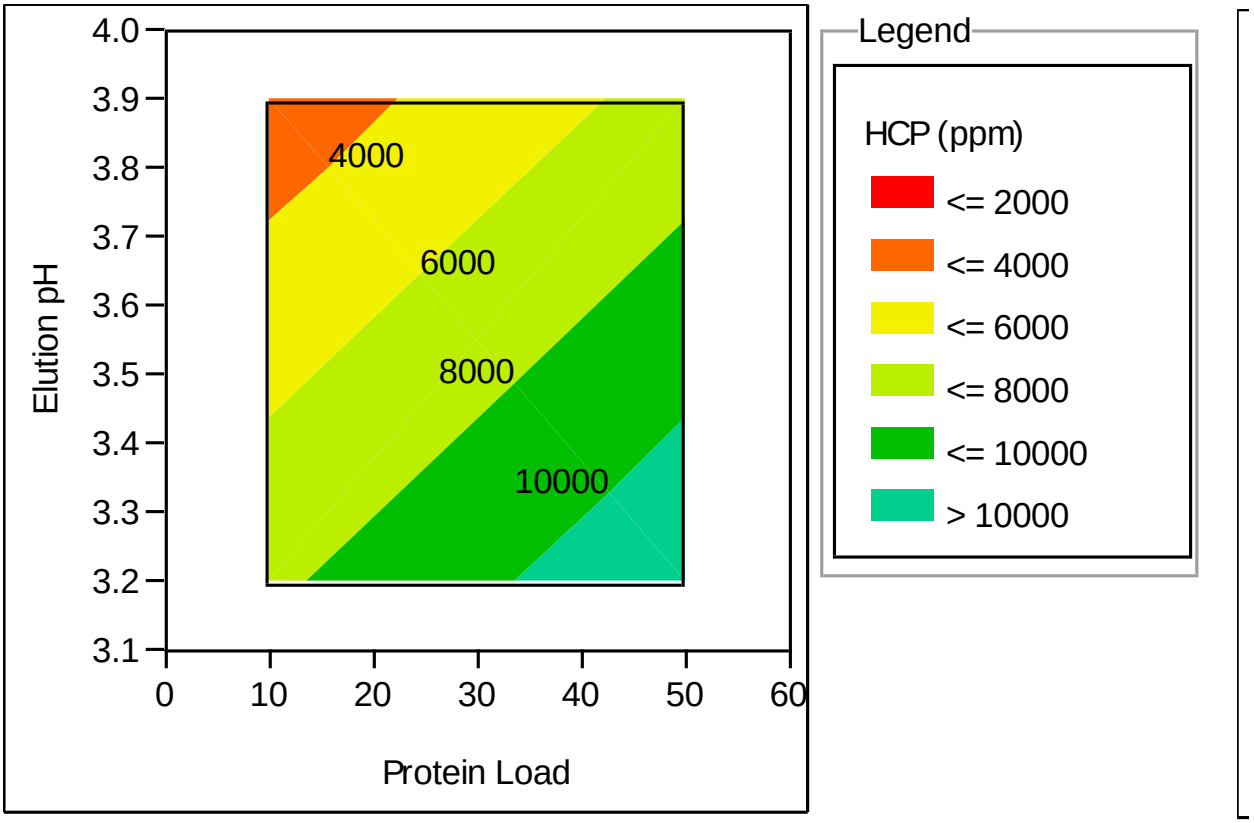

Figure 4.2 Predicted Protein A HCP (ppm) concentration as a function of Protein Load and Elution pH in Protein A chromatography step.

4.6.1.5 Univariate Studies

Based on the risk assessment results and prior knowledge, load concentration was not expected to interact with any other process parameter of this step thereby enabling it to be studied as a univariate process variable (Table 4.9). Results of the study showed that no impact was observed on step performance or product quality over the range tested (data not shown). Thus this parameter was classified as a GPP and included along with multivariate study results to fully describe the knowledge space for the Protein A step.

Table 4.9 Design and Results

Parameter	Testing Range	Severity Rating	Potential Interactions	Scientific Rationale
Load concentration	2.5-6 g/L	1	None	No impact or interaction is expected. An extended load volume due to a decrease in titer would only potentially cause displacement of impurities during the load phase (resulting in lower pool impurities). The two feedstocks that were used in the multivariate study were further evaluated by spiking and dilution studies to cover 2.5-6 g/L titers.

4.6.1.6 Process Ranges based on Platform Knowledge

Based on the risk assessment, the process parameters that were considered as not requiring further investigation are listed in Table 4.10. For these parameters, the extensive process knowledge and modular process performance claims justify the proposed acceptable ranges.

Table 4.10 Process Parameter Ranges Supported by Prior Knowledge and Modular Process Performance Claims

Parameter	Severity Rating	Prior knowledge (Mab ID)	Acceptable Range	Scientific Rationale
Bed height	4	X-Mab	10-30 cm	Platform knowledge shows no significant effect on product quality or process performance. There is potential at low bed height, high protein load and high flow rate to decrease yield and increase product pool impurities. Acceptable range is 10-30cm.
		Y-Mab	12-25 cm	
		Z-Mab	15-25 cm	
Eq/Wash pH	1	X-Mab	pH 6.6-7.6	Platform knowledge shows no significant effect on product quality or process performance. Therefore, the proposed buffer pH range should not affect the performance of this affinity resin. Acceptable range is pH 6.6-7.6.
		Y-Mab		
		Z-Mab		
Eq/Wash composition	1	X-Mab	(60-140%) Tris, NaCl	Platform knowledge shows no significant effect on product quality or process performance. Therefore, the proposed composition ranges should not affect the performance of this affinity resin. Acceptable range is 60-140% Tris, NaCl concentrations.
		Y-Mab		
		Z-Mab		
Start collection parameter	1	X-Mab	Start 0.1-1.0 OD	Platform knowledge shows no significant effect on product quality or process performance. The elution phase elutes the product and does not separate the product from impurities. Therefore the only potential impact to the process is decreased yield or collection of additional equilibration buffer in the product pool, but due to the steepness of the starting part of the elution peak neither of these outcomes will occur. Acceptable range is 0.05-1.0 OD.
		Y-Mab	Start 0.3-1.0 OD	
		Z-Mab	Start 0.05-0.5 OD	
Eq / Wash volumes	1	X-Mab	(90-110%)	Platform knowledge shows no significant effect on product quality or process performance. Acceptable range is 60-170%.
		Y-Mab	(90-110%)	
		Z-Mab	(60-170%)	

4.6.1.7 Summary of Process Parameter Classification and Ranges

Results of Protein A step characterization studies demonstrated that this step does not impact the distribution of product variant CQAs (e.g. acidic isoforms). Moreover, this step was shown to have robust process performance even when challenged with a wide range of feed stream inputs (HCP, DNA, Titer, and Viability).

The limit for maximum protein concentration in the Protein A pool is bound by the pH inactivation step requirements. Results show that the protein A step can consistently meet these requirements.

The Protein A operating conditions influence the HCP levels in the resulting product pool. Since subsequent steps (AEX and CEX) can reduce HCP to safe and consistent levels, the acceptable HCP output levels from the Protein A are linked to the operating conditions of these subsequent steps. A model defining this linkage is given in Section 4.7 “Linkage of Unit Operations”.

Risk analysis, process characterization studies and process performance history demonstrate that the Protein A step does not have any Critical process Parameter (CPPs). Only two parameters were linked to CQAs (Protein Load and Elution buffer pH) and were classified as WC-CPP based control capabilities to operate within the proposed design space. The classification of process parameters is summarized in Table 4.11.

Table 4.11 Variables, Ranges, Controls, and Parameter Classification

Parameter	Range Studied	Justification	Control	Classification
Protein load	10-50 g protein/L resin	Multivariate	Batch procedures, Skid control	WC-CPP
Elution buffer pH	3.2-3.9	Multivariate	Batch procedures	WC-CPP
Flow rate	100-300 cm/hr	Multivariate	Skid control	KPP
End collection	2.0-3.2 CV	Multivariate	Skid control	KPP
Temperature	15-30°C	Multivariate	Environmental control	GPP
Cell culture viability	10-90%	Multivariate	Batch record procedure	GPP
Resin lifetime (Resin A)	< 250 cycles	Univariate	Column use log	GPP
Load concentration	2.5-6 g/L	Univariate	Titer analysis	GPP
Bed height	10-30	Modular	Column use log	GPP
Eq/Wash pH	6.6-7.6	Modular	Batch record procedure	GPP
Eq/Wash composition	60-140% of target	Modular	Batch record procedure	GPP
Start collection parameter	0.05-1.0OD	Modular	Skid control	GPP
Eq/Wash volumes	60-70% of target	Modular	Skid control	GPP
Resin lifetime (Resin B)	200 cycles	Univariate	Column use log	GPP

4.6.1.8 Reuse/Lifetime Resin Studies

Column lifetime studies using the scale-down model for A-Mab established that the useful lifetime of the Protein A resin is expected to be at least 250 cycles. The resin lifetime study (Table 4.12) showed modest yield loss with extended use, but showed no change in HCP, aggregate or acidic variants. These data are consistent with published studies evaluating repeated use of this resin (Hahn et al 2006, Lute 2008).

Table 4.12 Protein A Resin Lifetime Study

Reuse Cycle Number	Yield (%)	HCP (ng/mg)	Acidic Variants	Aggregate %
6	97	8,100	9	2.2
20	97	7,900	10	2.4
70	97	6,500	11	2.0
130	94	9,500	11	1.9
170	94	8,800	8	2.5
204	91	8,300	9	2.1
250	90	7,900	9	2.2

4.6.1.9 Anticipated post-launch change: Different Source of Protein A Resin

The Protein A resin used for production of clinical and commercial scale lots (Resin A) is made by Vendor A. An alternate source of Protein A resin (Resin B, from Vendor B) has been qualified as an appropriate substitute based on results that demonstrate that there is no impact on product quality or significant effect on process performance.

The studies which established that process performance with Resin B is comparable to Resin A are summarized below. Resin B studies were conducted using the same scale-down model system used for Resin A, and have been shown to be representative of the process-scale performance of Resin B.

- Triplicate studies on multiple lots of clarified harvest run at midpoint of test conditions (Comparisons of removal of HCP, DNA, insulin, and ProA leaching)
- Leached Protein A removal by downstream polishing chromatography steps
- Multivariate study (similar to Section 4) establishing the new design space (with subtle changes to elution pH, protein load, etc.) and impact to the CEX design space with regards to HCP clearance.
- A full resin reuse study at lab-scale to support the claimed lifetime (250 cycles)
- In-process hold study of lab-scale product pools generated from Resin B

The resins are based on different matrices and Protein A ligands which have slight sequence differences. The design space for Resin B is therefore slightly different than for Resin A, as would be expected for resins having different properties such as pressure-flow hydraulics, dynamic binding capacities, resin reuse lifetimes, cleaning and sanitization solutions, and potentially the compatibility with column material of construction. A risk assessment was conducted to summarize the impact of

these differences, as well as the subtle differences in the performance of the two chromatographic resins.

In most cases, the levels of host-cell or media-derived impurities were similar or better for Resin B than for Resin A. For leached Protein A levels, however, for Resin B had modestly higher levels of leached Protein A (up to two-fold higher), yet subsequent processing steps removed the leached Protein A to comparable levels using an appropriate qualified assay, indistinguishable from material produced by Resin A. A multivariate study similar to that conducted on Resin A was conducted, with variables and process ranges established by a separate risk assessment. The Protein A product pool stability and resin reuse studies were also repeated for Resin B, to ensure that there were no changes in the mechanism or rate of product degradation upon substitution of the second resin. Because there are no claims for virus removal by this chromatography step, there are no data generated on virus clearance by Resin B. The previous modular claims were assessed for applicability, and additional studies were performed if warranted.

In the future, new Protein A resins (Resin C, Resin D, etc.) will become available which may be suitable for use in the commercial process based on a similar set of lab-scale studies to those described above.

4.6.2 Step 6: Low pH Viral Inactivation

Design space for the low pH viral inactivation step is based on modular viral clearance claims based on prior knowledge and A-Mab protein stability data.

The pH inactivation unit operation bridges the Protein A and cation exchange chromatography steps. The Protein A chromatography step provides a consistent product pool containing ≤20 g A-Mab/L, well within the design space maximum concentration of 35 g A-Mab/L. The pH inactivation operation consistently provides a process stream at pH 5.0 ± 0.2 to the cation exchange chromatography unit operation. The low pH step is linked to the Protein A and cation exchange steps as follows:

Table 4.13 Low pH Viral Inactivation Step Linkages

Input: Eluate from Protein A Affinity Chromatography	Output to Cation Exchange Chromatography
Protein concentration ≤ 20 g/L	Protein concentration ≤ 20 g/L
pH > 4.0	pH = 5.0 ± 0.2
Aggregate < 3.1%	Aggregate < 3.1%
Acidic variants ~ 10%	Acidic variants ~ 10%
HCP typically 7200 ng/mg	Same as input

Note: Precipitation of HCP often occurs during low pH inactivation and is removed during subsequent depth filtration. However, the clearance is not predictable and for the purposes of this case study HCP clearance will not be claimed in this step. Therefore, the HCP output level of the Protein A chromatography step will be assumed to carry through low pH inactivation to serve as the input for cation exchange chromatography.

4.6.2.1 Step Description

The purpose of the low pH inactivation step is to inactivate adventitious enveloped viruses that may be present in the Protein A chromatography product pool. After completion of the final Protein A cycle, the pools are combined and the pH is adjusted to 3.5 with acetic acid and held at ambient temperature for 30 to 60 minutes. The pH is then adjusted to 5.0, filtered and held for further processing by Cation exchange chromatography.

4.6.2.2 Prior Knowledge

Low pH viral inactivation step has been used extensively to manufacture three previous licensed antibodies (X-Mab, Y-Mab and Z-Mab) as well as many other therapeutic proteins. Moreover, the low pH step process conditions have remained essentially unchanged for these products and throughout the A-Mab development process. Thus, experience gained from the characterization of low pH inactivation studies constitutes prior product knowledge and may be applied directly to the A-Mab process. Because this is not a purification step, worst-case conditions have been identified to assess the stability of the antibody during the inactivation process. These worst case operating conditions involve holding the antibody at a higher concentration, lower pH, longer time and higher temperature than routinely specified in manufacturing. Following the worst-case low pH treatment, the product was tested for aggregation by SE-HPLC and for change in the acidic variant distribution by WCX-HPLC.

4.6.2.3 Scale-Down Model

Scale-down models have been used to characterize the process performance of the low pH inactivation step. Table 4.14 compares the scale factors, yield, aggregate and charge variants of the low pH inactivation product in the lab scale experiments and at large scale manufacturing scales. In order to qualify the model and to ensure proper performance at full scale, mixing studies were executed across all scales to ensure efficient mixing within the established time limits. The data indicate that the process is consistent and comparable across all manufacturing scales and that the laboratory model is representative of full scale manufacturing operations.

Table 4.14 Comparison of Low pH Inactivation Performance at Various Scales

Process	Low pH step Scale	Scale Factor	Mab (g/L)	Yield (%)	Aggregate (%)	Acidic Species (%)
Scale-Down Model (n=5)	30 mL	1	24 ± 2	95 ± 2	2.6 ± 0.1	10.3 ± 1.2
Tox (n=2) [a]	50 L	1,667	20 ± 3	92 ± 3	2.7 ± 0.3	10.5 ± 1.0
Phase 1 and 2 (n=3) [b]	93 L	3100	25 ± 2	94 ± 3	2.5 ± 0.2	11.1 ± 1.5
Phase 3 (n=5) [c]	900 L	30,000	24 ± 2	96 ± 2	2.5 ± 0.1	10.7 ± 0.8
Commercial (n=2) [d]	2700 L	90,000	24 ± 1	95 ± 2	2.5 ± 0.2	10.8 ± 1.2

[a] 2.5 g/L process, 500 L clarified harvest, 81% Protein A chromatography yield.

[b] Same as Tox process, 1000 L clarified harvest, 84% Protein A chromatography yield.

[c] New process 4.5 g/L, 5000 L clarified harvest, 83% Protein A chromatography yield.

[d] Same as Phase 3 process, but at 15,000 L.

4.6.2.4 Risk Assessment to Define Process Characterization Studies

A risk assessment was conducted based on prior knowledge, clinical production of A-Mab as well as clinical and commercial manufacture of three other antibodies, to design the process characterization studies. Two aspects were considered for these studies: 1. Impact on product quality; and 2. Impact on viral inactivation.

4.6.2.5 Characterization Studies to Assess Impact to Product Quality

The experiments were designed to test worst case process conditions on product quality and stability by assessing impact on aggregate (SE-HPLC) and charge heterogeneity (WCX-HPLC). Table 4.15 summarizes the process parameters, normal parameter ranges, worst-case study set points and the scientific rationale for the selection of the study set points.

Table 4.15 Low pH Inactivation – Impact on Product Quality Study Design Rationale

Process Parameter	Normal Manufacturing Target or Range	Worst Case Study Conditions	Conditions used for Virus Clearance	Scientific Rationale
pH	3.5 ± 0.1	3.2	3.2 – 4.0	Lower pH is expected to result in a greater tendency of the antibody to aggregate and may also result in changes to the charge variants. The lower pH will enhance the rate inactivation. Previous univariate experiments have indicated that antibody precipitation may occur at pH 3.1 or below. Therefore pH 3.2 was chosen as the lowest pH to assure precipitation did not occur during the study. The upper limit was defined by the highest pH studied in inactivation experiments.
A-Mab concentration	≤ 20 g/L	35 g/L	35 g/L	Higher mAb concentration may lead to a greater tendency to aggregate. Previous experience with the platform process has resulted in a maximum antibody concentration in the Protein A product pool of 31 g/L. The maximum A-Mab concentration in the study was set at 35 g/L in order to assess potential aggregation and to support future process yield improvements. The higher mAb concentration may inhibit the inactivation process.
Time	60 - 120 minutes	0-240 minutes	15-180 minutes	Longer hold times are expected to result in greater aggregation and may result in changes to the charge variant profile. Previous experience with the platform process indicates that product quality may begin to deteriorate after 180 minutes at these conditions. In order to gain kinetic data on the stability of A-Mab, samples were taken at time points up to 240 minutes. The maximum hold time was set at the longest time the antibody could be held at this condition without loss of acceptable quality

Table 4.15 Low pH Inactivation – Impact on Product Quality Study Design Rationale

Process Parameter	Normal Manufacturing Target or Range	Worst Case Study Conditions	Conditions used for Virus Clearance	Scientific Rationale
Temperature	19-23°C	25°C	15-25°C	Higher temperatures may result in greater aggregation and changes to the charge variant profile. The temperature in the inactivation tank is well controlled, and 25°C, a temperature greater than the normal operating condition, was studied to assure that the antibody was stable under normal operating conditions. Temperatures below the normal operating range of 19-23 degrees, (namely 15 degrees) were also studied to assess the effect of lower temperature on inactivation kinetics.

The experiment was executed in triplicate in a 30 mL laboratory model and the results are summarized in Table 4.16. A-Mab results show that over time there was a steady decline in monomer content with a corresponding increase in aggregated species which is consistent with results obtained other antibodies under comparable process conditions. The maximum acceptable hold time based on aggregate formation is derived from the capability of the CEX step to clear aggregate. Based on the characterization studies, the data indicate that the maximum acceptable hold time is 180 minutes based on an average aggregate content of 2.5 ± 0.2% and a corresponding 3 standard deviation upper limit of 3.1%. Step linkage studies have demonstrated that the subsequent cation exchange chromatography step has the capability to reduce this aggregate level to approximately 1%.

Also, a slight but not significant increase in the acidic variants was observed over the 240 minute time course. Overall, results show that there was no significant impact on product quality when A-Mab was held up to 180 minutes at worst case scenario conditions. Thus, based on product quality considerations, no quality-linked process parameters were identified for this step.

Table 4.16 Product quality results for worst-case scenario Studies

Process Hold Time (minutes)	Aggregate (%)	Acidic Species (%)
0	1.8 ± 0.2	10.7 ± 0.8
30	1.8 ± 0.3	10.6 ± 1.0
60	2.0 ± 0.2	10.9 ± 0.9
90	2.1 ± 0.2	10.9 ± 1.2
120	2.3 ± 0.3	11.1 ± 1.3
150	2.2 ± 0.3	11.0 ± 1.1
180	2.5 ± 0.2	11.3 ± 1.0
240	2.7 ± 0.5	12.0 ± 0.9

4.6.2.6 Hold Time Study

An additional study was conducted to determine the acceptable hold time for the low pH solution after completion of the inactivation, adjustment to pH 5.0 and depth filtration. A solution at the maximum A-Mab concentration of 35 g/L was held in stainless steel containers at the maximum hold temperature of 25°C for seven days. Samples were taken periodically and assayed for aggregation and changes in acidic variants. The results are given in Table 4.17. The data demonstrate that this process intermediate can be held at 25°C for seven days without significant degradation or impact to product quality.

Table 4.17 In-process Hold Study Results

Time (days)	Aggregate (%)	Acidic Species (%)
0	1.8 ± 0.2	10.9 ± 1.2
1	1.7 ± 0.2	11.1 ± 1.3
3	1.9 ± 0.3	10.9 ± 0.9
5	1.9 ± 0.2	10.7 ± 0.8
7	2.1 ± 0.3	10.6 ± 1.0

4.6.2.7 Characterization Studies to Assess Viral Inactivation

Based on the extensive viral clearance information derived from process characterization studies with X-Mab, Y-Mab and Z-Mab, we propose that additional virus spiking studies with A-Mab are not required to demonstrate the effectiveness of the low pH inactivation step.

The characterization studies conducted with three licensed monoclonal antibodies (X-Mab, Y-Mab, and Z-Mab) have demonstrated that the low pH inactivation step has a wide design space and robust process performance. For all 3 mAb processes, virus inactivation kinetics were comparable and process performance was consistent and robust over a variety of conditions of feed-streams, buffer composition and protein loads. These results provide a high level of assurance for viral clearance in the A-Mab process when operated under the established design space conditions for the low pH inactivation step. The details of process conditions and design space for this step are described below.

As described in the introduction section, modular viral clearance data was obtained from extensive viral inactivation characterization experiments conducted with three licensed monoclonal antibodies (X-Mab, Y-Mab, and Z-Mab). These studies included experiments in which the kinetics of inactivation of XMuLV were evaluated over a range of conditions (time, temperature, pH, and protein concentration), and included worst case conditions for viral inactivation: shorter time, lower temperature, higher pH and higher protein concentration. The results of these studies for average log reduction factor (LRF) are shown in Figure 4.3, and indicate that pH conditions between 3.2 and 4.0 for 60 minutes result in viral inactivation of greater than 6.6 LRF. The kinetic profiles for the three antibodies showed slightly lower rates of inactivation at lower temperature and higher pH, but the rate was only minimally affected by protein concentration.

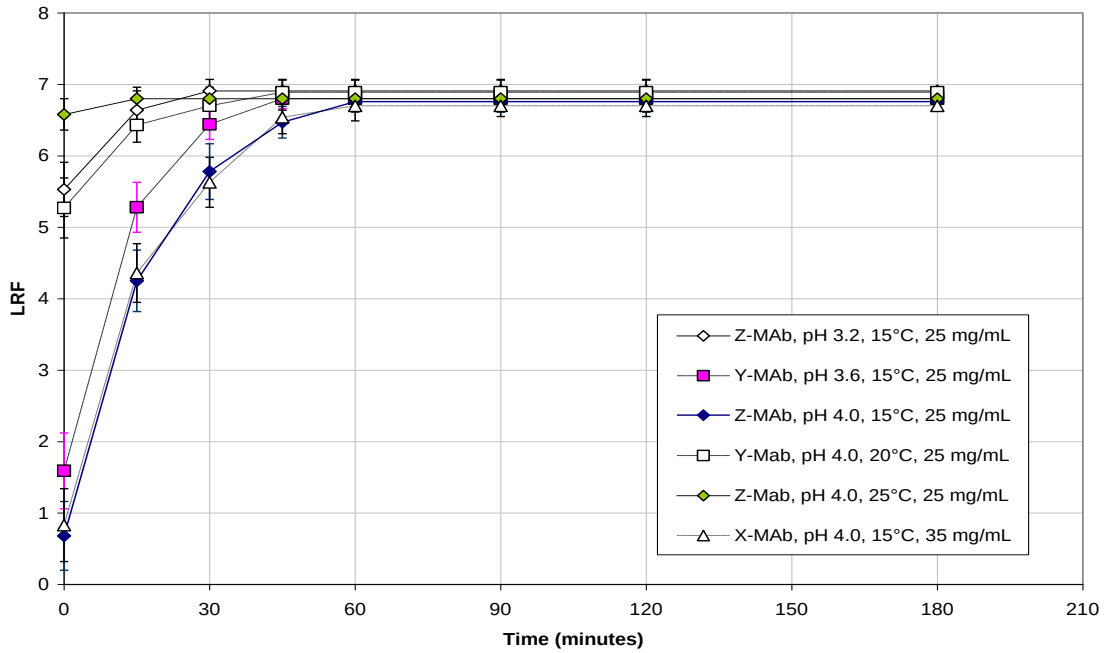

Figure 4.3 Kinetics of XMuLV Inactivation for X-Mab, Y-Mab and Z-Mab.

Note: LRF values exceeding 6.5 in Figure 4.3 represent "greater than" values because residual virus levels for those time points were below the limit of detection.

The results of these studies demonstrate that consistent and reproducible inactivation of XMuLV is achieved at operating ranges of pH 3.2-4.0, for 60-180 minutes at protein concentrations of ≤ 35 g/L. Within these ranges there were no conditions that resulted in loss of product quality. The results with previous mAb products also demonstrate that the pH inactivation step is matrix independent. The robustness of the viral inactivation results therefore support the application of a modular viral clearance approach to the establishment of the design space for the low pH virus inactivation step (Brorson 2003).

4.6.2.8 Summary of Process Parameter Classification and Ranges

Results show that both pH and time are important parameters to assure viral safety. pH was designated a critical process parameter (CPP) because the range is relatively narrow and pH values above 4.0 have not been demonstrated to effectively inactivate XMuLV within 60 minutes. Because time is readily controlled and had no adverse impact on the Quality Attributes over a broad range, it was designated a well-controlled critical process parameter (WC-CPP). Similarly, temperature is a WC-CPP because slightly lower rates of virus inactivation are observed at lower temperature, but it is readily maintained within the 15°-25° C that has been demonstrated to effectively inactivate XMuLV. On the other hand, protein concentration had little or no effect on inactivation kinetics, product aggregation or acidic variants and was therefore classified as a general process parameter (GPP). The acceptable ranges are summarized for each parameter in Table 4.18, along with the criticality classification.

Operating Parameter	Acceptable Range	Classification	Rationale	Control strategy
pH	3.2- 4.0	CPP	pH < 3.2 may lead to aggregation, pH > 4.0 not studied in clearance study	Batch record procedure
Time	60-180 min	WC-CPP	Longer times lead to aggregation, shorter times may result in incomplete inactivation	Batch record procedure
A-Mab concentration	≤ 35 g/L	GPP	No effect seen on stability or inactivation	Batch record procedure
Temperature	15-25°C	WC-PP	Lower temperatures may result in incomplete inactivation at short time and higher pH	Temperature control

Table 4.18 Acceptable Ranges and Criticality Assessment for low pH Viral Inactivation step

4.6.2.9 Design Space

The intersection of the acceptable operating ranges derived from the process characterization and viral clearance studies defines the design space for the low pH viral inactivation step and is shown graphically in Figure 4.4. Note that the design space is also constrained by the acceptable temperature range 15°-25° C.

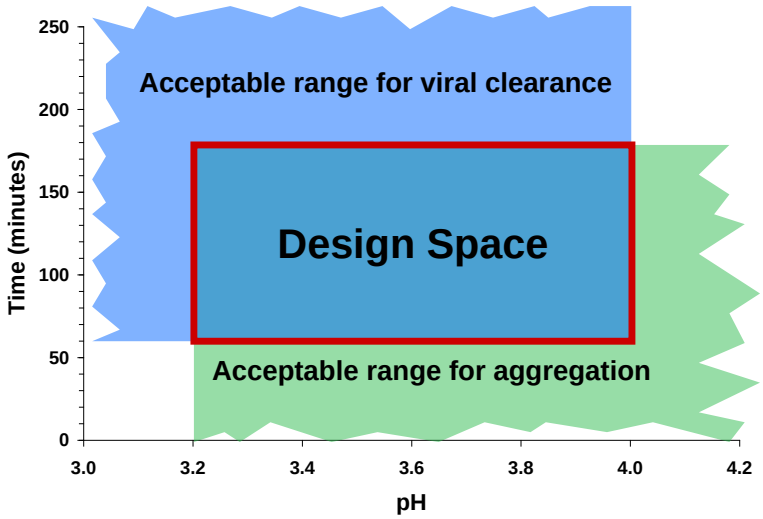

Figure 4.4 Graphical Representation of Design Space for the Low-pH viral inactivation step.

4.6.3 Step 7: Cation Exchange Chromatography

The design space for the Cation Exchange Chromatography (CEX) step is linked to the performance of the Pro A and AEX chromatography steps and is described in the form of a multivariate model in section 4.7 "Linkage of Unit Operations".

The CEX step is linked to the outputs of the Protein A /low pH inactivation and AEX steps as follows.

Table 4.19 Cation Exchange Chromatography Step Linkages

Input from Protein A/Low pH Viral Inactivation	Output to Anion Exchange Chromatography
Protein concentration ≤ 20 g/L	Protein concentration ~ 10 g/L
pH = 5.0 ± 0.2	pH = ~ 6.0
Aggregate < 3.1%	Aggregate < 0.8%
Acidic variants ~ 10%	Acidic variants ~ 10%
HCP ~ 7,200 ppm but may range from 3,000 to 12,000 ppm	~ 100 ppm but may range up to 170 ppm

4.6.3.1 Step Description

The CEX step utilizes a strong cation exchange resin operating in a bind and elute mode to capture the A-Mab. The step is operated with a step elution designed to provide separation of HCP and aggregate, while also providing clearance of DNA and leached ProA. Charge and glycosylation variants are unaffected across the step when operated within platform conditions. Although some viral clearance can be demonstrated across this step, no claims are made.

Following low pH treatment, the product pool is adjusted to pH 5.0 ± 0.2 in acetate buffer and loaded onto a pre-equilibrated 20 ± 3 cm bed height column at ambient temperature. The column is washed, and subsequently eluted by increasing pH and acetate concentration. The peak collection criteria are based on UV absorbance at 280/320 nm dual wavelength detection of the elution peak. Multi-cycle operations are allowed, in which case the column is re-equilibrated following regeneration for additional loading. After the final product cycle, the column is washed with and stored in storage buffer until the next use.

4.6.3.2 Scale-Down Model

A laboratory scale model has been developed and qualified as representative for use in characterization studies. Three runs were performed at lab scale, using feed material from development, pilot and manufacturing scales. Process performance (step yield, elution profiles, pool volume) and product quality (aggregate, HCP, and acidic species) were comparable across scales (Table 4.20). Column packing procedures have been optimized for each scale, and transitional analysis is used to assess column efficiency in between runs. Column packing/bed efficiency is well controlled and has been demonstrated to not have a significant effect on resolution across a wide

range. During pilot scale runs peak fractionation was executed and compared to lab scale fractions with very consistent results.

When the process is run at target values of controlled parameters, the quality and process performance is comparable across scales, demonstrating the linearity of process scale-up (Table 4.20) and the validity of the scale-down model.

Table 4.20 CEX Process Performance and Multiple Scales

	Scale up factor	Step yield (%)	Elution pool volume (CV)	Aggregate (%)	HCP (ng/mg)	% acidic species
Load material				1.8 ± 0.4	7000 ± 750	10 ± 2
Scale-down model (N=55)	1	90 ± 7	4.0 ± 0.4	0.7 ± 0.2	99 ± 22	9 ± 2
Pilot scale 500 L (N=2)	2000	89 ± 4	4.1 ± 0.2	0.6 ± 0.1	100 ± 30	8 ± 2
Pilot scale 5000 L (N=5)	8000	90 ± 5	4.2 ± 0.4	0.8 ± 0.2	105 ± 15	9± 2
Commercial scale 15000 L (N=2)	33,000	89 ± 5	4.2 ± 0.5	0.7 ± 0.1	90 ± 20	10 ± 2
Clearance factors				**2-3x**	**50-100x**	**0x**

4.6.3.3 Risk Assessment Used to Define Process Characterization Studies

Prior knowledge applied to the CEX step for the A-Mab process was derived from platform process experience and extensive process performance history with other mAbs. In addition, high resolution process characterization studies have been conducted for 3 previously licensed mAbs which have provided a solid scientific understanding of this step.

A risk assessment was performed based on this prior knowledge and results are presented in the risk matrix Table 4.21which lists the operating parameters known to have a significant impact on either process performance or drug substance quality. Attributes which are affected by more than one process parameter were included in multivariate studies to establish the impact and potential interactions of parameters.

Table 4.21 Risk Matrix for CEX Step

	Step yield (%)	Elution pool volume (CV)	Aggregate (%)	HCP (ng/mg)	% acidic species	Risk mitigation
Bed Height (cm)		X		X		Univariate
Equil/Load / Wash flow rate (cm/hr)						Not Required
Elution flow rate (cm/hr)	X	X	X	X		DOE
Load pH						Not Required
Protein load (g/Lresin)	X	X	X	X	X	DOE
Wash pH						Not Required
Load/wash conductivity (mS/cm)				X		DOE
Temperature (°C)						Not Required
Elution start collect						Not Required
Elution stop collect	X		X			DOE
Elution pH	X			X		DOE
Elution conductivity (mS/cm)	X					Not Required

4.6.3.4 Process Characterization Studies

The process parameters chosen for further characterization were based on the risk assessment described above. Platform conditions were used as a guide to select parameter set points. Small scale binding isotherms were generated for A-Mab to fine-tune the model utilized for the previous mAbs and identify parameter ranges for the characterization studies. The parameters and ranges evaluated in the DOEs are summarized in Table 4.22

Table 4.22 Process Parameters and Ranges evaluated in DOEs for CEX

Parameter	Low	Mid	High
Protein load (g/L resin)	10	25	40
Elution flow rate (cm/hr)	100	200	300
Elution stop collect (OD)	0.5	1.0	1.5
Elution buffer pH	5.8	6.0	6.2
Wash conductivity (mS/cm)	3.0	5.0	7.0
Load HCP (ng/mg)	3000	7500	12000
Aggregate	2.4	2.7	3.0

Resin lot-to-lot variability was assessed, and selected lots with low and high ionic capacity were evaluated to establish impact on binding. Load material was obtained from three 5000 L scale runs to cover all development studies. Also, worst case load material was obtained from the Protein A step, which ranged from 3000 to 12000 ppm of host cell proteins. Worst case concentrations of aggregate were generated by extended hold at low pH.

Product quality outputs of the process characterization experiments were HCP, aggregate, and charge variants. DNA and Protein A levels were also monitored throughout the process characterization studies.

In the multifactor study, elution stop collect, elution flow rate, load/wash conductivity, elution pH and protein load were the five controlled factors selected for a two level half fractional factorial experiment. To evaluate the effects of different starting levels of both HCP and aggregate, three separate lots of load material, each containing low, mid, and high levels, were treated as a separate factors (Table 4.22). A 2^{7-2} fractional factorial design was executed. The center point data were collected from ten runs at target conditions which spanned at least three lots of load material variability. Additionally, six runs were included to estimate the quadratic effects, if present, due to Protein Load, Elution Stop Collect, and/or Elution pH. A total of 48 runs was executed in this study. Low bed height and high temperature were evaluated in additional univariate studies

A statistical analysis was performed to assess the effects of the process parameters on each CQA. Statistically significant effects were detected and a predictive model developed for step yield, aggregate, and HCP. The multivariate experimental design (DoE) revealed the following parameter impacts on step performance and product quality:

- HCP levels were impacted by protein load, wash conductivity, and HCP levels in the input feed-stream. A significant interaction between protein load and wash conductivity was identified. Since the AEX step which follows CEX also clears HCP, further discussion of HCP and linkage with other steps is presented in the linkage section (4.7)
- Aggregate levels were impacted by protein load, elution stop collect, elution pH, and aggregate levels in the input feed-stream. A significant interaction between protein load and elution pH was identified as well as significant curvature due to elution stop collect and elution pH.
- Acidic species were slightly impacted by protein load. However, a model was not developed since acidic species (e.g. deamidation) are not considered critical and thus will not be used to define the design space.
- A statistical model was also developed for step yield as a function of protein load, Elution Stop Collect, and Load/Wash Conductivity, Elution Flow Rate and Elution pH. HCP Input and Aggregate Input were shown to have a slight effect on yield.
- The multivariate experiments also confirmed that DNA and Protein A levels are largely insensitive to the CEX process step.

The prediction profile displayed in Figure 4.5 shows the relative effects of each process or input parameter on yield, aggregate output, and HCP output. The red dashed vertical line that indicates the process operating conditions and the predicted values of the CQA on the Y- axis.

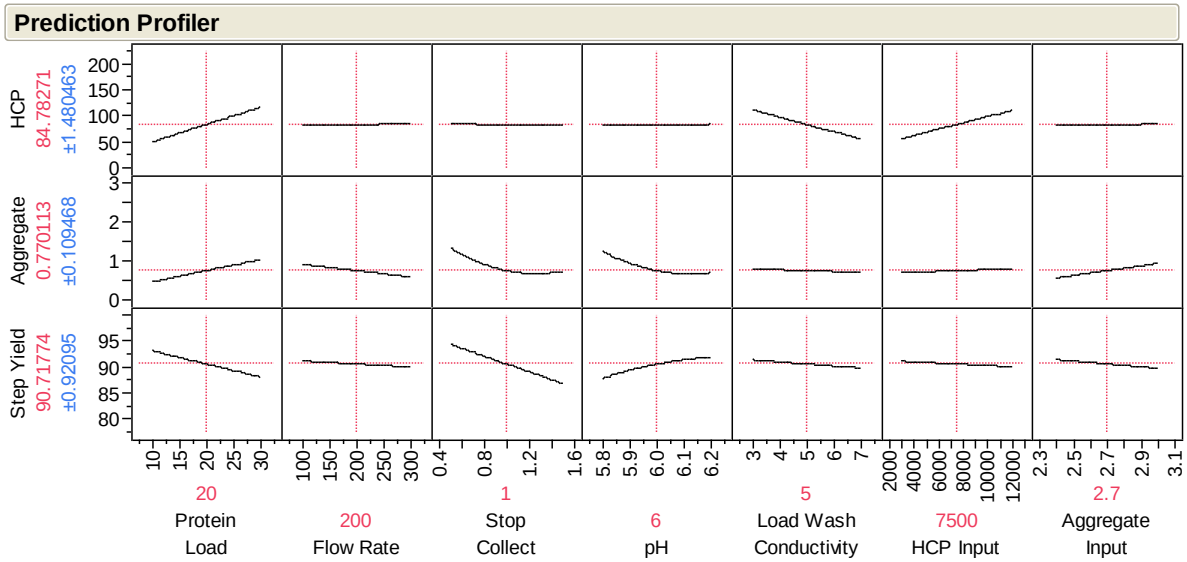

Figure 4.5 Process Characterization (DOE) Results for CEX Step: Prediction Profile based on Statistical Models

In addition, the univariate studies of bed height and temperature showed no significant impact on performance or product quality across the range tested (data not shown). A series of worst case runs was executed using operating conditions predicted to represent worst case for both product quality and yield; center-point conditions were used as controls. The outputs from these runs were subsequently used as input material for AEX characterization studies. Results from the CEX worst case studies confirmed model predictions and showed acceptable performance in all cases.

Since there are no further reduction/clearance steps for aggregate downstream of the CEX step, it is important to demonstrate that this step consistently reduces aggregate to acceptable levels for drug substance. For this purpose, the worst case conditions for aggregate levels were evaluated and models developed. Results show that even under the worst-case for load conditions the CEX step results in consistent aggregate reduction to levels below specifications. Based on linkage to the low pH inactivation step which is tightly controlled to ensure that aggregate levels do not exceed 3.1%, the CEX process will result in robust and acceptable aggregate reduction over all the process conditions evaluated in the study. Therefore, aggregate removal does not constrain the design space of the CEX step.

4.6.3.5 Summary of Process Parameter Classification and Ranges

Table 4.23 summarizes the parameters assessed for the CEX column chromatography step, with their respective classifications, rationale, and control strategy.

Table 4.23 Summary of Process Parameter Classification and Ranges for CEX Step

	Range Studied	Classification	Rationale	Control strategy
Protein load (g/Lresin)	10-30	WC-CPP	Higher load challenges will decrease resolution and increase levels of aggregate and HCP	Batch procedures, skid pump control
Load/wash conductivity (mS/cm)	3-7	WC-CPP	Decrease in load/wash conductivity decreases ability to clear HCP, but is well controlled and has downstream robustness	Batch procedures
Elution pH	6.0 ± 0.2	WC-CPP	Elution pH affects both yield and distribution of impurities, but is well controlled.	Batch procedures
Elution stop collect	1.0 ± 0.5 OD descending	WC-CPP	Extended stop collect leads to minor increase in aggregate levels in pool, but is well controlled	Skid control
Elution flow rate (cm/hr)	200 ± 100	GPP	High flow rate affects peak shape with minor impact on aggregate, and is well controlled	Skid control
Bed Height (cm)	20 ± 3	GPP	Bed height variation across range has minimal effect on residence time	Batch procedures
Equil/Load / Wash flow rate (cm/hr)	200 ± 100	GPP	Flow rate has previously been shown to have no significant impact across these steps	Skid control
Load pH	5.0 ± 0.3	GPP	Load pH has previously been shown to have no significant effect on performance	Batch procedures
Wash pH	5.0 ± 0.3	GPP	No significant impact across range, which is tightly controlled	Batch procedures
Temperature (°C)	15-30 °C	GPP	Temperature does not have a significant impact on process performance	Facility HVAC control
Elution start collect	0.4 ± 0.2 OD ascending	GPP	Acidic charge variants elute at front of peak, early start has small increase in this non-CQA	Skid control
Elution conductivity (mS/cm)	8.5 ± 2.0	GPP	Elution conductivity across range has no significant impact	Batch procedures

4.6.3.6 Reuse/Lifetime Study

The CEX lifetime study was not performed for A-Mab. Previous experience and process data generated with similar mAb products supports CEX resin re-use and demonstrates the effectiveness of the cleaning conditions used in the platform process. This approach is justified because the purity of the feed-stream after Protein A chromatography is sufficiently high and consistent that no significant differences are expected in the CEX process performance with different mAbs.

Small-scale lifetime studies with previous mAbs, with similar product quality profiles and comparable load pool characteristics, demonstrated that there are no changes in dynamic binding capacity up to 200 cycles. Results showed that there was no impact on process performance or impurity clearance over time, and no significant protein carryover in blank elutions. These results are consistent with experience that the product pools following Protein A chromatography are sufficiently pure such that fouling of the CEX resin over the course of lifetime has not been observed. Extended contact with cleaning/storage solutions has been shown to have no effect on ligand integrity. As there are no viral clearance claims for the AEX step, we propose that there is

no need to generate end-of-lifetime resin evaluation for A-Mab. In addition, commercial-scale verification of lifetime limits will not be executed unless they exceed the limits established based on previous mAb experience. Transitional analysis will continue to be used to assess column efficiency between runs at commercial scale.

4.6.3.7 Continued Process Monitoring

In order to verify the design space model using full scale data, multivariate analysis will be performed on an on-going basis throughout the lifecycle of the product.

Multivariate analysis will include process performance information such as process parameter values, buffer information, on-line data and product quality results. Results will be stored in a database and will support troubleshooting as well as process improvement activities. In addition, HETP and asymmetry will be controlled to consistent levels and monitored via transitional analysis to trigger column repacking when necessary.

4.6.4 Step 8: Anion Exchange Chromatography

The design space for the Anion Exchange Chromatography (AEX) step is linked to the performance of the Pro A and CEX chromatography steps and is described in the form of a multivariate model in section: "Linkage of Unit Operations". This section describes the use of prior knowledge to design A-Mab process characterization studies and support a modular approach to viral clearance.

The AEX step is linked to cation exchange and nanofiltration steps as follows.

Table 4.24 Anion Exchange Chromatography Step Linkages

Input from Cation Exchange Chromatography	Output to Small Virus Retentive Filtration
Protein concentration ~ 10 g/L	Protein concentration 7 - 8 g/L
pH ~6.0	pH 7.4-7.6
Aggregate < 0.8%	Aggregate < 0.8%
Acidic variants ~10%	Acidic variants ~ 10%
HCP ~ 100 ng/mg	HCP <12 ng/mg

4.6.4.1 Step Description

AEX chromatography is the final purification step in the A-Mab downstream process. It is operated in the flow-through mode binding impurities such as HCP, DNA and endotoxins to the resin (or membrane) while the antibody passes through. A viral clearance claim can be made for this step and details to support it are discussed below.

The column is packed with AEX resin to a height of approximately 20 cm. Prior to loading, the CEX product pool is adjusted to the appropriate pH and conductivity. Following equilibration and loading, the column is washed with equilibration buffer to collect the A-Mab product based on A_{280}. The entire mAb batch is typically processed in one cycle; however multiple cycles are acceptable where the AEX product pools are combined for subsequent processing. If multiple cycles are

required, the column is regenerated and re-equilibrated prior to subsequent cycles. After the final cycle, the column is regenerated, cleaned and stored.

4.6.4.2 Scale-Down Model

A scale down model for the AEX step was established following standard scale-down/up considerations for chromatography: the column size was scaled based on column diameter, with constant bed height, linear velocity, protein load and load volume/column volume ratio across the scales. This scale-up approach ensures that residence time and mass transport are constant across scales. Volumes of the equilibration, wash and other buffers are based on column volume thereby ensuring the same amounts are used proportionally at laboratory and production scales. A summary of the scale-up parameters is presented in Table 4.25.

Table 4.25 Scale-up parameters for AEX Chromatography Step

Column parameters	Laboratory scale	Pilot scale (5K L)	Manufacturing scale (15K L)
Bed volume (L)	0.015	72	509
Bed height (cm)	19-20	20 cm	20
Diameter (cm)	1.0	63 cm	180
Linear flow rate (cm/hr)	150-300	300 cm/hr	300
Loading volume (L)	0.057 – 0.060	189 – 282 L	1140-1666
Protein concentration (gm of mAb/L of resin)	50-200	~200	~200
Scale-up factor	1	4812	34140

Results show that the laboratory-scale AEX chromatography step performance is comparable to the full scale manufacturing (15000 L) scale process including the quality attributes of the AEX product (Table 4.26.) The residence time of the product on the columns and the elution profiles were comparable in both the laboratory and full scale production processes. Furthermore, by visual inspection the chromatograms were consistent and comparable for the individual small scale purification runs.

Table 4.26 Process Performance for the AEX chromatography step at different scales

Product Quality Attributes	Laboratory Scale		Commercial Scale (15K L)	
	Min	Max	Min	Max
HCP (ng/mg)	5.1	15.6	5.3	15.1
Acidic variant (%)	8	11	7	13.0
Yield (%)	87	100	95	100
Aggregate(%)	0.8	1.0	0.4	1.0

4.6.4.3 Risk Assessment used to plan process characterization studies

A Cause and Effect Matrix risk assessment was performed to categorize the operating parameters into three groups: i) parameters warranting multivariate evaluation, ii) secondary parameters whose ranges could be supported by univariate studies, and iii) parameters which would not require new studies, but instead would be employ ranges based on prior knowledge or modular claims established from previous products or literature studies.

Each process parameter was assessed based on the potential impact on quality attributes or process attributes. Each quality attribute was assigned a "Weight" score based on its impact to product quality or safety. The impact and weight score criteria are summarized in Table 4.27.

Table 4.27 Scoring of Process Parameters and Quality Attributes

Process Parameters		Quality Attributes	
Impact Score	**Ranking Criteria**	**Weight Score**	**Ranking Criteria**
10	Strong relationship known based on available data and experience	**10**	Established or expected direct relationship to product quality or safety (including mfg safety)
7	Strong relationship is expected	**7**	Unsure. Impact to product quality or safety or key business drivers expected
5	Not-so-strong relationship expected or unknown	**5**	Unlikely to impact product quality or safety
1	Known to not have a relationship	**1**	No product quality or safety impact expected

A cumulative score was then calculated for each parameter using **Equation 5**.

Equation 5

$$\text{Cumulative score} = \Sigma\,(\text{Impact of parameter} \times \text{Weight of quality attribute})$$

The cumulative scores in the Cause and Effect Matrix were used to identify the parameters and the experimental approach for process characterization studies. Results for an abbreviated Cause and Effect matrix for the AEX step are summarized in Table 4.28 . The parameters are ranked based on the calculated scores. Here only a selected subset of quality and process parameters is shown to exemplify the approach.

The cumulative scores represent the relative importance of the parameter for the unit operations, so parameters with high scores were considered to be high risk. Prior knowledge was used to prioritize and group parameters for multivariate experiments, for example, parameters with scores greater than 300 were studied in DOE-1.

DOE-2 studies were not carried out, as data from multivariate DOE experiments, using the same grouping of parameters and ranges performed for X-Mab, Y-Mab and Z-Mab was used to demonstrate the robustness of the AEX column packing, cleaning, regeneration procedures. Some high risk parameters were studied as a single variable (OFAT). Parameters with scores below 250 were not studied as they were considered to be low risk.

Table 4.28 Abbreviated Cause and Effect Matrix for the AEX Step

Weight	5	7	5	7	7	5	10	5	5	7	7	7	10	5		
Parameter	Fragment	Process Time	HMW (%)	Charge variant	HCP	Leach rProtein A	DNA	Equilibration final pH	Equilibration final Conductivity	Wash pH	Wash Conductivity	Column Reuse	Endotoxin	Monomer	Score	Exp't Strategy
Equilibration/Wash Buffer Conductivity	1	1	1		5	5	10	1	10	1	10	10	9	1	481	DOE-1
Equilibration/Wash Buffer pH	1	1	1	5	5	5	10	10	1	10	1	10	9	1	516	DOE-1
Q Load pH	1	1	1	5	5	5	10	1	1	1	1	10	9	1	408	DOE-1
Load Capacity	1	10	1	1	5	5	10	1	1	1	1	5	10	1	418	DOE-1
Peak Collection Start	1	5	1	1	5	5	5	1	1	1	1	1	5	9	295	DOE-1
Peak Collection End	1	5	1	1	5	5	5	1	1	1	1	1	5	9	295	DOE-1
Operating flow rate	1	10	1	1	5	5	9	1	1	1	1	1	9	1	370	DOE-1
Q Load Conductivity	1	1	1	1	5	5	10	1	1	1	1	1	10	1	327	DOE-1
Resin lot variability	1	1	1	1	5	5	9	1	1	1	1	5	9	1	335	OFAT
final pH setpoint	9	1	9	5	1	1	1	1	1	1	1	1	1	5	290	DOE-2
Bed Height	1	10	1	1	5	5	9	1	1	1	1	1	5	1	330	DOE-2
Integrity - Asymmetry	1	1	1	1	5	5	9	1	1	1	1	1	5	1	267	DOE-2
Integrity - HETP	1	1	1	1	5	5	9	1	1	1	1	1	5	1	267	DOE-2
Column Reuse #	1	1	1	1	5	5	9	1	1	1	1	1	5	1	267	DOE-2
Regeneration & Clean Flow Rate	1	1	1	1	5	5	9	1	1	1	1	1	5	1	267	DOE-2
Regeneration CV	1	1	1	1	5	5	9	1	1	1	1	1	5	1	267	DOE-2
Cleaning CV	1	1	1	1	5	5	9	1	1	1	1	1	5	1	267	DOE-2
Hold at pH 7.5	5	10	9	10	1	1	1	1	1	1	1	1	1	1	285	OFAT
Equilibration CV	1	10	1	1	1	1	1	10	10	1	1	1	1	1	252	DOE-2
Column storage solution/condition	1	1	1	1	1	1	1	1	1	1	1	10	9	1	242	
pH Adj - Acetic Acid addition rate	9	10	9	1	1	1	1	1	1	1	1	1	1	1	242	
pH Adj - Vessel Mixing RPM	9	10	9	1	1	1	1	1	1	1	1	1	1	1	242	
Hold Temperature at pH 7.5	5	1	9	10	1	1	1	1	1	1	1	1	1	1	222	
pH Adj - Process Temperature	9	1	9	5	1	1	1	1	1	1	1	1	1	1	207	
Q Load Volume	1	10	1	1	1	1	1	1	1	1	1	1	1	1	162	
Q Load mAb conc.	1	1	1	1	1	1	1	1	1	1	1	1	1	10	144	
Equilibration Buffer Concentration	1	1	1	1	1	1	1	1	1	1	1	1	1	1	99	

4.6.4.4 Process Characterization Studies for Purification using AEX Chromatography Resin

Following the risk assessment and parameter ranking, the AEX chromatography process parameters that were deemed most likely to affect product quality were chosen for further studies. These parameters are identified in Table 4.28 and were grouped into 2 DOE studies. The DOE-1 study was a fractional factorial design consisting of nineteen experiments in total with sixteen variable experimental runs and three center point, standard condition, runs (Table 4.29). One lot of feed material was used for these studies, with HCP content of 170ng/mg (the highest level of HCP observed in the CEX eluate).

Table 4.29 AEX DOE-1 Experimental Design

Parameter	High	Middle	Low
Eq/ wash 1 Buffer Cond (mS/cm)	5.6	3.6	1.6
Eq / wash 1 Buffer pH	7.8	7.5	7.2
Q Load pH	7.8	7.5	7.2
Protein Load (mg/mL)	293	164.4	46.3
Peak Collection start (OD)	2.0	1.0	0.1
Peak Collection end (OD)	2.0	1.0	0.1
Operating Flow rate (cm/hr)	450	300	150
Q Load Conductivity (mS/cm)	8.0	5.5	3.0

The process characterization studies were designed around the target and process control ranges used for clinical manufacturing at the 5K L scale. The ranges were expanded to 2 or 3X of the routine control ranges to assess process performance and impact on CQAs over a wider range and determine process robustness. The wider ranges also provided process understanding to support future potential process improvements and movement within the design space. Quality attributes considered were HCP, aggregate levels, and acidic species. Yield was also evaluated as a measure of process performance.

DOE-1 results showed that all product quality and process attributes were within acceptable limits for all process parameter ranges and combinations tested. Specifically:

- HCP levels were impacted by Equilibration/Wash 1 buffer conductivity and AEX load pH (Figure 4.6),
- Aggregate levels were slightly impacted by Equilibrium/Wash 1 conductivity and peak collection end. A model was constructed, data not shown here.
- Acidic species were slightly impacted by the load pH.. However, a model was not developed since acidic species (e.g. deamidation) are not considered critical and thus will not be used to define the design space.
- A statistical model was also developed for step yield as a function of Equilibration/Wash 1 conductivity and peak collection end.

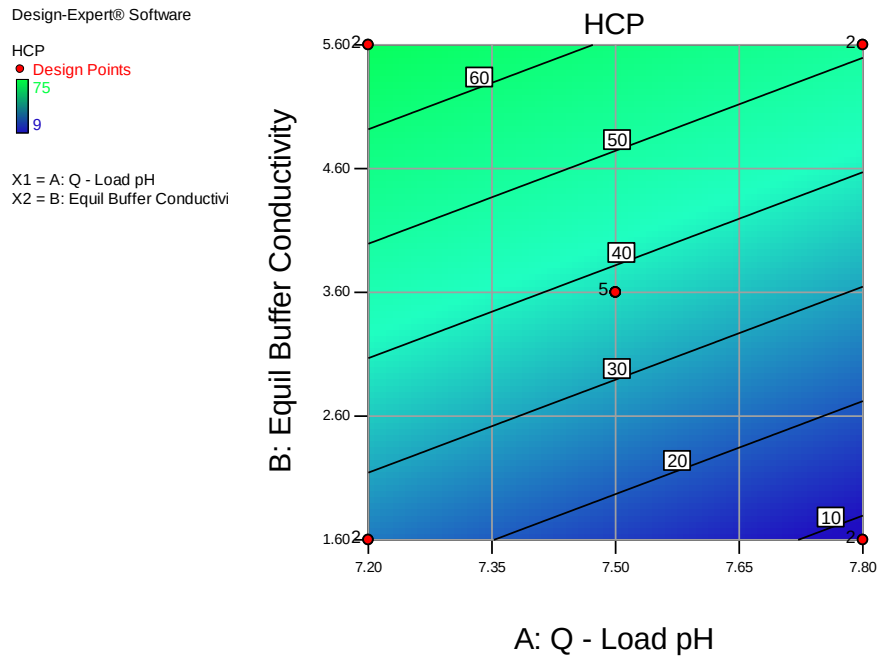

Figure 4.6 Effect of Equilibration/Wash 1 Buffer conductivity, AEX Load pH on HCP removal for input HCP of approximately 170 ng/mg

4.6.4.5 Process Characterization Studies for Viral Removal using AEX Chromatography Resin and AEX Membranes

The data presented in the AEX viral clearance section represent combined results for A-Mab and three other monoclonal antibodies for various process platform approaches. These include the generic platform process (similar steps such as AEX resin or AEX membrane, same sequence, run under the similar conditions) or a modular platform (similar steps, different sequence, run under similar conditions). AEX membranes are included in these studies to support a potential future change from resin to membrane configuration. This proposed change is justified because the mechanism of impurity removal and viral clearance is similar for both AEX configurations and both are operated in the flow-through mode.

The scale-down model for viral clearance studies was shown to be representative of the large scale production process based on operational parameters and performance. Virus-spiking experiments were carried out in duplicate. In each experiment, the load and elution fractions were analyzed for virus titer. Only the lower log reduction factor (LRF) from the duplicate experiments was used in the data calculations for overall process capability and the design space analysis.

A multifactorial DOE approach is not feasible for viral clearance studies due to the practical limitations in the number of individual virus-spiking experiments which can be run. Instead, viral clearance studies focused primarily on the main parameters (pH and conductivity) expected to impact viral clearance in AEX flow-through chromatography. These parameters were identified based on ion exchange adsorption mechanisms and prior product knowledge. In the studies, the conductivity and pH of the equilibration and wash buffers were kept the same as those of the load to simplify study design. Also, prior experience with multiple antibody products and platforms (data

not shown) has demonstrated that the effects of equilibration/wash buffer pH and conductivity are similar in nature and minor compared to the dominant effects of load pH and conductivity.

For the other operational parameters which were deemed most likely to affect product quality and process performance, the viral clearance studies were carefully designed to represent a worst-case conditions for virus clearance, based on scientific principles and prior product knowledge. As such, flow rates and protein loads used for viral clearance studies were higher than the maximum values used in the process characterization described above. Column bed height and residence time were set at the minimum operating values. Worst-case peak collection start and end (UV) criteria were used in all experiments (lowest UV for start and end to maximize inclusion of early and late-eluting virus).

The data in Figure 4.7 demonstrate the dependence of virus removal on the pH and conductivity of the anion exchange load, as well as the interactions between these parameters in the observed effects. The data also shows that there is little dependence of virus removal on the buffering salt system. Although the extent of the effects may differ slightly, viral clearance decreases as pH decreases and conductivity increases.

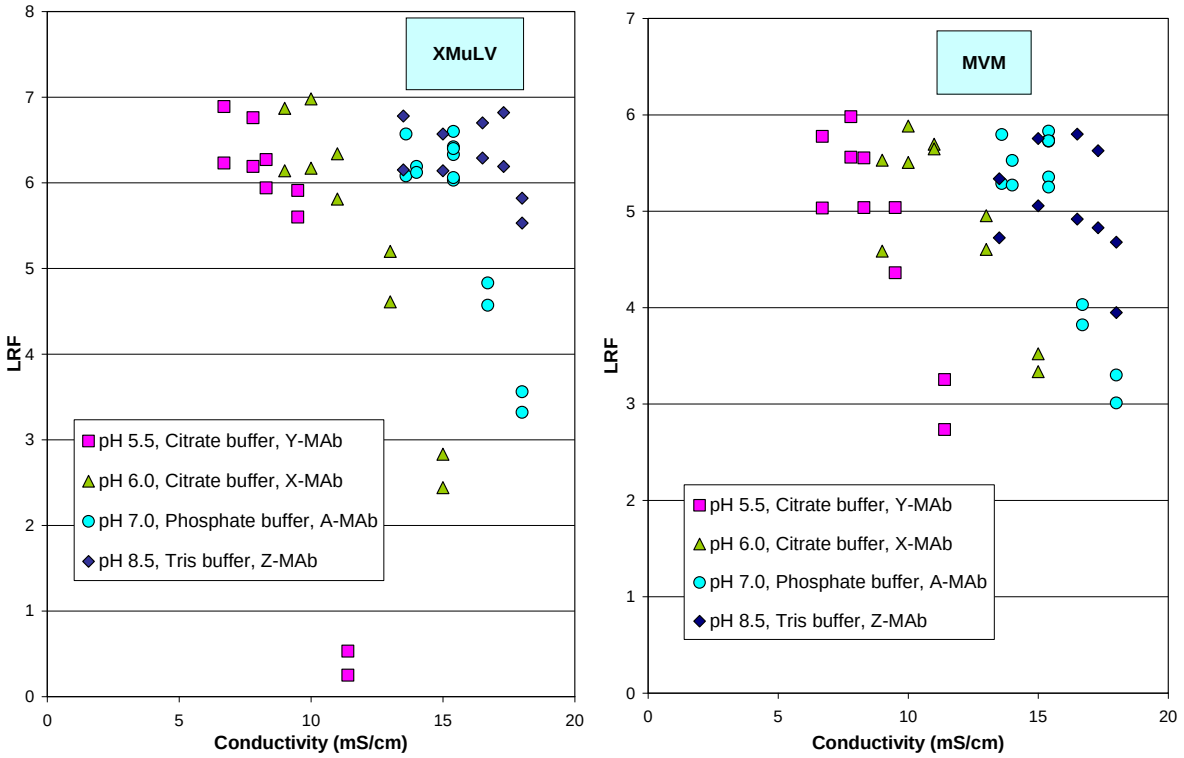

Figure 4.7 Effects of pH and Conductivity on Clearance of XMuLV and MVM in Anion Exchange Chromatography

Note: These are combined results for A-Mab and three other monoclonal antibody products

The results of the studies presented in Figure 4.7 and the interactions of the effects of pH and conductivity are summarized in the pH-conductivity design space diagram of Figure 4.8. Virus removal is maximized in the green shaded portions of the diagram, compared to the black shaded portion (at the extremes of low pH and high conductivity), where little if any removal of virus is

observed. Provided that pH and conductivity values are maintained in the green shaded area, LRF values of ≥ 5.5 for XMuLV and ≥ 4.0 for MVM are consistently achieved.

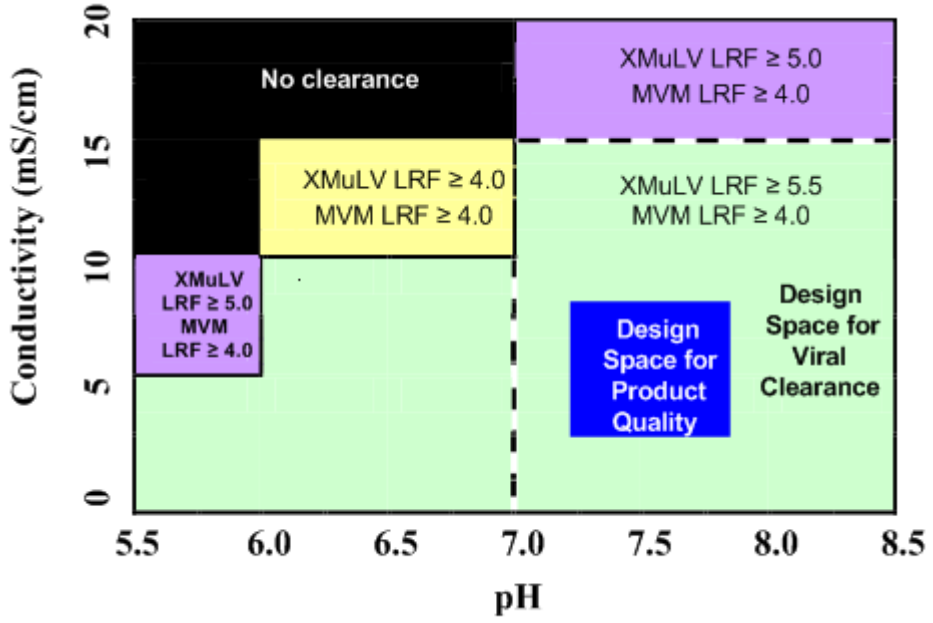

Figure 4.8 pH-Conductivity Design Space Diagram for Clearance of XMuLV and MVM in Anion Exchange Chromatography

Note: These are combined results for A-Mab and three other monoclonal antibody products. Design space for product quality as shown in the diagram is a representative simplified example; the actual pH range for this design space is dependent on the other chromatography steps as described in Section 4.7 on linkages between steps

The effect of protein concentration in the load was evaluated further in the viral clearance studies although it was not expected to have a significant impact on virus removal. This was done to verify the general modular applicability of the viral clearance design space across a range of purification process platforms. These studies were limited to a portion of the pH-conductivity design space defined by the dashed lines in Figure 4.8. To represent a worst case of low pH and high conductivity within this zone, constant values of pH 7.0 and 15 mS/cm conductivity were used for these additional viral clearance studies. Figure 4.9 shows that there is little or no dependence of virus removal on protein concentration, and all LRF values were within the ranges predicted by the design space diagram in Figure 4.8.

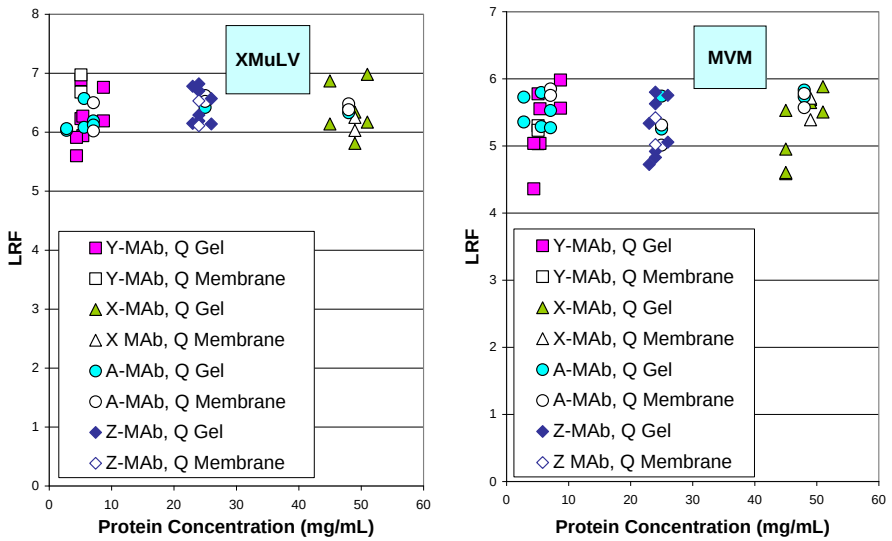

Figure 4.9 Effects of Protein Concentration on Clearance of XMuLV and MVM in AEX Chromatography at pH 7.0 and Conductivity 15mS/cm

Note: These are combined results for A-Mab and three other monoclonal antibody products using both AEX chromatography and AEX membrane.

The effectiveness of the AEX Membrane for the anion exchange chromatography step was assessed as a potential alternative to the current anion exchange AEX resin. Viral clearance studies were included as part of this evaluation, as shown in Figure 4.9, over a range of protein concentrations. For these studies, the load was pre-adjusted to pH 7.0 and 15 mS/cm conductivity to represent the worst case within the zone defined by the dashed lines in Figure 4.8. Protein loads on the AEX Membrane uniformly exceeded 10 gram/ml in these studies, providing a worst case value for the maximum AEX Membrane load in the manufacturing process. Figure 4.9 shows that virus removal on AEX Membrane is equivalent to that on AEX resin, and all LRF values were within the ranges predicted by the design space diagram in Figure 4.8.

Based on the results from the AEX characterization studies and prior knowledge, parameters linked to virus removal were identified and classified as WC-CPPs. The multivariate combination of WC-CPPs ranges that provide assurance of a LRF of ≥ 5.5 for XMuLV and ≥ 4.0 for MVM defines the acceptable operating space for viral clearance. It encompasses the parameters and ranges described in Table 4.30 which have been demonstrated to provide assurance of product quality.

Results from anion exchange studies with multiple antibodies purified via generic template process (similar steps such as both AEX resin and AEX membrane, same sequence, run under similar conditions) or purified via a modular process (similar steps, different sequence, run under similar conditions) show that the LRF values were similar. A generic and modular viral clearance design space based on these results is proposed.

Table 4.30 Summary of Process Parameter Classification and Ranges for Generic and Modular Viral Clearance in AEX step

Variable	Viral Clearance Acceptable Range	Classification (for viral clearance)
AEX load pH	≥ 7.0	WC-CPP
Protein Load (AEX Resin)	≤ 300 mg protein/mL resin	WC-CPP
Protein Load (AEX Membrane)	≤ 10 g protein/mL membrane	WC-CPP
Operating flow rate	≤ 450 cm/hr	WC-CPP
AEX load conductivity	≤ 15 mS/cm	WC-CPP
Equilibration / Wash 1 buffer conductivity	≤ 15 mS/cm	GPP
Equilibration / Wash 1 buffer pH	≥ 7.0	GPP
Peak collection start	≥ 0.1 OD (UV)	GPP
Peak collection end	≥ 0.1 OD (UV)	GPP
Load buffering salt system	Citrate, Phosphate, or Tris	GPP
Protein concentration in load	≤ 50 mg/mL	GPP
Claimed LRF values	≥ 5.5 LRF for XMuLV; ≥ 4.0 LRF for MVM	

4.6.4.6 Summary of Parameter Classifications and Ranges

A combination of multivariate, univariate and modular process characterization studies have generated a design space for the operation of the anion exchange chromatography step, as well as an approach to generating data to establish a design space for the AEX membrane as an alternative to the AEX resin. Based on a final risk assessment, the parameters, ranges, controls, and classification were defined and are summarized in Table 4.31. The viral clearance operating ranges (discussed above) are included in the table for comparison and are much broader than the operating ranges for quality.

Table 4.31 Summary of Design Space for AEX Step

Process Parameter	Acceptable Range for AEX Step	Acceptable Ranges for Viral Clearance [a]	Parameter Classification	Rationale	Control Strategy
Equilibration/Wash buffer conductivity (mS/cm)	1.6-3.6	≤ 15	WC-CPP	Multivariate Study	Batch record procedure
AEX load pH	7.2-7.8	≥ 7.0	WC-CPP	Multivariate Study, Modular Viral Clearance	Batch record procedure
Protein Load (g/L resin)	50-300	≤ 300	WC-CPP	Modular Viral Clearance Study	Batch record procedure
Operating flow rate (cm/hr)	≤ 450	≤ 450	WC-CPP	Modular Viral Clearance Study	Skid control
AEX load conductivity (mS/cm)	3.0-8.0	≤ 15	WC-CPP	Modular Viral Clearance Study [a]	Batch record procedure
Equilibration/Wash 1 buffer pH	7.2-7.8	≥ 7.0	GPP	Multivariate Study	Batch record procedure
Peak collection start A280	0.1-2 OD ascending	≥ 0.1 OD ascending	GPP	Multivariate Study	Skid control
Peak collection end A280	0.1-2 OD descending	≥ 0.1 OD descending	GPP	Multivariate Study	Skid control

[a] The viral clearance design space represents only the range of parameter values that will be considered to provide assurance of a LRF of ≥ 5.5 for XMuLV and ≥ 4.0 for MVM. The process must be run within the narrower design space for operation of the AEX step in order to assure control of other quality attributes.

[b] Range constrained by multivariate study.

4.6.4.7 Resin Reuse and Lifetime Study

AEX reuse studies and end-of-lifetime resin evaluations for A-Mab were not performed. Previous experience and process data generated with similar mAb products supports AEX resin re-use. Viral clearance at end-of-lifetime demonstrates no correlation of viral clearance characteristics with column age in the platform process.

The results of the resin lifetime study with X-Mab, Y-Mab and Z-Mab have demonstrated consistent performance of AEX chromatography resin over a total of 150 runs for product yield, elution performance, impurity removal, product quality attributes, and regeneration efficiency. Over the course of the re-use studies the product met all quality attribute acceptance criteria.

Also, studies were conducted with fresh and used resins with these three mAbs in a range of process platforms where chromatography resins were tested in viral clearance studies at early, middle and late stages of the resin lifetime (Figure 4.10). For the viral clearance studies, the load was pre-adjusted to pH 7.0 and 15 mS/cm conductivity to represent the worst case condition within the zone defined by the red dashed lines in Figure 4.8. The error bars in Figure 4.10 represent mean ± 1

standard deviation for the data from the combined lifetime studies. The data demonstrated that there is no correlation between viral clearance characteristics with resin age on reuse up to 150 cycles.

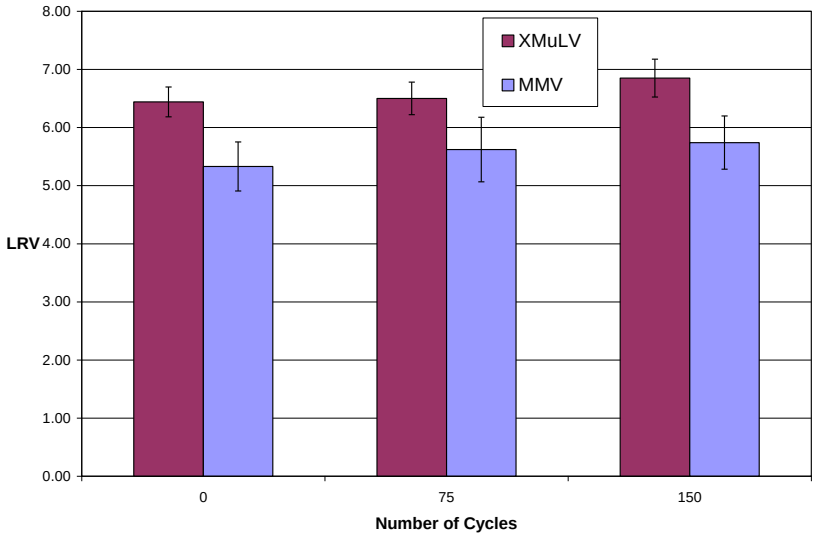

Figure 4.10 Effects of Resin Reuse on Clearance of XMuLV and MVM in AEX Anion Exchange Chromatography

Note: Combined results for three monoclonal antibody products.

4.6.4.8 Anticipated post-launch change: Other AEX Formats

Viral clearance studies support the implementation of AEX membranes into the process. A risk-based approach would be used to justify the proposed change.

The potential advantages of using a disposable AEX membrane include decreased overall processing time compared to the time required for packing, qualification and cleaning a chromatography column. In order to explore a possible change from AEX resin to AEX membrane, the following points were considered:

- An assessment of the design space/operating parameters of the membrane to ensure comparable A-Mab quality
- Triplicate studies on multiple lots of AEX load material run at midpoint of test conditions to compare A-Mab quality, impurity clearance and yield
- Multivariate study establishing the new design space and impact to the CEX and Protein A linkages with regard to HCP clearance
- In-process hold study of lab-scale product pools generated from the membrane.
- An assessment of viral clearance comparability as described above.

To demonstrate the feasibility of the proposed change, laboratory scale studies were conducted followed by pilot scale demonstrations. The AEX membrane process was first optimized based on the pressure flow characteristics and then a process characterization strategy similar to that of the AEX resin chromatography was designed based on achieving comparable product quality and performance characteristics. Results showed that A-Mab product quality was comparable from AEX resin and membrane processes (Table 4.32). Also, results showed that drug substance stability

from pilot scale AEX membrane process was comparable to drug substance from resin process (data not shown).

Table 4.32 Comparison AEX resin and membrane process performance

	Load (gm/L)	Yield (%)	HCP (ng/mg)
Load			170
AEX Resin	195	98.4	11.0
AEX Membrane	2,120	99.5	10.8

The numbers are average of three pilot scale (2000 L) runs.

Based on these results, a design space for the AEX membrane was defined (not shown). As expected, the ranges for process parameters were different between the resin and membrane formats since they have different surface chemistry and mass transfer characteristics.

Viral clearance studies (see AEX viral clearance section) also support implementation of AEX membranes; laboratory scale experiments with virus spiking studies demonstrated that comparable LRF of XMuLV and MVM were obtained for AEX resin and membrane formats, even at higher loadings on the membrane up to 10 g protein/mL membrane.

4.6.5 Step 9: Small Virus Retentive Filtration

The design space for this step is based on prior knowledge and A-Mab data. In this section, we use the designation of "F-Type" to describe the filter type used in the platform process.

The small virus retentive filtration (SVRF) step is linked to the anion exchange chromatography and ultrafiltration/diafiltration (formulation) steps as shown in Table 4.33. Product quality characteristics from the anion exchange chromatography are not changed by the SVRF except for a slight decrease in protein concentration due to dilution by the filter chase buffer volume.

Table 4.33 Small Virus Retentive Filtration Step Linkages

Input from Anion Exchange Chromatography	Output to Ultrafiltration and Diafiltration
Protein concentration 7-8 g/L	Protein concentration 6-8 g/L
HMW < 0.8%	HMW < 0.8%
Acidic variants typically 10%	Acidic variants typically 10%
HCP typically <12 ng/mg	HCP typically <12 ng/mg
pH ~7.4-7.6	pH ~7.4-7.6
Conductivity 2.7-6.3 mS/cm	Conductivity 2.7-6.3 mS/cm

4.6.5.1 Step Description

Small virus retentive filtration is a dedicated orthogonal virus removal step in the purification process. It is used to minimize risk of potential virus contamination through physical removal of viruses by a combination of size exclusion and depth filtration. Although the structure and mechanism of virus removal may differ with different filter types, these filters are effective for the

removal of small non-enveloped viruses such as Porcine Parvovirus (PPV; 18-24 nm) or Minute Virus of Mice (MVM; 18-24 nm) as well as larger more complex enveloped viruses such as Xenotropic Murine Leukemia Virus (XMuLV; 80-130 nm).

The AEX pools are combined (if necessary) and filtered through sterile small virus retentive filters which are pre-washed with AEX elution buffer. The filters are then rinsed with additional amount (chase volume) of the same buffer to maximize product recovery. The load conditions vary based on the filter type. After the buffer chase, the filters are further rinsed prior to integrity-testing according to procedures specific for each filter type.

4.6.5.2 Prior knowledge and Risk Assessment Used to Plan Process Characterization Studies

Based on extensive prior experience, a risk assessment for A-Mab determined that the small virus retentive filtration step was not expected to cause detectable variation in product quality and therefore only viral clearance studies were required.

Results from small virus retentive filtration studies with multiple antibodies purified via the platform process (similar steps in the same order run under similar conditions) or purified via a modular process (similar steps in a different order run under similar conditions) show that the LRF values were similar, thus supporting modular claims for viral reduction.

The small virus retentive filtration step has been used extensively for the manufacture of several other antibodies. Extensive experience has been gained from the characterization of virus filtration studies for 3 mAbs with "Type F" filters. This prior knowledge can be applied directly to the A-Mab process because the mechanism of virus clearance is identical and no differences in performance are expected with A-Mab.

From studies with three other mAbs as well as information supplied by the filter manufacturer, filtration load volume, chase volume, and filtration pressure were identified as process parameters that potentially impact the effectiveness of the virus removal for "Type F" filters. In the evaluation of this step, both the filtration volume and the filter pressure were higher than the levels routinely used in commercial manufacturing.

Previous risk assessments were also conducted to determine whether multifactorial or univariate studies were required to assess the impact of filtration process parameters on quality attributes other than viral clearance. Quality attributes most likely to be affected by this process step are aggregate and fragmentation. Process characterization results showed that no aggregation or fragmentation were detected in any of the different mAbs following virus filtration (data not shown) and thus demonstrating that the small virus retentive filtration step does not impact product quality characteristics other than virus load.

4.6.5.3 Scale-down Model

A laboratory scale system using small virus retentive filters "Type-F" with small surface areas was qualified as a model of the manufacturing-scale process. The laboratory models represented scale-down factors between 1,000 and 4,000. The laboratory scale filter units are manufactured with the same structural features and housing design as the units used in full scale manufacturing. The laboratory model was operated based on well-established scaling principles for dead-end filtration. The qualification of the model verified that the initial and final transmembrane flux and operational parameters exhibited a similar profile compared to the full scale virus filtration. Process performance results from four different mAbs (including A-Mab) at different operation scales are

summarized in Figure 4.11. The data demonstrate that for the virus filter used in this study (Type-F), minimal flux decay is observed at filtration volumes as high as 124 L/m^2, suggesting there is no appreciable degree of pore plugging.

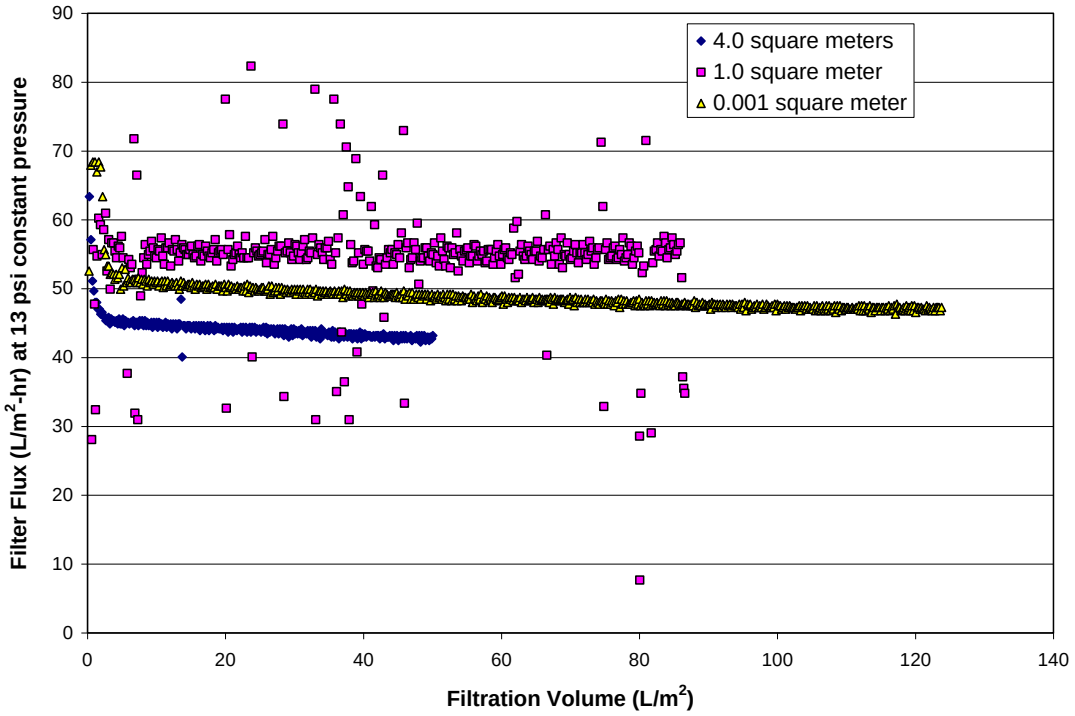

Figure 4.11 Comparison of Filtration Process Performance (Filter Flux at Constant Pressure as a function of Filtration Volume) at various filter scales.

(Note: Results correspond to data from four mAbs, including A-Mab)

Product recovery with the laboratory scale virus filtration system was comparable to pilot and full-scale manufacturing systems, with recoveries ≥ 98%. Results also showed that product quality results were comparable across scales (not shown). Taken together, the results show that laboratory scale model accurately represents the full-scale system and is suitable for use in viral clearance studies.

4.6.5.4 Process Characterization Studies

Laboratory scale model results demonstrated that the process did not induce aggregation, fragmentation or denaturation of the product based on SE-HPLC analysis before and after virus filtration (Figure 4.12). Similar results were observed for studies conducted with different size/area filters. Also, no effect on aggregate or fragmentation levels was observed when product was filtered twice thus supporting a re-filtration step if required in the event of a filter integrity failure.

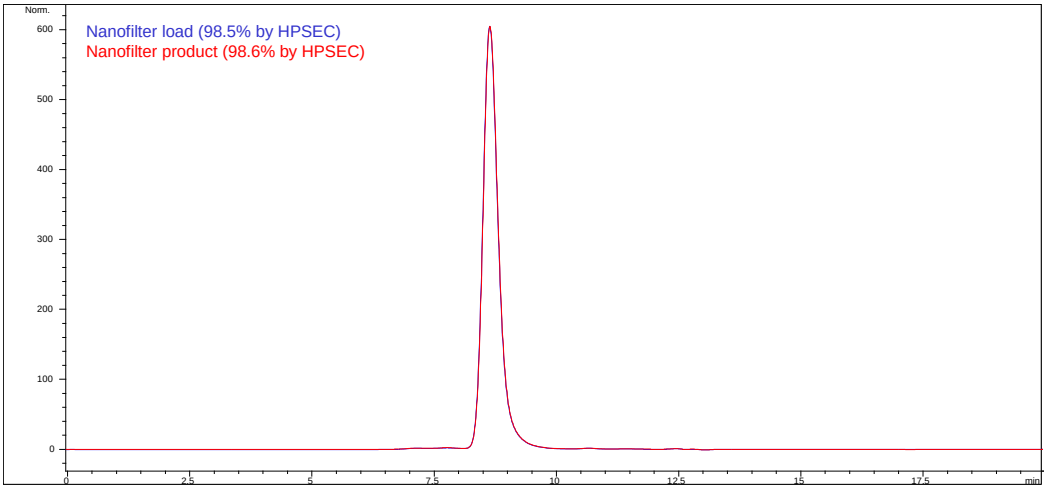

Figure 4.12 SE-HPLC Chromatograms for A-Mab before and After Virus Filtration

Virus removal by small virus retentive filtration was evaluated for two model viruses, Xenotropic Murine Leukemia Virus (XMuLV) and Minute Virus of Mice (MVM) using "Type F" filters. For all MVM studies, the virus-spiked load material was first passed through a pre-filter to remove any potential aggregated virus that could contribute to an overestimate of virus removal. In addition, four successive filtrate fractions were collected to evaluate the possibility of breakthrough as a function of the volume load for this step. These studies were conducted with four different monoclonal antibody products, including A-Mab, in a range of process platforms (sequence of process steps). Results presented in Figure 4.13 and Figure 4.14 demonstrate that there is little or no dependence of virus removal on protein concentration or buffer characteristics, including buffer salt species, pH, and conductivity.

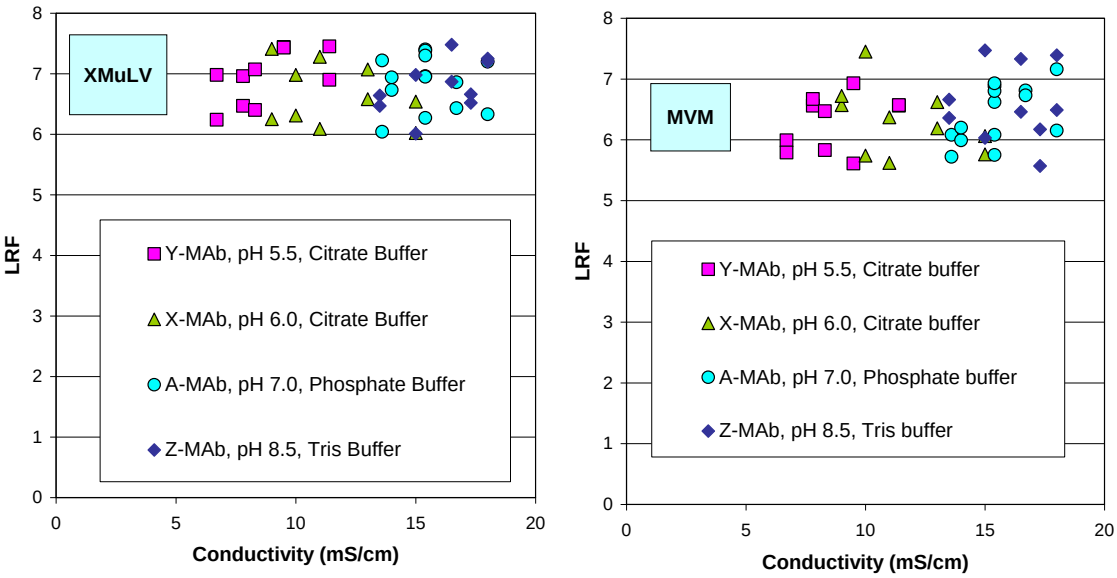

Figure 4.13 Effects of Buffer pH, Conductivity and Salt Species on Clearance of XMuLV and MVM in Virus Filtration

Note: Combined results for A-Mab and three other antibody products.

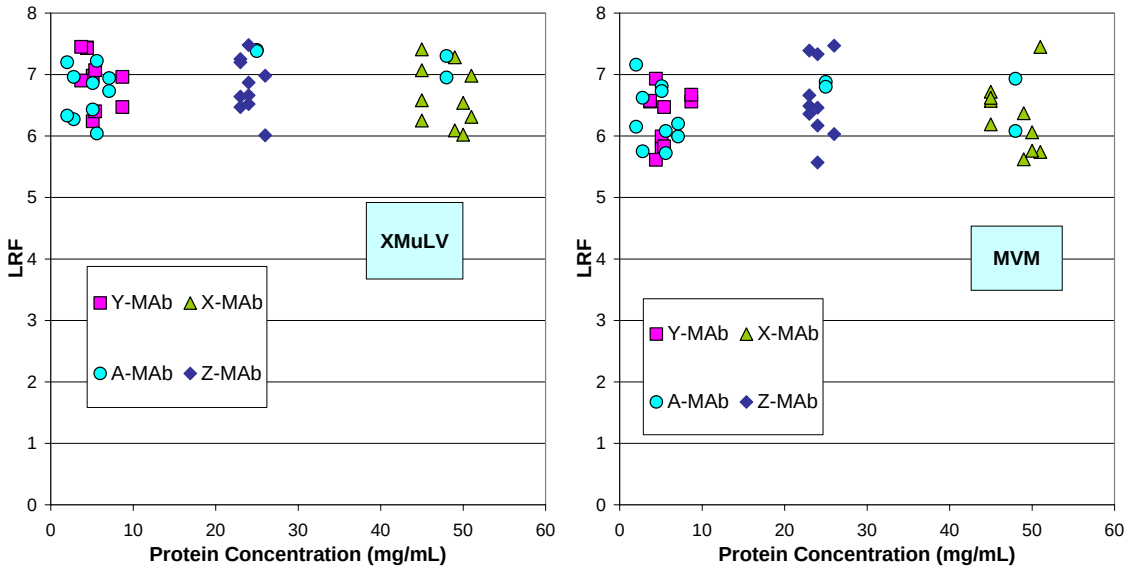

Figure 4.14 Effects of Protein Concentration on Clearance of XMuLV and MVM in Virus Filtration

Note: Combined results for A-Mab and three other antibody products.

Based on process characterization studies, the LRF achieved for MVM in the small virus retentive filters decreases slightly with increasing load volume. To further characterize this observation, MVM breakthrough was assessed as a function of load volume challenge. For these studies, virus was first spiked into the load sample. Four equal volumes of filtrate were collected (0-35 L/m^2, 35-70 L/m^2, 70-105 L/m^2, 105-140 L/m^2) and pools representing the cumulative filtrate at each volume were assayed for virus titer. The results showed that the LRF decreased as volume increased (Figure 4.15). The third fraction included the chase volume which represents only a small fraction (typically about 1%) of the total volume. The fourth fraction collected showed a LRF of 3.91 compared to ≥ 4.62 LRF in the first three fractions. For the MVM viral clearance study, the chase buffer volume was only 3% of the third filtrate fraction. The operating pressures varied somewhat in the various studies shown in this figure, but all pressure values were within the range that is known to have no impact on LRF reduction based on studies performed by the filter manufacturer.

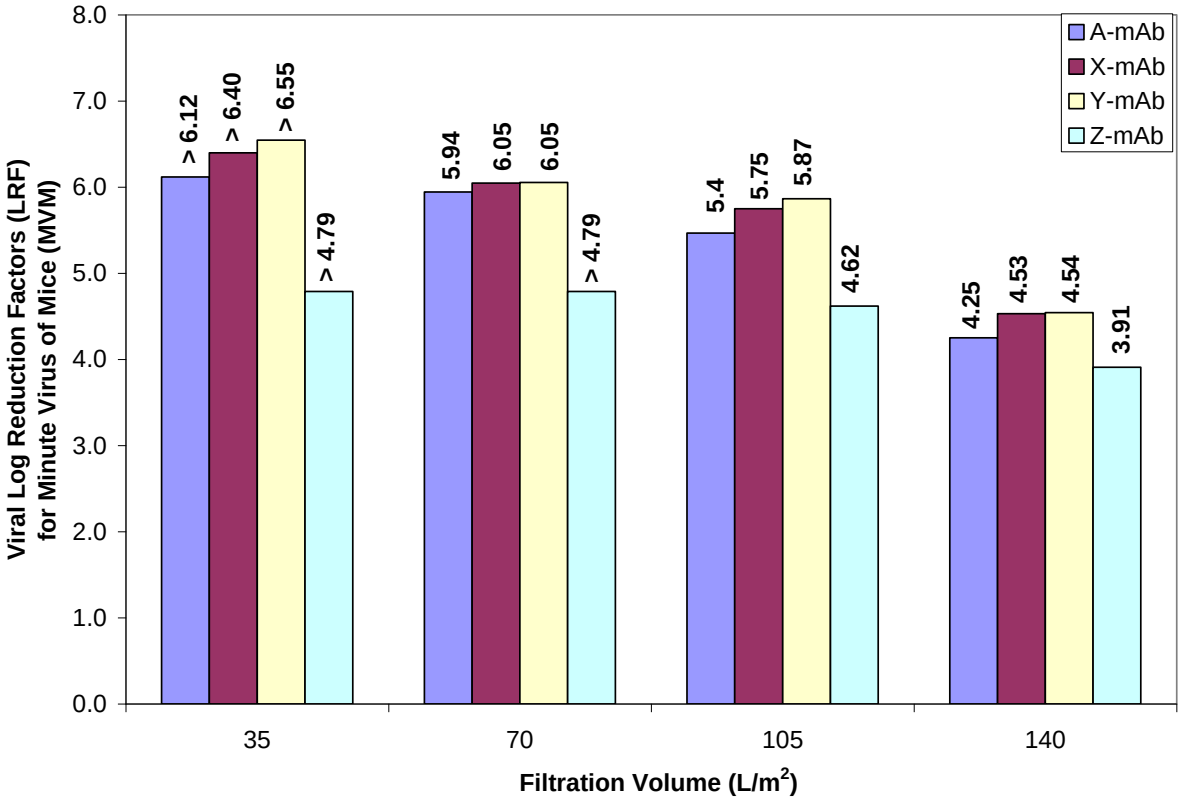

Figure 4.15 Effect of Filtration Volume on LRF of Minute Virus of Mice Filtered through Small Virus Retentive Filters for Four Monoclonal Antibody Products

These results confirm that the total filtration volume is important for assuring effective removal of virus. Because the volumetric load is easy to control, it was classified as a WC-CPP. Based on MVM clearance results, a volumetric load of $\leq$ 105 L/m^2 (including the chase volume) was selected as the upper limit for the "Type-F" filters used for A-Mab. It should be noted that although virus breakthrough may be observed for MVM at higher volumetric loads, no breakthrough has been observed with XMuLV in any mAb processes that use this type filter, even under conditions when the typical load volumes are exceeded.

Operating pressure limits are based on manufacturer recommendations for each filter type. To represent worst-case conditions, all viral clearance studies were carried out at pressures that exceeded the operating pressures limits but remained within the manufacturer's limits. Results from the filter manufacturer's studies at significantly higher pressures indicated that pressure may be important for assuring effective virus removal especially at high filtration volumes. Since pressure is easy to control it was classified as a WC-CPP and not a CPP.

4.6.5.5 Summary of Parameter Classifications and Ranges

In order to assure viral safety, there are two important process parameters that need to be controlled: volumetric load and filtration pressure. Since both are easy to control and have minimal impact on LRF when operated within their acceptable ranges, they were classified as WC-CPPs. Also, the filter integrity test is critical as a procedural control (Table 4.34).

The overall design space with normal operating ranges, acceptable ranges and their criticality is provided in Table 4.34. As previously noted, these conditions have exhibited robust reproducibility

of the LRF values for both generic template and modular processes. The body of virus filtration data obtained for multiple antibodies and multiple processes supports the use of this design space for modular or generic viral clearance assurance. Working within the design space will not be considered as a change, and will be considered to provide assurance of a LRF of ≥ 4.62 for both MVM and XMuLV, regardless of buffer composition, pH, and conductivity, or protein concentration up to 50 mg/mL.

Table 4.34 Summary of Design Space

Parameter	Design Space	Justification	Control	Classification
Pressure	Filter Specific	Modular	Batch procedures	WC-CPP
Filtration volume	Filter Specific	Modular	Batch procedures	WC-CPP
Integrity test	Pass	Modular	Filter integrity test	Procedural Control

4.7 Linkage of Unit Operations

The design space for the downstream process is described in terms of a multivariate statistical model that links process performance for Protein A, CEX ad AEX steps. The approach used to create a linkage model is exemplified through HCP clearance.

The preceding sections show how the process parameters affect the outputs of each individual unit operation. In order to create a design space for the entire process, we need to understand how the individual unit operations interact. All three chromatography unit operations remove HCP, so the full design space for parameters that influence HCP cannot be determined for a single step in isolation from the other steps. One solution is to set arbitrary in-process limits on HCP at each step, which would then determine acceptable parameter ranges for each step. While this approach is simple, it unnecessarily constrains the design space. Instead, a design space approach similar to that shown in ICH Q8(R2) Appendix 2C was taken where the acceptable range of one parameter is dependent on the values of other parameters.

The DOE experiments resulted in empirical models for each step which predict HCP clearance as a function of the incoming HCP level and the process parameters which had a significant impact on that step. These models were combined algebraically to predict the HCP level in the drug substance as a function of all the significant input parameters in the process. This model is shown in Equation 6. As more process knowledge is gained, this model will be verified as part of the ongoing control strategy for the process.

Equation 6

$$\begin{aligned} HCP_{AEX} &= \left(24100 + 117x_{p1} - 5650x_{p2}\right) \\ &\times \left(1 + \exp(5.2 - 0.056x_{c1} - 0.032x_{c2} + 0.007x_{c1}x_{c2})\right)^{-1} \\ &\times \left(1 + \exp(-5.40 + 1.10x_{a1} - 0.40x_{a2})\right)^{-1} \end{aligned}$$

$$where$$

$$x_{a1} = AEX\ Q\ Load\ pH$$

$$x_{a2} = AEX\ Equilibration\ Wash\ Buffer\ Conductivity$$

$$x_{c1} = CEX\ \mathrm{Pr}otein\ Load$$

$$x_{c2} = CEX\ Load\ Wash\ Conductivity$$

$$x_{p1} = \mathrm{Pr}oA\ \mathrm{Pr}otein\ Load$$

$$x_{p2} = \mathrm{Pr}oA\ Elution\ pH$$

This model was verified at small scale by experimentally linking all the steps at target and extreme conditions. The results predicted by Equation 6 correlate well with the measured values (Table 4.35). Thus, the model used to establish the design space was confirmed through experimental verification in the representative scale models and considered robust and predictive of performance at commercial scale.

Table 4.35: Comparison of Model Predictions and Experimental Results for HCP Clearance Across Downstream Process

Experiments	Experimental Process Conditions						HCP Results (ng/mg)	
	ProA Protein Load	ProA Eluate pH	CEX Protein Load	CEX Load Wash Conduct.	AEX Load pH	AEX Elution Wash Conduct.	Measured Values	Model Predicted Values
Target Conditions	30	3.55	20	5	7.5	3.6	**< 12**	**14**
Extreme Conditions: outside Design Space	50	3.2	30	3	7.2	5.6	**92**	**86**
Off-Target Conditions #1	10	3.9	10	7	7.8	1.6	**<20**	**2**
Off-Target Conditions #2	50	3.9	10	3	7.2	5.6	**32**	**29**
Off-Target Conditions #3	10	3.2	30	7	7.2	5.6	**33**	**26**

In order to provide assurance that the operational settings of the process parameters will reliably produce HCP levels below the specification limit, the uncertainty of the prediction must be considered and accounted for. This includes process, measurement and sampling variation as well as uncertainty of the model itself (parameter estimates, parameters studied, form of the model). For this case study a 99.5% prediction interval was added to the mean predicted HCP levels to reflect the desired level of assurance in the design space that specifications will be met. The design space is therefore represented by the inequality,

Equation 7 $$HCP_{AEX} + PI \leq 100 ng/mg$$

where PI = Prediction Interval, subject to the constraint that none of the individual process parameters exceed the ranges tested in the DOE studies in Table 4.36. For a more detailed discussion on the determination of the prediction interval, the interested reader is referred to the Appendix, Section 4.12.

Table 4.36 Limits of Experimental Knowledge

Parameter	Knowledge space (experimental range)
Protein A Chromatography	
Protein load	10-50 g protein/L resin
Elution buffer pH	3.2-3.9
Cation Exchange Chromatography	
Protein load	10-30 g/L resin
Load / wash conductivity	3-7 mS/cm
Anion Exchange Chromatography	
Equilibration / Wash conductivity	1.6-5.6 mS/cm
Load pH	7.2-7.8

The allowable combination of process parameters may be obtained by substituting Equation 6 into Equation 7. Therefore, the resulting design space is the combination of conditions predicted to meet the 100 ng/mg HCP specification 99.5% of the time.

Another way to represent the multivariate equation is graphical depiction of contour plots (Figure 4.16). The green areas represent the design space and correspond to conditions that meet the HCP criteria. The red area represents conditions that do not meet the HCP criteria and thus are outside the design space. Each individual graph is a function of the AEX parameters, at set CEX and Protein A conditions, and each set of 4 graphs represents high and low conditions for CEX parameters at a set Protein A condition.

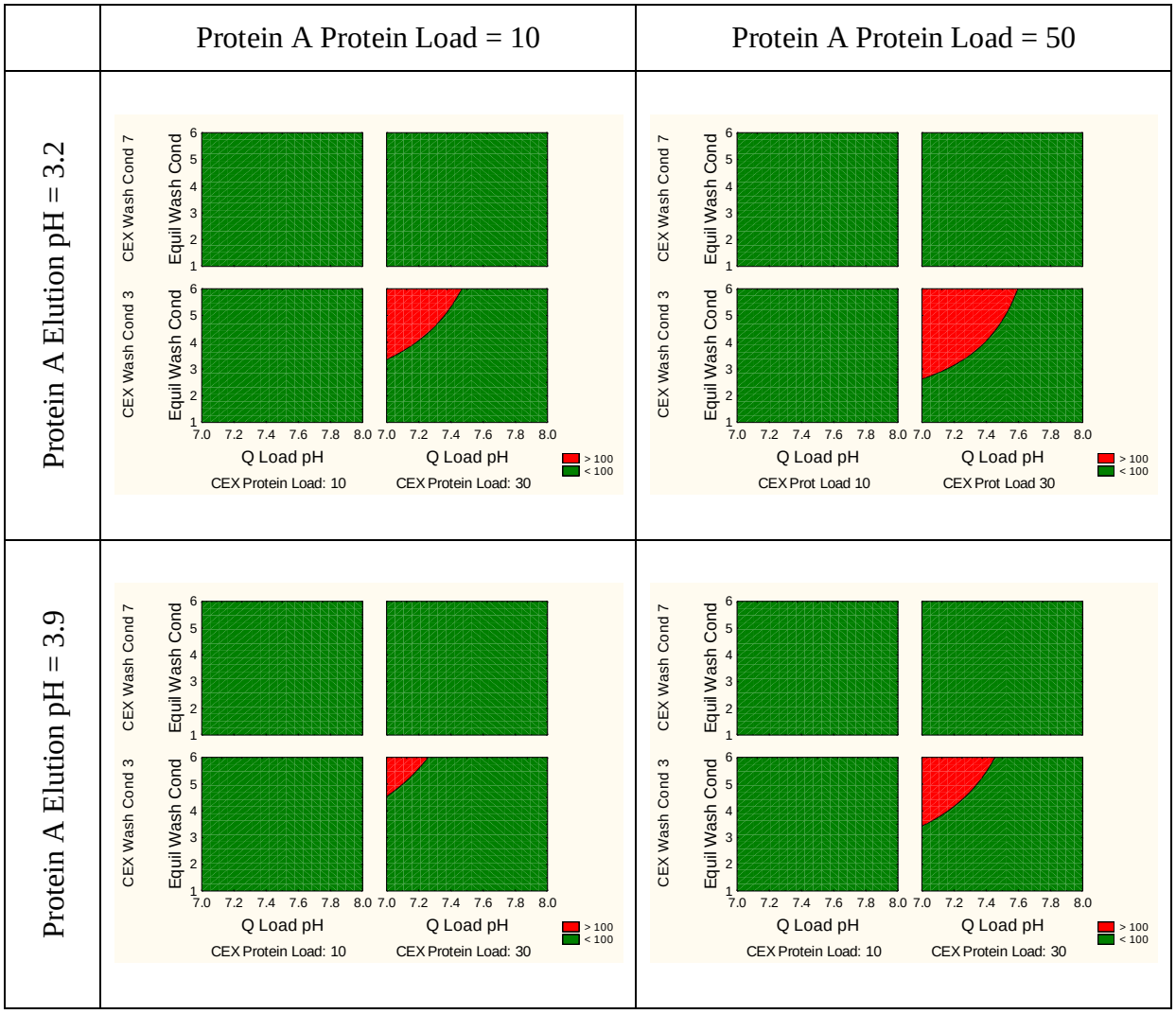

Figure 4.16 Linkage Between Pro-A and CEX Unit Operations Showing 99.5% Prediction Limit for HCP

Equation 7 defining the design space for HCP can be translated into linear ranges defining a control space for routine manufacturing operations. This control space lies within the design space, and allows operational flexibility to adjust the target and ranges for these six parameters within the broader design space. Any movement within the design space would be checked against Equation 7 to ensure acceptable product quality would be obtained. The figure below (Figure 4.17) represents three possible scenarios for setting linear acceptable ranges to illustrate this flexibility.

In the first row of graphs, the full range of Protein A and CEX parameters are allowed, which restricts the AEX parameters to the region shaded green in the control space. In the second row of graphs, the full range of Protein A and CEX protein load is allowed, however restrictions are placed on the Protein A elution pH and CEX wash conductivity, to allow full use of the AEX linear ranges. In the third row of graphs, allowing full range of the Protein A and AEX parameters requires CEX to be operated within a tighter window to ensure that HCP specifications are met. These three scenarios illustrate the flexibility of the operational space using the multivariate design space. The specific approach used will be process and product specific, taking into account the manufacturing facility design and equipment and operational considerations.

1. In the first row, if the entire DOE design spaces (unlinked) for ProA and CEX are employed, then the AEX is restricted (green area) by the model in both Load pH and Equil/Wash Conductivity resulting in a smaller operating space for the AEX step.

2. In the second row, restricting the Elution pH for ProA and the Wash Conductivity for CEX allows the entire AEX design space from the DOE to be used.

3. In the third row, using the entire design space for ProA, but restricting both Load and Wash Conductivity for CEX allows the entire DOE design space for AEX to be used

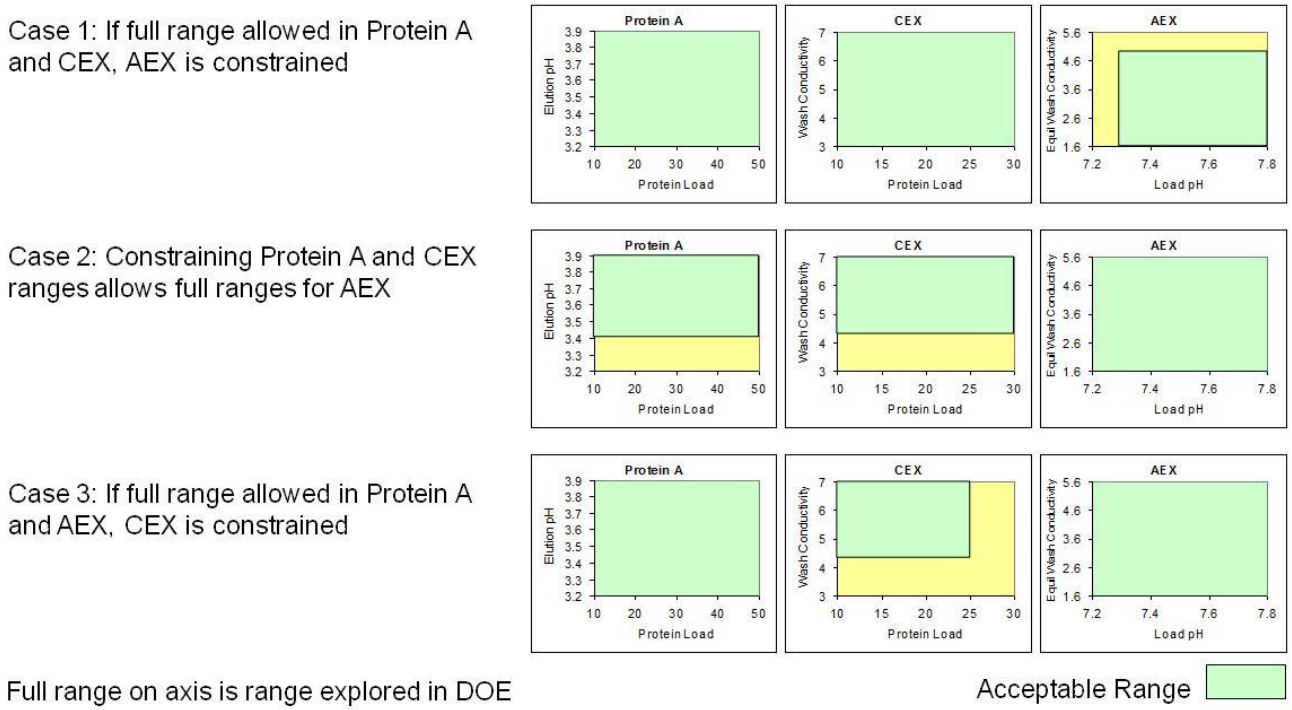

Figure 4.17 Examples of Design Space Interactions

4.8 Summary of Downstream Process Design Space

Detailed process characterization studies using representative laboratory scale models have allowed a thorough assessment of unit operations to establish an overall design space that provides a high degree of assurance that the Quality attributes (exemplified here by HCP and aggregate levels) will consistently meet their respective acceptance criteria.

The following table (Table 4.37) summarizes the design space for the purification process by unit operation. The design space includes CPPs and WC-CPPs for each step, including the viral clearance design space for those steps with viral clearance claims, and the supporting rationale. The control strategy for each process step is also presented.

Table 4.37 Downstream Process Design Space

Parameter	Range	Justification	Control Strategy	Classification
Protein A Chromatography				
Protein load	10-50 g protein/L resin, constrained by Equation 7	Multivariate Study	Batch procedures, Skid control	WC-CPP
Elution buffer pH	3.2-3.9, constrained by Equation 7	Multivariate Study	Batch procedures	WC-CPP
Low pH Inactivation				
pH	3.2- 4.0	Aggregation and viral inactivation considerations	Batch procedures	CPP
Time	60-180 min	Aggregation and viral inactivation considerations	Batch procedures	WC-CPP
Temperature	15°-25°	Aggregation and viral inactivation considerations	Batch procedures	WC-CPP
Cation Exchange Chromatography				
Protein load	10-30 g/L resin. constrained by Equation 7	Multivariate Study	Batch procedures, Skid control	WC-CPP
Load / wash conductivity	3-7 mS/cm, constrained by Equation 7	Multivariate Study	Batch procedures	WC-CPP
Elution pH	6.0 ± 0.2	Multivariate Study	Batch procedures	WC-CPP
Elution stop collect	1.0 ± 0.5 OD descending	Multivariate Study	Skid control	WC-CPP
Anion Exchange Chromatography				
Equilibration / Wash conductivity	1.6-3.6 mS/cm, constrained by Equation 7	Multivariate Study	Batch procedures	WC-CPP
Load pH	7.2-7.8, constrained by Equation 7	Multivariate Study, Generic and Modular Viral Clearance	Batch procedures	WC-CPP
Load conductivity	3.0 – 8.0 mS/cm	Generic and Modular Viral Clearance Study[a]	Batch procedures	WC-CPP
Protein load	≤ 300 g/L resin	Generic and Modular Viral Clearance	Batch procedures	WC-CPP
Flow rate	≤ 450 cm/hr	Generic and Modular Viral Clearance	Batch procedures	WC-CPP
Small Virus Retentive Filtration				
Pressure	Filter Specific	Generic and Modular Viral Clearance	Batch procedures	WC-CPP
Filtration volume	Filter Specific	Generic and Modular Viral Clearance	Batch procedure	WC-CPP
Integrity test	Pass	Generic and Modular Viral Clearance	Filter integrity test	Procedural Control

[a] Range constrained by multivariate study. Acceptable range for viral clearance is conductivity ≤ 15 mS/cm and pH ≥ 7.0.

4.9 Control Strategy for Downstream Process

In this case study, the design space and control strategy for the downstream process have been limited to include process parameters that are linked to a sub-set of product quality attributes. In a real case scenario, the control strategy would be based on all relevant product quality attributes.

The proposed control strategy for the downstream process has a dual purpose: 1) Ensure product quality and safety, 2) Ensure that the commercial manufacturing process is consistent and robust.

Product quality and safety are ensured by controlling all quality-linked process parameters (CPP and WC-CPP) within the limits of the design space. Process consistency is ensured by controlling key process parameters (KPPs) within established limits and by monitoring relevant process attributes.

A summary of the control strategy for the commercial A-Mab downstream process is presented in Figure 4.18.

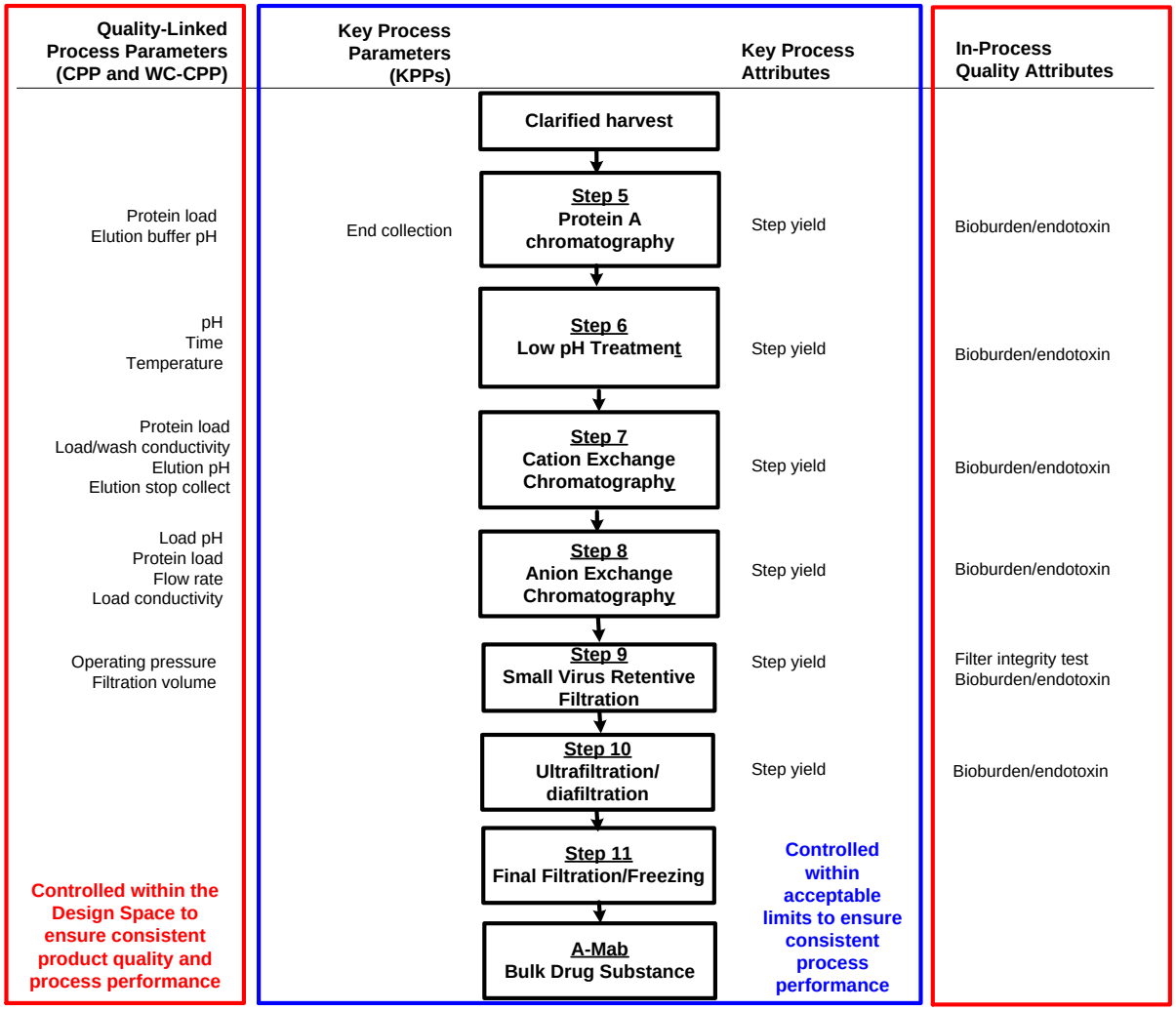

Figure 4.18 Overview of Control Strategy for Downstream Manufacturing Process

4.10 Viral Clearance Summary

The cumulative results of the virus removal and inactivation studies for A-Mab and three other monoclonal antibody products are shown in Table 4.38.

Table 4.38 Viral Clearance for A-Mab and three other Monoclonal Antibody Products

Process Step	XMuLV Log_{10} Reduction (Total PFU)	MVM Log_{10} Reduction (Total PFU)
Low pH Treatment	> 6.6	NT
Anion Exchange Chromatography	> 5.5	> 4.0
Small Virus Retentive Filtration	> 4.6	> 4.6
Total viral log reduction	> 16.7	> 8.6

PFU = plaque forming unit, NT = not tested

4.10.1 Safety Factor Calculation

The safety margin for endogenous virus is determined by comparison of the calculated retrovirus-like particles per dose based on quantitative transmission electron microscopy (TEM) in the absence of clearance. The viral clearance determined by spiking studies with scale down models and the virus model, XMuLV, for the purification process is used to calculate an overall clearance factor for the process. This calculation is shown below.

The theoretical viral contamination per dose in the absence of clearance is calculated based on the harvest titer (4.5 g/L), purification yield (70%), and TEM measurement ($< 2.1 \times 10^6$ particles/mL). The worst case assumption is that all the particles observed by TEM are potentially infective. The process has been designed to ensure a wide margin of safety in the ability of the downstream process to clear XMuLV.

Also required for the analysis is the amount of harvest per dose, which is based on the anticipated dose of 900 mg (10 mg/kg for a 90 kg adult), the A-Mab titer at of unprocessed harvest (4.5 mg/mL) and the overall downstream process yield of 70%. This calculation is given below, resulting in 286 mL unprocessed harvest/dose of A-Mab.

$$\text{Maximum harvest volume/dose} = \frac{900 \text{ mg A-Mab/dose}}{0.70 \times 4.5 \text{ mg A-Mab/mL}} = 286 \text{ mL/dose}$$

- Maximum viral load by TEM = $< 2.1 \times 10^6$ particles/mL

Therefore the theoretical viral contamination/dose without clearance would be:

$$286 \text{ mL/dose} \times (<2.1 \times 10^6) \text{ particles/mL} = (<6.00 \times 10^8) \text{ particles/dose} = (<8.78) \log_{10}$$

The minimum clearance in the purification process is 16.7 $\log_{10}$ for XMuLV.

Therefore, the calculated number of virus particles per dose is:

$$(< 6.00 \times 10^8) \text{ particles/dose} \div 10^{16.7} = (< 6.00 \times 10^{-10}) \text{ particles/dose}$$

or fewer than 1 virus particle per 1.67×10^9 doses (= 9.22 $\log_{10}$).

Therefore, for five monoclonal antibodies, the purification process provides an adequate safety margin of > 9.22 $\log_{10}$ for the removal of endogenous virus particles.

4.10.2 Viral Safety Risk Assessment

A risk assessment for viral safety was conducted based on the United States Food and Drug Administration (US FDA) Points to Consider (PTC in the Manufacture and Testing of Monoclonal Antibody Products for Human Use; PTC in the Characterization of Cell Lines Used to Produce Biologicals) and International Conference on Harmonisation (ICH) guidance documents (Q5A(R1): Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin). An outline of the risk assessment conducted to assure viral safety is summarized below:

- A-Mab is produced in Chinese hamster ovary (CHO) cells using animal component free (ACF) growth medium, nutrient feeds and supplements. In addition, CHO is a well characterized cell line used for the production of other clinical monoclonal antibody products.

- The A-Mab master cell bank (MCB) and working cell bank (WCB) were characterized and shown to be free from adventitious virus contaminants.
- Measures are in place to ensure the safety of raw materials used in the manufacturing process. Any animal derived components used in the medium preparation of the research cell bank, the MCB, and the media for cell cultivation are sourced from low BSE risk countries that have bans in place against ruminant-to-ruminant feeding.
- The capacity of the Drug Substance purification process to remove or inactivate viruses as potential adventitious agents was assessed using a scaled down purification process. The results obtained for five antibodies purified using similar purification steps with well characterized mechanisms of removal or inactivation showed that there is less than 1 retrovirus particle for every 1.67×10^9 doses of antibody, thus presenting a minimal risk to patient safety. This assessment is based on three of five purification steps namely, low pH treatment, anion exchange chromatography and small virus retentive filtration. Prior product knowledge indicates that cation exchange chromatography usually removes approximately 2 logs of XMuLV and Protein A chromatography exhibits robust removal of 4-6 logs based on the flow through fraction from a spiked load (without the low pH elution). Collectively, these steps typically remove 4-8 logs of XMuLV, resulting in an overall 12-18 log safety margin with a minimal risk to patient safety.

4.10.3 Process-related Impurity Clearance

For biotechnology-derived products, a safety risk assessment is used to evaluate the risk associated with process-related impurities. Process-related impurities can be subdivided into two categories in this risk assessment. The first includes host cell-derived and bioactive substances such as host cell DNA, host cell protein, medium supplements such as protein hydrolysates from plant or microbial sources, or residual Protein A from chromatography resins used in the purification process. A second category includes non-bioactive components such as anti-foam that may pose a potential safety risk.

The risk from these impurities can be evaluated using an impurity safety factor (ISF) calculation (Tool #3 in CQA Section). The ISF is the ratio of the impurity to the LD_{50} (or other toxicity measure) to the maximum amount of an impurity potentially present in dose. Other measures such as the no observed adverse effect level (NOAEL) or low observed adverse effect level (LOAEL) can also be used.

$$\mathbf{ISF = LD_{50} \div Level\ in\ Product\ Dose}$$

The risk assessment process is outlined in Figure 4.19.

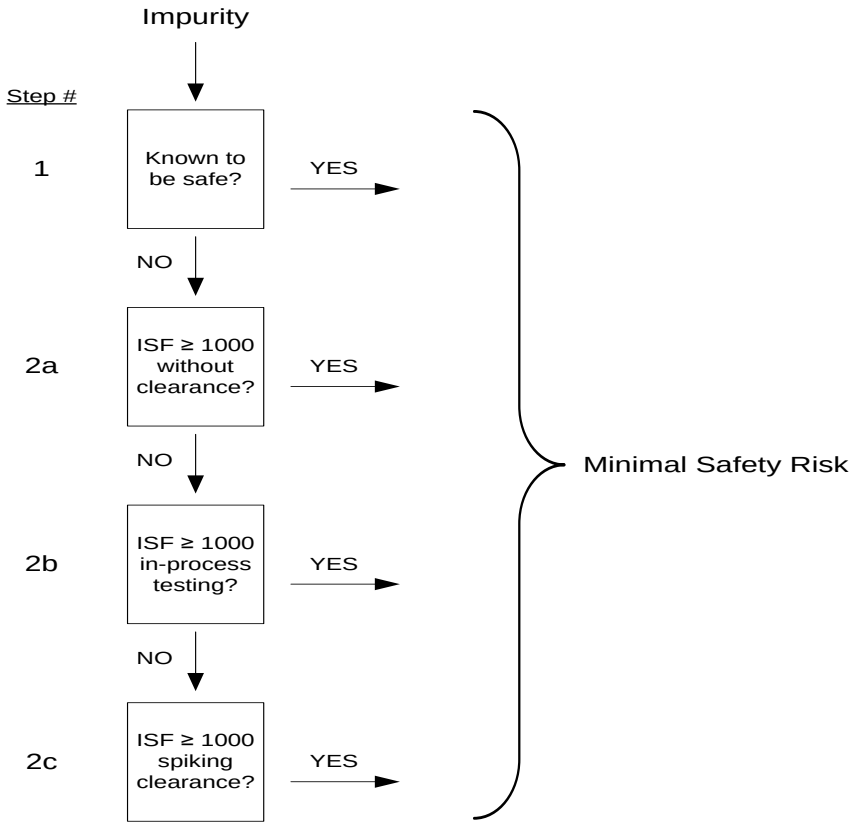

Figure 4.19 Impurity Safety Assessment Strategy

The risk analysis tool described above was used to assess whether the impurity safety factors were sufficient without additional studies or process modification. The impurities evaluated were all contained in the cell culture media and included insulin, methotrexate, Antifoam C and Pluronic F68. Measures of toxicity are known, or can be estimated, for each of these materials and were used in the risk analysis.

The measures of toxicity, levels of the impurities in the production cell culture medium, and the initial ISFs, assuming complete co-purification of the impurities with A-Mab, are given in Table 4.39. Under these assumptions, only insulin met the minimum IFS of 1000 and additional studies were required for Pluronic F68, Antifoam C and methotrexate. Prior studies with the platform process have log reduction factor (LRF) of at least three for insulin over the Protein A chromatography step (Step 2a). Therefore, insulin was not included in the subsequent clearance studies.

Table 4.39 Impurity Levels, NOAEL, and ISF for Cell Culture Impurities

Impurity	Concentration in Production Cell Culture Medium	Amount per Dose	LD_{50}, NOAEL [a] Oral Dose	ISF
Pluronic F68	1 mg/mL	286 mg	90 g [b]	314
Antifoam C [c]	0.1 mg/mL [d]	28.6 mg	125 mg [e]	4.37
Methotrexate	0.9 µg/mL	0.257 mg	1.17 mg	4.1
Recombinant human insulin	1 µg/L	0.286 µg	0.327 mg	1143

[a] Based on a 90 kg adult

[b] Based on an LD_{50} of 1000 mg/kg (i.v. rat) in a 90 kg adult.

[c] After clarification. The level in the production bioreactor is a maximum of 100 mg/L.

[d] Limit of detection of the assay.

[e] Non-prescription oral dose.

Process mapping showed that Pluronic F68 has an LRF of > 2.9 over the Protein A step resulting in an ISF of > 249,000 (Step 2b). The sensitivities of the assays for methotrexate and Antifoam C were insufficient to determine the effectiveness of clearance by process mapping. Therefore, the clearance of these two cell culture impurities over Protein A chromatography was determined with spiking studies using the laboratory model developed for the process characterization.

The assay for Antifoam C was not sufficiently sensitive to demonstrate an ISF > 1000 by Protein A chromatography alone. Therefore, spiking studies on the clarification operation were required (Step 2c). A scale-down model of the final 0.45/0.2 µm filter in the harvest clarification filter train was developed that had a comparable load (L harvest/m^2) to the manufacturing scale. Clarified harvest was then spiked with Antifoam C and filtered. Assay of the load and filtrate provided an LRF (log reduction factor) that was combined with the Protein A LRF for the Antifoam C clearance.

The results of the clearance studies are given in Table 4.40. All process-related impurities had an ISF > 50,000 when prior knowledge, process mapping and spiking study results were included in the ISF calculation.

Table 4.40 ISF Adjusted for Process Clearance

Impurity	LRF	ISF (Without Clearance)	ISF (With Clearance)
Pluronic F68	2.9 [a]	314	2.49×10^5
Antifoam C	4.3	4.37	8.72×10^5
Methotrexate	4.1	4.1	5.16×10^4
Recombinant human insulin	3.0 [b]	1143	1.14×10^6

[a] Mapping study.

[b] Knowledge from prior studies.

4.11 Biblogrpahy

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4.12 Appendix: Combining the Models for a Series of Purification Steps and the determination of prediction interval for HCP

As presented in the case study some gaps in understanding were identified based on scientific review and risk assessment. Experimental studies were designed to determine the effects and interactions of process parameters on the outputs from each downstream purification step. As a result of these studies the process parameters having a significant effect on the outputs were revealed and highlighted. These effects were shown graphically as a contour plot in the Pro-A and AEX section, and with a prediction profiler in the CEX section.

The data obtained from the experiments on each step were originally analyzed without considering the linkages to subsequent downstream purification steps. However, to predict the performance of a series of purification steps a slightly different approach must be taken that considers the three independent steps as one process. When viewed as a process more comprehensive design space can be developed. In the new approach a logistic model is utilized to help combine what was learned from the three independent studies into a single model that describes and predicts the multi-step process. One assumption is that process parameters from different steps do not interact. The model will then be tested at large scale to see if it is a reliable predictor of future performance.

There are three downstream steps that reduce the amount of host cell protein (HCP). The degree of HCP removal is often measured as log-clearance (log(Initial/Final)). For the sake of modeling and analysis the inverse of this ratio, where $p_i = HCP_{out} / HCP_{in}$, will be used to represent the proportion of HCP remaining after each purification step. By applying the logit transformation the proportion is now bounded between 0 and 1. An advantage of using the logistic model is that it will not predict values greater than 1 or less than zero for p_i, and the form of the model is linear. The model for the CEX and AEX purification step can be represented in this manner. However, a logistic model for the Pro-A step is not utilized since the incoming amount of HCP (from the harvested cell pool) does not affect the HCP exiting the ProA step. In other words, the output is not proportional to the input. Another benefit of using a logistic model is that the coefficients for each model can be combined in a fairly straightforward manner to describe the entire sequential process. The logit transformation is first applied to the experimental data for CEX and AEX and then analyzed to find the best model for each purification step. The resulting models are shown below.

PRO-A STEP

$$HCP_{ProA} = \alpha_p + \beta_{p1} x_{p1} + \beta_{p2} x_{p2}$$

where

α_p = ProA intercept

x_{p1} = ProA Protein Load

x_{p2} = ProA Elution pH

CATION EXCHANGE (CEX) STEP

$$p_c = \frac{HCP_{CEX}}{HCP_{proA}}$$

$$\log\left[p_c / 1 - p_c\right] = \alpha_c + \beta_{c1}x_{c1} + \beta_{c2}x_{c2} + \beta_{c12}x_{c1}x_{c2}$$

where

$\alpha_c = \text{intercept}$

$x_{c1} = \text{CEX Protein Load}$

$x_{c2} = \text{CEX Load Wash Conductivity}$

ANION EXCHANGE (AEX) STEP

$$p_a = \frac{HCP_{AEX}}{HCP_{CEX}}$$

$$\log\left[p_a / 1 - p_a\right] = \alpha_a + \beta_{a1}x_{a1} + \beta_{a2}x_{a2}$$

where

$\alpha_a = \text{intercept}$

$x_{a1} = \text{AEX Q Load pH}$

$x_{a2} = \text{AEX Equilibration Wash Buffer Conductivity}$

COMBINED PRO-A, CEX, AND AEX MODEL

Before the models are combined the models must first be converted back into the original units so that the proportion remaining after the CEX and AEX step can be predicted.

$$\hat{p}_c = 1/(1 + e^{-(\alpha_c + \beta_{c1}x_{c1} + \beta_{c2}x_{c2} + \beta_{c12}x_{c1}x_{c2})})$$

$$\hat{p}_a = 1/(1 + e^{-(\alpha_a + \beta_{a1}x_{a1} + \beta_{a2}x_{a2})})$$

The proportion remaining after all the downstream purification steps will be the product of the proportions coming from each individual purification step. Also note that the proportion remaining after CEX and AEX involves HCP amounts from all three steps.

$$\hat{p}_c \hat{p}_a = \frac{1}{1+e^{-(\alpha_c+\beta_{c1}x_{c1}+\beta_{c2}x_{c2}+\beta_{c12}x_{c1}x_{c2})}} \times \frac{1}{1+e^{-(\alpha_a+\beta_{a1}x_{a1}+\beta_{a2}x_{a2})}}$$

$$\frac{HCP_{CEX}}{HCP_{ProA}} \times \frac{HCP_{AEX}}{HCP_{CEX}} = \frac{1}{1+e^{-(\alpha_c+\beta_{c1}x_{c1}+\beta_{c2}x_{c2}+\beta_{c12}x_{c1}x_{c2})}} \times \frac{1}{1+e^{-(\alpha_a+\beta_{a1}x_{a1}+\beta_{a2}x_{a2})}}$$

To complete the combined model the HCP_{CEX} terms cancel each other out and the HCP_{ProA} can be replaced by its predictive equation. So, as shown below, by combining the models in this manner the amount of HCP remaining after all three steps can be predicted from the significant process parameters identified. No incoming or intermediate measurements of HCP are required since the ProA step output is not proportional to the HCP input.

$$HCP_{AEX} = (\alpha_p + \beta_{p1}x_{p1} + \beta_{p2}x_{p2}) \times \left(1+e^{-(\alpha_c+\beta_{c1}x_{c1}+\beta_{c2}x_{c2}+\beta_{c12}x_{c1}x_{c2})}\right)^{-1} \times \left(1+e^{-(\alpha_a+\beta_{a1}x_{a1}+\beta_{a2}x_{a2})}\right)^{-1}$$

The parameter coefficients estimated from the original analysis of Pro-A data may simply be inserted into the equation. However, CEX and AEX experiments had to be re-analyzed using the transformed data to obtain the parameter coefficients needed for the combined model. The final combined model below may be used for predictive purposes.

$$\begin{aligned} HCP_{AEX} &= \left(24100 + 117x_{p1} - 5650x_{p2}\right) \\ &\times \left(1+\exp(5.2 - 0.056x_{c1} - 0.032x_{c2} + 0.007x_{c1}x_{c2})\right)^{-1} \\ &\times \left(1+\exp(-5.40 + 1.10x_{a1} - 0.40x_{a2})\right)^{-1} \end{aligned}$$

PROVIDING ASSURANCE

In order to provide assurance that the operational settings of the process parameters will reliably produce HCP levels below the specification limit the uncertainty of the prediction must be considered and accounted for. This includes process, measurement and sampling variation. Additionally, the uncertainty of the model (parameter estimates, parameters studied, form of the model) contributes to the uncertainty of predicting the output HCP levels. Among the various ways to account for the uncertainty are prediction and confidence intervals, tolerance intervals, and Bayesian derived intervals. In this case study a 99.5% prediction interval is chosen to reflect the desired level of assurance that specifications will be met. The following describes how the variance of the final HCP is estimated and the prediction interval calculated.

First, let's start by letting y_i represent the linear models used in the case study. Suppose

$$y_1 = HCP_{ProA} = \left(\beta_{p0} + \beta_{p1}x_{p1} + \beta_{p2}x_{p2} + e_p\right) \quad (1)$$

$$y_2 = \log\left(\frac{HCP_{\text{CEX}} / HCP_{\text{ProA}}}{1 - HCP_{\text{CEX}} / HCP_{\text{ProA}}}\right) = \left(\beta_{c0} + \beta_{c1}x_{c1} + \beta_{c2}x_{c2} + \beta_{c12}x_{c1}x_{c2} + e_c\right) \quad (2)$$

$$y_3 = \log\left(\frac{HCP_{\text{AEX}} / HCP_{\text{CEX}}}{1 - HCP_{\text{AEX}} / HCP_{\text{CEX}}}\right) = \left(\beta_{a0} + \beta_{a1}x_{a1} + \beta_{a2}x_{a2} + e_a\right) \quad (3)$$

where

$x_{p1} = \text{ProA Protein Load}$, $x_{p2} = \text{ProA Elution pH}$

$x_{c1} = \text{CEX Protein Load}$, $x_{c2} = \text{CEX Load Wash Conductivity}$

$x_{a1} = \text{ProA QLoad pH}$, $x_{c2} = \text{CEX Equilibrium Wash Buffer Conductivity}$.

A linear model was estimated by using the logit transformation on the HCP data. The models must now be inverted back to a proportion so that combination of the models for each step makes more sense and the overall variance of HCP_{AEX} can be determined.

Let $p_c = HCP_{\text{CEX}} / HCP_{\text{ProA}}$ and $p_a = HCP_{\text{AEX}} / HCP_{\text{CEX}}$. From equations (2) and (3) we have

$$p_c = \frac{\exp(y_2)}{1 + \exp(y_2)}$$

and

$$p_a = \frac{\exp(y_3)}{1 + \exp(y_3)}.$$

Now

$$HCP_{\text{AEX}} = HCP_{\text{ProA}} \cdot \frac{HCP_{\text{CEX}}}{HCP_{\text{ProA}}} \cdot \frac{HCP_{\text{AEX}}}{HCP_{\text{CEX}}} = y_1 \cdot p_c \cdot p_a = f\left(y_1, y_2, y_3\right) \tag{4}$$

where

$$f\left(y_1, y_2, y_3\right) = y_1 \cdot \frac{\exp(y_2)}{1+\exp(y_2)} \cdot \frac{\exp(y_3)}{1+\exp(y_3)} \tag{5}$$

At this point we can use the delta method to approximate the variance of HCP_{AEX}. To carry out the delta method some calculus must be used. First note that

$$\frac{\partial \exp\left(x\right)/\left(1+\exp\left(x\right)\right)}{\partial x} = \exp\left(x\right)/\left(1+\exp\left(x\right)\right)^2 .$$

It follows that

$$\frac{\partial HCP_{\text{AEX}}}{\partial y_1} = \frac{\partial f\left(y_1, y_2, y_3\right)}{\partial y_1} = \frac{\exp(y_2)}{1+\exp(y_2)} \cdot \frac{\exp(y_3)}{1+\exp(y_3)}$$

$$\frac{\partial HCP_{\text{AEX}}}{\partial y_2} = \frac{\partial f\left(y_1, y_2, y_3\right)}{\partial y_2} = y_1 \cdot \frac{\exp(y_2)}{\left(1+\exp(y_2)\right)^2} \cdot \frac{\exp(y_3)}{1+\exp(y_3)}$$

$$\frac{\partial HCP_{\text{AEX}}}{\partial y_3} = \frac{\partial f\left(y_1, y_2, y_3\right)}{\partial y_3} = y_1 \cdot \frac{\exp(y_2)}{1+\exp(y_2)} \cdot \frac{\exp(y_3)}{\left(1+\exp(y_3)\right)^2} .$$

Next assume that the three purification steps are independent of one another and the covariance matrix of $\underset{\sim}{y} = \left(y_1, y_2, y_3\right)'$ is diagonal. More specifically,

$$Var(\underset{\sim}{y}) = \begin{bmatrix} Var\left(\hat{y}_{1,\,\text{Prediction}}\right) & 0 & 0 \\ 0 & Var\left(\hat{y}_{2,\,\text{Prediction}}\right) & 0 \\ 0 & 0 & Var\left(\hat{y}_{3,\,\text{Prediction}}\right) \end{bmatrix}$$

is used for calculating a prediction interval of individual (batch) values. In this case study the prediction covariance matrix is utilized. The delta method then yields

$$Var\left(HCP_{\mathrm{AEX}}\right)=Var\left(f\left(y_1,y_2,y_3\right)\right)=\sum\left(\frac{\partial f}{\partial y_i}\right)^2 Var\left(y_i\right)$$

$$=\left(\frac{\exp(y_2)}{1+\exp(y_2)}\cdot\frac{\exp(y_3)}{1+\exp(y_3)}\right)^2 Var\left(y_1\right)+\left(y_1\cdot\frac{\exp(y_2)}{\left(1+\exp(y_2)\right)^2}\cdot\frac{\exp(y_3)}{1+\exp(y_3)}\right)^2 Var\left(y_2\right)$$

$$+\left(y_1\cdot\frac{\exp(y_2)}{1+\exp(y_2)}\cdot\frac{\exp(y_3)}{\left(1+\exp(y_3)\right)^2}\right)^2 Var\left(y_3\right)$$

$$=\left(\frac{\exp(y_2)\exp(y_3)}{\left(1+\exp(y_2)\right)\left(1+\exp(y_3)\right)}\right)^2\left(Var\left(y_1\right)+\frac{y_1^2\cdot Var\left(y_2\right)}{\left(1+\exp(y_2)\right)^2}+\frac{y_1^2\cdot Var\left(y_3\right)}{\left(1+\exp(y_3)\right)^2}\right)$$

The variances in the above equation are defined by

$$Var\left(\hat{y}_{1,\,Prediction}\right)=Var\left(b_{p0}+b_{p1}x_{p1}+b_{p2}x_{p2}+e_p\right)=\left(1+\underset{\sim}{x}_p'\left(X_p'X_p\right)\underset{\sim}{x}_p\right)\sigma_p^2$$

$$Var\left(\hat{y}_{2,\,Prediction}\right)=Var\left(b_{c0}+b_{c1}x_{c1}+b_{c2}x_{c2}+b_{c12}x_{c1}x_{c2}+b_{c12}x_{c1}x_{c2}+e_c\right)=\left(1+\underset{\sim}{x}_c'\left(X_c'X_c\right)\underset{\sim}{x}_c\right)\sigma_c^2$$

$$Var\left(\hat{y}_{3,\,Prediction}\right)=Var\left(b_{a0}+b_{a1}x_{a1}+b_{a2}x_{a2}+e_a\right)=\left(1+\underset{\sim}{x}_a'\left(X_a'X_a\right)\underset{\sim}{x}_a\right)\sigma_a^2$$

To calculate the upper 99.5% prediction interval the linear form of the y_i terms (as used in the case study) are inserted into the equation, and $(1+x_i'(X'X)^{-1}x_i)*s_i^2$ is used for the $Var(y_i)$. To simplify further some additional assumptions are made about the leverage, $h_i = x_i'(X'X)^{-1}x_i$, so that each point in the experimental region has the same influence on the prediction interval. We are mainly concerned about the prediction limits near the factorial "corners" of the experimental region. Therefore, a conservative approach is to use the leverage of the factorial runs where the leverage, and thus the prediction variance, will be greatest. In the following the leverage chosen for each experimental design matrix is substituted into their respective variances. Additionally, an estimate for σ_i^2 is obtained from analysis of the experimental design where the MSE = s_i^2 . This represents the unexplained variance in the experiment. Also, the standard normal curve is used to determine $z_{.005}$ = 2.575. Thus, to determine the prediction interval we have the following

$$U95PL_HCP_{AEX} =$$

$$HCP_{AEX} + z_{.005} * \sqrt{\left(\frac{\exp(y_2)\exp(y_3)}{(1+\exp(y_2))(1+\exp(y_3))}\right)^2 \left(Var(y_1) + \frac{y_1^2 \cdot Var(y_2)}{(1+\exp(y_2))^2} + \frac{y_1^2 \cdot Var(y_3)}{(1+\exp(y_3))^2}\right)}$$

And, with a little algebra, the variance term can be simplified to the following:

$$U95PL_HCP_{AEX} =$$

$$HCP_{AEX} + z_{.005} * \sqrt{(HCP_{AEX})^2 \left(\frac{Var(y_1)}{y_1^2} + \frac{\cdot Var(y_2)}{(1+\exp(y_2))^2} + \frac{\cdot Var(y_3)}{(1+\exp(y_3))^2}\right)}$$

In the above HCP_{AEX} is the predicted value. The y_i terms can be substituted with their previously defined terms to complete the calculation. This can be implemented in a spreadsheet or other software package to determine the 99.5% prediction limit for HCP_{AEX} for various combinations of operating parameters.

$$U95PL_HCP_{AEX} =$$

$$HCP_{AEX} + 2.575 * \sqrt{HCP_{AEX}^2 \left(\frac{(1.18)400_p^2}{HCP_{\text{ProA}}^2} + \frac{(1.11)0.15_c^2}{(1+(\hat{p}_{CEX}/(1-\hat{p}_{CEX})))^2} + \frac{(1.18)0.15_a^2}{(1+(\hat{p}_{AEX}/(1-\hat{p}_{AEX})))^2}\right)}$$

5 Drug Product

The drug product for A-Mab is based on a well established platform with extensive process and performance history. The formulation composition is based on an existing formulation that has served other antibodies and no further optimization was done for A-Mab. Only verification of the suitability of the design space established for previous products was required.

Similarly the manufacturing process leverages prior knowledge from other antibodies to guide process development. The design space for the compounding step is based on scale-independent process parameters and thus is applicable to all scales of operation. The derivation of scale independent parameters is provided as an Appendix to this section. A scale-independent approach is also presented for the filling step.

As a second appendix, a Fault Tree Analysis is provided as an example of a process to support a comprehensive risk assessment of potential failure points of a process.

Key Points from Drug Product Section

- Only a very limited formulation development exercise is necessary for A-Mab since platform formulation and prior knowledge gained from previous mAbs is leveraged
- Predictive mathematical models can be applied to vessels used for compounding to deliver a scale, facility and equipment-independent process
- QbD principles can be used to enhance understanding of the relationship between sterile filtration parameters and product quality to establish a process platform that fits A-Mab as a “next in class” molecule
- A risk-based approach and the use of DoE to create an engineering design space for filling pumps
- Fault Tree Analysis enables a comprehensive risk assessment for the overall A-Mab drug product process and supports a mitigation strategy for highest assurance of process performance
- Table 5.1summarizes the elements of QbD that will be exemplified in this case study versus the traditional approaches to drug product development.

Table 5.1 QbD Compared to Traditional Approach

Quality by Design Approaches Exemplified in the A-Mab Drug Product	Traditional Drug Product Development Approaches
Leverage of a previous formulation design space where platform composition and conditions have proven history. For A-Mab, only verification is needed through limited DoE studies	No leveraging of class knowledge or platform formulation design space. A-Mab is treated as 1st in class. Extensive DoEs and wide ranging studies
Extensive use of prior knowledge of unit operations, supported by both multi-variate or univariate risk-based verification	Prior knowledge used and both multi-variate and univariate experiments conducted, but without formal risk-based assessment
Establish predictive relationships between process parameters and product quality attributes by iterative scientific steps applied to many products and/or using statistically designed experiments for unique products. Acceptable operating conditions expressed in terms of a design space or as PARs, the choice being based on an understanding of potential for interactions.	Some experiments conducted using single-variable approaches, potentially overlooking parameter interactions. Acceptable operating ranges expressed as univariate Proven Acceptable Ranges with no systematic understanding of interaction potential
Process development leverages platform knowledge through systematic application of risk management tools.	Process development based on established industry precedents.
Rational approach to establishing a control strategy supported by thorough process/product understanding. Control strategy focuses on critical control points and control of critical process parameters.	Control Strategy based on prior experience and precedent. Product quality controlled primarily by end-product testing
Operating ranges applicable to multiple operational scales. Predictability and robustness of process performance at multiple scales is ensured by defining an engineering design space	Process performance at multiple scales is demonstrated through empirical experience and end-product testing.
Lifecycle approach to process validation which includes continuous process verification to demonstrate that process remains in state of control. Continual improvement enabled Use of multivariate (MVA) approaches for process verification.	Process validation based on limited and defined number of full-scale batches. Primary focus on corrective action. Process performance generally monitored using single variable approaches

5.1 Quality Target Product Profile

The Quality Target Product Profile for A-Mab is listed in Table 5.2. The Critical Quality Attributes listed (aggregates, fucose content, galactosylation and HCP), are followed throughout the upstream, downstream and drug product manufacturing processes. The quality attribute that is the focus for the drug product development of A-Mab from this list is aggregation. Additionally, attention is paid to sub-visible and visible particles as these are related to aggregation. Other drug product-specific quality attributes, e.g. oxidation products, fragmentation products and color are usually considered in the broader context of drug product development, but will not be addressed here in this case study.

Table 5.2 Quality Target Product Profile (QTPP) for A-Mab

Product attribute	Target
Dosage Form	Liquid, single use
Protein content per vial	500 mg
Dose	10 mg/kg
Concentration	25 mg/mL
Mode of administration	IV, diluted with isotonic saline or dextrose
Viscosity	Acceptable for manufacturing, storage and delivery without the use of special devices (for example, less than 10 cP at room temperature).
Container	20R type 1 borosilicate glass vials, fluro-resin laminated stopper
Shelf life	≥ 2 years at 2-8°C
Compatibility with manufacturing processes	Minimum 14 days at 25°C and subsequent 2 years at 2-8°C, soluble at higher concentrations during UF/DF
Biocompatibility	Acceptable toleration on infusion
Degradants and impurities	Below safety threshold, or qualified
Pharmacopoeial compliance	Meets pharmacopoeial requirements for parenteral dosage forms, colorless to slightly yellow, practically free of visible particles and meets USP criteria for sub-visible particles
Aggregate	0-5%
Fucose content	2-13%
Galactosylation (%G1 + %G2)	10-40%
HCP	0-100 ng/mg

5.2 Formulation Selection

The drug product formulation and presentation are entirely conventional and leverage extensive prior knowledge gained from the development of previously licensed antibodies (X-Mab, Y-Mab and Z-Mab). The drug product manufacturing process for A-Mab also follows a set of unit operations that have been successfully applied to several liquid formulation monoclonal antibody products.

The prior knowledge gained from these previous products indicates that A-Mab has no risk of biochemical and biophysical instability to the target manufacturing conditions, storage conditions and transportation as measured by aggregation, sub-visible and visible particles.

The formulation components, function and composition are shown in Table 5.3.

Table 5.3 Formulation Description

Component	Amount	Function	Quality Standard
A-Mab	500 mg	Active ingredient	In-house specification
Sucrose	1.8 g	Isotonicity agent	Compendial
Acetic Acid	20 mM	Buffering agent	Compendial
Polysorbate 20	2 mg	Surfactant	Compendial
Sodium Acetate	q.s. to pH 5.3	pH adjustment	Compendial
WFI	q.s. to final volume of 20 mL	Solvent	Compendial

The manufacturing steps from A-Mab drug substance to packaged and labeled drug product are shown in Figure 5.1.

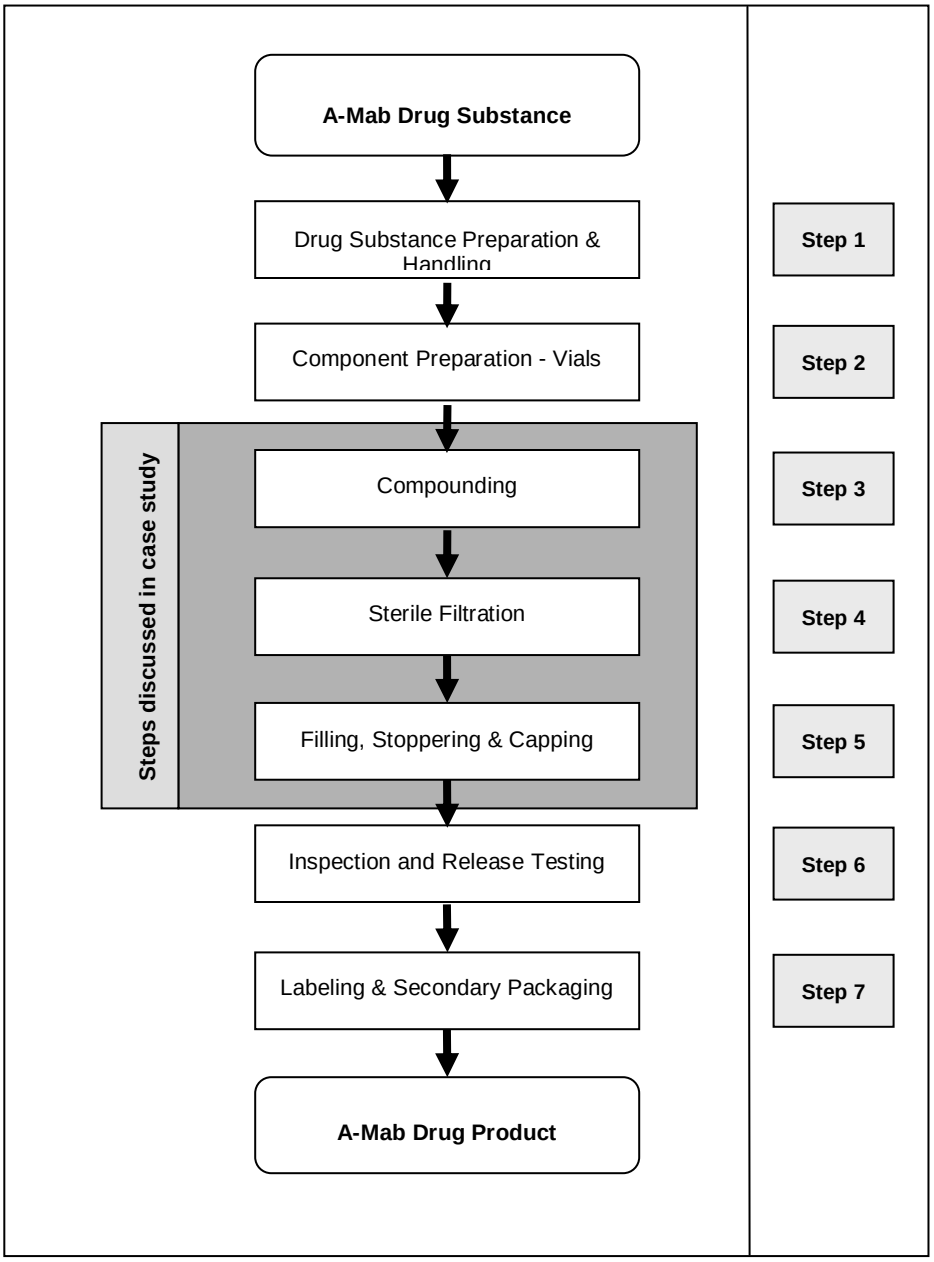

Figure 5.1 Drug Product Manufacturing Process Steps

5.2.1 Prior Knowledge and Initial Risk Assessment

A risk analysis was used to establish which variables and unit operations were likely to have the greatest impact on product quality. This initial risk assessment is shown in below in Table 5.4.

Table 5.4 Initial Risk Assessment for Formulation and Unit Operations

	Variables and Unit Operations			
DP CQAs	**Formulation Composition**	**Compounding**	**Sterile Filtration**	**Filling and stoppering**
Aggregate	High	High	High	High
Sub-visible particles	High	High	High	High
Visible particles	High	High	High	High
Fucose content	Low	Low	Low	Low
Galactosylation (%G1 + %G2)	Low	Low	Low	Low
HCP	Low	Low	Low	Low

Fucose content, Galacosylation and HCP are all CQAs of the drug substance and are controlled during the manufacture of the drug substance. Prior knowledge demonstrates they are not affected by the drug product manufacturing process. Therefore aggregation, sub-visible and visible particles will be the primary focus.

5.2.2 Verification of the drug product composition

This section describes how a reduced set of experiments was used to verify a formulation composition design space that had been established from previously approved antibodies

A cause and effect matrix risk assessment was conducted to rank the criticality of the A-Mab formulation parameters, Table 5.5. The quality attributes used were aggregates, visible and sub-visible particles.

Table 5.5 Formulation Composition Risk Assessment

	Weight factor	10	10	5	
	Quality attribute / Parameter	Purity: aggregation	Purity: visible particles	Purity: subvisible particles	Weighted score
Formulation Composition	pH	10	10	10	250
	A-mAb concentration	10	10	10	250
	Polysorbate 20 concentration	5	10	10	200
	Fill Volume	5	7	7	155
	Acetate concentration	5	5	5	125
	Primary container DS	5	5	5	125
	Raw material impurities	5	5	5	125
	Sucrose concentration	5	1	1	65
	20R DP primary container	1	1	1	25

The outcome of the risk assessment was as expected based on prior knowledge. The highest ranked parameters (pH, A-Mab and polysorbate 20 concentration) from the risk assessment were screened as the main causes of aggregation and particle formation by experiments conducted at approximately two times the expected formulation limits (for DS and DP) based on prior knowledge. The experimental design used was a fractional factorial in order to determine the critical formulation parameters for further characterization. The critical formulation parameters were identified as pH and protein concentration for aggregation and polysorbate 20 level for particulate matter. The flow diagram for the formulation selection strategy is shown in Figure 5.2.

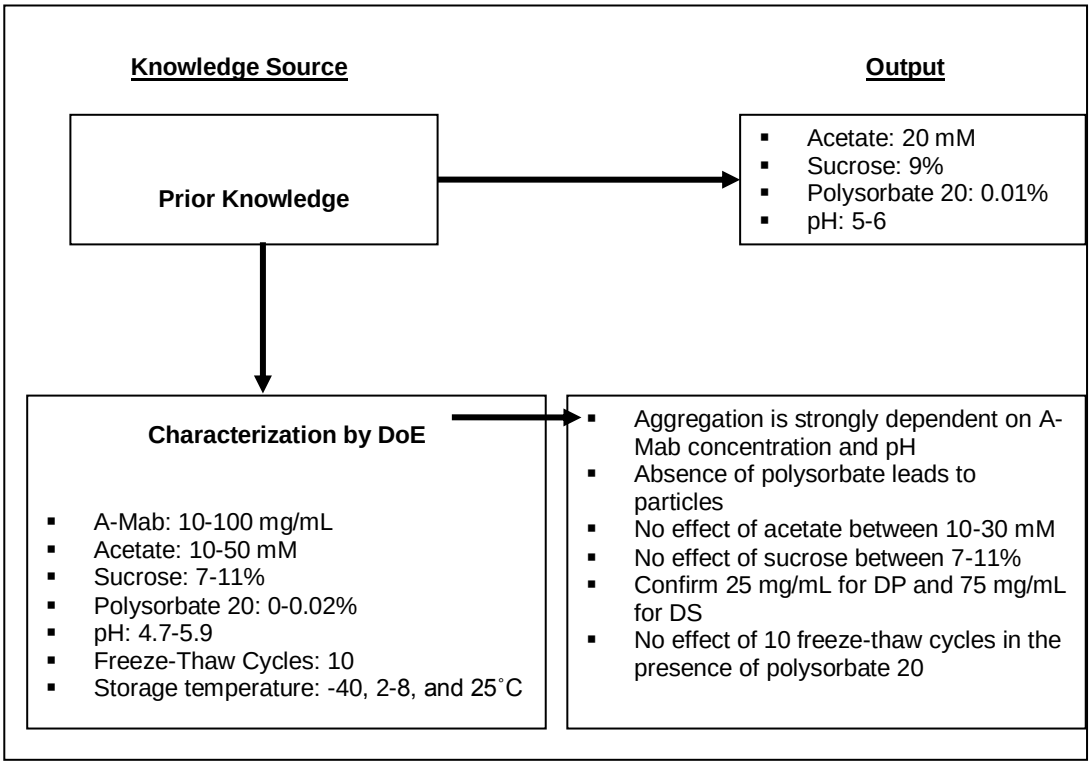

Figure 5.2 Schematic Flow Diagram Showing the Formulation Selection Strategy

The results of these experiments demonstrated that A-Mab behaves identically to monoclonal antibody products X-Mab, Y-Mab and Z-Mab. The formulation knowledge space around all the formulation parameters was then further verified using a central composite design. A full factorial model was completed for the anticipated formulation composition ranges around the drug product critical formulation parameters, which were pH, and protein and polysorbate 20 concentrations.

The pH, protein concentration and polysorbate 20 were found to have the most significant effects during the characterization of the formulation, and these formulation parameters were further explored using a central composite design. Samples were tested after 3 months storage at 5˚C±3 and 40˚C. Aggregation and sub-visible particulate matter (2, 10 and 25 um) as a function of pH, polysorbate 20 and protein concentration are shown in Figure 5.3.

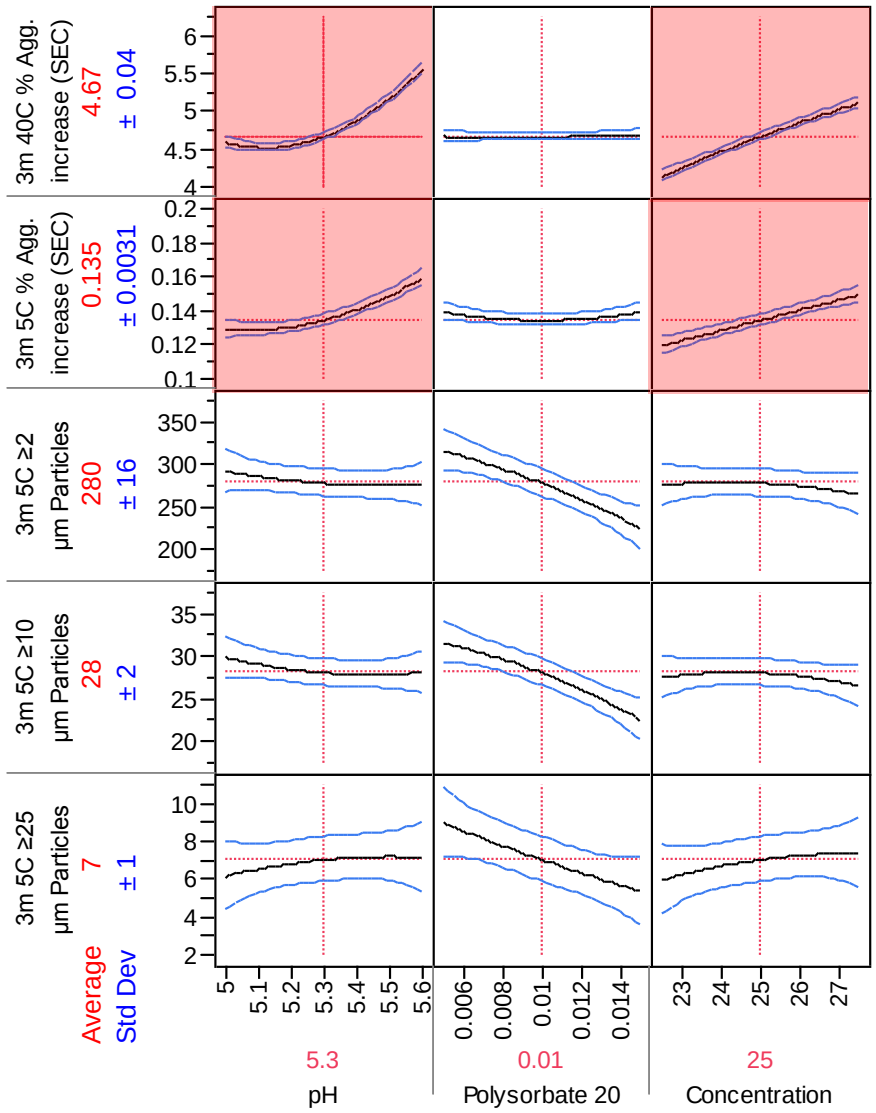

Figure 5.3. Formulation Characterization Studies

Aggregation increased above pH 5.3 for storage at 40˚C and was dependent on protein concentration. Change in sub-visible particles over the polysorbate 20 concentration range of 0.005-0.015% was not significant and was consistent with prior knowledge from commercial products X-Mab, Y-Mab and Z-Mab. Since aggregation is dependent on pH and protein concentration, the response surface was mapped for these parameters versus aggregation after 3 months storage at 40 °C, (Figure 5.4). A very weak interaction between pH and concentration is observed. However, there is no suggestion of any further interactions. Due to the dependence of aggregation on pH, the formulation range for pH should not exceed 5.6.

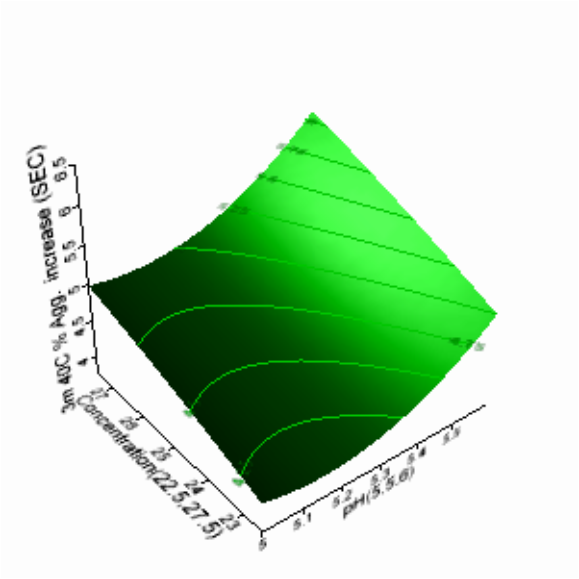

Figure 5.4. Response surface for aggregation after 3 months at 40°C as a function of pH and protein concentration

Stability studies were conducted at 2-8, 25, and 40°C, but decisions were made based on 40°C data. For A-Mab, the Tm for the C_H2 and C_H3 domains are 71°C and 83°C, respectively, and are well above the 40°C storage temperature. Therefore, degradation from global unfolding is not expected to play an important role at 40°C. However, local unfolding may be significant and decisions based on accelerated data always need to be verified post-decision with lower temperature data. For A-Mab, preliminary investigations suggested that the temperature dependence of stability could be modeled using Arrhenius kinetics. Upon further investigation, aggregation was determined to follow Arrhenius kinetics up to 40°C and deviate at 50°C.

Prior knowledge from X-Mab, Y-Mab and Z-Mab products demonstrated that effective control of protein particulates is obtained at polysorbate concentrations at or about 0.01%. However, significant increase in particulates was not observed at levels down to 0.005% throughout the shelf-life of these products. For A-Mab, a confirmatory study was conducted with Polysorbate 20 at two levels (0.005% and 0.01%). The fill volume for these experiments was chosen to be appropriate to the final drug substance container configuration. Polycarbonate carboys, fluorinated HDPP containers, and cryovessels were investigated. The containers were frozen at -40 °C. A freeze-thaw cycle consisted of freezing for at least 24 hours followed by a static thaw at room temperature. After each cycle, the aggregation properties were determined by SE-HPLC and the sub-visible particles were measured. The containers were also inspected for presence of any visible particles and were found to remain visually clear after 10 freeze-thaw cycles.

SE-HPLC analysis of drug substance which included 0.01% polysorbate 20 showed that aggregation did not increase and that A-Mab was stable up to 10 cycles of freeze-thaw. No increase in sub-visible particle counts (≥ 2 µm , ≥ 5 µm ≥ 10 µm and ≥ 25 µm) from time zero were seen after 10 freeze-thaw cycles. SE-HPLC was used to monitor stability after storage for 52 weeks at -40°C of A-Mab drug substance (with 0.01% polysorbate 20) in polycarbonate carboys, fluorinated HDPP containers, and cryovessels. No significant change in the formation of high molecular weight

species from time zero was noted after this length of time in any of the containers tested. Any of these containers can be used to store drug substance.

The results for 0.005% were similar to those for 0.01% polysorbate 20 thereby demonstrating that A-Mab behaves identically to previous candidates and showing that the lower limit of 0.005% polysorbate 20 is sufficient to protect against particle formation.

None of the formulation component variations showed any evidence of interactions apart from the very weak interaction found for pH and concentration. Therefore, based on the information for previous commercial monoclonal antibody products and the confirmatory studies conducted, a formulation design space can be constructed and represented as the Table 5.6 shown below.

Table 5.6 Formulation Design Space

		Design Space Lower Limit	Design Space Upper Limit	Target
Drug Substance	pH	4.7	5.6	5.3
	Acetic acid/Acetate (mM)	10	30	20
	Sucrose (% w/vol)	5	13	9
	Polysorbate 20 (% w/vol)	0.005	0.02	0.01
	A-Mab concentration (mg/ml)	65	85	75
Drug Product	pH	4.7	5.6	5.3
	Acetic acid/ Acetate (mM)	10	30	20
	Sucrose (% w/vol)	5	13	9
	Polysorbate 20 (% w/vol)	0.005	0.02	0.01
	A-Mab concentration (mg/ml)	20	30	25

5.3 Manufacturing Process Development

For the purpose of this case study only the compounding, sterile filtration and filling steps are considered.

5.3.1 Step 3: Compounding

The compounding step for A-Mab was derived from prior process knowledge from multiple marketed mAbs and verified with A-Mab. The scale model for compounding vessels discussed in Appendix 1 to this section demonstrates how a scale-independent process can be developed.

5.3.1.1 Definition of Target Process

The compounding step (Figure 5.5) is critical to delivering the exact formulation composition, designed for biopotency, processing and storage stability in the final drug product container and packaging. The compounding step begins with raw material excipients and drug substance and ends with the bulk drug product formulation. The proposed batch size for this process in this case study is 50-1500 L.

The process begins with the preparation of the diluent to reduce the A-Mab concentration from the drug substance level of 75 mg/mL to the drug product level of 25 mg/mL. The first step is the addition of water for injection (WFI) into the diluent preparation tank. The buffer species are added next; sodium acetate (excipient 1) and acetic acid (excipient 2) are mixed until dissolved. Next, the sucrose (excipient 3) is added and dissolved followed by the polysorbate 20 (excipient 4). The pH is tested on-line and adjusted, if necessary to the target pH. The final weight (volume) of the solution is adjusted with WFI and the diluent mixed.

The drug substance is weighed into the compounding vessel. The drug substance can be used from a single container or lot or pooled from multiple containers and lots. The diluent is added to the drug substance through a filter to the targeted final weight of the bulk drug product formulation. The pH, conductivity and A-Mab concentration are measured on-line to confirm complete mixing and conclusion of the compounding step.

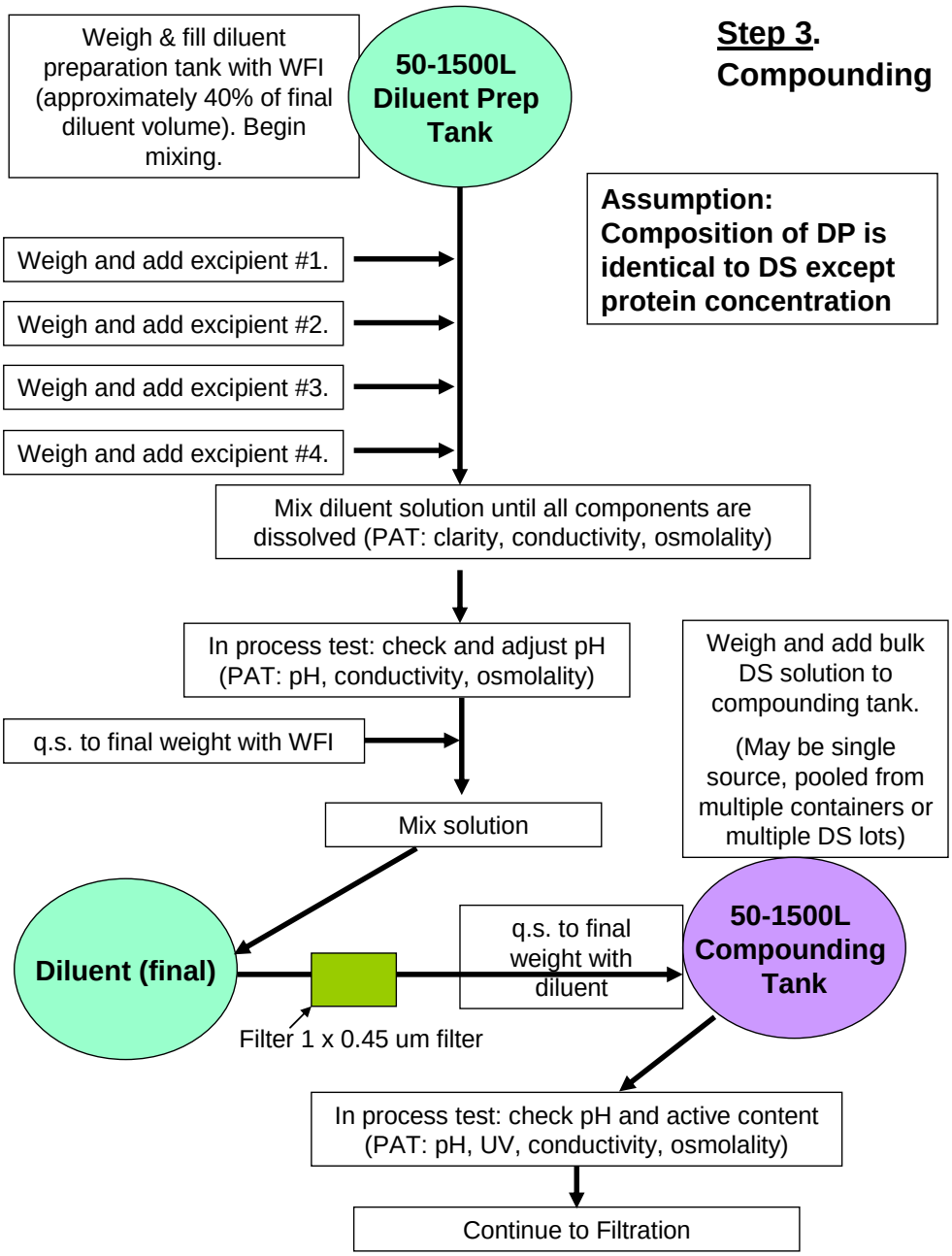

Figure 5.5 Process Flow Schematic of Step 3, Compounding

5.3.1.2 Development History - Prior Knowledge and Design Space

Compounding is a standard step in the production of mAbs. Development information from previous products can be applied directly to A-Mab with only verification of applicability being required. This section summarises the prior knowledge from development studies as well as from batches made for non-clinical and clinical studies for multiple marketed mAbs. The design space proposed for A-Mab in Table 5.7 is, in fact, identical to the equivalent design spaces approved for X-Mab, Y-Mab and Z-Mab.

Table 5.7 Summary of Prior Knowledge for Compounding and Design Space

Parameter	Experience Base	Design space	Summary of Knowledge
Diluent Prep			
Mixing speed	X-Mab, Y-Mab and Z-Mab	75-150 rpm	Prior experience at 50-1000 L scale shows that the process ranges given here are suitable to dissolve the excipients completely. The mixing time required for complete dissolution in this operating range is < 30 minutes.
Temperature of WFI		15-25°C	
Storage and Handling of Polysorbate 20			
Fill volume	X-Mab, Y-Mab and Z-Mab, E-Mab, F-Mab	0.46-0.97 PS20 volume/Head Space volume ratio	Prior experience shows that greater headspace volume corresponds to higher peroxide value. Less Peroxide content is seen with a lower air content in the headspace. Based upon this experience, a low headspace volume and nitrogen overfill with storage at controlled room temperature is recommended.
Head space		Air to Nitrogen	
Drug Substance Dilution			
Temperature of Drug Substance	X-Mab, Y-Mab and Z-Mab	5-25°C	Prior experience of dilution at 50 to 150 L scale showed that these temperatures and mixing speed and time did not impact the aggregate levels of the mAbs. This experience was gained in multiple tank and stirrer geometries.
Mixing Time		3-600 minutes	
Mixing Speed		5-100 rpm	
Hold Times for drug substance, bulk drug product and diluent			
Time	X-Mab, Y-Mab and Z-Mab	0-10 days for Drug substance 0-5 days for bulk drug Product	Prior experience shows that the impact of hold time is through the metals leached from the containers and any bioburden that may occur. The impact of metals needs to be studied for each mAb since this may be product specific. The maximum level of metals observed was 15 ppm Fe.
Temperature of Drug Substance/Product		5-25°C	
Container		EVA/PE/SS*	

* EVA – ethyl vinyl acetate, PE – polyethylene, SS – stainless steel

5.3.1.3 Initial Risk Assessment and Classification of Input Process Parameters

A risk assessment utilizing a cause and effect matrix was used and the results shown in Table 5.8. The highest scores in red were considered highest priority. The rank order for A-Mab is exactly the same as for previous antibodies.

Table 5.8 Initial Cause and Effect Risk Assessment Table for the Compounding Step, Including Diluent Preparation, Dilution of Drug Substance, and Bulk Drug Product Preparation

Rank	10	10	7	10			
Quality attribute / Parameter	Protein Content Uniformity	Monomer	Particulate Matter - Visible	Particulate Matter - Sub Visible	Score	Experimental Strategy	Classification
Temperature - DS dilution	5	7	7	7	239	DOE/modeling	CPP
Mixing time - DS dilution	7	7	7	7	259	DOE/modeling	CPP
Mixing Speed - DS dilution	7	7	7	7	259	DOE/modeling	CPP
Impeller/mixer configuration - compounding tank	7	7	7	7	259	DOE/modeling	WC-CPP
Compounding tank size	5	5	5	5	185	DOE/modeling	WC-CPP
Hold time of DS @ RT	1	5	5	5	145	Prior knowledge	CPP
Hold time bulk DP @ RT	1	5	5	5	145	Prior knowledge	CPP
Hold time of diluent @ RT	1	1	1	1	37	Prior knowledge	GPP
Temperature - diluent - dissolution	1	1	1	1	37	Prior knowledge	GPP
Mixing time - dissolution	1	1	1	1	37	Modeling	GPP
Mixing speed - dissolution	1	1	1	1	37	Modeling	GPP
Order of excipient addition	1	1	1	1	37	Prior knowledge	GPP
Diluent tank size	1	1	1	1	37	Prior knowledge	GPP

The classification of the parameters was performed on the basis of the above analysis. Two parameters were classified as WC-CPPs since they are inherently well controlled. Once an impeller configuration and tank size has been selected based upon experimentation and modeling, they are fixed. Changing product demand may change scale, but these parameters do not vary during the execution of the compounding step itself.

The rest of this section will focus on the CPPs and WC-CPPs only. Two sets of studies will be described that cover the CPPs and the WC-CPPs:

(1) elucidation of the dilution system (tank sizes and configuration by modeling) and dilution operating parameters (temperature and mixing speed by DOE)

(2) confirmation of prior knowledge based hold times of DS and bulk DP

5.3.1.4 Application of scale-up and mixing model to drug substance dilution system

Dilution of A-Mab bulk drug substance with diluent to produce bulk drug product has been chosen to exemplify the use of models for mixing and scale-up. Data obtained at 50 L scale have been used to predict operating conditions at 500 L and 1500 L scales. The approach used for A-Mab was based on chemical engineering principles commonly used in other industries for Newtonian fluids. The Section provides the results from studies done at the various scales to verify the output of the model

In this case study, two extreme operating conditions from the 50 L tank were used for scale-up. One was the minimum time at low temperature and low speed to obtain complete mixing. The other was a maximum time at room temperature at highest speed that was shown to not cause degradation of the product.

Scale up of mixing is dependent on the geometry of the vessel and mixer and process parameters, such as mixing speed and solution temperature, since the flow pattern of the fluid is impacted by

these parameters. The actual details of the model developed are provided in the Appendix Section 5.8. A key element of this approach is the dimensional analysis allowing the calculation of dimensionless numbers.

5.3.1.5 Scale Data to Verify Model Output

Data at the 50 L, 500 L and 1500 L scales were collected during process demonstration batches. The 500 L and 1500 L tanks were configured as per model output. The studies were performed at two temperatures at mixing speeds specified by the model. Protein concentration and aggregate levels were monitored during the process. No decrease in monomer was noted in any case.

Graphical representation of the protein concentrations are given below.

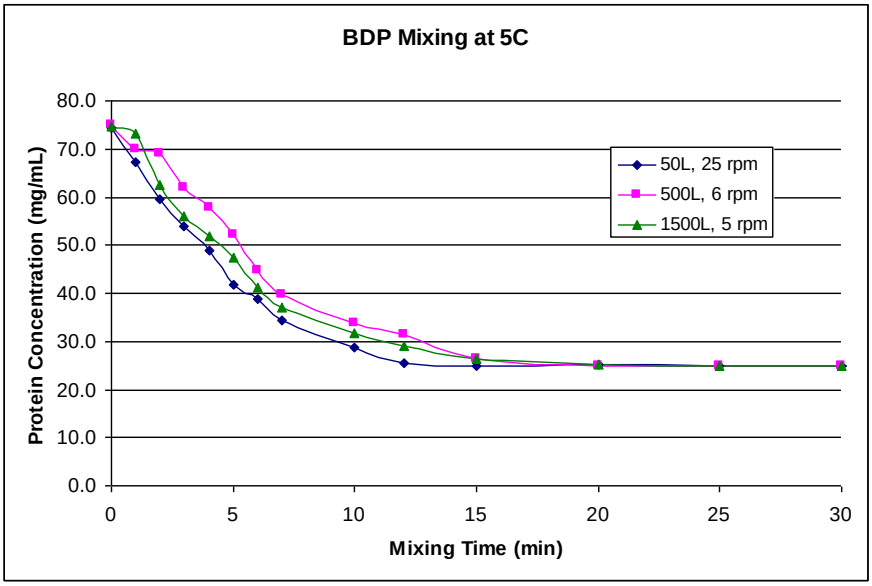

Figure 5.6 Mixing at 5°C for the 50 L, 500 L and 1500 L vessels of A-Mab Solutions During the Compounding Step of Drug Substance Solution Dilution with Prepared Diluent

Mixing speeds for the 500 and 1500 L vessels are based on the modeling completed with a 50 L vessel.

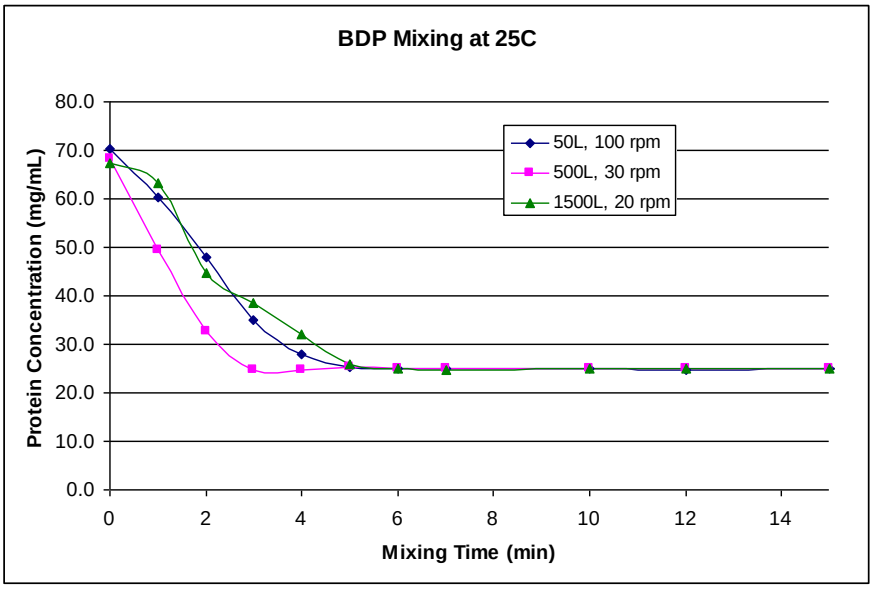

Figure 5.7 Mixing at 25C for the 50 L, 500 L and 1500 L vessels of A-Mab Solutions During the Compounding Step of Drug Substance Solution Dilution with Prepared Diluent

Mixing speeds for the 500 and 1500 L vessels are based on the modeling completed with a 50 L vessel.

5.3.1.6 Confirmation of prior knowledge - Hold times and temperatures of drug substance/product

Prior experience demonstrated that drug substance hold times of up to 10 days at room temperature followed by up to 5 days for the bulk product caused no product quality deterioration.

The A-Mab hold-times were confirmed at scale during engineering runs. Using worst-case scenarios, the longest allowable hold-time was used to manufacture the engineering batch at room temperature. This verifies the part of the design space with the greatest risk to the quality parameters. The drug substance hold time of 10 days at room temperature was followed by bulk drug product hold time of 5 days at room temperature. After completion of each hold time step, the holding tanks were aseptically sampled. The drug substance and bulk drug product were tested for bioburden, aggregate (monomer and sub-visible particles) and oxidation. Metal content in solution was measured after each hold time for information only (Table 5.9). The results show that all acceptance criteria are met under worst case conditions, verifying the applicability of the prior knowledge.

Table 5.9 Drug Substance and Bulk Drug Product Hold Time Study Results

Drug Substance after Hold Time			Design Space: 0- 10 days at 5 – 25°C		
Study parameter: 10 Days at RT in SS	**Bioburden**	**Aggregates**	**Oxidation**	**Subvisible Particulates (HIAC)**	**Fe ion Content (ppm)**
Acceptance Criteria for quality parameters	<10 CFU/100 mL	< 5%	< 10%	per Compendia	< 15 ppm
Lot 1	0 CFU/100 mL	2.0	2.6	2006/mL ≥ 2μm 1420/mL ≥ 5μm 125/mL ≥ 10μm 56/mL ≥ 25μm	< 0.2
Lot 2	0 CFU/100 mL	2.1	2.8	3245/mL ≥ 2μm 1653/mL ≥ 5μm 269/mL ≥ 10μm 85/mL ≥ 25μm	< 0.2
Lot 3	0 CFU/100 mL	1.8	2.8	1908/mL ≥ 2μm 999/mL ≥ 5μm 103/mL ≥ 10μm 32/mL ≥ 25μm	< 0.2
Subsequent Bulk Drug Product Hold Time			**Design Space: 0- 5 days at 5 – 25°C**		
Study parameter: 5 Days at RT in SS	**Bioburden**	**Aggregates**	**Oxidation**	**Subvisible Particulates (HIAC)**	**Fe ion Content (ppm)**
Acceptance Criteria for quality parameters	≤10 CFU /100 mL	< 5%	< 10 %	per Compendia	< 15 ppm
Lot 1	0 CFU/100 mL	2.0	2.3	1222/mL ≥ 2μm 521/mL ≥ 5μm 123/mL ≥ 10μm 15/mL ≥ 25μm	< 0.2
Lot 2	0 CFU/100 mL	2.2	3.1	2325/mL ≥ 2μm 1267/mL ≥ 5μm 234/mL ≥ 10μm 34/mL ≥ 25μm	< 0.2
Lot 3	0 CFU/100 mL	2.0	2.9	2721/mL ≥ 2μm 802/mL ≥ 5μm 175/mL ≥ 10μm 22/mL ≥ 25μm	< 0.2

5.3.1.7 Compounding: Design Space, Control Strategy and Final Risk Assessment

The extensive prior knowledge, initial risk assessment, scale model studies and verification studies led to the generation of a design space ensuring a uniform product concentration for the

compounding operations. The studies confirm that the sub-unit operations in the compounding step do not impact aggregation or particle formation.

Table 5.10 Compounding: Design Space and Control Strategy

Process Parameter	Range	Justification	Control Strategy	Classification
Temperature - DS dilution	5 - 25°C	covers typical operations without increase in aggregate - study at scale	Batch record procedures	WC-CPP
Mixing time - DS dilution	3-600 min	covers typical operations without increase in aggregate - study at scale	Batch record procedures	WC-CPP
Mixing Speed - DS dilution	5 - 100 rpm	covers typical operations without increase in aggregate - study at scale	Batch record procedures	WC-CPP
Hold time of drug substance @ RT	0 - 10 days	covers typical operations without increase in aggregate, oxidation or bioburden - hold time based on prior knowledge and experimental confirmation at scale	Batch record procedures	WC-CPP
Hold time bulk drug product @ RT	0 - 10 days	covers typical operations without increase in aggregate, oxidation or bioburden - hold time based on prior knowledge and experimental confirmation at scale	Batch record procedures	WC-CPP

A final risk assessment was performed after putting the above design space and control strategy in place and is shown in Table 5.11 below. Based on the experiments and enhanced process knowledge, as well as an evaluation of the control strategy, all parameters earlier classified as CPPs are now re-classified as WC-CPPs.

Table 5.11 Compounding: Final Risk Assessment and Parameter Classification

Rank	10	10	0	10		
Quality attribute / Parameter	Protein Content Uniformity	Monomer	Particulate Matter - Visible	Particulate Matter - Sub Visible	Score	Final Classification
Temperature - DS dilution	1	1	1	1	37	WC-CPP
Mixing time - DS dilution	1	1	1	1	37	WC-CPP
Mixing Speed - DS dilution	1	1	1	1	37	WC-CPP
Hold time of DS @ RT	1	1	1	1	37	WC-CPP
Hold time bulk DP @ RT	1	1	1	1	37	WC-CPP

5.3.1.8 Life-Cycle Management

The details outlined in the compounding section demonstrate the use of prior knowledge and models for scale-up to allow flexibility in the manufacture of A-Mab drug product batches from 50 to 1500 liters in size. The model has been confirmed experimentally and will not require further experiments at scale.

Hold times confirmed here are similar to other marketed company mAbs and extend the platform knowledge for this operating parameter to A-Mab.

5.3.2 Step 4: Sterile Filtration

In this section, a systematic (QbD) approach is described that can be used to define a sterile filtration process platform based on three monoclonal antibodies (X, Y and Z-Mab) through small scale experiments, and show how this platform is applied to A-Mab as a "next in class" molecule, requiring only limited experimental work.

5.3.2.1 Introduction

Although the failure of the sterile filtration step in final bulk drug product processing is recognized as "fatal", it is not typically subjected to the same systematice rigor of robustness studies as the other unit operations. Hence, there is no body of data generated that links process parameters and product quality attributes for monoclonal antibodies in our prior knowledge that demonstrates enhanced process and product understanding for sterile filtration.

To establish the design space for the sterile filtration process platform, multivariate experiments looking at properties of material of construction of the filter e.g. adsorption of active and excipients, and effect of leachants, combined with fluid dynamics dependency of flow velocity, contact time and processing temperature are considered. However, these are not discussed in detail here to define the design space.

Figure 5.8 shows a schematic flow chart of the steps outlined in this case study to establish the sterile filtration process platform to develop a useful prior knowledge database on which to base the A-Mab process.

Figure 5.8 Schematic of the steps presented to establish a sterile filtration process platform and apply it to A-Mab.

5.3.2.2 Definition of Target Process

This section describes the sterile filtration process for A-Mab. The process is designed to cover batch sizes from 50 L to 1500 L total volume of bulk drug product solution. The filtration operation starts directly after compounding. The formulated bulk drug product solution is filtered through one 0.45 µm pre-filter and one 0.22 µm filter in series for bio-burden reduction into a sterile holding tank and held until filling under nitrogen atmosphere. The solution is then sterilized by filtration through two 0.22 µm filters in series into the reservoir of the filling operation which is the next processing step. The process scheme of the sterile filtration process is illustrated in Figure 5.9

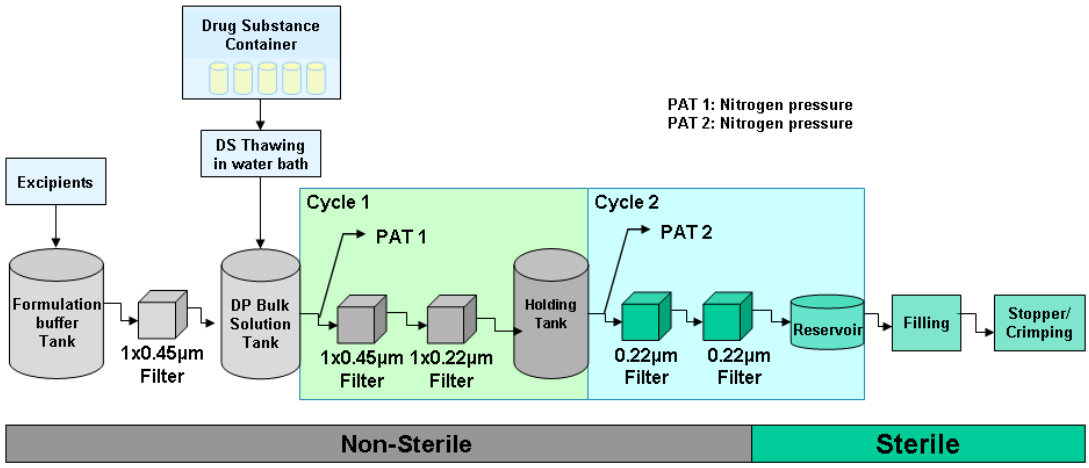

Figure 5.9 Target Process Scheme and PAT Tool for Monitoring Nitrogen Pressure

5.3.2.3 Identification of process parameters

As illustrated in the scheme shown in Figure 5.9, the sterile filtration process can be subdivided into to two separate sub-unit operations (cycles 1 and 2) that can be performed several times during sterile filtration processing. The two key aspects of both cycles are filtration then holding. Based on these steps, all parameters that have a potential effect on cycle 1 and cycle 2 were identified as illustrated in the fishbone diagram in Figure 5.10..

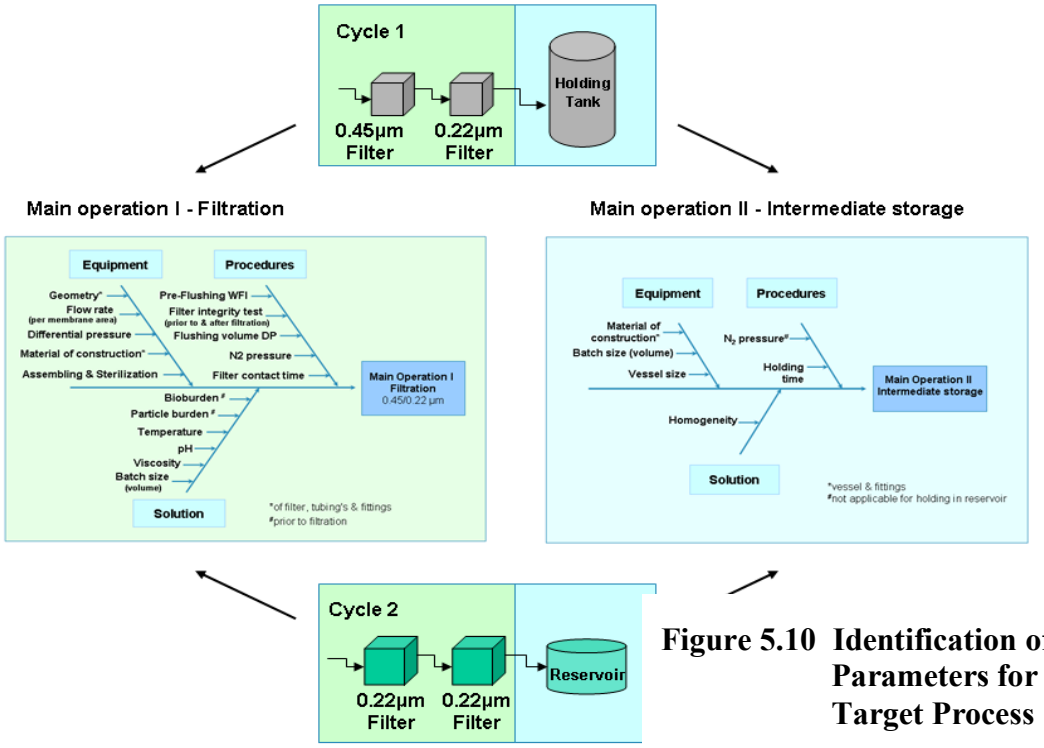

Figure 5.10 Identification of Process Parameters for the Target Process

5.3.2.4 Enhancement of Prior Knowledge

Knowledge on standardized methods described in the literature (especially on scale-up that can be leveraged to identify the optimal filter parameters for a given process and scale), (PDA 1998, FDA 2004), together with the available prior knowledge from formulation and process development (e.g. preclinical and clinical supply, and commercial manufacturing) of X, Y- & Z-Mabs, is the source for the initial risk assessment of the identified process parameter.

5.3.2.5 Initial Risk Assessment

Based on the defined target process and the identified process parameters, the initial risk assessment, using a cause and effect analysis tool, provided a ranking order of which process parameters were most critical for the small scale studies using X-, Y- & Z-Mab. The parameters ranked high and medium were explored in the design space studies.

Table 5.12 shows the ranking criteria used for the initial risk assessment. For this case study, the main product quality attributes used in the risk assessment are aggregates, sub-visible and visible particles. However, bacterial endotoxins and sterility are also considered due to their direct relevance to the sterile filtration process. Table 5.13 shows only those results, ranked high or medium. Parameters ranked with low criticality level are not shown.

Table 5.12 Criteria for the Ranking Used in the Risk Assessment

Designation		Criteria/Rationale
Criticality Score	**Criticality Level**	
47 ≤ Score ≤88	Low	No parameter scores above 4
89 ≤Score ≤136	Medium	At least one parameter scores 5
Score ≥ 137	High	At least one parameter scores 7

Table 5.13 Initial Risk Assessment for Filtration Unit Operations

Rank	10	7	10	10	10	NA	NA
Quality Attribute / Parameter	Aggr.	Visible particles	Sub-visible particles	Bacterial Endotoxin	Sterility	Rationale / Comment	Score
Cycle I - Filtration (0.45 µm/ 0.22 µm and 0.22 µm/ 0.22 µm)							
Filter integrity test (prior to and after filtration)[1]	1	7	7	10	10	Impact on particulate matter, endotoxin and sterility	329
Flushing (Pre-run) volume DP bulk solution	5	10	10	1	1	Particles shedding from the filter	240
Pre-flushing volume WFI	5	10	10	1	1	Effect of particles and oxidative species might influence the formation of aggregates or sub-visibles	240
Bioburden prior to filtration	1	1	1	7	7	Bioburden level can effect Bacterial Endotoxins and Sterility	167
Flow rate per membrane area	7	1	5	1	1	Level of induced shear stress can lead to aggregation	147
Material of construction[1]	1	5	5	1	1	Adsorption of formula components onto the filter membrane, particle shedding and leachables from the filter	115
Filter size (membrane area)	1	1	1	1	7	Filter size dependent on batch size	107
Filter contact time	1	1	1	1	7	Contamination due to microbial growth through the membrane	107
Cycle II - Intermediate storage							
Material of construction[1]	7	7	7	1	1	Adsorption of formula components and leachables can induce aggregation and fragmentation	209
Homogeneity of the solution[1]	1	7	7	1	1	Solution homogeneity affects filter performance	149

[1]Process parameters used in this risk assessment that cannot be adjusted and/or have to meet vendor specifications will not be considered for the definition of the design space.

5.3.2.6 Establishment of the Process Platform

The outcome of the initial risk assessment determined the essential characterization studies to establish the sterile filtration process platform and define the design space using X, Y and Z-Mabs. The steps taken in executing the characterization studies are shown schematically in Figure 5.11. The experiments are classified into three modules: 1. pre-selection, 2. characterization of fluid flow characteristics and 3. materials compatibility assessment.

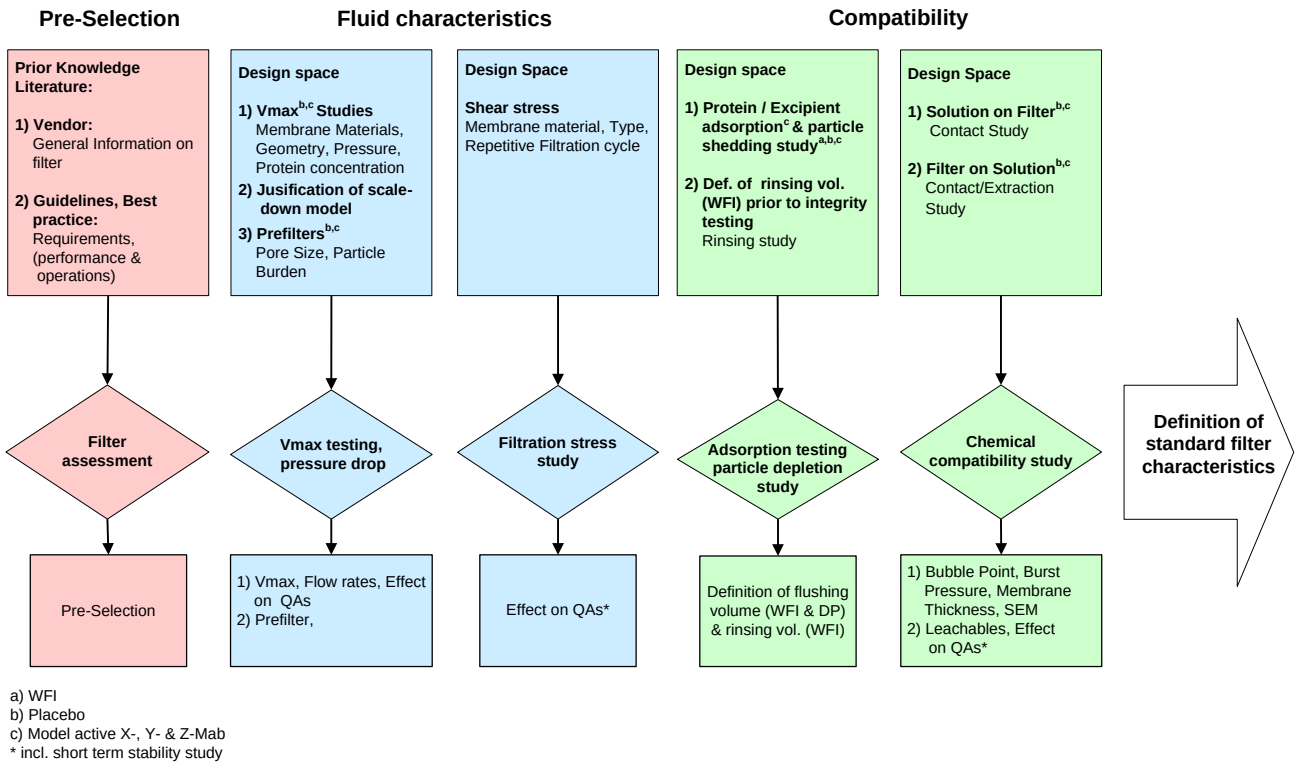

Figure 5.11 Schematic for sterile filtration characterization program to establish the process platform using X, Y and Z-Mabs.

5.3.2.7 Characterization Program

Consistent results of the characterization program using X, Y and Z-Mabs established a process platform design space. These results determined filter characteristics that ensure the best sterile filtration performance and establish standard filter configurations to be used with "next in class" monoclonal antibodies. In this case study, two filter configurations, PES and cellulose operated in combination of 0.45µm followed by 0.2µm pore sizes met the optimal characteristics determined by the study.

5.3.2.8 Design Space of the Platform Process

The sterile filtration process platform design space, established using X, Y and Z-Mabs is summarized in Table 5.14. This platform design space allows the use of only a limited set of experiments to verify the design space for A-Mab. These verification studies follow a modular

approach, illustrated in Figure 5.12, focusing on fluid characteristics and drug product specific filter compatibility.

Table 5.14 Summary of the Design Space for Platform Sterile Filtration Process

Process Parameter	Quality Attribute Impact	Design Space Upper Limit	Design Space Lower Limit	Target
Pre-run flushing volume with DP bulk solution	Vis.- and sub-visible particles, Aggregation	PES: 0-5 L Cellulose: 0.8 L	PES: 0.1 L Cellulose: 0.2 L	PES: 0.3 L Cellulose: 0.5 L
Pre-flushing volume WFI	Vis.- and sub-visible particles, Aggregation	50 L	10 L	20 L
Bioburden level prior to filtration	Sterility	≤ 10 CFU / mL	0 CFU / mL	≤ 10 CFU / mL
Flow Rate per membrane area (Flux)	Aggregation, Vis. and sub-visible particles	PES: 217 L/min/m^2 Cellulose: 142 L/min/m^2	PES: 60 L/min/m^2 Cellulose: 35 L/min/m^2	PES: 60 L/min/m^2 Cellulose: 35 L/min/m^2
Filter size (Membrane area per filtration volume based on Vmax80%)	Sterility, Bioburden	PES: 5.6×10^{-4} m^2 / L Cellulose: 9.4×10^{-4} m^2 / L	PES: 3.1×10^{-4} m^2 / L Cellulose: 6.5×10^{-4} m^2 / L	PES:3.9×10^{-4} m^2 /L Cellulose: 7.4×10^{-4} m^2 / L
Filter contact time (Filtration of bulk drug product)	Sterility	24 h	30 minutes	≤ 12 h

5.3.2.9 Strategy for the Design Space for A-Mab using the established Process Platform

The specific studies necessary to verify the design space for A-Mab, based on the sterile filtration process platform, are closely aligned with the filter characterization program used for X-,Y- and Z-Mabs. The first module of A-Mab (product specific) design space verification assesses the fluid characteristics of the filter. The second module verifies the drug product specific compatibility of the formulation with the filter e.g., materials of construction, particle shedding. If the data obtained from those two modules demonstrate comparability with the design space of X-,Y-, and Z-Mab, the process platform is justified and therefore can be applied to A-Mab.

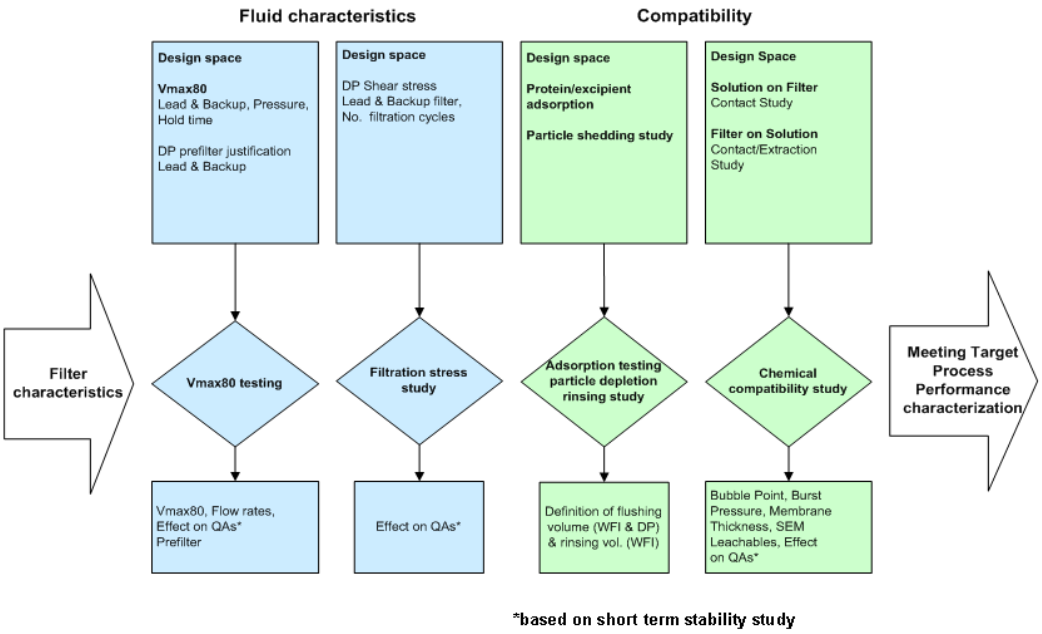

Figure 5.12 Scheme for application of platform design space to A-Mab processing

5.3.2.10 Process Demonstration/Verification

Based on the modular approach to characterize the sterile filtration operations, the enhanced prior knowledge and process and product understanding, on-going continuous process verification could be established to monitor the process executed at full scale.

5.3.2.11 Control Strategy

The control strategy for the sterile filtration process, based on the established platform process and its application to A-Mab is shown below in Table 5.15. The studies executed to define the design space for X-, Y-, Z- & A-Mab provide deeper understanding of impact of the process parameters on the product quality attributes and guided the designations of critical process parameters (CPP), well-controlled critical process parameters (WC-CPP), key performance parameters (KPP) and general process parameters (GPP). According to these designations, the control strategy is defined to

mitigate the impact of the given parameters by operation within the design space and the drug product specification testing done for product release.

Table 5.15 Designation of Process Parameters for Sterile Filtration Unit Operations and proposed Control Strategy

Parameter	Designation	Control Strategy
Main operation I - Filtration (0.45 µm/0.22 µm and 0.22 µm/0.22 µm)		
Flushing (Pre-run) volume DP bulk solution	WC-CPP	Controlled within design space (pre-run) volume DP solution [a]
Pre-flushing volume WFI	WC-CPP	Controlled within design space WFI pre-flush volume [a]
Bioburden prior to filtration	WC-CPP	Release testing of raw materials (excipients & drug substance)
Flow rate per unit of membrane area	WC-CPP	Filter specification (C of A) N2 pressure[a] controlled within the design space (PAT)
Filter size (membrane area)	WC-CPP	Filter specification - depends on batch size
Filter contact time	KPP	Controlled within quality system [a]

[a] Documented in batch record

5.3.3 Step 5: Filling, Stoppering, and Capping

5.3.3.1 Definition of Target Process

After sterile filtration, the A-Mab formulated bulk solution is transferred to a surge tank, followed by standard filling and stoppering procedures. The A-Mab-filled vials are stoppered with Teflon-coated rubber stoppers at the end of the filling line, and capped with aluminum/plastic flip-off crimp seals. After capping, the vials are inspected for particulates and content clarity. The vials that pass inspection are stored at 2-8°C until needed. They will then be transferred for labeling and packaging.

A general process flow illustrated in Figure 5.13 highlights the core unit operations: filling, stoppering, capping, inspection and packaging. Preparatory steps for the stoppers and vials that involve washing, sterilization or depyrogenation are considered supportive processes and are not included in the scope of these studies.

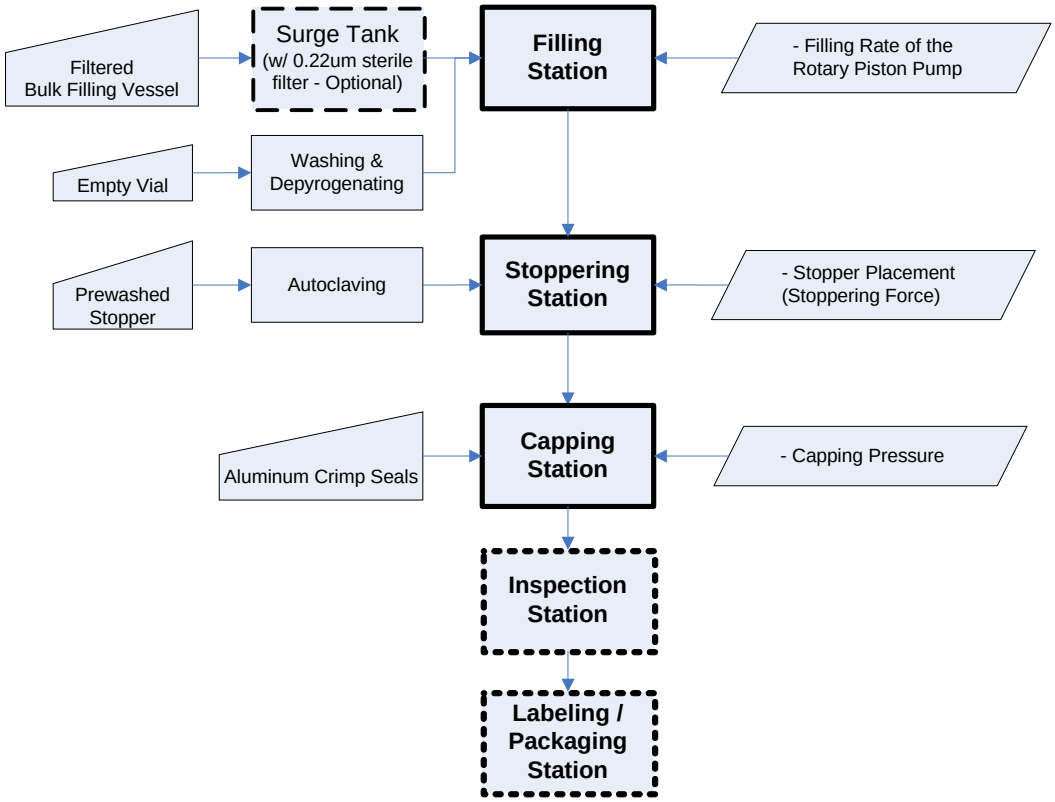

Figure 5.13 Process Flow Diagram of the Filling, Stoppering, and Capping Processes

The target filling line facility is intended to have a 6 head rotary piston pump. Typical fill speed is at 25 vials per minute per pump head. Typical batch size is 1,500 L of A-Mab formulated drug product which can be filled in approximately 9 hours. This processing time is within the limit of 28 hours fill duration validated by media fill challenge. For pilot scale studies, a batch size of 50 L is filled in approximately 20 minutes with the same filler system. This batch size represents the low end of the production range.

5.3.3.2 Prior Knowledge

The four most commonly used fill pumping systems in the industry are rotary piston pump, rolling diaphragm pump, peristaltic pump, and time pressure pump. These various pumping mechanisms produce different levels of mechanical stresses on the protein solutions, such as shear, agitation and cavitation. Each of these stresses may lead to antibody denaturation and subsequent aggregation and/or formation of particulates.

The scope of the pre-manufacturing scaled development work related to filling involved evaluating the impact of pumping mechanism on product quality as well as the effects of stoppering and capping conditions on product vial integrity. In this case study, it is proposed that the knowledge and experience gained from these historic development studies may be applied to support A-Mab and other monoclonal antibodies with similar formulations.

Filling operations are usually scaled up by increasing the numbers of pump heads, not by increasing the pump speed or the size of the pump. Therefore, for process characterization, a single pump head can be considered as a "scaled down model". This model has been employed in over 20 development cases for different monoclonal antibody products in the past and has demonstrated to be representative of at-scale operations. By carrying out some of the characterization studies at worst case conditions; it is readily apparent whether a predetermined design space can be applied to the current product (A-Mab).

Although the studies described in this case study were conducted using a rotary piston pump filling system, the same experimental procedures may be applied to other pumping systems, such as rolling diaphragm and peristaltic pumps. Fill accuracy is not part of the scope and is addressed during equipment validation.

5.3.3.3 Risk Ranking, Process Characterization, CPP Determination, and Control Strategy

The first step was to perform an initial risk ranking to select the relevant process parameters of the core unit operations that may have an impact on A-Mab drug product attributes (e.g. aggregates, particulates, container integrity, and etc.). For each product attribute, identification is performed using a Risk Ranking and Filtering (RRF) tool that assesses each process parameter for its main effect and potential interaction effects with other process parameters. The severity score determines the type of characterization studies performed (i.e. univariate or multivariate).

The determination of the main and interaction effect relies on process knowledge by technical experts, understanding of the physical process, historical manufacturing data, and development. Main effect and interaction effect impact ranks are multiplied to generate an overall Severity score. For filling, the main product attribute considered is aggregation. For stoppering and capping, the main product attribute considered is seal integrity. The scoring criteria are summarized in Table 5.16. Definitions for the relative impact descriptions and ranking are provided in Table 5.17.

Table 5.16 Scoring Criteria for Risk Ranking

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

Table 5.17 Definition of Main Effect Impact and Scoring

Impact Description	Definition	Score
No Impact	Effect causes variation in process output which is not expected to be detectable (e.g., no effect or within assay variability)	2
Minor Impact	Effect causes variation in process output which is expected to be within acceptable range	4
Major Impact	Effect causes variation in process output which is expected to be outside acceptable range (can be near edge of failure)	8

Effect is considered for variation of parameter across a proposed design space range.

An example of this risk ranking study to gauge the impact of the rotary piston filler process parameters (presumptive CPPs) on protein aggregation is shown in Table 5.18.

Table 5.18 Risk Ranking Study for the Rotary Piston Filler Process Parameters on Protein Aggregation

Process Parameter	Proposed Design Space Range		Main Effect Score	Rationale for (M)	Inter-action Score	Rationale for (I)	Severity Score	Potential Interaction Parameters	Recommended Characterization Studies
	Low	**High**	**(M)**	**Main Effect**	**(I)**	**Interaction Effect**	**(M x I)**		
Pump Speed/ head (vpm)	10	40	8	Shear effects and foaming due to air interaction may cause aggregation	4	Other parameters may exacerbate foaming effects	**32**	Temperature, Fill volume, nozzle position	Multivariate study with fill temperature, nozzle diameter, and nozzle position
Fill Temperature (°C)	2	20	2	A-Mab has good stability even at RT	4	May have additive effect	8	Pump speed	See pump speed study
Nozzle Diameter (mm)	1	2	4	Diameter affects jetting of solution leaving nozzle	4	May have additive effect	16	Pump speed	See pump speed study
Nozzle Position (mm)	0.5	2.5	4	Height affects amount of air interaction	4	May have additive effect	16	Pump speed, nozzle diameter	See pump speed study
Fill Volume (L)	40	2000	8	Volume affects number of pump strokes. Product in between piston and wall may be over stressed leading to aggregation	4	May have additive effect	**32**	Pump speed	Multivariate study with pump speed and number of strokes per pump head

5.3.3.4 Process Characterization

The results of the RRF exercise recommended that a multivariate filling study and a multivariate pumping recirculation study be performed. A similar exercise can be performed for the stoppering and capping operations (not shown). The resulting list of recommended process characterization studies based on this type of risk assessment is shown in Table 5.19. The ultimate goal of these studies is to build a knowledge space for the unit operation, hence to define an acceptable design space and control space. Outcome from these studies will help address typical process optimization needs in the future and demonstrate understanding of the manufacturing process.

Table 5.19 Modular Process Characterization Study

Development Activities	Study Objective	Processing Parameters
Pumping Study	To evaluate effects of the rotary piston pumping mechanism by using worst case recirculation scenarios on the formulated A-Mab, hence to fully understand the extent of shear damage.	• Pump speed • # of piston strokes
Filling Study	To evaluate the effect of filling parameters by varying pumping speed along with its nozzle position using sterile, filtered A-Mab.	• Pump Speed • Temperature • Nozzle ID • Nozzle Position
Seal Integrity Study	To demonstrate seal integrity of its dosage vial by varying stoppering and crimping process parameters.	• Stoppering Force • Crimping Pressure • Line Speed
Engineering Runs	To verify the results of filling and capping 20 cc vials containing A-Mab under various full scale operating conditions within the given limits.	• Pump Speed • Stoppering Force • Crimping Pressure

An example of a design of a filling study is presented next.

5.3.3.4.1 Experiment Design - Filling Study

A single head rotary piston pump was operated at its highest and slowest speed to fill product into vials. In order to assess filling nozzle effects, the position and the size of the nozzles were also varied. The nozzle position was defined as the insertion depth of the nozzle tip into the vial during filling. The nozzle ID was measured at the opening canal. Furthermore, temperature effect during filling was also evaluated by conducting the experiments at both room temperature and 2-8°C in a cold room environment. Although the filling line in the actual manufacturing facility is designed to

operate at room temperature, the product is maintained at 2-8°C during the fill. The range of different processing parameters studied in a DOE screening format is illustrated in the Table 5.20.

Table 5.20 Filling Study DOE

Number	Pattern	Temperature (°C)	Nozzle ID Size (mm)	Nozzle Position (mm)	Pump Speed (Unit / min)
1	++−−	20	2	0.5	10
2	+−0−	20	1	1.5	10
3	++−0	20	2	0.5	25
4	−−−−	5	1	0.5	10
5	++00	20	2	1.5	25
6	−+−0	5	2	0.5	25
7	−−00	5	1	1.5	25
8	−−++	5	1	2.5	40
9	−+−+	5	2	0.5	40
10	−++−	5	2	2.5	10
11	+−+0	20	1	2.5	25
12	++++	20	2	2.5	40
13	−+0−	5	2	1.5	10
14	−++0	5	2	2.5	25
15	+−−+	20	1	0.5	40
16	++0+	20	2	1.5	40
17	−+0+	5	2	1.5	40
18	+++−	20	2	2.5	10

+ represents the higher limit within a specific range
- represents the lower limit within a specific range
0 represents the mid-point within a specific range

5.3.3.4.2 Filling Study Results

Table 5.14 is a summary of the DOE study. The size of the dark sphere represents the magnitude of aggregation; the larger the sphere, the greater the measured aggregation. From all the parameters evaluated in this study, pumping speed was determined to be the critical process parameter affecting A-Mab aggregate generation.

Within the knowledge space, a design space and control space were defined where protein aggregation is controlled yet providing adequate scope for manufacturing optimisation.

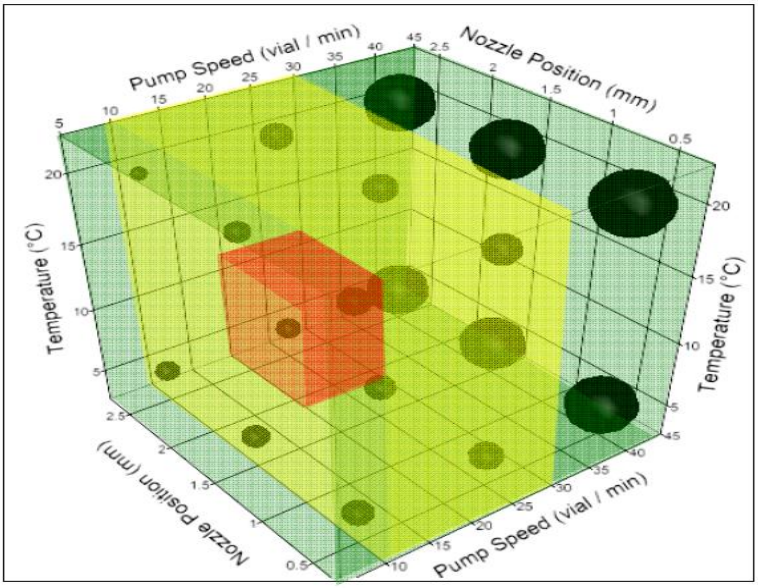

Figure 5.14 Knowledge Space Matrix from A-Mab Filling Study

Green zone represents the overall knowledge space, yellow zone represents the design space, red zone is the control space

The proposed design space is as follows: Temperature between 2-20°C; Pump speed between 10 to 30 vpm per head; nozzle ID between 1 to 2 mm and nozzle position between 0.5 to 2.5 mm.

Results from this DOE study may be used for future products. Instead of repeating this study, tests may be performed under worst case conditions (++++) of the design space (i.e. 20°C, 30 vpm, nozzle diameter of 1 mm and nozzle position at 0.5 mm). If no product impact is observed under these conditions, then one may conclude that the design space shown also applies to the new product.

5.3.3.5 Identification of Site Specific Critical Process Parameters

Using the data from the characterization studies, an FMEA risk ranking was performed to identify site specific CPPs and Table 5.21 lists the process parameter risk analysis for filling, stoppering, and capping.

Table 5.21 FMEA Risk Ranking of a Specific Site

Process Parameter	Potential Failure Mode	Potential Failure Effect(s)	Risk Assessment				Control Strategy	Final Parameter Designation
			Severity	Occurrence	Detection	RPN		
Pumping Speed	Greater than maximum allowed speed	Aggregation splashing	6	6	6	216	Actual speed of specific filler is documented during each fill accuracy check. Defined acceptable operating range from design space	WC-CPP
Stopper force	Not fully seated (for liquid products)	Loss of seal integrity	10	10	2	200	Raised stopper detection in the filler. Define acceptable operating range from design space.	WC-CPP
Stopper placement	Unstoppered vial exits filler	Loss of seal Integrity	10	4	6	240	Stopper detection system catches this failure mode prior to capping. Visual detection can be effective -needs to be in SOP/batch record, final vial inspection.	WC-CPP
Capping Pressure	Outside the validated range	Critical defects - cracked vials, non-critical defects.	8	2	10	160	Trained operators. SOPs that specify machine set-up. Settings recorded at set up. Define acceptable operating range from design space.	WC-CPP

A risk priority number (RPN) was calculated by considering severity, occurrence, and detection, as defined in Table 5.22, Table 5.23, and Table 5.24 for product vial manufacturing. Both product impact and fill accuracy are considered for this analysis. Process parameters that have severity ≥ 8 or RPN > 72 are classified as CPPs. The CPP definitions are described in Table 5.25.

Table 5.22 Severity Evaluation Criteria

Effect	Criteria	Rank
Very High	Effect of parameter deviation causes definite impact to product quality; the lot(s) need(s) to be rejected.	10
High	Effect of parameter deviation will probably cause impact on product quality. One of the following or both occur: Discrepancy is initiated and product may be assessed after significant supplemental testing, which may include accelerated stability. Significant re-processing of batch required.	8
Moderate	Effect of parameter deviation potentially causes impact to product quality. One of the following or both occurs: Discrepancy is initiated and product may be assessed after supplemental t=0 testing. Minor re-processing of batch required	6
Slight	Effect of parameter deviation is unlikely to impact product quality. Both of the following occur: No supplemental testing is required, but a memo may be issued to address the discrepancy and release the lot. No re-processing of batch required.	4
Low/None	Effect of parameter deviation has no impact to product quality; no remediation/re-processing of batch is required.	2

Table 5.23 Occurrence Evaluation Criteria

Occurrence	Effect	Rank
Very high	The parameter failure occurs on the order of once every 100 units or greater	10
High	The parameter failure occurs on the order of once every 1000 units	8
Moderate	The parameter failure occurs on the order of once every 2000 units	6
Low	The parameter failure occurs on the order of once every 5000 units	4
Minimal	The parameter failure occurs on the order of once every 10000 units or lower	2

Table 5.24 Detection Evaluation Criteria

Detection	Criteria	Rank
None	This failure will not be detected with in-process or CofA testing	10
Low	In-process testing controls or monitoring will not detect this failure, but CofA testing will catch this failure.	8
Moderate	In-process testing or monitoring will not catch this failure during the unit operation, and detection is delayed several downstream unit operations, but prior to CofA testing.	6
High	The failure may or may not be detected by in-process controls or monitoring, but would definitely be detected in the next downstream operation.	4
Very High	The failure can be immediately and readily detected by inspection, in-process testing or monitoring controls, prior to downstream unit operation.	2

Table 5.25 Criteria to Determine CPP Designation

RPN Result	CPP Designation
RPN ≤ 48 (Low risk) and severity < 8	Parameter is not considered a CPP.
RPN > 48 and ≤ 72 (Medium risk), and severity < 8	Parameters are further analyzed for CPP designation. The analysis may include historical data review, literature review and assessment of manufacturing control range vs acceptable range. Corrective action may also be required to reduce RPN by reducing occurrence and/or improving detectability.
Severity ≥ 8 or RPN > 72 (High Risk)	Parameter is a CPP. In order to change the classification of this parameter to non-critical, additional actions, analysis, or controls must be considered to reduce the RPN result or severity rating.

The risks identified in this assessment exercise may be mitigated by implementing the control strategies listed in Table 5.21. This results in these CPP's classified as WC-CPPs. Note equipment preparation i.e., CIP (clean-in-place) and (SIP steam-in-place) is not in the scope of this evaluation. They will be handled separately through equipment qualification and validation programs.

Another type of risk assessment that may be performed is fault tree analysis (FTA). FTA is well suited to identify the operating conditions, operator's practice and processing environment in these core unit operations that may lead to a given failure mode. An example of the use of this analysis for aggregate formation is discussed in the FTA section.

5.3.3.6 Process Demonstration/Process Qualification

Finally, pilot scale batches are conducted to confirm the characterization study results. Process demonstration for A-Mab drug product filling, stoppering, and capping operation was covered by three separate engineering runs at 50 L batch scale using a 6 head pump system available in the filling facility. Each run took approximately 20 minutes to complete. The intent of these runs was to assess overall machinability of the filling line, the seal integrity of the primary packaging, the performance of the rotary piston pump, and the behavior of the A-Mab drug product solution during filling operation. Results from these runs allow setting the specific parameters within the design space that will provide assurance of proper operation during process qualification.

Critical process parameters that were identified in the design space (i.e., pumping speed, capping pressure, and stoppering force) will be analyzed. The primary goal is to verify the results of filling and capping 20 cc vials containing A-Mab under various full scale operating conditions within the given limits. These runs will cover the extreme conditions as well as a targeted set point within the control space defined in the pumping study, filling study, and seal integrity study.

Table 5.26 outlines the critical parameters under study for the three engineering runs. Samples will be drawn from the beginning, middle, and the end of each run in order to capture all possible effects in a full production timeframe. The success of these engineering runs will be measured by the ability to successfully process the three batches of A-Mab under the specific scenarios without compromising product attributes and seal integrity. Furthermore, outcome from these engineering runs will provide a high level of assurance that the manufacturing process can be validated to produce consistent, pre-determined quality attributes for final product that meet release criteria. Samples produced in these engineering runs are not intended for use in humans.

Table 5.26 Processing Parameters Outline of Engineering Runs

Engineering Run Number	Conditions	Pumping Speed (unit/min)	Capping Pressure (psig)	Stoppering Force (lbf)
1	Worst	30	30	50
2	Best	20	50	80
3	Target	25	40	65

The validation of all ancillary facilities, utilities, equipment and processes, e.g., clean rooms, water systems, sterilizers, and cleaning processes are outside the scope of this engineering study plan.

5.3.3.6.1 Process Demonstration and Qualification Results

Three batches of A-Mab bulks were filled at the 50 L scale at the conditions outlined in Table 5.26. Results from these engineering runs were found to be acceptable and consistent in terms of processing capability and compatibility. Samples drawn from different time points during the fills did not show any noticeable differences. The percentage of aggregate as a function of time for the three engineering runs is plotted in Figure 5.15. All samples passed seal integrity testing. Based on

these pilot scale results and the characterization studies, the proposed design space for filling, stoppering and capping is found to be robust and will provide drug product with acceptable levels of aggregation and vial closure integrity.

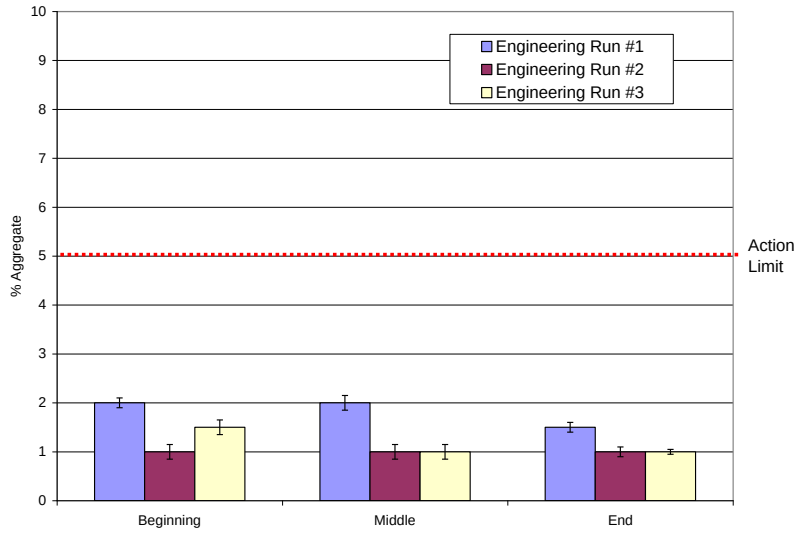

Figure 5.15 Engineering Run Result Summary for A-Mab

5.3.4 Life Cycle Management

Based on prior knowledge, filling throughput of A-Mab can be increased by increasing the number of pump heads on the filling line. Design space and control space of the critical process parameters derived from this case study can be applied to the throughput scale-up directly.

In the case of transferring filling operation from one site to another, different pumping mechanisms may be introduced. Rolling diaphragm pump, peristaltic pump, or time pressure filling pumps are the most commonly used filling system in processing biopharmaceutical products. Previous experience with other protein drug products shows that a rotary piston pump produces the highest amount of shear to the proteins. Thus, using a rotary piston pump for A-Mab pumping and filling studies as reported in this case study can be considered the worst case scenario to define a design space. However, if deemed necessary through further risk assessment, some additional pumping studies and filling studies would be conducted for A-Mab with the new pump system using similar approach to those described in the previous section.

5.4 Step 6: Inspection and Release Testing

The vials are 100% inspected for particulate matter, cracks and other defects.

5.5 Step 7: Labeling and Secondary Packaging

The vials are labeled and packed into cartons. The packed vials are stored at 5 ± 3°C.

5.6 Summary of Overall Drug Product Process Control Strategy

A summary of the control strategy for the A-Mab drug product process is presented in Figure 5.16. The flow diagram shows what is monitored during processing and the link between process parameters, product quality attributes and process attributes for each unit operation of the drug product process. The critical process parameters (CPPs and WC-CPPs) are controlled within the design space(s) for delivery of consistent product quality.

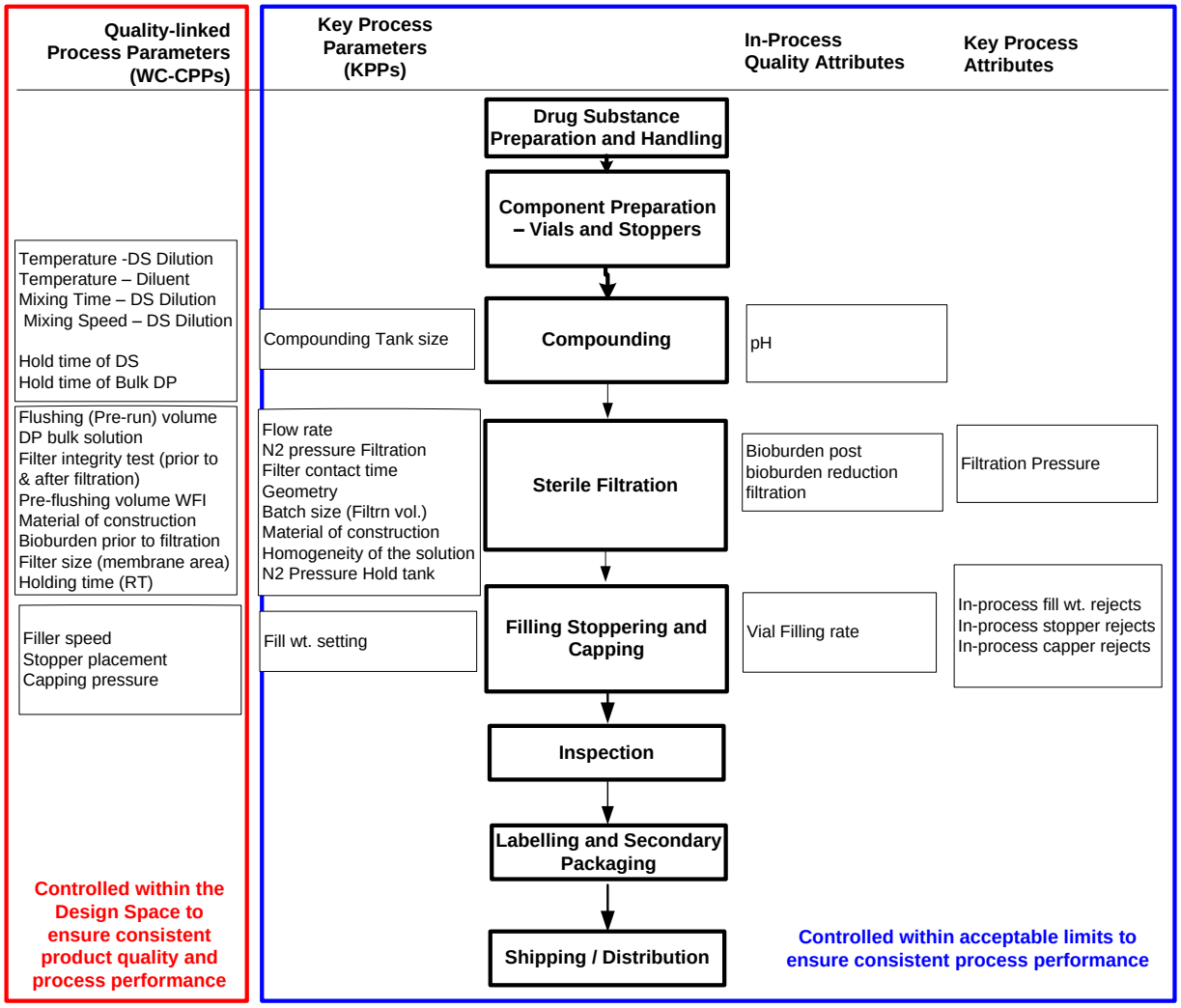

Figure 5.16 Overview of the Control Strategy for Drug Product Process

5.7 Bibliography

FDA. "Guidance for Industry: Sterile Drug Products produced by Aseptic Processing", Current Good Manufacturing Practice, FDA Sep. 2004." 2004.

PDA. "PDA technical Report No. 26, "Sterilizing Filtration of Liquids", .*J. Pharm. Sci. and Technol. 52 (1 Supp)*, 1998 .

5.8 Appendix 1: Scaling Models and Experimental Studies for Compounding

This Appendix is for those interested in understanding the dimensionless analysis that supports the case study assertions that the compounding can be run at any scale between 50 and 1500L. Additionally the Appendix shows how the model translates where mixer geometries change.

Generally, there are two methods that can be used for process scale-up of a unit operation: 1) The standard engineering technique by using dimensionless numbers and 2) the numerical approach by using computational fluid dynamic software. Only the standard engineering method was used here. A key element of this approach is the dimensional analysis allowing the calculation of dimensionless numbers. The model utilizes the fluid properties of A-Mab formulation to determine operating parameters and dimensions for scale-up.

5.8.1 Dimensional Analysis

The impact of a mixer in a given compounding vessel is described by the mixer power P and mainly influences the mechanical stress, which acts on dissolved molecules. Two dimensionless numbers are commonly used to describe mixing behavior in a stirred tank.

Newton (or Power) number is the ratio of resistance force to inertial force and is given by the formula:

$$Ne = \frac{P}{\rho \cdot d_{mixer}^{5} \cdot n^{3}}$$

Reynold's number is the ratio of inertial forces to viscous forces and is given by the formula:

$$\mathrm{Re} = \frac{n \cdot d_{mixer}^{2}}{\nu}$$

(P = Mixer power applied in Watts, n = mixing speed in rpm; ρ = density in kg/m^3; ν = kinematic viscosity in m^2/s)

These numbers can be utilized to design the experimental studies in the lab and for scale-up.

5.8.2 Outline of Scale-up Procedure

When scaling-up between vessels of similar geometries (similar d_{mixer}/d_{vessel}- and the $h_{filling\ level}/d_{vessel}$), the following general procedure is applied:

1) Characterize the small-scale vessel for mixing characteristics using various fluids and/or various mixing parameters and create plots between Re and Ne as well as Re and Θ.n where Θ is the mixing time to achieve target uniformity. Such experiments require knowledge of the mixing vessel's "dead spots" where sampling is performed. Mixing is started as soon as diluent addition begins. Plots such as shown in Figure 5.18 and Figure 5.19 are developed.
2) Once appropriate conditions have been identified in the lab-scale vessel, the parameters are used to determine target operating parameters for the 500 and 1500 L scale tanks.

a) From small-scale vessel information, set scale-up criterion (P/V constant or Θ constant)

b) For larger scale tanks, calculate P

c) Calculate n using knowledge of torque (M_d) as a function of mixer speed for each tank

d) Calculate Re and obtain Θ using correlation in Figure 5.18.

3) After identifying the operating parameters for the larger tanks, the values are confirmed by appropriate experiments.

5.8.3 Scale-Up from Small Scale with Similar Tank and Mixer Geometries

The scale-up of the compounding vessel is demonstrated in the following section.

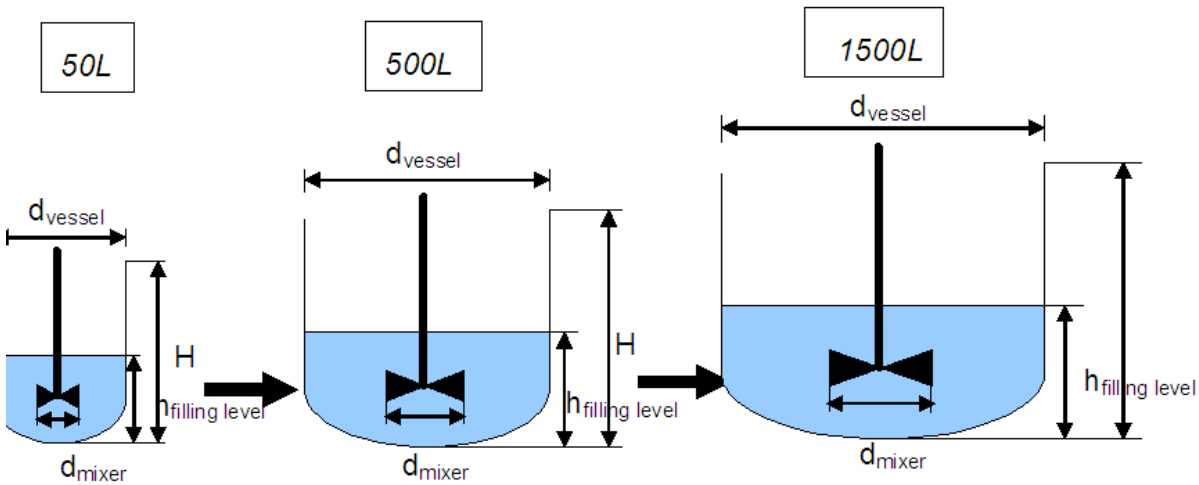

Figure 5.17 Intended scale of compounding vessels for the manufacturing of A-Mab

5.8.4 Characterization of small-scale vessel performance

The small-scale experimental studies are performed in a 50 L mixing vessel to determine the process parameters of an accepted range for the larger vessels. The relevant parameters of the small-scale vessel are summarized in Table 5.27 along with the solution parameters.

Table 5.27 Small scale Vessel (50 L)

Process parameter	n= various (e.g., 100 rpm)
Fluid parameters	ρ=1.03g/ml η=2.5mPas $\nu=2.43\cdot10^{-6}\,m^2/s$
Mixer geometry	d_{mixer} = 150mm
Vessel geometry	d_{vessel} = 408mm $h_{filling\ level}$ = 432mm (V_{fluid} = 50 L)

A correlation, as shown in Figure 5.19, is developed between the Ne and Re for the specific tank and mixer configuration by performing lab studies with test fluids at various mixing speeds. For a certain fluid and rotational speed n, the Re number can be calculated. The torque on the mixer to maintain the speed, M_d, is measured and the mixer power calculated by the expression, $P = 2 \cdot \pi \cdot n \cdot M_d$. From this, a Ne number value is calculated. This Ne-Re pair is plotted in Figure 5.18. The Re number is varied by changing the viscosity of a test fluid or the rotational speed of the mixer and corresponding Ne values are obtained to create the correlation shown. This correlation forms the basis of the scale-up. The mixing behavior of the mixer as measured in the small-scale tank is given in Figure 5.19, where Θ is the mixing time. The intensity of mechanical impact is made up of the mixer speed and time of mixing. Therefore, each product solution has to be analyzed for its sensitivity to impact at small-scale.

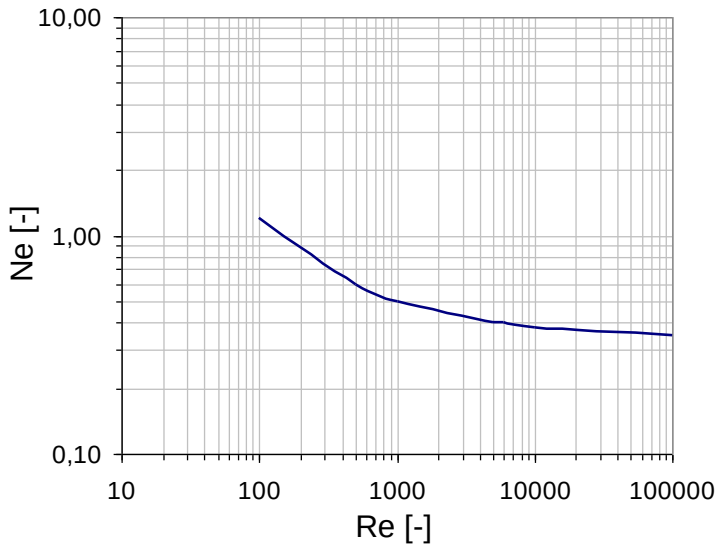

Figure 5.18 Characterization of the Power Input of the Impeller Mixer

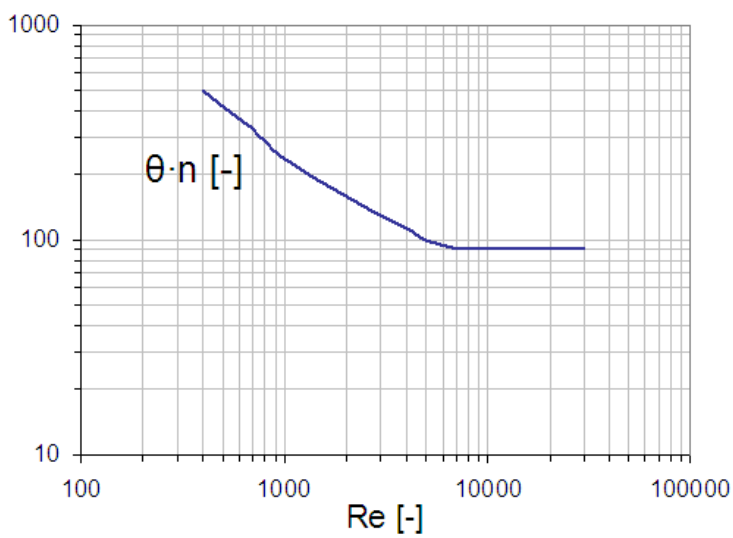

Figure 5.19 Mixing Behavior of the Impeller Mixer Correlation Developed in the 50 L Scale Tank

5.8.5 Scale-up Criterion

For the scale-up of a compounding vessel during manufacture of A-Mab a constant specific power impact: $\frac{P}{V} = \text{const}$ criterion (and therefore a comparable mechanical stress at both scales), so called Büche`s theorem, or a constant mixing time Θ criterion can be selected. In this case, a constant P/V-ratio is used as the scale-up criterion.

For a given rotational speed of the mixer (e.g., n=100 rpm) in the small-scale vessel the Re number can be calculated:

$$\text{Re} = \frac{n \cdot d_{stirrer}^2}{\nu} = \frac{\frac{100}{60s} \cdot (0.150m)^2}{2.43 \cdot 10^{-6} \frac{m^2}{s}} = 15432$$

By the use of the correlation (Figure 5.19) the Ne number can be determined:

Ne=0.38.

Given Ne, the mixer power can be calculated:

$$\text{Ne} = \frac{P}{\rho \cdot d_{stirrer}^5 \cdot n^3} \rightarrow P = \text{Ne} \cdot \rho \cdot d_{stirrer}^5 \cdot n^3 = 0.38 \cdot 1030 \text{kg/m}^3 \cdot (150 \cdot 10^{-3} \text{m})^5 \left(\frac{100}{60s}\right)^3 = 0.138 \text{ W}$$

This sets the parameter for the scale-up as shown below:

Scale-up criterion: $\frac{P}{V} = \text{const} = 2.76 \text{W/m}^3$ (comparable stress situation)

5.8.6 Scale-up to 500 L Scale

The geometric similarity of the mixer-vessel configuration means that the d_{mixer}/d_{vessel}- and the $h_{filling\ level}/d_{vessel}$ - ratio are constant between small-scale and larger scale. For the small-scale tank:

$$\frac{d_{stirrer}}{d_{vessel}} = \text{const} = 0.368$$

and $$\frac{h_{filling_level}}{d_{vessel}} = \text{const} = 1.059$$

The 500 L tank parameters are in Table 5.28. In order to produce a shear stress level in the 500 L tank that is similar to that in the 50 L scale, using the above geometry criteria, a size for the mixer in the 500 L tank is obtained → d_{mixer}=313mm

Table 5.28 500 L Tank Parameters

Mixing speed	n=? <Output from application of scale-up model>
Fluid parameter	ρ=1.03g/ml η=2.5mPas $\nu=2.43\cdot10^{-6}m^2/s$
Mixer geometry	d_{mixer}=? <Output from application of scale-up model>
Vessel geometry	d_{vessel}=850mm $h_{filling\ level}$=881mm (V_{fluid}=500 L)

With the scale-up criterion: $\frac{P}{V} = const = 2.76W/m^3$, the mixer power for the pilot-scale can be calculated:

$$P=2.76W/m^3\cdot500\ L=1.38W$$

The rotational speed is then given by the experimental data of the mixer using measurement of torque M_d as a function of mixing speed and the relation, $P = 2\cdot\pi\cdot n\cdot M_d$. This gives a 500 L scale mixer speed of → n=30rpm

At this speed, the 500 L scale tank (with a mixer of dimensions calculated above) provided the comparable mechanical impact as in the 50 L scale vessel at 100 rpm. The 500 L-tank speed calculation can be repeated for any small (50 L)-scale tank speed that is considered appropriate from a shear impact perspective.

The Re number and the experimental data on mixing time (Figure 5.19) enable an estimation of the mixing time in the 500 L scale tank, when scaled-up on the basis of constant P/V ratio.

100 rpm at 50 L scale ⇒ Re (50 L) =15432

30 rpm at 500 L scale ⇒ Re (500 L) =20158

For both scales $\theta\cdot n$=90, and therefore the mixing time in the 50 L scale is θ=60s and in the 500 L plant θ=180s.

5.8.7 Scale-up to 1500 L Tank

The above procedure is repeated when scaling to the 1500 L scale tank. The 1500 L tank dimensions are provided in Table 5.29

Table 5.29 1500 L Tank Dimensions

Mixing speed	n=? <Output from application of scale-up model>
Fluid parameter	ρ=1.03g/ml η=2.5mPas $\nu=2.43\cdot10^{-6}m^2/s$
Mixer geometry	d_{mixer}= ? mm <Output from application of scale-up model>
Vessel geometry	d_{vessel} = 1226 mm $h_{filling\ level}$ = 1299 mm (V_{fluid} = 1500 L)

5.8.8 Application to Diluent Mixing with Bulk Drug Substance to Produce Bulk Drug Product

A-Mab drug substance dilution was studied at the 50 L, 500 L and 1500 L.

Operating parameters found acceptable representing the widest range for lack of impact on bulk drug product of A-Mab are used below as input into the model approach.

Table 5.30 Dimensions of 50 L Compounding Vessel

50 L Tank	5°C, 25 rpm, 60 minutes	25°C, 100 rpm, 600 minutes
Performance Criteria for range of speed/time	Provides uniform product	No degradation seen
Re	3858 (impact of temp on ρ, η neglected)	15432
Ne	0.42	0.38
P	$2.38\cdot10^{-3}$ W	0.138 W
P/V Criterion	0.0475 W/m^3	2.76 W/m^3
d_{mixer}/d_{vessel}	0.368	
$h_{filling_level}/d_{vessel}$	1.059	

Based on the above criterion generated at 50 L scale, the corresponding operating parameters for 500-L tank are estimated (dimensions in Table 5.31).

Table 5.31 Dimensions of 500 L Compounding Vessel

500 L Tank	5°C	25°C
d_{mixer} (d_{vessel} = 850 mm)	313 mm	
$h_{filling_level}$ (d_{vessel} = 850 mm)	881 mm	
P/V Criterion (from above)	0.0475 W/m^3	2.76 W/m^3
P	0.02375 W	1.38 W
n (= P/2.π.M_d)	6 rpm	30 rpm
Re	4032	20158
Θ ·n	110	90
Θ	18 min	3 min

Similarly, the corresponding operating parameters for 500 L tank are also estimated (dimensions in Table 5.32).

Table 5.32 Dimensions of 1500 L Compounding Vessel

1500 L Tank	5°C	25°C
d_{mixer} (d_{vessel} = 1050 mm)	451 mm	
$h_{filling_level}$ (d_{vessel} = 1050 mm)	1299 mm	
P/V Criterion (from above)	0.0475 W/m^3	2.76 W/m^3
P	0.071 W	4.968 W
n (= P/2.π.M_d)	7 rpm	30 rpm
Re	7190	230816
θ·n	95	90
Θ	19 min	5 min

These parameters were subsequently tested in experiments performed at scale.

5.8.9 Summary of Results

The results from the scale-up data suggest that the model approach developed from the small scale vessel is appropriate for scale-up activities. The completion of mixing in comparison to the model estimates is given in Table 5.33 below. The differences in mixing time as compared to the model ranged from 0 to 20%.

Table 5.33 Differences Between Predicted and Actual Mixing Times Required for Compounding Vessels from 50 to 1500 L

Temp/Tank Size	50 L	500 L			1500 L		
	Actual (min)	Predicted (min)	Actual (min)	Difference from Model (%)	Predicted (min)	Actual (min)	Difference from Model (%)
5°C	15	18	20	11%	19	20	5%
25°C	5	3	3	0%	5	6	20%

5.8.10 Scale-up when Tank Geometry is not the same

When the shape of the compounding vessel in the manufacturing plant (see Figure 5.20) or the type of mixer is different than the previously studied vessels, the Ne-Re- and the mixing diagram cannot be used. The characteristics of the flow pattern in the vessels are different. Basically, a vessel has to be designed for small-scale and the power input and mixing behavior have to be analyzed.

Table 5.34 1500 L Tank Dimensions of Different Geometry

Mixing speed	n = ?
Fluid parameter	ρ=1.03g/mL η=2.5mPas $\nu=2.43\cdot10^{-6}m^2/s$
Mixer geometry	d_{mixer} =475mm
Vessel geometry	d_{vessel} =1226mm $h_{filling\ level}$ =1271mm (V_{fluid} =1500 L)

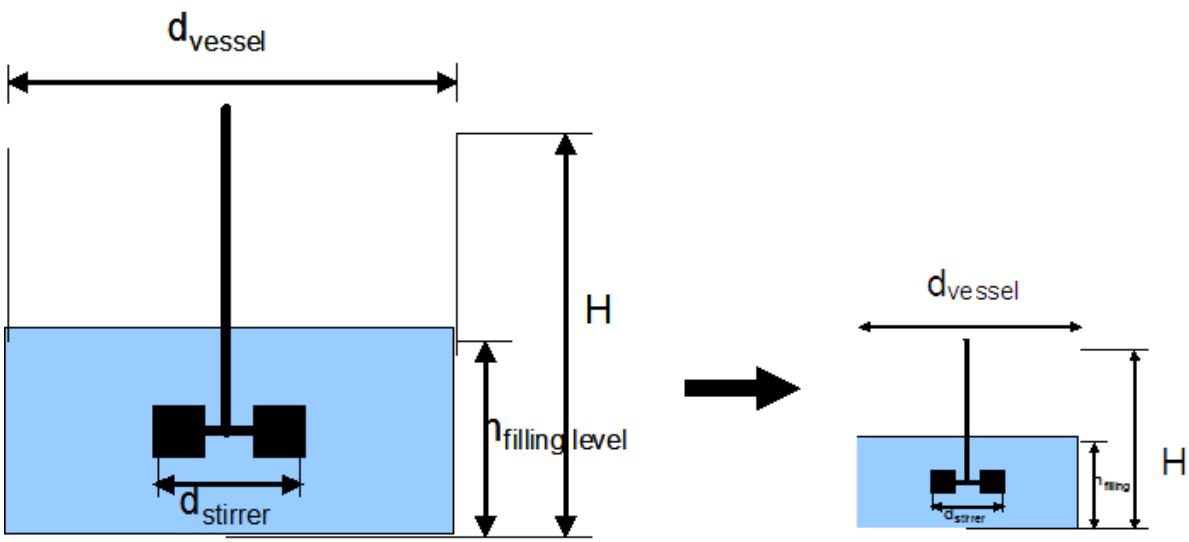

Figure 5.20 Scale down of Manufacturing Plant

A scale-up or scale-down model is defined by the use of a scale coefficient. The scale-up/-down coefficient, which is defined by $\mu = \frac{d_{Plant}}{d_{Model}}$ should be as small as possible (values of 10 lead to an accuracy of measurement of ±10%).

In the above example, the small-scale vessel diameter is set to 400 mm. Therefore, the scale-down coefficient is μ=3.1, which is an acceptable value.

Using the scale-up rule: $\frac{d_{stirrer}}{d_{vessel}} = const = 0.368$

the mixer diameter for the small-scale setup can be calculated: d_{mixer}= 451mm.

The filling level $h_{filling\ level}$ is given by $\frac{h_{filling_level}}{d_{vessel}} = const = 1.059$,

and therefore $h_{filling\ level}$ in the 50 L scale is 423.6 mm (fluid volume is about 53 litres).

Once a small-scale vessel has been designed and manufactured, the power input (Ne-Re relation) and the mixing studies should be performed to obtain the accepted rotational speed in terms of stress impact in the manufacturing plant (as described in the previous section).

5.9 Appendix 2. Fault Tree Analysis

This Appendix is for those readers who are interested in the use of Fault Tree Analysis as a tool to assist in risk assessment of unit operations and its documentation.

To aid in documenting the logic and process behind a QbD development approach, Fault Tree Analysis (FTA) was utilized. FTA is a deductive top-down risk assessment tool used to identify potential contributing causes that can lead to a defined undesirable outcome. For a comprehensive assessment of a Monoclonal Antibody Drug Product process, FTA would be performed for all CQAs. To exemplify this approach, aggregation was selected as the top-level failure event.

A fault tree diagram is used to show the logical branches from the single undesired outcome at the top of the tree (aggregation) to the potential root causes or initiating events at the bottom of the tree. This allows for a holistic assessment of gating and control of CQA failures that encompasses a design space of possible Critical Process Parameters (CPPs) that includes: personnel, materials, manufacturing processes, methods and environment. In this way a CQA such as aggregation can be used to thread together Drug Product process unit operations using comprehensive design space control.

5.9.1 Tree Construction

Having an accurate and comprehensive understanding of the process was the first step of the FTA. This involved obtaining and reviewing current, updated and accurate process flow diagrams, procedures, specifications, and requirements documentation. The next step involves brainstorming from the top-level event (i.e., how can a particular CQA fail at this particular step) and identifying the events that lead directly to the top-level event. Each higher-level event that occurs is decomposed until the bottom-level initiating events are derived. Once the bottom-level events are identified, the trees are assessed from the bottom-up. Here, a review of the process on the basis of process impact via personnel, machinery, procedures, materials, and the environment was performed. For this project, there were Fault Trees established for the process unit operation steps related to Drug Substance, Compounding, Sterile Filtration, Filling and Inspection.

5.9.2 Analysis

After the fault trees are constructed and reviewed, the controls or gating is defined. Within each level of the fault trees, combinations of fault modes are described and the controls are depicted as logical operators (“AND” gates or “OR” gate). Here, controls are those elements resident in the process that are used to help monitor, mitigate, or eliminate the fault from occurring. The gates are used to interconnect the lower-level events that contribute to the top-level or high-level event. Gates are assigned with respect to three elements: 1) strength, 2) frequency and 3) risk and a value from 1-3 (3 being most) is assigned.

5.9.3 Results

A summary of the FTA results for all unit operations is captured in Table 5.35, with details of the critical initiating events shown in Table 5.36. The comprehensive, classified results for Filling and Inspection process step are captured in Table 5.37, Table 5.38 and Table 5.39, and Table 5.39; and

the associated Fault Tree is visualized in Figure 5.21. FTA of all drug product unit operations identified 64 initiating events, 51 important events and 0 non-critical/operative events leading to aggregation for the process. The bottom-level initiating events were identified and categorized in the following order of priority:

8) Operator Error
9) Equipment Failure
10) Inappropriate Requirements
11) Unable to Perform
12) Maintenance Failure / Error
13) Instrument Failure
14) Calibration Failure
15) Environmental Monitoring

The Drug Substance preparation and handling step was found to have the highest number of the potential initiating events followed by Sterile Filtration, Compounding, Filling and Inspection. Within the Drug Substance preparation and handling step, there is a significant potential for aggregation as there were no in-process steps for monitoring aggregation. The Drug Substance preparation and handling step, for the purpose of the A-Mab process, essentially consisted of storage condition failure at the refrigeration and freezing of the starting material.

Table 5.35 Summary of Results

Process Step	Number of Initiating Events Leading to Aggregation			
	Critical	Important	Non-Critical/Operative	Total
Drug Substance	26	20	0	46
Compounding	10	15	0	25
Sterile Filtration	15	11	0	26
Filling and Inspection	13	5	0	18
Total	64	51	0	115

Table 5.36 Events that initiate aggregation: Summary of Critical Results

Critical	Drug Substance	Compounding	Sterile Filtration	Filling and Inspection	Total
Inappropriate Requirements	5	1	1	0	**7**
Operator Error	14	3	9	5	**31**
Equipment Failure	5	1	4	3	**13**
Instrument Failure	0	2	0	0	**2**
Maintenance Failure / Error	1	1	0	1	**3**

Table 5.36 Events that initiate aggregation: Summary of Critical Results

Critical	Drug Substance	Compounding	Sterile Filtration	Filling and Inspection	Total
Control System Failure	1	0	0	2	**3**
Unable to Perform	0	1	1	1	**3**
Calibration Failure	0	1	0	0	**1**
Environmental Monitoring	0	0	0	1	**1**
Total					**64**

5.9.4 Recommendations for Mitigation

5.9.4.1 General, for All Unit Operations

- There are a number of instances for operator error. For instance the operator does not follow the specified or relevant procedures. Batch documents are to be assessed to include the additional cross-check by another operator and/or supervisor and subsequent verification by QA.
- For the various equipment and instrument potential failures; verify the adequacy of the associated operation, maintenance, and instrument performance with respect to the identified initiating event.

5.9.4.2 Drug Substance Preparation and Handling

- The cryovessel and carboy physical storage location in the applicable chambers will be assessed relative to the potential for fully loaded temperature chamber excursions.
- The gaskets use, performance, and maintenance will be re-evaluated. Work with the manufacturer to assess the current mean-time-between failures and potential verifications that can be implemented during the receipt inspection as well as normal operations.
- The cryovessel and carboy storage chamber as well as the freeze thaw unit performance data and procedures will be reviewed for potential issues related to leaving the freezer door open too long as well as lighting control (i.e., maintenance and operations). Calibration and instrument data and procedures assessed to identify improvements for the temperature control, monitoring, and verifications.

5.9.4.3 Compounding

- Procedures and batch documentation related to the UV, pH, and conductivity instruments (PAT) reviewed and assessed to identify continuous improvements of performance, monitoring and the interval for calibration.
- The mixer performance data and procedures reviewed for potential failure, (i.e., availability, maintenance and operations). Calibration and instrument data and procedures assessed to identify improvements for the speed control, monitoring, and verifications.

- The Compounding process workflow assessed for constraints and optimized for down time (i.e., related to shift change).
- The HVAC and environmental monitoring performance and instrument calibration data evaluated for potential occurrences of temperature excursions.

5.9.4.4 Sterile Filtration

- The cleaning procedures and performance data assessed for the adequacy of cleaning intervals as well as to further define the cleaning requirements in more detail.
- The pressure regulator and filters performance data and procedures related to operation and maintenance reviewed for adequacy (i.e., interval for replacement and frequency for inspections).
- The process workflow assessed for constraints in order to identify improvement and the mitigations for “down-time” or delays in the line set-up. Includes the reviews of the performance data and procedures related to maintenance, calibration, and operation.

5.9.5 Filling and Inspections

An example of the critical, important and noncritical initiating events for aggregation failure during filling, stoppering and capping is shown in Table 5.36. Filling line operation, maintenance, and calibration data and procedures evaluated for potential process workflow constraints and areas of improvement. This will focus on the automated inspection machine, spinner, and rotary pumps.

5.9.6 Aggregation Testing

There currently are no steps in place within or between the applicable process steps to monitor aggregation. There is no mechanism to monitor the aggregation potential formation in the drug product processing steps between Drug Substance to Inspection. The current in-process controls are not specific to aggregation. The controls currently in-place are essentially good GMP systems involving storage controls (temperature) and batch record processing with the follow-on check-the-checker verification testing. As the greatest potential for aggregation to occur is related to the operator interaction in the process and equipment failures, improvements in this area are recommended. Installing controls within the process would mitigate the number of critical initiating events. Additional aggregation testing will be put in place after Drug Substance thaw and after Compounding and monitored by SEC and HIAC Royco. Data will be assessed and it will be determined whether to introduce an aggregation PAT (Process Analytical technology) step permanently.

5.9.7 Conclusions

Capturing and assessing information and the relationships between unit operations were accomplished by using FTA as demonstrated here. Tools such as Design of Experiments (DOE) have limited use for practical reasons. For many Drug product unit operations small-scale models are actually more worst-case and some unit operations are not scaleable. The ability to demonstrate comprehensive process control becomes more critical as the process edges are not as well defined. FTA provides a holistic approach for the evaluation of the full drug product process.

Table 5.37 Process Risk Assessment Summary (1 of 3)

FILLING and INSPECTION

Criticality Classification: Critical (Quantity = 13 Initiating Events)

Type of Failure - Initiating (Bottom Level) Event	Description	Specifics	Mitigation
Operator Error	(5) Operator error in that the operator doesn't follow the specified procedure. Although there were controls in-place with "check-the-checker" to help mitigate this risk, the number of occurrence or repetition of the operator error equaled seven. Therefore, since this high number potential is tied to the operations of a single operator in set-up of the needle position.	Set-up error of the needle height recording which will potentially cause splash or spraying on the vial Set-up of the filling speed too fast (causing spraying / splashing on vial wall) Set-up of the filling speed too fast (causing shearing of material) Set-up of the spinning speed too high or too long Set-up of the light exposure too long (Set-up time)	Where a sole operator is performing such a task, a second operator / supervisor, cross check will be applied to the process step to help mitigate the potential impact and issue. Batch documents will be revised to include the improvements.
Control System Failure	(2) Filling speed doesn't alarm when filling speed limit is exceeded.	Filling speed alarm	Filling machine operation, calibration, and maintenance data will be evaluated. Improvements will be made to the relevant SOPs.
Unable to Perform	Physically unable to perform the process steps as a result of delays that are observed as other products are completing their processes (hence delaying the process).	Production breaks	Process workflow will be analyzed for "constraints" and the current workflow will be optimized via the Lean 6 Sigma program execution.
Maintenance Failure	Maintenance error or failure related to the rotary pumps which causes shredded particles from pumps to be introduced.	Rotary pumps	The Rotary Pump operation and maintenance data will be evaluated. Improvements will be made to the relevant SOPs.
Environmental Monitoring	Environmental monitoring failure in that the foreign particles are not observed from the rotary pump.	Environmental monitoring of the rotary pumps	The Environmental monitoring operation data and procedures will be evaluated. Improvements will be made to the relevant SOPs.

Table 5.37 Process Risk Assessment Summary (1 of 3)

FILLING and INSPECTION **Criticality Classification: Critical (Quantity = 13 Initiating Events)**			
Type of Failure - Initiating (Bottom Level) Event	**Description**	**Specifics**	**Mitigation**
Equipment Failure	Filling Line fails	Filling Line	The Filling operation, calibration, and maintenance data will be evaluated. Improvements will be made to the relevant SOPs.
Equipment Failure	Spinner failure causes the speed to exceed limit.	Spinner Speed	The Spinner operation, calibration, and maintenance data will be evaluated. Improvements will be made to the relevant SOPs.
Equipment Failure	Automated inspection Machine failure causes too much light.	AIM failure	The AIM operation, calibration, and maintenance data will be evaluated. Improvements will be made to the relevant SOPs.

Table 5.38 Process Risk Assessment Summary (2 of 3)

FILLING and INSPECTION **Criticality Classification: Important (Quantity = 5 Initiating Events)**			
Type of Failure - Initiating (Bottom Level) Event	**Description**	**Specifics**	**Mitigation**
Operator Error	Check-the-checker error in the review and reporting of data in the batch record.	Needle height recording in the batch record Filling speed too fast (causing spraying / splashing on vial wall) Filling speed too fast (causing shearing of material) Spinning speed too high or too long Light exposure too long (Set-up time)	In addition to the check-the-checker in the batch record, which is typically a second operator or supervisor, an additional QA review check will be included in the batch record.

Table 5.39 Process Risk Assessment Summary (3 of 3)

FILLING and INSPECTION **Criticality Classification: Non-Critical (Quantity = 0 Initiating Events)**			
Type of Failure – Initiating (Bottom Level) Event	**Description**	**Specifics**	**Mitigation**
Repetition of Contributing Events	NA	NA	NA

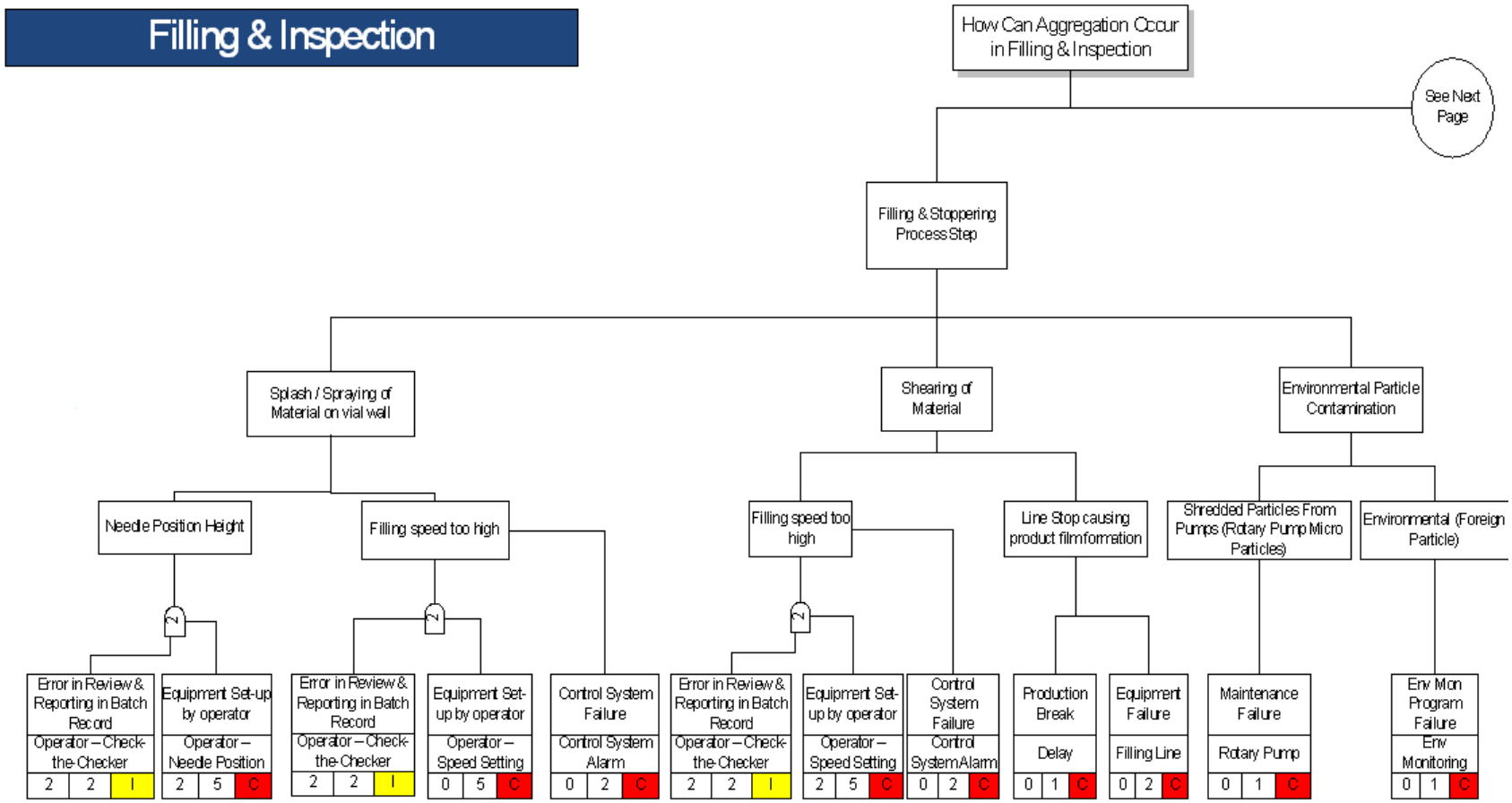

Figure 5.21 A-Mab Fault Tree for Aggregation (1 of 2)

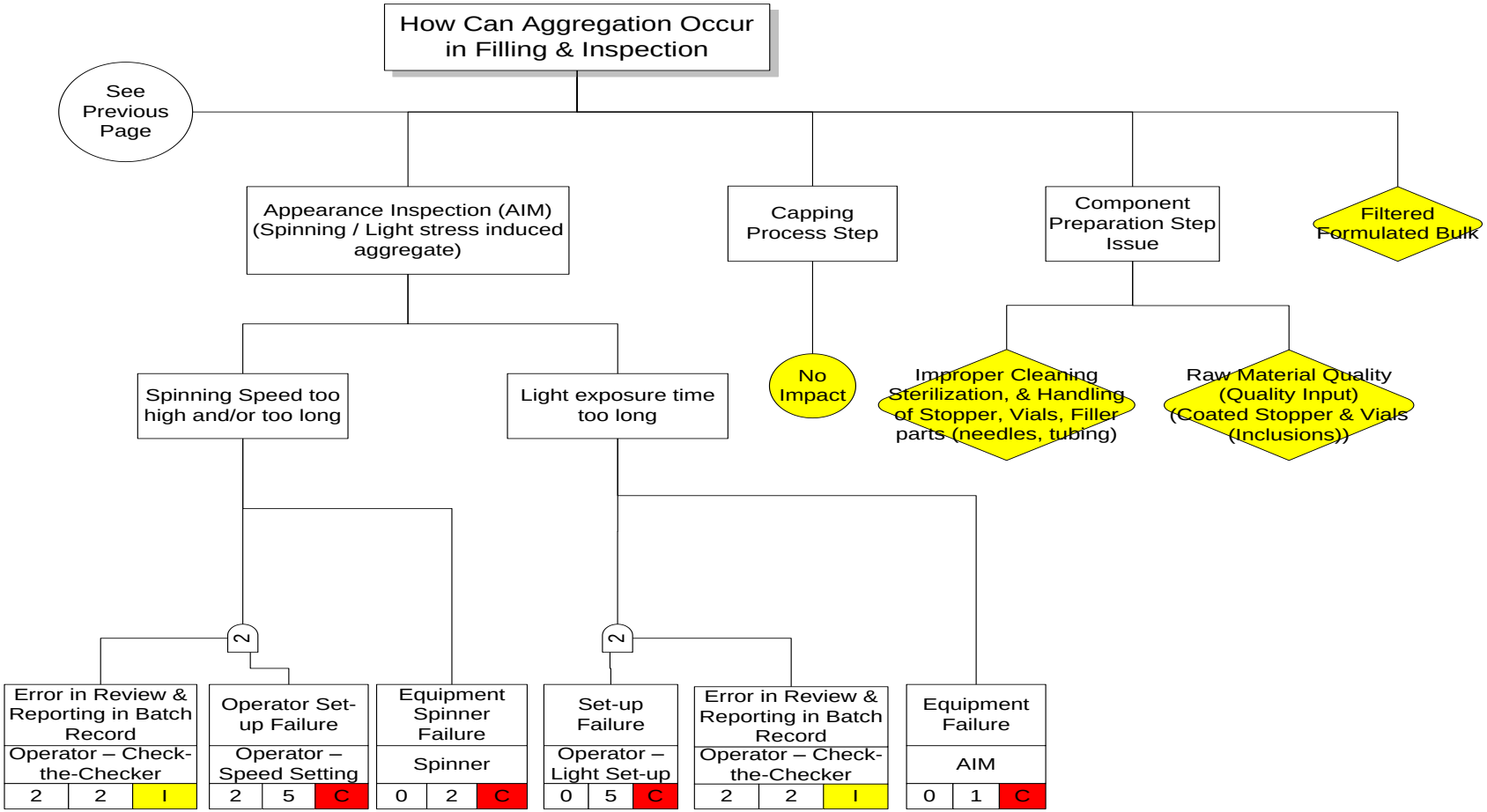

Figure 5.22 A-Mab Fault Tree for Aggregation (2 of 2)

6 Control Strategy

The Control Strategy for A-Mab integrates input material controls, procedural controls, process parameter controls, in-process testing, release testing, characterization and/or comparability testing and process monitoring to provide a high degree of assurance that the product quality specifications are met.

The level of control for each individual quality attribute is determined on the basis of the criticality level of the attribute and a risk assessment of the capability of the process to consistently deliver product that meets the acceptance criteria for each attribute. Based on this risk assessment results, a rational control strategy is formulated for each quality attribute by choosing the appropriate control elements. Thus it is the sum of the individual control strategies that represent the overall process control strategy for A-Mab.

This section describes the process and approaches used for the risk assessments that underpin the control strategy for A-Mab. Examples of process capability assessments are provided as well as justification for Drug Substance and Drug Product Specifications and characterization testing.

Based on the enhanced product and process understanding, specification tests are significantly reduced compared to traditional approaches. Some specification testing has been moved to in-process tests (including PAT) while other tests were eliminated because operation within the process design space provides a high degree of assurance that the process will deliver consistent product quality.

Characterization testing performed during process monitoring and/or comparability assessments is also described. The continuous process monitoring scheme will verify that the control strategy is performing as expected and remains appropriate.

Example control strategies are provided for aggregation, glycosylation, host cell proteins, deamidated isoforms and viral clearance. These examples include application of the Process Capability Risk Assessment and summaries of the specific control strategy elements and the rationale for including or not including them in the final control strategy.

Key Points

1. The overall control strategy is risk-based and takes into account both product and process understanding to ensure that the acceptable ranges for CQAs are always maintained.
2. The overall control strategy includes testing (in-process, release, characterization/ comparability and process monitoring) and controls (input materials, procedural and process parameter).
3. Level of testing and controls is commensurate with risk. Risk is determined by the Criticality Level of the CQA, the process capability (or probability that a CQA would fail at a given step) and the probability of detection of a CQA failure.

6.1 Introduction

A science and risk based approach based on product and process understanding has been applied to define a robust control strategy for A-Mab which provides a high degree of assurance that the acceptable ranges for critical quality attributes are maintained (Table 2.29). The Control Strategy integrates a number of elements including input material controls, procedural controls, process parameter controls, in-process testing, specification testing, characterization/comparability testing and process monitoring applied as appropriate.

For the purposes of this case study, it is assumed that appropriate quality systems are in place to assure compliance with current Good Manufacturing Practices.

The Control Strategy for the commercial manufacturing of A-Mab is based on an overall risk assessment where each CQA is evaluated independently to ensure that the proposed control strategy will deliver each CQA within its acceptable ranges established for safety and efficacy. A Failure Mode and Effects Analysis (FMEA) approach was used (see Figure 6.1)

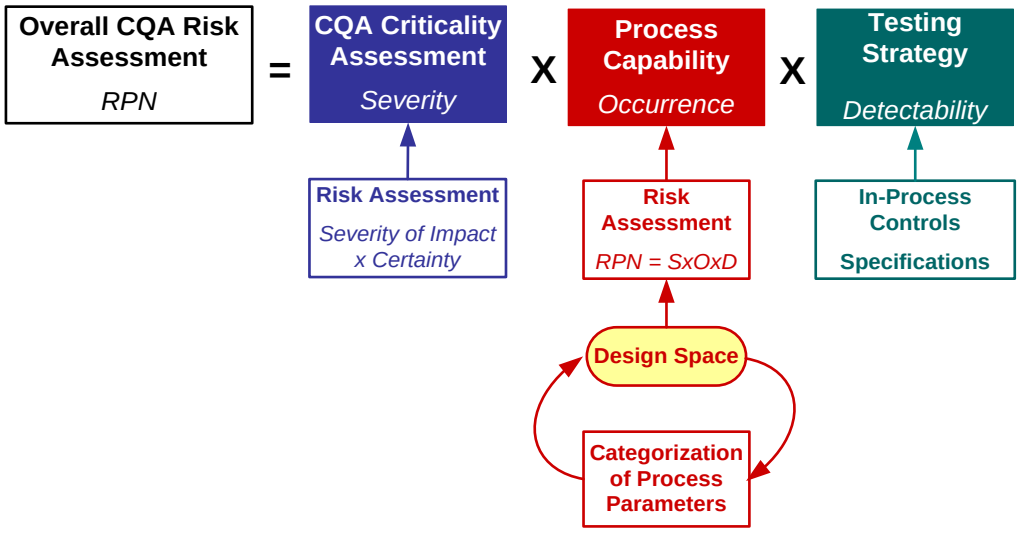

Figure 6.1 Overall Risk Assessment for each CQA based on A-Mab Control Strategy

1) The Severity (S) of failure corresponds to the Criticality Level of the CQA which was determined based on the impact to safety and efficacy and the certainty of the knowledge used to establish that impact. A detailed description of the criticality assessment is presented in Section 2 (Design of Molecule and Quality Attributes Assessment). The Criticality Level used for this FMEA was based on the assessment done using Tool #1 and summarized in Table 2.4.
2) The probability of Occurrence (O) or frequency of failure was determined based on process capability analysis to assess the risk that a CQA could exceed its acceptable limits. A description of capability assessment is presented in Section 6.2 (Process Capability).
3) The probability of Detection (D) of failure is based on the proposed testing strategy which includes in-process controls and end-product testing (specifications).

This risk based approach can be used in an iterative fashion to design the overall control strategy. For instance, more critical quality attributes may require more stringent control. If the probability of process failure is too high, the design space can be narrowed to improve the process capability. If an

attribute can be detected, there is an option of controlling the attribute by a combination of process capability and testing. If an attribute cannot be detected (e.g., virus clearance), process design and control strategy must ensure that the process is capable of controlling the attribute. Examples of testing strategies are presented through specific CQA examples in Section 6.5 (Example Control Strategies section).

The overall risk assessment presented in Table 2.28 represents the cumulative process and product understanding for A-Mab. At the core is the development of a manufacturing process that consistently and reproducibly operates within the established design space and delivers product that meets CQAs. To develop such a process, the approach used was based on understanding the relationships between input process parameters and output attributes in each unit operations over the entire manufacturing process. For this, multiple systematic risk assessments were conducted throughout the development lifecycle to identify process steps, material attributes, equipment design and operation parameters that would be most likely to impact drug substance and/or drug product-CQAs (Figure 6.2).

Early risk assessments (Risk Assessments 1 and 2) were used to guide process development activities and included prioritization of process steps and parameters for optimization and characterization. Experimentation was carried out with univariate and multivariate studies (*e.g.*, DoE) as appropriate using scale-down models to establish parameter-attribute relationships, identify robust operating conditions and acceptable process ranges. Results from the DOE studies provided an understanding of the multidimensional relationships between input process parameters and output quality attributes. Additionally, clinical manufacturing experience provided understanding of process performance and process control at various operational scales. This information served as the basis for risk assessments conducted for each unit operation to define the design space and a draft control strategy (Risk Assessment #3). Capability for clearance of some impurities is very high and minimal product-specific data was necessary to assess the process capability risk (e.g., DNA and MTX clearance). Modular claims based on prior knowledge from similar operations were deemed sufficient to justify control. Knowledge gained from full scale manufacturing of A-Mab and prior knowledge with commercial operations with other mAbs were then used for the overall risk assessment (Risk Assessment #4) that serves as the basis for the proposed final control strategy.

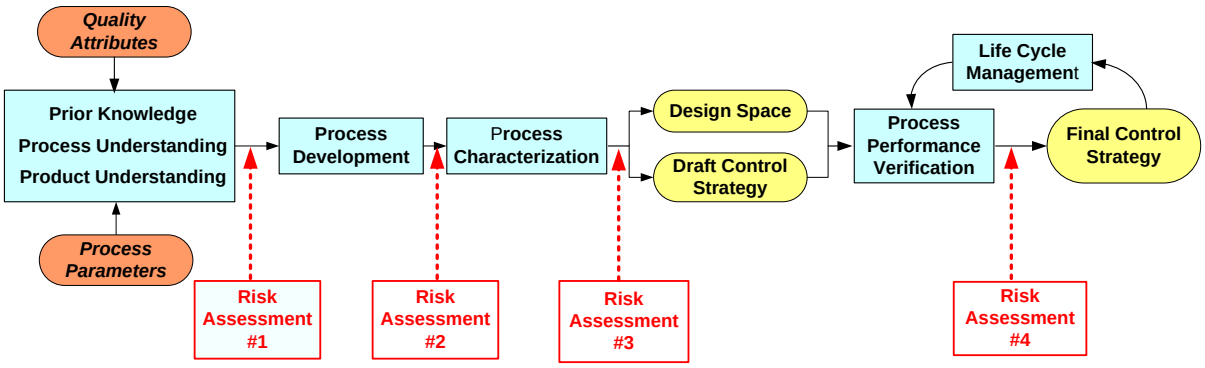

Figure 6.2 Risk Assessment Approach Used through A-Mab Development Lifecycle

As illustrated in Figure 6.1 the design space is underpinned by the categorization of process parameters. This categorization is based on the potential impact on CQAs and is re-evaluated throughout the development lifecycle via iterative risk assessments (Risk Assessments 3 and 4) that build on cumulative process and product understanding (Figure 6.2). The final assessment of criticality for process parameters was performed according to the decision logic outlined in Figure

6.3. Although there is a binary decision process at each decision point (e.g., Yes vs No impact on CQAs, high vs low risk), in reality the criticality of process parameters represents a continuum based on the significance of the impact on CQAs and the ability to control the parameter within the design space. For instance, both a critical and key parameter can impact product quality, but the categorization depends on the degree of potential impact. If the impact is minor and not practically significant, or if there is significant redundancy in the process, the parameter could be classified as key. For the purposes of this case study, the continuum of process parameter criticality was divided according to the diagram in Figure 6.4.

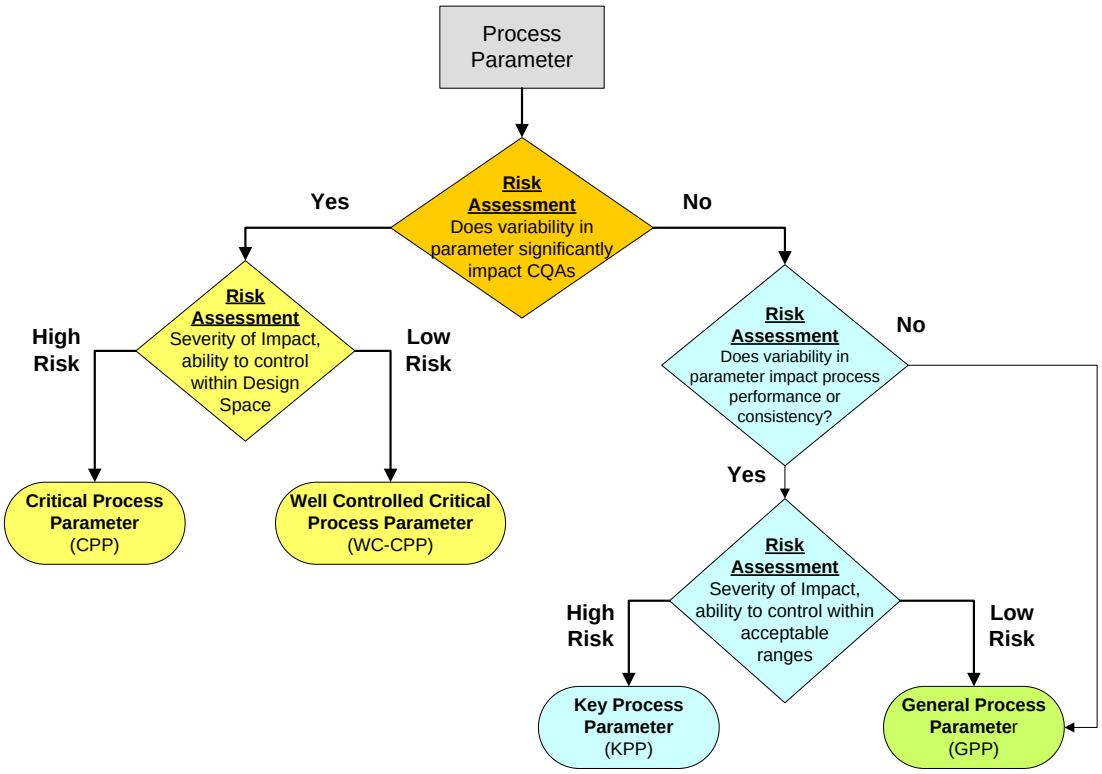

Figure 6.3 Final Categorization of Input Process Parameters for A-Mab Control Strategy

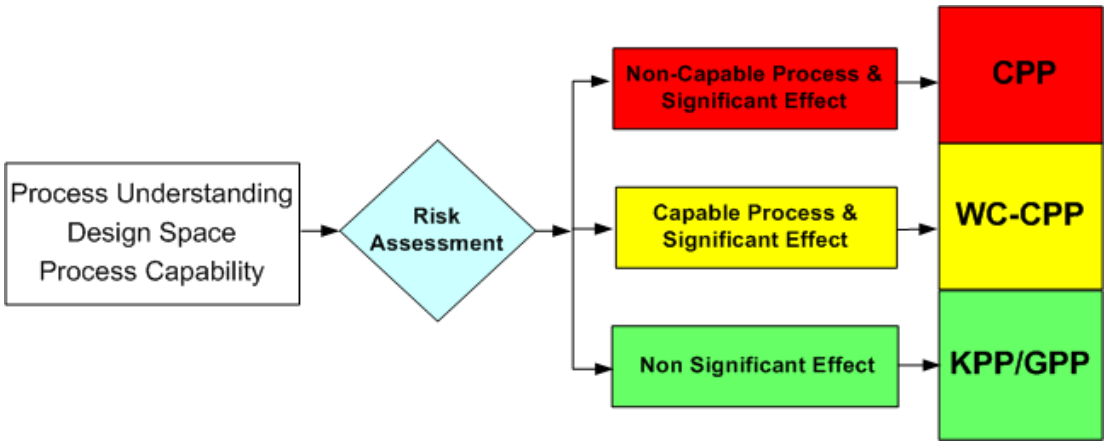

Figure 6.4 Categorization of Criticality for Process Parameters

Capable Process = Parameter is well controlled within the design space and/or acceptable range
Significant Effect = Parameter meaningfully affects CQA, either through exceeding the limit of failure or by considerably affecting the CQA

Based on this assessment, process parameters that significantly impact CQAs were categorized either as Critical Process Parameter (CPP) or Well Controlled Critical Process Parameter (WC-CPP).

Process parameters that do not significantly impact product quality but are important to ensure consistent process performance were categorized as either Key Process Parameter (KPP) or General Process Parameter (GPP) depending on level of risk to process performance. Examples of process parameter classification are provided in the process sections (Upstream, Downstream and Drug Product).

Definitions:

1. Critical Process Parameter (CPP) and Well-Controlled Critical Process Parameter (WC-CPP). Both, CPPs and WC-CPPs, are process parameters whose variability have 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 has a low risk of falling outside the design space.

A CPP has a high risk of falling outside the design space.

Here, the assessment of risk is based on a combination of factors that include equipment design considerations, process control capability and complexity, the size and reliability of the design space, ability to detect/measure a parameter deviation, etc.

2. Key Process Parameter. An adjustable parameter (variable) of the process that, when maintained within a narrow range, ensures operational reliability. A key process parameter does not affect critical product quality attributes.

3. General Process Parameter. An adjustable parameter (variable) of the process that does not have a meaningful effect on product quality or process performance.

It is important to note that the ability to properly categorize process parameters and accurately assess the significance and effect of the variability of a parameter on CQAs depends on process/product understanding and the size of the characterized process space (i.e., knowledge space). The design space is a subset of the knowledge space that is known to result in acceptable values for the Critical Quality Attributes. Typically, the process is operated within a more limited control space which lies within the design space.

6.2 Process Capability

A tool was developed to assess the risk that each process step will fail to achieve its intended purpose. This tool was first used for Risk Assessment #3 (Figure 6.2) to define a draft control strategy and subsequently in Risk Assessment #4 (Figure 6.2) to establish the final control strategy. For the latter, the RPN was rescaled to a range to 1-10 and used to calculate the "occurrence" score in the overall FMEA risk assessment for each CQA as presented in Table 6.1.

6.2.1 Process Capability Scoring Tool: Risk Assessment by FMEA.

The scoring criteria used in this assessment are described below and the corresponding scales are summarized in Table 6.1.

Severity(S): Scored based on the potential impact of the step on the Critical Quality Attribute in context of the overall process.

Occurrence (O): Scored based on the probability of the Critical Quality Attribute exceeding the acceptable range if this step fails to achieve its intended purpose.

Detection (D): How well can the failure of the Critical Quality Attribute be detected prior to completion of the step.

The acceptable output range for a QA at each step corresponds to the range that has been proven to work based on DOE studies and prior knowledge. Where linkages between steps exist, the acceptable output of a step is based on what the downstream process steps can handle.

Table 6.1 Process Capability Scales for Severity, Occurrence and Detection

Scoring	1	3	10
Severity (S) What is the potential impact if the step fails to meet its intended purpose?	Will not lead to failure of DS or DP to meet quality targets	Could lead to failure of DS or DP to meet quality targets however other steps mitigate impact	Will lead to failure of DS or DP to meet quality targets; other steps cannot mitigate impact
Occurrence (O) What is the probability that the acceptance limits for the step will be exceeded.	High capability or ability to react to assure attribute remains within acceptable range; Few WC-CPP's, no CPP's	Multiple WC-CPP's, no CPP's	Influenced solely by CPP's
Detection (D) What is the likelihood of detection of step failure?	Can readily detect	Indirect ability to detect	Cannot readily detect

The overall score (RPN=SxOxD) was calculated for each unit operations and process capability was assessed based on the criteria summarized in Table 6.2.

Table 6.2 Scoring for Process Capability Risk Assessment

RPN Score (= S x O x D) For Risk Assessment #3	Risk of Quality Attribute Failure in Step	Process Capability	Score for Risk Assessment #4
≥ 100	High	Low	10
31-90	Medium	Medium	3
≤ 30	Low	High	1

6.3 A-Mab Control Strategy

The A-Mab control strategy was designed to ensure that the manufacturing process consistently delivers product that meets established quality attribute ranges that ensure drug safety and efficacy. As summarized in Figure 6.5, the overall control strategy is based on a detailed knowledge of both the product and the process and integrates a number of elements including input material controls, procedural controls, process parameter controls, in-process testing, specification testing, characterization/comparability testing and process monitoring applied as appropriate.

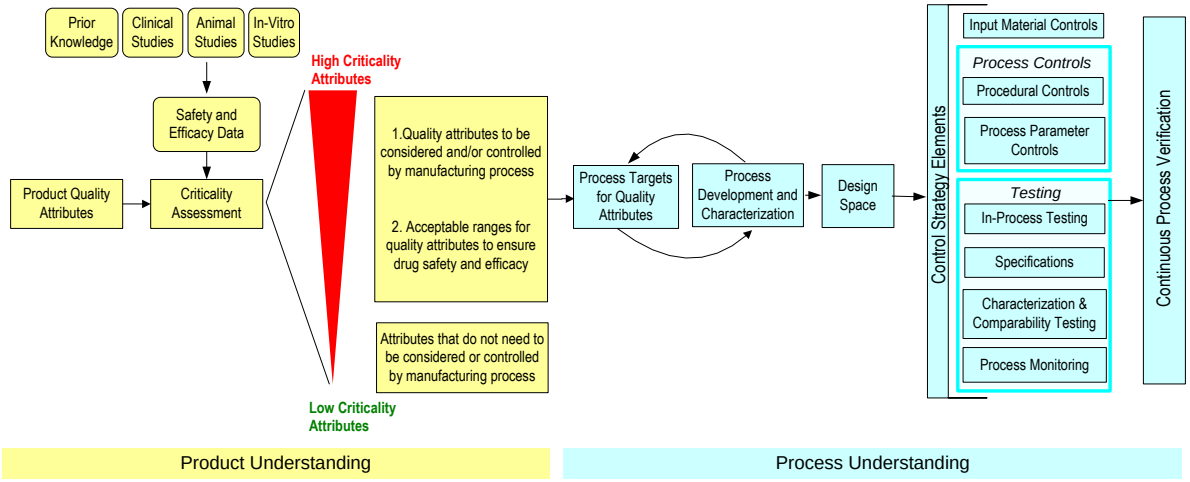

Figure 6.5 The control strategy is based on a rational approach that links process understanding to product quality requirements (product understanding)

For the purposes of this case study, only a selected number of quality attributes were considered to define the Control Strategy. Normally, the approaches presented here would be expanded to include all critical quality attributes. Quality attributes considered here include: aggregates, glycosylation (afucosylation and galactosylation), deamidated isoforms, viral clearance and host cell proteins. These attributes were selected to illustrate the application of QbD principles based on one or more of the following criteria: criticality, detectability and linkage across multiple unit operations. The corresponding ranges for the A-Mab process are summarized in Table 6.3. The basis for the acceptable range for each of the quality attributes is summarized in Table 2.29 in Section 2.7. The target threshold for viral clearance was selected in accordance with ICH Q5A and EMEA Guidance on Viral Safety.

Table 6.3 Quality Attribute Ranges for A-Mab Process

Quality Attribute	Criticality Level (Tool #1)	Acceptable Range Based on Safety and Efficacy	Range Assured by Design Space
Aggregates	60*	0-5%	NMT 5%
aFucosylation	60*	2-13%	2-11%
Galactosylation	48*	10-40%	20-40%
Deamidated Isoforms	4	NA	NA
Viral Clearance	ND*	NLT 6 LRV	NLT 6 LRV
Host Cell Proteins	36*	NMT 100 ppm	NMT 100 ppm

ND = score not determined; *Critical Quality Attributes

6.3.1 Elements of the Control Strategy

The Control Strategy for A-Mab is comprised of input material controls, process control elements and testing control elements. Descriptions of these are provided in Table 6.4.

Table 6.4 Control Strategy Elements for A-Mab

Control Element	Description
Input Material Controls	These are controls pertaining to raw materials, excipients, components etc. used in manufacturing operations, including supplier quality management, raw material qualification and raw material specifications. The case study does not address risk assessment or control strategy supporting input material controls.
Process Control Elements	
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	Process parameters that are linked to Critical Quality Attributes (CQAs) and include Critical Process Parameters (CPPs) or Well Controlled Critical Process Parameters (WC-CPPs) that must be controlled within the limits of the design space to ensure product quality. Process parameters linked to process performance (KPPs and GPPs) that must be controlled to ensure process consistency.
Testing Control Elements	
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. In-process tests include acceptance criteria.
Specification (Lot 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. Certain attributes will also be monitored as part of the stability program.
Characterization and/or Comparability Testing	Testing of certain attributes outside of lot release testing for the purposes of intermittent process monitoring or 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.

Figure 6.6 provides a summary of the process control points and associated parameter categorization and testing strategy for the A-Mab drug substance and drug product. Note that the table includes only a limited number of unit operations and product quality attributes for illustrative purposes of the case study. In a real situation, all unit operations and product quality attributes would be considered.

Product Quality Attribute	CQA	Production Bioreactor	Protein A	Low pH/VI	CEX	AEX	Nanofiltration	UF/DF	Compounding	Filtration	Filling, stopper, cap	Testing elements
Aggregate	Yes	**Form**	**Removal**	Risk to form	**Removal**	**Removal**			**Risk to form**		**Risk to form**	LR
Deamidated isoforms	No	**Form**										
Oligosaccharide	Yes	**Form**										PM
Process-related Impurities												
CHO HCP	Yes	**Form**	**Removal**	Removal	**Removal**	**Removal**						PM
DNA	No	**Form**				**Removal**						None
Protein A	No		**Form**		**Removal**	**Removal**						None
Viral safety	Yes			**Inactiva-tion**		**Clear-ance**	**Clear-ance**					Bioreactor IPC

CPP	Operation includes a parameter(s) that must be tightly controlled to achieve CQAs	IPC	In-process control testing
WC-CPP	Operation includes a WC-CPP affecting a quality attribute	LR	Lot release
KPP	Operation includes a KPP impacting a process attribute	PM	Process monitoring
GPP	Operation includes no parameters with significant impact on QA or PA		
	Where CPPs and KPPs are present in the same operation, coloring highlights the highest level of control required.		

Figure 6.6 Process Control Points Parameter Categorization and Testing Strategy

6.4 Rationale for Selection of Testing Control Elements

A discussion on the testing control elements for the A-Mab control strategy is provided below. Additional details on the control strategies for the selected attributes (aggregates, oligosaccharide profile, HCP, viral purity and deamidated isoforms) are discussed in the Example Control Strategies section.

The science and risk based approaches utilized for the development of A-Mab along with application of prior knowledge and platform experience, provided opportunities to create a control strategy that incorporates a variety of different control elements. Due to the increased process and product understanding, there is less need to rely on specifications (final lot release testing), which is only one element of a control strategy, as a means to ensure consistent product quality. A natural consequence of this holistic view to establishing a control strategy for A-Mab is the exclusion of certain drug substance/product attributes that typically might be included as part of lot release testing under a non-QbD development paradigm and assuring control in other ways. This section describes the rationale for selecting the testing control elements used in the A-Mab drug substance and drug product control strategies.

6.4.1 Specification Tests

Specifications have been established to confirm drug substance and drug product quality and testing is performed using validated analytical procedures that have been shown to be suitable for this intended purpose. Design space and process experience from manufacture at multiple scales, including batches at the proposed commercial scale and facility have confirmed a robust and reproducible manufacturing process. Appropriate acceptance criteria were established considering this process knowledge along with stability assessments and data obtained for lots used in nonclinical and clinical studies. Specification tests for A-Mab Drug Substance and Drug Product are presented in Table 6.5 and Table 6.6, respectively.

Table 6.5 Drug Substance Specification (Final Lot Release)

Attribute	Test	Acceptance Criteria	Release	Stability [a]
Identity [b]	CEX	Consistent with reference standard and inspection of chromatogram for new peaks	Yes	No
Monomer	HPSEC	NLT 97%	Yes	Yes
Aggregates	HPSEC	NMT 3%	Yes	Yes
Endotoxin (LAL)	USP <85>	NMT 12.5 EU/mL	Yes	No

NLT = not less than; NMT = not more than

[a] Stability specification is for end of shelf-life.

[b] Identity chromatograms will be inspected for new peaks to monitor charge heterogeneity.

Table 6.6 Drug Product Specification

Attribute	Test	Acceptance Criteria	Release	Stability [a]
Identity [b]	CEX	Consistent with reference standard and inspection of chromatogram for new peaks	Yes	No
Biopotency	ADCC Bioassay	70% to 130% relative to reference standard	Yes	Yes
Monomer	HPSEC	NLT 95%	Yes	Yes
Aggregates	HPSEC	NMT 5%	Yes	Yes
Color	Ph. Eur. 2.2.2	Colorless	Yes	Yes
Clarity	Ph. Eur. 2.2.1	Clear	Yes	Yes
Particulate Matter	Visible particles (Visual)	Essentially free of visible particles	Yes	Yes
	Sub-visible particles USP <788>	Meets USP <788> requirement	Yes	Yes
Endotoxin (LAL)	USP <85>	NMT 12.5 EU/mL	Yes	No
Sterility	USP <71>	Meets USP <71> requirements	Yes	No

NLT = not less than; NMT = not more than

[a] Stability specification is for end of shelf-life.

[b] Identity chromatograms will be inspected for new peaks to monitor charge heterogeneity.

6.4.2 Justification for Specification Testing

Identity: A CEX HPLC method is conducted at lot release of drug substance and drug product to confirm the identity of A-Mab. The method separates the main charged isoforms of A-Mab that are considered to be product-related substances as defined in ICH Q6B. The resulting chromatographic profile is specific to A-Mab and unambiguously distinguishes it from other monoclonal antibodies manufactured by the sponsor. The spectrum of isoforms contained in the reference chromatogram for A-Mab represents acidic and basic isoforms that were assigned a low criticality by the CQA risk

assessment. Quantitative changes in these expected peaks at levels typically observed during routine manufacturing are not expected to have a practical impact on the safety and efficacy of A-Mab. However, the chromatogram is inspected to ensure a consistent profile with the reference standard and the absence of any new peaks. A quantitative definition of 'new peaks' is included in the CEX test method. Charged isoforms of A-Mab do not increase when stored at recommended conditions; therefore, this attribute does not need to be monitored on stability.

Biopotency: The potency of A-Mab is determined on drug product only using an *in vitro* cell based bioassay for antibody-dependent cellular cytotoxicity (ADCC). This bioassay is consistent with the proposed mechanism of action for this monoclonal antibody. This confirmation of functional activity is more relevant to be conducted on the finished dosage form since it is the drug product that is ultimately provided to the end user. Forward processing of drug substance without this test does not pose risk to the patient since it is conducted prior to lot release of the finished dosage form.

ADCC has nonetheless been roughly correlated with afucosylation *in vitro*. Thus, measurement of potency by ADCC also provides an indicator for this quality attribute that might impact Fc effector function. Potency is reported relative to a reference standard and the limit of NLT 70% and NMT 130% is consistent with assay capability and product knowledge. The bioassay is stability indicating and will be included in stability testing protocols.

Monomer and Aggregates (Purity): A SE-HPLC method is conducted on both drug substance and drug product at lot release and as part of the stability programs to quantify the levels of A-Mab monomer and soluble aggregated species in the drug substance. Soluble aggregates do have a high risk of potentially impacting safety and efficacy. Aggregated species can form in upstream, downstream and drug product processing without demonstrated means to provide clearance. Moreover, levels increase over the drug product shelf-life. Therefore, confirmation of purity by routine lot release and stability testing of monomer and aggregates has been implemented as part of the overall control strategy. The acceptance criteria of NLT 97% monomer and NMT 3% aggregates for drug substance and NLT 95% and NMT 5% aggregates for drug product are based on product knowledge, process capability, stability data and nonclinical and clinical experience.

The extensive product knowledge obtained throughout development indicates that there are no other A-Mab impurities having the potential to impact safety or efficacy. Therefore, a secondary orthogonal purity method is not required for specification tests. However the CEX method used for identity does provide an indirect means of accessing purity by evaluating the charge isoform profile.

Color, Clarity and Particulate Matter: Lot release testing for these quality attributes are not included for Drug Substance. A-Mab Drug Substance has been shown to be stable when stored at the recommended conditions and changes in color, clarity or particulate matter do not occur over time. These tests are appropriate for A-Mab drug product since the manufacturing operations are controlled to minimize extraneous particulate matter and stability testing is performed to confirm control of intrinsic proteinaceous particles and/or changes to color and clarity. Furthermore, A-Mab drug product must meet these compendia requirements.

Color is determined for drug product using test methodology described in Ph. Eur. 2.2.2. The specification conforms to the criteria for a colorless solution. Testing is performed at lot release and is included in stability testing programs. Clarity is determined for drug product using test methodology described in Ph. Eur. 2.2.1. The specification conforms to the criteria for a clear solution. Testing is performed at lot release and is included in stability testing programs. Two tests are performed on drug product for determination of particulate matter at lot release and as part of stability testing programs. The first test is conducted by visual inspection for evaluation of visible

particles. A specification of essentially free of visible particles is consistent with parenteral solutions for injection and the known attributes of A-Mab drug product. The second test is conducted for evaluation of sub-visible particles using test methodology and criteria described in USP <788>. Sub-visible particle enumeration by USP <788> is generally recognized for parenteral solutions for injection.

Endotoxin: Bacterial endotoxin is measured on both drug substance and drug product using the kinetic test methodology described in <USP 85>. The specification is consistent with USP requirements and is in accordance with the acceptable calculated Tolerance Limit in the FDA guideline, "Validation of the Limulus Amebocyte Lysate Test as an End Product Endotoxin Test for Human and Parenteral Drugs, Biological Products, and Medical Devices, 1987.

Sterility: Sterility testing of drug product is conducted at final lot release using USP <71>.

6.4.3 In-Process Testing

For certain quality attributes, in-process testing was selected as the appropriate means of control. This approach eliminates the need for testing at final lot release and is entirely consistent with ICH Q6B guidance. More importantly, the use of in-process testing provides opportunities to incorporate PAT to provide real-time monitoring of quality attributes, and applications were implemented wherever feasible. Table 6.7 and Table 6.8 show the in-process tests for A-Mab drug substance and drug product, respectively.

Table 6.7 In-Process Tests for Drug Substance

Attribute	Test	Acceptance Criteria	Comments
Protein Content	UV (280 nm)	NLT 65 and NMT 85 mg/mL	PAT post UF/DF. Acceptance criterion defines the minimum concentration to enable dilution to the target drug product concentration and the maximum concentration where stability is maintained.
pH	pH probe	5.0-5.6	PAT post UF/DF. Acceptance criterion defines the pH range affording maximum stability of A-Mab.
Bioburden	USP <61>	NMT 5 cfu/mL	Conducted on purified drug substance. Acceptance criterion based on prior knowledge with similar monoclonal antibodies manufactured using the platform process.
Mycoplasma [a]		Negative	Tested on unprocessed bulk bioreactor
Adventitious Viral Agents [a]		Negative	Tested on unprocessed bulk bioreactor
MMV [a]	PCR	Negative	Tested on unprocessed bulk bioreactor
Bioburden	USP <61>	Confirmed no contamination	Tested on unprocessed bulk bioreactor

[a] Tests conform with FDA Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products (1997) and the European Union (EU) Biotech Guideline 3A204A: Production and Quality Control of Monoclonal Antibodies Section 8.1 states that each lot of unprocessed bulk should be tested for mycoplasma, adventitious viruses and species specific viruses.

Table 6.8 In-process Tests for Drug Product

Attribute	Test	Acceptance Criteria	Comments
Protein Content	UV (280 nm)	95.0-105.0% of label claim	PAT Compounding Technology incorporated into compounding vessel and transfer lines.
Extractable Volume	Fill Weight	NLT 90% of label claim	PAT Filling and stoppering Measurement in real-time during filling adapted to fill line operation.
pH	pH measurement	5.0-5.6	PAT Compounding Technology incorporated into compounding vessel and transfer lines. Acceptance criterion defines the pH range affording maximum stability of A-Mab.
Osmolality	USP <785>	NLT 260 and NMT 320 mOsm/kg	PAT Compounding Conductivity measurement is a possible surrogate, but does not address contribution due to non-ionic species. In-line direct osmolality measurement by freezing point depression is possible. Acceptance criterion ensures isotonicity of drug product.
Polysorbate 20	HILIC CAD	±20% of target	Controlled by batch record. Mass balance across DP unit operations confirmed by off-line testing during development.
Sucrose	HILIC CAD	±20% of target	Controlled by batch record. Variability across DP unit operations confirmed by off-line testing during development.

6.4.4 Justification for In-Process Testing

PAT applications for measuring protein content and pH of drug substance and drug product have been implemented. PAT equipment, calibration and maintenance procedures used in downstream and drug product processing for content and pH measurement have been aligned to ensure consistency of output results. The remaining in-process tests for drug product are part of the overall product protection strategy. An additional PAT application for confirmation of isotonicity has been implemented in the A-Mab drug product process. The approach will either use conductivity or in-line freezing point depression measurement. Excipient levels are controlled by batch record with the acceptable ranges established by off-line testing during development.

6.4.5 Characterization Tests performed during Process Monitoring and/or Comparability Testing

The control strategy employs the use of process monitoring for selected quality attributes. This testing strategy is conducted routinely to trend product quality and process performance. Frequency of monitoring is periodically reviewed and adjusted based on observed trends. Data are evaluated relative to alert and/or action limits as appropriate. Characterization testing of selected quality attributes may be performed intermittently to confirm comparability in the case of movements within or changes outside the design space.

Oligosaccharide (afucosylation and galactosylation): Although afucosylation was determined to be a high criticality attribute, the ability of the process to control this post-translational modification was judged to be robust, thus removing the need to test for this attribute at lot release. Levels of afucosylated and galactosylated glycans were shown to be influenced solely by the production bioreactor. No process clearance or further modification is expected in downstream processing or drug product manufacture, and the attribute is not stability indicating. Input material and procedural controls are in place to ensure quality of raw materials and cell line. Control of the identified process parameters within the limits of the design space ensures consistency of afucosylation and galactosylation. In addition, process monitoring of afucosylated and galactosylated glycans has been implemented to trend consistency. Characterization of the complete oligosaccharide profile will be conducted to confirm comparability as needed. See also the discussion for this quality attribute provided in the Example Control Strategies section. Note that sialylation, high mannose content and non-glycosylated heavy chain were also determined to be CQAs. However, for the purposes of this case study, these attributes were not further considered.

HCP, DNA, Leached Protein A, Pluronic F68, Antifoam C, Methotrexate and Recombinant Human Insulin: Process parameter controls are used to ensure consistent clearance of these process-related impurities. The ability of the process to remove impurities has been demonstrated, relieving the need for lot-to-lot assessment. Characterization testing for selected process-related impurities may be conducted to confirm comparability as needed to support a process change that might affect these attributes. Note only HCP, DNA, methotrexate and leached Protein A were assessed for criticality. Only HCP was identified as a CQA.

6.5 Control Strategy Verification/Lifecycle Management

A continuous process monitoring scheme will be put in place to verify that the control strategy is performing as expected and remains appropriate.

Multivariate statistical process control (MSPC) will be used to monitor the performance of the upstream production bioreactor and selected downstream unit operations. Parametric data, both online and at-line, will be used to feed the MSPC. This monitoring tool provides a heightened level of sensitivity compared to traditional univariate approaches as it ensures that the correlation among all process variables included in it remains consistent over batches.

In addition, the control strategy employs the use of process monitoring for selected attributes. This testing strategy is conducted routinely to trend product quality and process performance. Frequency of monitoring is periodically reviewed and adjusted based on observed trends. Data will be control-charted using Statistical Process Control (SPC) and subject to alert/action limits. Characterization testing of selected quality attributes may be performed intermittently to confirm comparability.

Approximately thirty batches of the commercial process at scale are needed to calibrate the MSPC model and the control charts on selected attributes. Selected characterization testing will be conducted on all batches before the MSPC is established to provide the necessary data for the model and to establish the attributes control charts. After this, both monitoring tools will be used to monitor the process, with the MSPC performed on every batch.

Since both, the MSPC on parametric data and the control charts on selected attributes only apply to the current control space; they will be re-established whenever there is a movement within the design space. Re-establishing the MSPC or the control charts might not take the same number of batches after a movement within the design space. The complete control strategy verification scheme and its lifecycle management are illustrated in Figure 6.7.

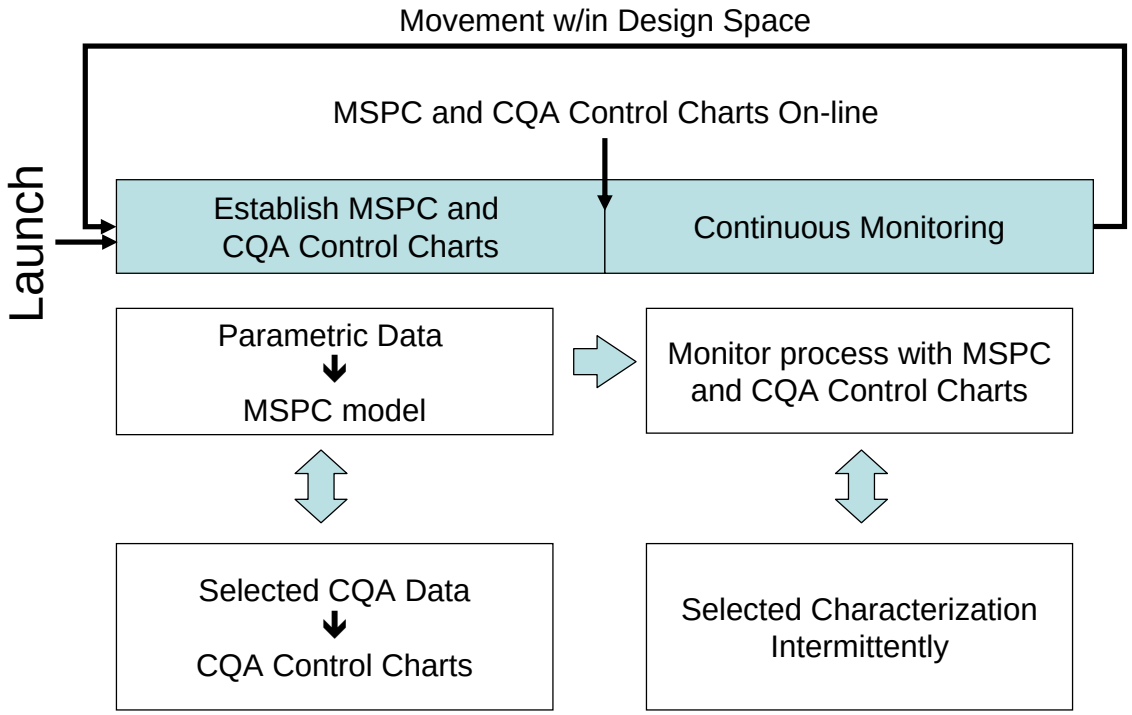

Figure 6.7 Control Strategy Verification Scheme

6.6 Example Control Strategies

6.6.1 Establishing the Control Strategy for Glycosylation

Molecular engineering (i.e., glycoengineering) to specifically influence the Fc oligossacharide profile was not incorporated into the design of the A-Mab. Therefore, this Quality Attribute was evaluated in conjunction with upstream process development since oligosaccharide heterogeneity is dependent on mode of production and culture conditions. No process clearance or further modification is expected in downstream processing or DP manufacture. The oligosaccharide profile is not a stability indicating attribute. Specific analytical assays were developed to support upstream process development and to evaluate product quality for batches used in nonclinical and clinical studies. An oligosaccharide profiling method utilizing CE-LIF (capillary electrophoresis-laser induced fluorescence) was developed and qualified for characterization of the oligosaccharide profile. This assay is capable of resolving and quantifying a broad spectrum of glycan structures.

Two additional *in vitro* cell-based bioassays were developed and qualified to enable collection of biological activity data related to ADCC and CDC effector functions as a means to assess Fc-oligosaccharide structure-function relationships.

A risk assessment was performed to evaluate the impact of glycosylation on the safety and efficacy of the product. Process performance and capability were not taken into consideration for this assessment. The outcome of this risk assessment resulted in a designation of 'High' criticality for certain oligosaccharide structures. For the purposes of this case study, sialylated, high mannose and other glycan structures including non-glycosylated forms identified as part of A-Mab oligosaccharide profiling were not considered, and the control strategy for glycosylation focused specifically on galactosylated and afucosylated structures which are known to impact CDC and ADCC effector functions, respectively. Note that high mannose glycans are also afucosylated. The ranges for other glycan structures are supported by nonclinical and clinical experience.

Since the production bioreactor has the greatest potential to influence the oligosaccharide profile and consequently impact safety and efficacy, a risk assessment was performed to evaluate the impact of each process parameter associated with this unit operation. The process parameters identified by this risk assessment as having the greatest impact on the oligosaccharide profile were further examined in upstream process characterization studies. Following completion of these studies, a final risk assessment was conducted to categorize input parameters. No Critical Process Parameters (CPPs) associated with the production bioreactor were identified since all parameters are well controlled and have demonstrated robust process operation. Thus, all quality-linked process parameters for the production bioreactor were classified as WC-CPPs and include: temperature, pH, dissolved carbon dioxide, culture duration and osmolarity.

Data from upstream process development for A-Mab (design space DOEs, scale-up lots, etc.), demonstrates that the levels of galactosylated and afucosylated glycans are maintained within the range of clinical experience by controlling the identified WC-CPPs. The design space limits for each of these parameters are discussed in the Upstream Process Development section.

A process capability risk assessment was conducted for the production bioreactor and the results are shown in Table 6.9. A low risk (RPN 30) was determined driven primarily by the fact that there are no other steps downstream that can mitigate impact of the oligosaccharide profile, but compensated by multiple process parameter controls (WC-CPP's) at the production bioreactor and a high degree of detectability.

Table 6.9 Process Capability Risk Assessment for Oligosaccharide Profile

Step	Severity	Occurrence	Detection	RPN
Production Bioreactor	10 Other steps cannot mitigate impact	3 Five WC-CPP's	1 Ability to detect	30 Low

The final control strategy for glycosylation takes into consideration additional key information obtained throughout the A-Mab development program. These include:

- The ranges for afucosylated and galactosylated structures are supported by nonclinical and clinical experience.
- *In vitro* bioassay data for ADCC and CDC effector functions demonstrates that potency is maintained within 70-130% over the range of experience for both

afucosylated and galactosylated glycan structures. The 70-130% potency range is supported by clinical experience.

- The ADCC bioassay included for drug product lot release testing indirectly measures consistency of afucosylated structures.
- Prior knowledge obtained with other monoclonal antibodies manufactured using the platform upstream process show similar consistency of oligosaccharide profile over multiple commercial lots.

Even though the criticality of afucosylated glycans is rated high, there is only one unit operation that impacts this quality attribute and the process capability risk is low. The extensive upstream development studies have identified the process parameters that influence the oligosaccharide profile and all of these are well controlled. Therefore, the levels of afucosylated and galactosylated glycan structures in A-Mab will be controlled at the production bioreactor by maintaining the identified WC-CPPs within limits of the design space, and routine testing will not be part of the drug substance lot release specification. To ensure consistency, testing for these specific glycan structures will be included as part of process monitoring. Process monitoring at the production bioreactor may include collection of complete oligosaccharide profiling data to support lifecycle verification of consistent operations. The frequency of monitoring will be reviewed and adjusted based on trends. Oligosaccharide profiling will also be included as part of characterization and comparability testing to verify movement within the design space or of process changes outside of the defined design space. A summary of the integrated control strategy for the oligosaccharide profile is presented in Table 6.10.

Table 6.10 Summary of Control Strategy for Oligosaccharide Profile

Control Strategy Element	Included Y/N	Rationale
Input Material Controls	Y	Routine analysis of raw materials by appropriate analytical methods ensures the required level of quality prior to use.
Procedural Controls	Y	A quality system is in place for raw materials management and maintenance of the cell line.
Process Parameter Controls	Y	Control of WC-CPPs within the limits of the design space ensures consistency of the oligosaccharide profile within the range of clinical experience.
In-process Tests	N	In-process testing of unpurified drug substance obtained from the production bioreactor to confirm the oligosaccharide profile is not required as long as the WC-CPPs are controlled within the limits of the design space.
Specification	N	Routine lot release testing of purified drug substance to confirm the oligosaccharide profile is not required as long as the WC-CPPs are controlled within the limits of the design space. The oligosaccharide profile is not stability indicating.
Process Monitoring	Y	Process monitoring of the drug substance oligosaccharide profile will be conducted to support lifecycle verification of consistent operations. The frequency of monitoring will be reviewed periodically and adjusted based on trends.
Characterization and Comparability	Y	Oligosaccharide profile data will be collected to support comparability.

6.6.2 Establishing the Control Strategy for Aggregate Level

Aggregation is an undesirable event that is common to all proteins, including antibodies, and can be rooted in many different causes. Control of aggregation is, therefore, more complicated than for most attributes. Aggregate level is a critical quality attribute and is stability indicating. Acceptance criteria for both drug substance and drug product, and hence the CQA range used in determining the design space, were established considering the acceptable range that ensures safety and efficacy (see Table 6.3).

HPSEC is the routine method for quantitation of monomer content and to observe aggregate peaks in drug substance and drug product due to the high accuracy and precision of the method. Orthogonal methods, including SDS-PAGE, analytical ultracentrifugation (AUC) and HPSEC with a MALLS detector were used for characterization of A-Mab samples to assure aggregated forms were not present but outside of the window of analysis by HPSEC and as part of the method qualification. Both reducing and non-reducing SDS-PAGE were used to qualitatively determine the level of disulfide-bridged covalent dimer.

Development of the strategy to control aggregate level utilized iterative risk assessments based on platform knowledge and product-specific data. Aggregation may generally be viewed as a symptom of some type of stress to the protein. Individual proteins vary from one another in their sensitivity to specific stresses. Typically, extremes of temperature, pH, osmolality, mechanical shear, covalent degradation, exposure to hydrophobic surfaces or interfaces have the potential to encourage protein aggregation (Manning et al., 1989). Further, risk is greater as protein concentration is increased and the level of aggregate tends to increase on storage of solutions, whether that solution is a process intermediate or a formulated product. Thus, control of aggregation depends on a holistic approach. Measurable characteristics of a protein are closely associated with specific risks of aggregation. Thus, before selection of the A-Mab primary amino acid sequence as a clinical candidate, the propensity for aggregation under a variety of buffer matrices and stressed environments was evaluated and used as a candidate selection criterion and to inform initial risk assessments. Manufacturing process risk has been minimized by a combination of process design, to minimize aggregate formation, inclusion of an operation capable of aggregate removal following the steps with the highest risk of generating aggregates, plus analytical controls to assure consistent process performance and assure patient safety. HPSEC is used as the primary analytical method due to its sensitivity and accurate quantitation of various forms along with its throughput and suitability for a quality control lab.

The process used to manufacture A-Mab is a “platform” process designed to minimize aggregates and other risks that was refined during development of several prior IgG drug candidates. That prior platform knowledge informed process design and early product-specific process risk assessments, and was supported by product-specific experimental designs. In summary, there is risk of aggregate formation at multiple steps in the process and aggregates are removed/cleared effectively at the cation exchange step. Aggregation occurs on storage as a solution, especially when the mAb is concentrated and aggregation rate is affected by the buffer pH, matrix and temperature. This understanding guided development of the control strategy for operations, development of the drug product formulation and analytical control. The conclusion of the product-specific risk assessment was that the highest risks of aggregation are in the bioreactor, the low pH viral inactivation step, pumping of formulated drug product and during the shelf life of the drug product.

A process capability risk assessment was conducted for the unit operations that pose the highest risk to aggregate formation and the results are summarized in Table 6.11.

Table 6.11 Process Capability Risk Assessment for Aggregate Level

Step	Severity	Occurrence	Detection	RPN
Production Bioreactor	1 Downstream steps clear most	3 Two WC-CPP's	10 No ability to detect	30 Low
Low pH/VI	3	3 One WC-CPP	10 No ability to detect, but have downstream clearance	90 Medium
Cation Exchange	10 Last clearance step; no downstream clearance	3 Two WC-CPP; clearance limit of failure is unknown	10 No ability to react except at release	300 High
Filling	10 No downstream clearance	10 Three CPP; two WC-CPP	10 No ability to react except at release	1000 High

Aggregate may be formed at each of several steps and can be removed in the CEX ion exchange chromatography step. Thus, formation of aggregates during steps prior to CEX is primarily a threat to process yield and economics. Severity of impact was rated as 10 unless there are downstream steps that can clear aggregate. Therefore all steps prior to CEX were rated as either 1 or 3 and as 10 for CEX and filling. The process was scored as having moderate control risk over both generation and removal of aggregates when viewed at the CEX effluent mainstream. However, there is no capability to remove aggregates after CEX chromatography, so even small amounts of aggregate formed at any point thereafter affects the drug product. Therefore the overall process has moderate control over formation of aggregates and moderate ability to clear aggregates (two CPPs; see under Downstream), thus the overall process capability was scored as "high risk" under "occurrence" as the integrated process control strategy is developed. Thus, while the process delivers appropriate aggregation control when operated well within the process design space/limits, release testing is necessary to assure that an undetected process deviation does not compromise product quality.

An integrated control strategy has been developed based on the process risks and mitigations, above, and is summarized in Table 6.12. Detailed descriptions of process capability can be found in the respective sections describing the upstream, downstream and drug product processes.

Table 6.12 Integrated Control Elements for Aggregation

Unit Operation	Process Capability Risk	Input Material Testing	Procedural controls	In-Process Tests	Specification	Process Monitoring	Stability Testing	Characterization Testing
Vial Thaw and Seed								
N-1 Seed Bioreactor								
Production Bioreactor	Medium							
Clarification								
Protein A								
Low pH/VI	Medium		X			X [a]		X
CEX	High		X			X [a]		
AEX								
Small Virus Retentive Filtration								
TFF								
Drug Substance					X		X	X
Freeze			X					
Compounding		X						
Filling	Medium		X					
Finishing					X		X	X

[a] Process monitoring supports lifecycle verification of consistent operations; the frequency of monitoring will be reviewed periodically and adjusted based on trends.

6.6.3 Establishing the Control Strategy for Host Cell Proteins

Host cell protein (HCP) is a process-related impurity originating from the host cell line, consisting of a complex mixture of intracellular and secreted proteins which are released into the cell culture medium during the cell culture process. HCP has been rated as a high criticality quality attribute because of the potential immunogenicity of non-human proteins. The target HCP level in the Drug Substance is 100 ng/mg or less in the Drug Substance to ensure safety of the Drug Product.

HCP originates during the cell culture process and is cleared during the downstream purification process. HCP levels are not affected by the Drug Product manufacturing process, nor do the levels change during storage of Drug Substance or Drug Product. Therefore, the control strategy for HCP will be focused on the Drug Substance process only. The approach to controlling HCP levels has been to conduct risk assessments and process characterization studies to identify the process steps and parameters that influence HCP generation and removal, followed by definition of a design space and control ranges to ensure that HCP levels in the drug substance remain below 100 ng/mg. A dependency was found between the Protein A and Cation Exchange chromatography steps wherein the Cation Exchange step could not in all instances clear high challenge levels of HCP down to target levels. Options are presented to constrain either the Protein A design space or the Cation Exchange design space, or to employ in-process testing, to ensure robust control of HCP. Details of these risk assessments and characterization studies can be found in the Upstream and Downstream process sections.

HCP is measured by an ELISA assay which was developed specifically for the CHO host cell line and platform purification process. During its development, the method was qualified against 2-D gels and Western blots as providing a response to a broad range of host cell proteins; therefore the method can be considered to provide an accurate assessment of overall mass levels of host cell protein residuals in the product. The assay has been validated for linearity, precision, accuracy, and robustness to enable its use in process characterization and validation studies.

The overall process capability risk for HCP was assessed by considering the results of the process characterization studies for the production bioreactor, Protein A, AEX, and CEX steps, as shown in Table 6.13.

Table 6.13 Process Capability Risk Assessment for HCP

Step	Severity	Occurrence	Detection	RPN
Production Bioreactor	3 Downstream steps will clear HCP	1 Only one KPP impacts HCP	3 Cell viability is surrogate for HCP	9
Protein A	3 Downstream steps will clear HCP	1 Two WC-CPP impacting HCP	10 No detection possible without in-process test	30
Cation Exchange	3 Downstream steps will clear HCP	1 Two WC-CPP impacting HCP	10 No detection possible without in-process test	30

Table 6.13 Process Capability Risk Assessment for HCP

Step	Severity	Occurrence	Detection	RPN
Anion Exchange	3 No downstream steps to clear HCP, however, input levels are typically below target for DS	1 Two WC-CPP impacting HCP	10 No detection possible without in-process test	30

The overall process capability risk was assessed to be 30 (low risk), primarily driven by the presence of redundant clearance steps in the purification process and by the ability to tightly control parameters affecting HCP in all process steps, thereby greatly reducing the risk of occurrence of failures.

Despite the high criticality ranking for HCP, the low process capability risk for HCP results in a low overall risk to the patient, so procedural and process parameter controls will be the primary means for assuring control of HCP levels. To address the dependency between the Protein A and Cation Exchange design spaces, the option has been taken to constrain one or the other design space to avoid the need to perform in-process testing. However to demonstrate that HCP levels are well controlled upon scale-up of the process, HCP will be monitored in-process and in the Drug Substance during initial at-scale runs in the commercial facility. In addition, HCP will be monitored over the course of chromatography column lifetime at production scale to ensure that column performance remains consistent with respect to HCP removal. The control strategy elements for HCP are summarized in Table 6.14.

Table 6.14 Summary of Control Strategy for HCP

Control Strategy Element	Included Y/N	Rationale
Input Material Control	N	No specific tests are performed on raw materials to control HCP levels, since materials do not impact HCP generation or removal
Procedural Controls	Y	Procedures are in place to ensure operation of the process within the defined control space. WC-CPPs that are controlled procedurally include harvest time, column loading, and buffer preparation for various process steps.
Process Parameter Controls	Y	Control of KPPs and WC-CPPs at the Production Bioreactor, Protein A, Anion Exchange, and Cation Exchange steps within the design space ensures control of HCP generation and removal by the process
In-process testing	N	In-process tests are not required since HCP is well controlled by the maintaining process parameters within their defined ranges
Specification	N	The process has been characterized at small scale and at full scale for its capability to control both the generation and removal of HCP to levels well below the target range. In addition, HCP is a medium criticality quality attribute so the risk to the patient is extremely low.

Table 6.14 Summary of Control Strategy for HCP

Control Strategy Element	Included Y/N	Rationale
Process monitoring	Y	HCP will be assessed during initial at-scale runs to ensure consistent control at production scale. Also HCP levels will be monitored in-process at periodic intervals as part of the column lifetime protocols. This monitoring may be discontinued once robustness of column performance over the resin lifetime is demonstrated.
Characterization and Comparability	Y	Process changes may impact the generation or clearance of HCP by the process, so it is necessary to evaluate HCP levels when changes are made.

6.6.4 Establishing the Control Strategy for Deamidated Isoforms

The mechanism and conditions conducive to the formation of deamidated isoforms are widely known and well understood. This knowledge, in conjunction with the low criticality of the quality attribute, has eliminated the need for in-process testing or extraordinary process control.

To fully understand the process and determine the appropriate process control strategy, levels of deamidation observed in designed process characterization experiments were assessed. Clinical batch data as well as process history were also considered. Based on the body of process data as a whole, it was determined that the level of control on the process for other more critical attributes far exceeded the level of control needed to maintain deamidation. Due to the evidence of very low criticality of this attribute, acceptable levels of deamidation is not limited by levels seen in material used in clinical trials.

Finally, since release testing includes a CEX identity assay, shifts in the profile would be detectable, indicating that a process consistency investigation might be warranted.

6.6.5 Establishing the Control Strategy for Viral Clearance

The risk assessment for viral clearance is described in Section 4 (Downstream Manufacturing Process Development). Process capability results indicate that the A-Mab downstream process is robust and that it consistently reduces viral loads to levels that are well below safety concerns. Thus, the risk of viral clearance failure is very low.

The control strategy to ensure viral safety is based on operating the 3 viral clearance steps (low pH virus inactivation, AEX chromatography and small virus retentive filtration) within the established design spaces. Working within the design space of each step provides a high degree of assurance that viral clearance targets are met.

In addition, the potential presence of adventitious virus is checked by testing cell culture samples at the end of each production bioreactor step. These tests are part of the batch release specification.

References

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7 Regulatory Section

ICH Q8(R2) provides that when a company chooses to apply quality by design and quality risk management (ICH Q9) in the context of an appropriate pharmaceutical quality system, opportunities arise for enhanced science- and risk-based regulatory approaches (ICH Q10). The regulatory section is provided to stimulate discussion about how the knowledge and data exemplified in this case study can be used to create risk-based regulatory strategies for product licensure, and management of changes to the manufacturing process.

This section will discuss the following regulatory propositions:

1. An enhanced understanding of product attributes based on prior knowledge, preclinical and clinical data, linked to demonstrated understanding of the process can result in a more rational basis for design of the overall control strategy.
2. 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 regulatory approval and oversight (i.e., are considered "regulatory commitments"). Other parameters not associated with CQAs are controlled and monitored in the quality system to ensure process and product consistency, but are not considered regulatory commitments.
3. The design space is based on development data generated from small scale lots up to commercial scale lots. This data in its entirety can form the basis for process qualification and validation when coupled with a program of continued process verification.
4. An iterative, risk based approach for managing changes to the manufacturing process can be implemented by leveraging the original approach for creating a design space by linking process parameters to critical quality attributes.
5. Movement within a design space, based on the documented lack of effect on critical quality attributes, can be managed within the quality system.
6. For movement outside of a design space, the outcome of the risk assessment exercise will facilitate determination of the data required to support the change. The level of regulatory oversight required for the change should be proportional to the level of risk identified

Some examples of specific application of these principles with regard to managing changes outside of a design space are provided as a stimulus to discussion in an appendix to the section.

7.1 Definition of Critical Quality Attributes and Development and Management of a Control Strategy

Proposition 1: An enhanced understanding of product attributes based on prior knowledge, preclinical and clinical data, linked to demonstrated understanding of process results in a more rational basis for design of process controls and product specifications

In the QbD paradigm, control strategies are based on the scientific understanding of the linkage between product quality attributes and the safety and efficacy of the product.

In this case study, CQAs were identified by ranking product quality attributes based on their potential impact on clinical performance using all available relevant product knowledge. The results of this assessment were:

1. The identification of the critical quality attributes (CQAs) that must be considered and controlled by the manufacturing process
2. Establishment of numeric ranges for each CQA that, based on overall product knowledge, are consistent with the product's desired performance

The list of CQAs and their respective ranges were then used to define the design space and an overall control strategy that assures the quality of all CQAs when the process operates within the confines of the design space. The control strategy includes objective identification of the few higher ranked CQAs that should also be tested in-process or at lot release during routine manufacturing.

Thus, the QbD approach provides a rational and science based approach to linking product specifications to clinical relevance. This differs greatly from the current practice of setting numerical acceptance limits based solely on clinical trial experience and/or process capability and assay performance considerations

Likewise, in the QbD paradigm, adjustments to the product's specifications throughout the lifecycle should be based on the same rational evaluation of clinical relevance rather than on the traditional approach of basing adjustments on statistical analysis of manufacturing performance at target process conditions. In the traditional approach as process control capabilities increase and sources of variability are eliminated, the quality outputs and thus specification limits become even tighter. This traditional approach ensures that process performance and quality outputs are consistent, but fails to consider clinical relevance and the linkage of the specifications to the approved design space.

In QbD applications, approved product specifications should not change unless significant new data related to clinical outcomes became available. However, continued assurance of consistent process performance and identification of any potential out-of-trend results would be assured by applying process-capability analysis as part of the continued process verification approach managed through the quality system.

7.2 Presentation and Regulatory Impact of CPPs and Design Space in Filings

Proposition 2: 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 regulatory approval and oversight (i.e., are considered "regulatory commitments"). Other parameters not associated with CQAs are controlled and monitored in the quality system to ensure process and product consistency, but are not considered regulatory commitments.

Regulatory commitments are the elements of the file that will not change without health authority agreement. The health authority interaction can range from annual notification to approval prior to implementation.

Identification of critical quality attributes and linkages with process parameters provides a strong rationale for limiting the regulatory commitments only to those process parameters with a potentially significant impact on CQA(s). Design spaces are comprised of acceptable ranges or equations for the CPPs and WC-CPPs identified for each unit operation. These design space elements are considered regulatory commitments. The design space may also require regulatory control of critical raw materials; however, this was not explored in the case study. If a unit

operation has only one CPP or WC-CPP, that parameter is considered a regulatory commitment. The limited list of regulatory commitments does not imply a lack of oversight or control, since all parameters, including KPPs and GPPs are identified, controlled and managed within the quality system.

An understanding of the overall process development history incorporating risk assessments and process design decisions is important in the overall evaluation and justification of the product and process controls with regard to a regulatory submission. In order to understand the basis for the overall process and product control strategy the following information would be provided:

- A summary of the process development history and understanding that is the basis for selection of the routine operating parameters for each unit operation and their classification as critical, key or general for each unit operation.
- A list of CPP, WC-CPPs, critical inputs and raw materials that comprise the design space, and therefore the regulatory commitments, for each step.
- KPPs and GPPs are also described for each step, but because these parameters do not result in any practical impact on the product's critical quality attributes, they are not included in the design space and not considered regulatory commitments.
- High level process descriptions for unit operations where no CPPs or WC-CPPs are identified will be provided, but a specific design space will not be provided. This is exemplified in the case study by the thaw, seed-train and N-2 and N-1 steps presented in the upstream section.
- The current set of operating conditions (i.e., control space) will be described , but this information is provided for information only.

An important consideration is the amount of data that is required in order to understand the process development and risk assessment summaries. The data presented in this case study includes detailed information that exemplifies the types of logic that must be built into risk assessments used to identify areas of high risk to product and patient, as well as the rigor required of the tools used to perform the data analysis. This may represent an extreme level of data and detail not required or useful with regard to a regulatory submission.

7.3 Process Qualification and Validation

Proposition 3: The design space is based on development data generated from small scale lots up to commercial scale lots. This data in its entirety can form the basis for process qualification and validation when coupled with a program of continued process verification.

A design space is justified based on process-specific and historical (platform) data collected over a wide range of scales. The majority of the data will result from small scale experimentation with the balance being supported by clinical and commercial production scales. Continued process verification can be considered an integral part of the process to assure that the important process parameters associated with CQAs are maintained.

The approach to process qualification lots differs in this case study from the traditional 3 or more "pre-filing" validation lots by allowing commercial scale lots produced at any time during development to be used to assure that the process, when operated within the proposed commercial design space meets all in-process controls and release test limits intended for the commercial scale process.

These lots:

1. Are accepted as suitable based on a Quality approved protocol that specifies the required process conditions, analytical testing and criteria to be met for lots to be considered representative of the commercial production.
2. The number of lots required is risk-based and dependent on the amount of process understanding available and an assessment of the information needed to appropriately augment the development data. There is no arbitrary requirement for a certain number of lots.
3. They can be run using various target set points, provided the targets are contained within the proposed design space.

In this case study, since no commercial scale production was performed during clinical development, two commercial scale lots were manufactured prior to the license application to gather additional data to support the design space. However, if a sponsor had already produced commercial scale lots during clinical development using a documented process that met all the requirements of the commercial process, no additional commercial lots would be needed prior to the filing.

Small scale development lots can be utilized to demonstrate process robustness and consistency provided appropriate product characterization testing has been performed on these lots to ensure that they meet the criteria established for the CQAs. For example robustness of process design and independence from thaw to thaw variation, are judged through the robustness and inclusiveness of the development data with regard to these issues, rather than on including > 2 thaws in the traditional "demonstration campaign" of 3-5 consecutive runs. Information on these lots would be included in the Process History data set, and analytical results would be included in the appropriate section of the CTD.

Design spaces are not confirmed at scale at the edges of the ranges. Confidence in the small scale models is sufficient to allow direct application and approval of the design space based on a combination of the following:

1. engineering knowledge including first principles and model verification through limited ranges
2. the strength of the overall Process Design data set that indicates that appropriate process performance was achieved as the process was scaled, and
3. the historical platform knowledge derived from the scale-up of similar processes

There are other ways that confidence in the design space could be provided. Collection of development data at scale using various targets for parameters within the design space may be particularly useful.

An important component of this approach is the use of a Continued Process Verification & Improvement (CPV) program to further demonstrate expected process performance within the design space. CPV at commercial scale is managed within the quality system and enables continued improvement in addition to ongoing assessments of process capability and product consistency. Data collected as a result of investigation of manufacturing deviations also enhances process understanding and increases confidence in the design space model. Process monitoring data will be collected at pre-defined intervals to review process performance and to assess if a change in the process control is warranted. A general description of the Process Verification program linking the

process knowledge at filing to the CPV program will be included in the CTD in the sections describing process validation

7.4 Risk Based Approach and Lifecycle Management

Proposition 4: An iterative, risk based approach for managing changes to the manufacturing process can be implemented by leveraging the original approach for creating a design space linking process parameters to critical quality attributes.

In regulatory submissions employing a QbD approach, it is important to distinguish between the information provided to demonstrate product and process knowledge vs. commitments made under the license that impact day to day operations and the management of post-approval change.

Throughout this case study detailed information is provided to demonstrate the fundamental understanding of the process and product:

1) Full characterization of the molecule,
2) Definition of its CQAs and development of scientifically relevant specifications
3) Differentiation between process parameters with a significant impact on the CQAs and all others.

The overall risk assessment as outlined in the control strategy section (Figure 5-2) is used to support lifecycle management. The key element to consider when the risk assessment is repeated is to analyze proposed movements within or changes to the design space, is whether the proposed change falls within criteria and limits defined by the previous assessment. Categorization of reporting requirements would be commensurate with potential risk. The highest levels of regulatory oversight are reserved for change scenarios with a potential to impact CQAs. A discussion on our approach to assessments and categorization of change, within and beyond an approved design space, is provided in the following sections.

7.4.1 Movement within the Design Space

Proposition 5: Movement within a design space, based on the documented lack of effect on critical quality attributes, can be managed within the quality system.

As stated in ICH Q8(R2), working within the design space is *not considered a change* (from a regulatory filing perspective). However, as stated in ICH Q10, from a pharmaceutical quality system standpoint, *all changes* should be evaluated by a company's change management system.

Planned movement within a design space does require a prospective assessment of the risks associated with the particular move to be performed within the quality system and a conclusion that the proposed change is supported by the existing product and process knowledge. Movement within the design space is managed without a regulatory notification requirement because the space has already been assessed and approved.

Movement within the design space is exemplified by multiple examples in the case study demonstrated not to have an impact on the CPPs and WC-CPPs, such as a change in drug substance or product scale, increase in column size and appropriate redefinition of column load or reset of a process parameter target in the design space.

7.4.2 Changing the Design Space

Proposition 6: For movement outside of a design space, the outcome of the risk assessment exercise will facilitate determination of the data required to support the change. The level of regulatory oversight required for the change should be proportional to the level of risk identified

Change outside the design space must be evaluated on a case-by-case basis to determine the appropriate data package and regulatory approval pathways. We envision that the systematic process risk assessments described for the approval of the original process provide the roadmap to assess the impact of changes outside the design space. As with the designation of CQAs and CPPs, we believe that process change risk occurs on a continuum, and as a result, regulatory oversight should be tied to that continuum.

Figure 7.1 illustrates an approach for assessment of change for a given unit operation under a QbD paradigm. The proposed change would be evaluated for its impact on the originally defined design space, and the outputs for the specific unit operation. The impact of changes to a given unit operation must be evaluated for potential impact to the specific step and subsequent steps. Changes that impact multiple unit operations would be considered higher risk, and require appropriate evaluation before being deemed acceptable. More complex changes would require higher levels of regulatory oversight.

As an example of a simple change to a single unit operation, a new Protein A resin was implemented (Section 4.6.1), resulting in only minor changes to the design space parameters and no effect on acceptable outputs for that step, based on previous knowledge of attribute criticality.

A more complex scenario, but one still limited to a single unit operation, would be a situation where technology has improved. For example, the original process employed an anion exchange resin column and the manufacturer would like to replace it with an anion exchange membrane. The design space for the membrane, which would be established following the same principles as used to develop the resin design space, would be different than that of the resin, and the physical/chemical properties (i.e., equipment, buffer, buffer volume) are different. However, the resin and membrane employ the same separation mode, data demonstrate that the outputs of the steps have acceptable quality attributes, and the input requirements of the subsequent nanofiltration step are met. As a result, the potential impact of this technology change is limited to the process step. This change would require regulatory notification because a new critical material (the membrane) has been employed and a new design space was created for that step. However, analysis shows that the critical output of the step has not changed, thus the risk level would be deemed low and implementation would be immediate. An example of this type of technology change and how it would be evaluated is provided in the Appendix.

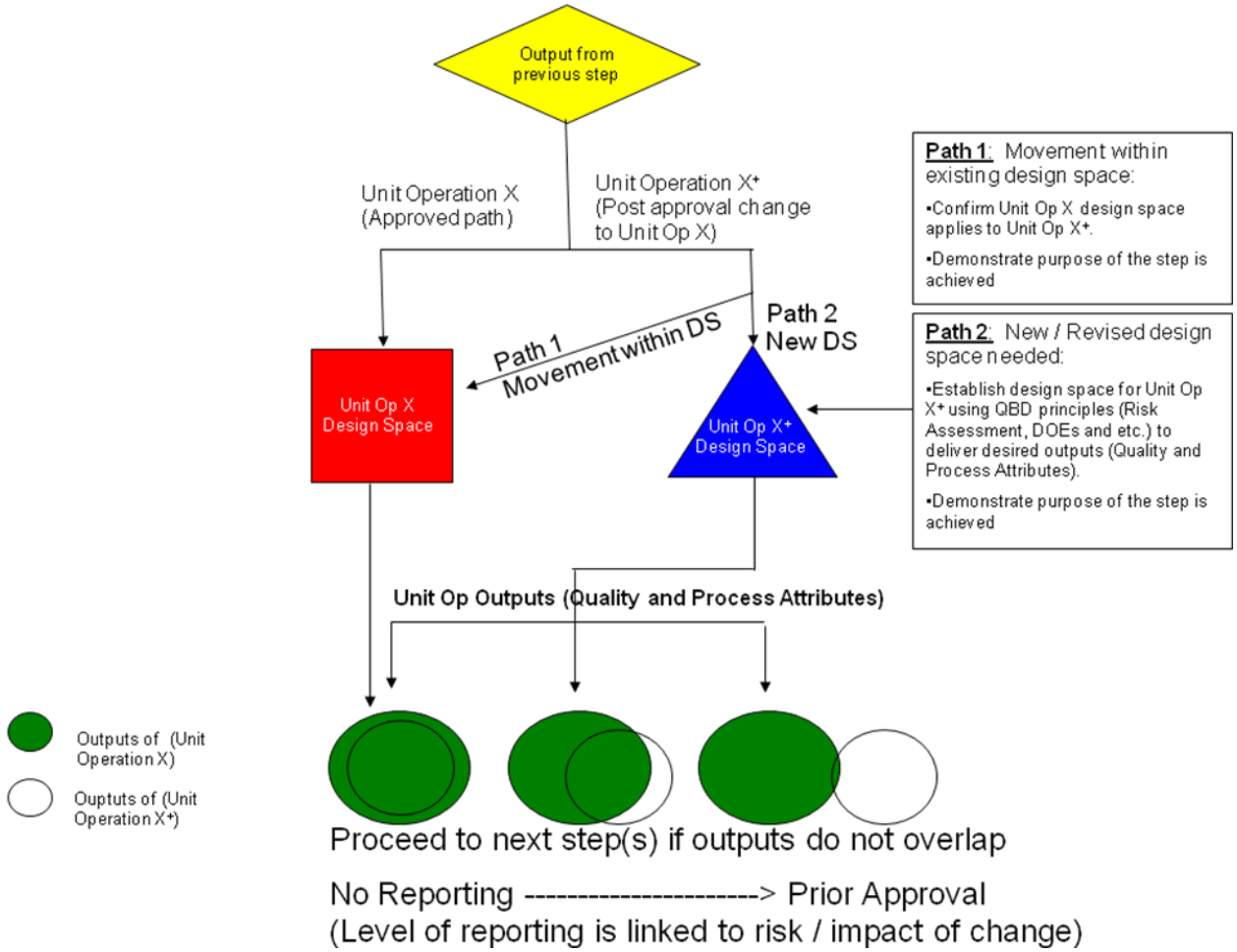

Figure 7.1 Change Assessment Diagram

7.4.3 Assessment of Risk and Continuum of Process Change

Establishing the initial design space creates the framework for movement/change. The scope of the knowledge presented in the original filing provides the foundation for assessing risk and for projecting the type of information that would be provided to justify the change. There could be greater latitude for less regulatory oversight based on the level of risk associated with the change due to the knowledge around the original filing. Some changes would be associated with greater potential risk and would require greater oversight such as those associated with an impact on a critical quality attribute. Assessment of risk is independent of regulatory system or region. Approaches for regulatory oversight differ among regions but generally are consistent that a greater degree of risk is associated with greater oversight. For the purpose of stimulating the discussion these elements have been captured in the discussion and table below but are not meant to be prescriptive with regard to any particular regulatory system.

A matrix building on the above discussion and illustrating changes of various complexities across the spectrum is provided in Table 7.1. Changes that effect individual -vs multiple unit operations, as well as changes that have no impact on product quality –vs- changes that may impact a CQA are captured. It represents one approach for how changes could be evaluated based on the inherent level

of risk. The Table captures multiple facets that would be evaluated as part of any change; and is intended to show the next level of detail beyond the high level view at the single unit operation level depicted in the flowchart. Since there are multiple alternative paths through the flowchart and the Table, the Table is illustrative, but not all inclusive. All sites where product introduction is proposed are authorized to manufacture mAbs. In the top row the risk continuum is shown from A through F with A being the lowest anticipated risk and F being the highest (Green = Low risk, Yellow = Med Risk, Red = High Risk). The lower portion of the table shows that the level of additional testing required to support the change would increase as the risk related to the change increases.

In the table, change is assessed based on a number of different criteria such as type of change, process fit, impact on critical process parameters, and number of unit operations affected. In the top row the risk continuum is shown from A through F with F being the highest risk. Unit operation outputs and the ability of the drug substance to meet in process and lot release criteria are assessed to categorize the change. The lower portion of the table shows that the level of additional testing required supporting the change increases as the risk related to the change increases. For the purpose of this discussion it is assumed that all sites where product introduction is proposed are authorized to manufacture mAbs. The different approaches for reporting the process change are also suggested in the last row of the table.

For a like-for-like change (risk level A) where the process fit, critical process parameters, and step outputs remain the same, and there is no impact on subsequent operations, no additional product characterization, stability or clinical studies would be required. Based on the assessment, no regulatory filing is required prior to implementation, although the change would be reported in an annual product update. In contrast, a risk level F change that creates a new design space and impacts multiple steps would require evaluation of in process test and lot release testing as well as full characterization testing to establish comparability. Depending on the outcome of those tests, additional non-clinical or clinical data could be required. In this circumstance, full review of all relevant data and agency approval would be required prior to implementation of the change.

In many situations, a change to the design space will not result in a change to the acceptable ranges of the CQAs (columns A-E). The change will involve an enhancement of process knowledge that extends the design space beyond its previously tested limits, or seeks to substitute new technology or materials with similar performance capabilities. In these cases reapplication of the original risk assessment processes to the proposed changes should result in the review of all relevant process parameters for critical impact on the product's quality attributes and provide assurance that the process control strategy is adequate to ensure the reliability of the revised process. In these cases, the level of risk is minimized and associated with a less stringent regulatory oversight.

However, certain changes may be more complex than others, either because of their poorly understood mechanism of action (for example a peptone replacement, categorized as column F) or because they impact multiple unit operations (columns D-F). For more complex changes, these would be associated with a greater potential risk requiring a more stringent regulatory oversight. For instances where the ranges of the CQAs themselves are being revised, it is anticipated that a comprehensive data package, including relevant non-clinical and clinical data may be required.

Assessment of any change requires knowledge of the risk assessment and testing utilized in characterizing the process change. In order to provide some additional detail regarding the above discussion, a number of process changes are discussed along with protocols for assessment in the appendix.

Table 7.1 Potential Regulatory Pathways for Risk-Based Approaches to Change Management

Risk Continuum	A =Low	B	C	D	E	F = High
INPUTS Raw Material/ Technology Equipment/ Site/ Scale	Like for Like	Minor Change	New Material, Technology	DS/DP Site Change	New Material, Technology, multiple changes	New Material, Technology, multiple changes
Process/ Engineering Fit Risk Assessment	Meets criteria	Minor Changes	Major Changes	Meets criteria	Major Changes	Major Changes
CPPs/WC-CPPs	Parameters unchanged	Minor change	New Design Space	Parameters Unchanged	New Design Space	New Design Space
OUTPUTS Acceptable for next step	Meets Criteria	Meets Criteria	Meets Criteria	Meets Criteria	Does Not Meet Criteria	Does Not Meet Criteria
# Unit Ops Impacted	Single	Single	Single	Multiple	Multiple	Multiple
Meets Lot release criteria and in process criteria	Yes	Yes	Yes	Yes	Yes	Some IPC criteria require minor modification
Extended Comparability Required	No	No	Yes, selected testing . Results meet criteria	Yes, full testing, Results meet criteria	Yes,, full extended testing, Results meet criteria	Yes,, full extended testing, some minor differences observed
Additional drug substance stability	NA	NA	Yes/ (annual lot)	Yes/ (annual lot)	Yes, data provided	Yes, data provided
Additional drug product stability	NA	NA	Yes, for DP changes only, annual lots	Yes, for DP changes only, annual lots	Yes, DP changes only	Yes, DP changes primarily
Supportive Clinical/Non-clinical	No	No	No	No	No	Maybe
Example Agency Reporting Category	Reported in Annual Update		Reported prior to implementation, no approval required		Reported prior to implementation , expedited review timeframe	Reported prior to implementation, routine review timeframe

If lot release tests fail, need for non-clinical, clinical data increases

"Extended testing" includes agreed upon tests beyond lot release that assure CQA consistency, including assurance of viral clearance.

Stability data would include accelerated & real time data

Depends on results of comparability exercise

Categorization based on what is justified by risk and knowledge. Suitable reporting categories may not yet be available in all regions.

7.5 Detailed Protocols: Appendix 1

7.5.1 Protocol for Change in the Source of Protein A Resin

The following is an example of a change to be included in an Expanded Change Protocol (ECP) and/or Regulatory Agreement/Post Marketing Plan and will be used in conjunction with the internal change management system to introduce a new source of Protein A resin into the A-Mab manufacturing process. The change will be reported as defined in the Table 7.1

- A technical report will be prepared to document internally the results of the experiments conducted (see below) to establish the unit operation design space for the new resin and demonstrate that the purpose of the step as defined in the initial submission is achieved. The report will include discussion of the design space and an assessment of the impact on subsequent steps to provide the supporting documentation to demonstrate that acceptable product quality is achieved.
- Process risk assessment will be conducted at the Protein A step or beyond as necessary using an approach similar to that described in 4.6.1and as outlined below:
  - Outputs from the previous step remain unchanged (preceding steps are not impacted by the change in resin)
  - Purpose of the step remains unchanged and demonstration that new resin achieves purpose of the step
    - A lab scale model, similar to the one used as the scale-down model for the manufacturing process scale step, will be used to qualify the new Protein A resin. The following studies will completed
      1) A multivariate DOE study (similar to that described in section 4 of the Protein A process description) will be performed to confirm or modify the existing design space.
      2) A study using multiple lots of clarified harvest will be conducted to evaluate the impact on product and process related impurities. The product and process related impurities will be controlled within the historical ranges. The outputs would be deemed acceptable if they can be handled in the model linking subsequent steps provided in the downstream section.
      3) Appropriate confirmation of resin reuse to support the claimed lifetime
      4) Confirmation that the registered Protein A assay is appropriate for testing leached Protein A from the new resin
      5) Confirmation that the leachable / extractable profile of the new Protein A resin is suitable for the intended use. This evaluation will include evaluating removal by downstream polishing steps.
      6) Stability study of lab-scale drug substance pools generated from use of the new resin
    - At scale data will be collected concurrently with introduction of the new resin into the manufacturing process, testing will be guided by the risk assessment.

7.5.2 Protocol for Replacement of the Anion Exchange Resin with a Membrane

The following is an example of a change to be included in an Expanded Change Protocol (ECP) and/or Regulatory Agreement/Post Marketing Plan and will be used in conjunction with the internal change management system to introduce a new membrane technology into the A-Mab

manufacturing process. The change along with the appropriate supporting information described below will be reported as defined in Table 7.1 (Potential Regulatory Pathways for Risk-Based Approaches to Change Management).

- A technical report will be prepared to document the results of the experiments conducted (see below) to establish the unit operation design space for the new membrane and demonstrate that the purpose of the step as defined in the initial submission is achieved. The report will include the critical/key process parameters for the new membrane, an assessment of the impact on subsequent steps, and provide the supporting documentation to demonstrate that product quality is not negatively impacted as a result of the technology change.
- A multivariate DOE study (similar to that described in Section 4.6.4 will be performed to establish the design space for the membrane. Lab scale runs using multiple lots of feed stock from CEX chromatography will be conducted to evaluate the impact on product and process related impurities. The product and process related impurities will be controlled within the historical ranges.
- Purpose of the step remains unchanged and demonstrate that new technology achieves purpose of the step including:
    1) Process related Impurities previously demonstrated to be impacted by this step are at or below the upper statistical limit of historical data (DNA, HCP, leached Protein A, etc.). Product related Impurity profile is consistent pre / post change
    2) Process outputs (i.e., volume, pH, salt, concentration, and etc.) of the new technology are for subsequent process steps.
    3) Viral reduction of the manufacturing process with the new technology complies with ICH Q5A. The outputs would be deemed acceptable if they can be handled in the model linking subsequent steps provided in the downstream section and provide adequate safety margin.
    4) Confirmation that the leachable / extractable profile of the membrane is suitable for the intended use.
    5) Stability study of lab-scale product pools generated from use of the new membrane.

At scale data will be collected concurrently with introduction of the membrane into the manufacturing process to confirm that the intended purpose of the step is achieved and that product quality is maintained. Drug substance batches produced using the membrane technology will comply with all registered specifications (Demonstrate no impact to drug substance CQAs).

7.5.3 Site Change for A-Mab Drug Substance or Drug Product

The following is an example of a change to be included in an Expanded Change Protocol (ECP) and/or Regulatory Agreement/Post Marketing Plan. Under the current 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 an established quality system and a successful inspection history.

With the implementation of QbD, the expanded process and product understanding serves as a foundation, supporting the sponsor’s ability to introduce change on a risk-based approach. The design space resulting from this evaluation is independent of the manufacturing location. Engineering knowledge has been captured explicitly for the bioreactor and for the drug product tanks to address scale. Where it is possible to waive the Pre-Approval Inspection (PAI), based on a

specific manufacturing sites satisfactory biennial inspection history or recent PAI for a similar type of product, introduction of a new manufacturing site poses minimal risk.

The body of data that served to support the licensed control strategy can likewise be utilized to support a post-approval site change. The verification of a new drug substance manufacturing site for A-Mab with 1 X 25,000 L is proposed.

Change Protocol - Drug Substance

The requirements for the establishment of a new drug substance manufacturing site, relative to the previously approved A-Mab process, are provided below. This change is categorized according to the Process Change and Regulatory Impact Table.

- Assessment of change and potential impact on design space, CQAs and step outputs (i.e., Risk Assessment). Design space modification as necessary.
- Risk analysis of the bioreactor engineering and design
- Refinement of the control strategy with regard to KPPs, GPPs for each unit operation
- Comparability of unit operation outputs
- Comparability of the drug substance lot via extensive physicochemical characterization (including accelerated and real time stability)
- For the specific example in the case study the engineering model supports making the change and reporting as shown in Table 7.1. Demonstration of performance within the approved design space with 1 lot assuming the only changes are related to KPPs and GPPs.
- Continued process verification at the new site.

There is no requirement to repeat viral clearance studies under the proposed Change Protocol since the verification of the approved design space for the downstream unit operations is a criterion for a successful site change, thus confirming the relevance and validity of the existing clearance data.

A detailed description of process parameter characterization for each unit operation was presented in the sections describing the upstream and downstream processes. Only process parameters linked to product quality (CPPs and WC-CPPs) were used to define the limits of the approved design space. These parameters ensure product quality; hence, confirmation of the originally approved design space is a requirement for (refer to Figure 7.1). On the other hand, process parameters linked to process performance are included in the control strategy and must be controlled and/or monitored to ensure process consistency, yet the control strategy may differ from the originally approved site to allow for optimization and continuous process improvement.

In summary, a post-approval site change for A-Mab drug substance manufacturing is contingent on verification of the design space and CQAs; it is acceptable to define a new control strategy given that KPPs and GPPs are optimized for process performance, i.e., consistency and robustness.

The verification of a new drug product manufacturing site for A-Mab with a limited number of runs is proposed.

Change Protocol - Drug Product

The requirements for the establishment of a new drug product manufacturing site, relative to the previously approved A-Mab process, are provided below. This change is categorized from a notification through a prior approval change contingent on the risk assessment.

- Assessment of change and potential impact on design space, CQAs and step outputs (i.e., Risk Assessment). Revision as necessary.
- Comparability of the engineering and design analysis.
- Demonstration of equivalence to the approved design space. Verification of established process parameters (CPPs, WC-CPPs)
- Refinement of the control strategy with regard to KPPs, GPPs for each unit operation
- Comparability of unit operation outputs
- Verify control of critical attributes tested in-process and conformance with release acceptance criteria
- Comparability of the drug substance via extensive physicochemical characterization (including accelerated and real time stability) and confirmation of CQAs

In summary, a post-approval site change for A-Mab drug product manufacturing is contingent on verification of the design space and CQAs; it is acceptable to define a new control strategy given that KPPs and GPPs are optimized for process performance, i.e., consistency and robustness.

8 Glossary

Term	Explanation	Source (s)
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.	Q6b
Action limits	An action limit is an internal (in-house) value used to assess the consistency of the process at less critical steps. These limits are the responsibility of the manufacturer.	Q6b
Capability of a Process	Ability of a process to realise a product that will fulfill the requirements of that product. The concept of process capability can also be defined in statistical terms. (ISO 9000:2005)	Q10
Commitment batches	Production batches of a drug substance or drug product for which the stability studies are initiated or completed post approval through a commitment made in the registration application.	Q1a(R2)
Comparability Bridging Study	A study performed to provide nonclinical or clinical data that allows extrapolation of the existing data from the drug product produced by the current process to the drug product from the changed process.	Q5e
Contaminants	Any adventitiously introduced materials (eg, chemical, biochemical, or microbial species) in the drug substance/drug product not intended to be part of the manufacturing process.	Q6b
Continuous Process Verification	An alternative approach to process validation in which manufacturing process performance is continuously monitored and evaluated.	Q8(R2)
Control Space	Region within the design space that defines the operational limits (for process parameters and input variables) used in routine manufacturing. The control space can be a multidimensional space or a combination of univariate process ranges.	CMC-BWG
Control Strategy	A planned set of controls, derived from current product and process understanding, that assures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control.	Q10
Critical	Describes a process step, process condition, test requirement, or other relevant parameter or item that must be controlled within predetermined criteria to ensure that the API meets its specification.	Q7
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.	Q8(R2)

Term	Explanation	Source (s)
Critical Process Parameter	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.	Q8(R2)
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. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post approval change process. Design space is proposed by the applicant and is subject to regulatory assessment and approval.	Q8(R2)
Detectability	The ability to discover or determine the existence, presence, or fact of a hazard.	Q9
Drug product (Dosage form; Finished product)	A pharmaceutical product type that contains a drug substance, generally in association with excipients. Drug substance (Bulk material): The drug substance is the material which is subsequently formulated with excipients to produce the drug product. It can be composed of the desired product, product-related substances, and product- and process-related impurities. It may also contain excipients and other components, such as buffers.	Q6b
Edge of Failure	The boundary to a variable or parameter, beyond which the relevant quality attributes or specification cannot be met.	
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."	Q8(R2)
General process parameter	An adjustable parameter (variable) of the process that does not have a meaningful effect on product quality or process performance. A key process parameter does not affect critical product quality attributes. Ranges for GPPs are established during process development, and changes to operating ranges will be managed within the quality system.	CMC-BWG
Harm	Damage to health, including the damage that can occur from loss of product quality or availability.	Q9
Hazard	The potential source of harm (ISO/IEC Guide 51).	Q9
Impurity	Any component present in the drug substance or drug product that is not the desired product, a product-related substance, or an excipient (including added buffer components). It may be either process- or product-related.	Q6b
In-Process Control also called Process Control	Checks performed during production in order to monitor and if necessary to adjust the process and/or to ensure that the intermediate or API conforms to its specifications.	Q7
In-process tests	Tests which may be performed during the manufacture of either the drug substance or drug product, rather than as part of the formal battery of tests which are conducted prior to release.	Q6a

Term	Explanation	Source (s)
Intermediate	For biotechnological/biological products, a material produced during a manufacturing process that is not the drug substance or the drug product but for which manufacture is critical to the successful production of the drug substance or the drug product. Generally, an intermediate will be quantifiable and specifications will be established to determine the successful completion of the manufacturing step before continuation of the manufacturing process. This includes material that may undergo further molecular modification or be held for an extended period before further processing.	Q5c
Key Process Parameter (KPP)	An adjustable 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.	CMC-BWG
Knowledge Management	Systematic approach to acquiring, analyzing, storing, and disseminating information related to products, manufacturing processes and components.	Q10
Knowledge Space	Multi-dimensional region encompassing internally and externally derived knowledge. Relating to properties of API, formulation design, quality of raw materials, process type, etc. Explored and/or modeled, relevant to the product under development.	CMC-BWG
Lifecycle Product lifecycle	All phases in the life of a product from the initial development through marketing until the product's discontinuation. All phases in the life of the product from the initial development through marketing until the product's discontinuation.	Q8(R2) Q9
Normal Operating Range (NOR)	A defined range, within the Proven Acceptable Range, specified in the manufacturing instructions as the target and range at which a process parameter is controlled, while producing unit operation material or final product meeting release criteria and Critical Quality Attributes.	PQRI
Performance Indicators	Measurable values used to quantify quality objectives to reflect the performance of an organisation, process or system, also known as "performance metrics" in some regions.	Q10
Pharmaceutical Quality System (PQS)	Management system to direct and control a pharmaceutical company with regard to quality.	ICH Q10
Process Analytical Technology (PAT)	A system for designing, analyzing, and controlling manufacturing through timely measurements (ie, during processing) of critical quality and performance attributes of raw and in-process materials and processes with the goal of ensuring final product quality.	Q8(R2)
Prior product knowledge	The accumulated laboratory, nonclinical, and clinical experience for a specific product quality attribute. This knowledge may also include relevant data from other similar molecules or from the scientific literature.	CMC BWG

Term	Explanation	Source (s)
Process Control	See In-Process Control.	Q7
Pilot Plant Scale	The production of a recombinant protein by a procedure fully representative of and simulating that to be applied on a full commercial manufacturing scale. The methods of cell expansion, harvest, and product purification should be identical except for the scale of production.	Q5b
Potency	Potency is the measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute of the product which is linked to the relevant biological properties.	Q6b
Process-related impurities	Impurities that are derived from the manufacturing process. They may be derived from cell substrates, culture (eg, inducers, antibiotics, or media components), or from downstream processing (eg, processing reagents or column leachables).	Q6b
Process Robustness	Ability of a process to tolerate variability of materials and changes of the process and equipment without negative impact on quality.	Q8(R2)
Product-related impurities	Product-related impurities are molecular variants of the desired product arising from processing or during storage (eg, certain degradation products) which do not have properties comparable to those of the desired product with respect to activity, efficacy, and safety.	Q6b
Product-related substances	Product-related substances are molecular variants of the desired product which are active and have no deleterious effect on the safety and efficacy of the drug product. These variants possess properties comparable to the desired product and are not considered impurities.	Q6b
Proven Acceptable Range	A characterised range of a process parameter for which operation within this range, while keeping other parameters constant, will result in producing a material meeting relevant quality criteria.	Q8(R2)
Quality	The degree to which a set of inherent properties of a product, system or process fulfils requirements.	Q9
Quality Attribute	A molecular or product characteristic that is selected for its ability to help indicate the quality of the product. Collectively, the quality attributes define the adventitious agent safety, purity, potency, identity, and stability of the product. Specifications measure a selected subset of the quality attributes.	Q5e
Quality by Design	A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.	Q8(R2)
Quality Control (QC)	Checking or testing, that specifications are met.	Q7

Term	Explanation	Source (s)
Quality risk management	A systematic process for the assessment, control, communication, and review of risks to the quality of the drug product across the product lifecycle.	Q9
Raw material	Raw material is a collective name for substances or components used in the manufacture of the drug substance or drug product.	Q6b
Real-time release	The ability to evaluate and ensure the acceptable quality of in-process and/or final product based on process data, which typically include a valid combination of assessed material attributes and process controls.	Q8(R2)
Reference standards/materials	In addition to the existing international/national standards, it is usually necessary to create in-house reference materials. — In-house primary reference material: A primary reference material is an appropriately characterized material prepared by the manufacturer from a representative lot(s) for the purpose of biological assay and physicochemical testing of subsequent lots, and against which in-house working reference material is calibrated. — In-house working reference material: The in-house working reference material is a material prepared similarly to the primary reference material and is established solely to assess and control subsequent lots for the individual attribute in question. It is always calibrated against the in-house primary reference material.	Q6b
Risk	The combination of the probability of occurrence of harm and the severity of that harm (ISO/IEC Guide 51).	Q9
Risk analysis	The estimation of the risk associated with the identified hazards.	Q9
Risk assessment	A systematic process of organizing information to support a risk decision to be made within a risk management process. It consists of the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards.	Q9
Risk evaluation	The comparison of the estimated risk to given risk criteria using a quantitative or qualitative scale to determine the significance of the risk.	Q9
Severity	A measure of the possible consequences of a hazard.	Q9
Specification - Release	The combination of physical, chemical, biological and microbiological tests and acceptance criteria that determine the suitability of a drug product at the time of its release.	Q1a(R2)
Specification	A specification is a list of tests, references to analytical procedures, and appropriate acceptance criteria with numerical limits, ranges, or other criteria for the tests described, which establishes the set of criteria to which a drug substance or drug product or materials at other stages of their manufacture should conform to be considered acceptable for its intended use.	Q6b

Term	Explanation	Source (s)
Testing plan	A determination as to whether routine monitoring, characterization testing, in process monitoring, stability testing, or no testing is conducted as a part of the overall control strategy.	CMC-BWG
Well controlled 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 and which has a low risk of falling outside the design space.	CMC-BWG