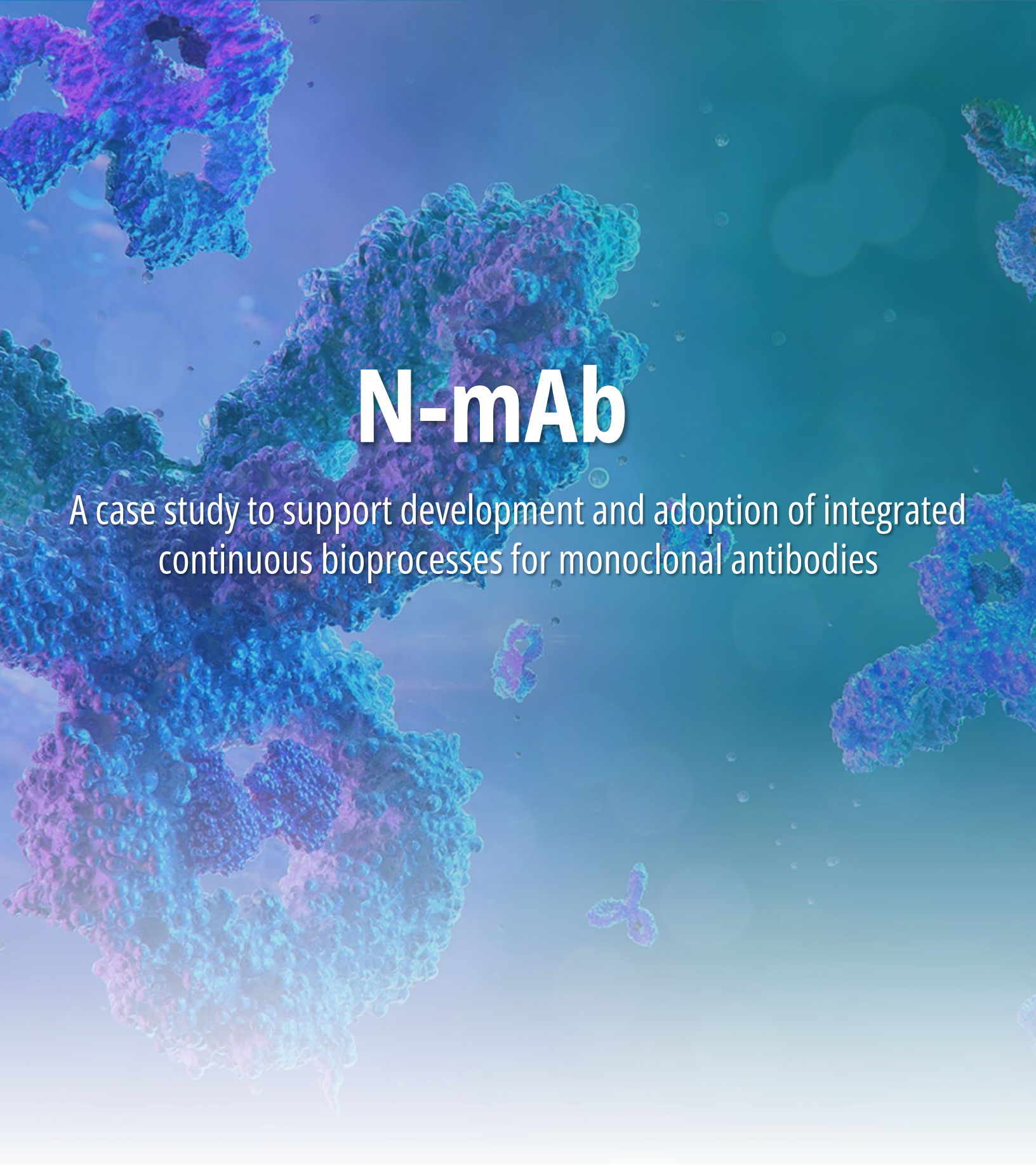

N-mAb

A case study to support development and adoption of integrated continuous bioprocesses for monoclonal antibodies

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Version 1.0

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> *"We encourage publication of future refinements of these tools and advances in their applications for the benefit of the larger industrial and regulatory community. Sharing of such information could result in superior approaches to product license applications in the future, as well as streamlining of late-stage product development and the review and approval phases of the biologics product lifecycle*."
>
> Brian Kelley, 2016

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EXECUTIVE SUMMARY

In early 2020, NIIMBL convened a group of 55 individuals across 22 organizations, including large and small biopharmaceutical companies and government agencies such as NIST and FDA, to discuss an update to the A-Mab case study. The A-Mab case study (2009) was created to stimulate discussion around how the core principles of quality by design (QbD) could be applied to the process development of a monoclonal antibody with examples of a multitude of real-world scenarios, as opposed to a singular approach. A-Mab was very successful in generating that discussion and impacted how process development was executed across the industry. This case study, named N-mAb, is focused on supporting the development and adoption of integrated continuous bioprocesses for monoclonal antibodies. The document follows the evolution of an integrated control strategy, from early clinical through process validation and commercial manufacturing, as process understanding increases, with a focus on elements that are unique to integrated continuous bioprocesses. Examples were developed based on an informed mock dataset, generated from consensus industry experiences. Also included is a discussion of challenges involved in managing quality in real time, as necessitated by a continuous process.

The goals for the case study included 1) providing a teaching and learning tool for both industry and regulators around adoption of advanced manufacturing process technologies for mAbs, complete with examples of process options and process analytical technologies that can drive an integrated control strategy, 2) enabling effective approaches for continuous improvement within process development and commercial manufacturing, and 3) provoking and challenging current thinking to stimulate discussion, advanced new concepts, and generate shared understanding and terminology.

The N-mAb document is structured to provide details and guidance on key activities that occur during development and manufacturing, and that lead to an integrated control strategy based on continually updated process knowledge. **Chapter 1** outlines the framework process while **Chapter 2** discusses process design decisions for the different upstream and downstream options. **Chapter 3** considers characterization of an integrated continuous bioprocess. Full-scale performance demonstration, as discussed in **Chapter 4**, is an essential component of development for both the process and the control strategy. **Chapter 5** specifically addresses the prevention, removal, and detection of viral and microbial contaminants. **Chapter 6** discusses the three main stages of process validation that occur throughout development. **Chapter 7** ties together threads from all previous chapters into an integrated control strategy, integrating knowledge from early development through commercial manufacturing, with considerations for new analytical approaches to ensure product quality. **Chapter 8** addresses the challenges of managing the quality aspects of an integrated continuous process in real time. Lastly, **Chapter 9** looks ahead at future directions for process, analytical, and control technologies.

During its development, N-mAb stimulated significant discussion and we hope it will continue to provide a starting place to advance new concepts around adoption of integrated continuous bioprocesses.

Chapter 1

Introduction and Framework

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

1 Introduction and Framework

1.1 Background - A-Mab and beyond

In August 2008, representatives from Abbott, Amgen, Eli Lilly & Company, Genentech, GlaxoSmithKline, MedImmune, and Pfizer came together as the CMC-Biotech Working Group to develop *the A-Mab case Study* (CMC Biotech Working Group, 2009). The goal was to "help advance the principles contained in ICH Q8(R2), Q9 and Q10, focusing on the principles of Quality by Design" as applied to the development of a monoclonal antibody (mAb) (CMC Biotech Working Group, 2009). These guidance documents had recently been published and the intent was "to create a case study that would stimulate discussion around how the core principles contained in these guidelines would be applied to product realization programs, with a multitude of real-world scenarios, as opposed to a singular approach." The process architecture for A-Mab consisted of a fed-batch upstream and a batch downstream. As part of one of their primary objectives, the authors of A-Mab sought not to create a work that was prescriptive but rather one that would stimulate discussion, debate, and learning as both industry and regulators moved forward with process development for new therapeutics. To that end, it was very successful: A-Mab became a standard guide that companies used to reframe their development efforts, whether they followed its approaches in detail or not. Multiple follow-on publications came out shortly afterwards expanding on points from A-Mab, and A-Mab was used in a number of teaching environments, including for the training of graduate students at multiple universities. The scope of large molecule therapeutics, and especially mAbs, has also increased significantly since 2009: 28 mAbs had been approved prior to 2009 while over 80 mAbs were approved between 2009 and 2022 (The Antibody Society, 2022), and the overall market value of mAb products increased from ~$30 billion to over $160 billion (Lu et al., 2020)

At roughly the same time, a number of conference presentations focused on a revived interest in a perfusion-based upstream process that could be connected to a downstream process that also ran in a continuous manner. The term "integrated continuous bioprocess" (ICB) was used to describe this process design (Warikoo et al., 2012) and it represented an advance in thinking from the perfusion processes that had been used to manufacture a number of approved biotherapeutics dating back to the early 1990s.

At that time, capacity and cost considerations were creating drivers for the development of more advanced manufacturing process technologies, several of which could be described by the general heading of "integrated continuous bioprocesses" (ICB) (Konstantinov & Cooney, 2015; Warikoo et al., 2012). Despite promising results presented by some companies adopting ICB, more widespread acceptance across the industry was slow. The slow pace of acceptance of new technology was also identified as an issue during the Active Listening sessions sponsored by the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) between industry representatives and the FDA, and case studies similar to A-Mab were identified as a useful tool for advancing development (Mantle & Lee, 2020). Following up on this, in late 2019, the Regulatory Considerations Committee (RCC) of NIIMBL proposed creating an update to A-Mab, which would focus on the manufacture of a prototype mAb by an integrated, continuous process. In February 2020, industry leaders from 14 major biopharmaceutical manufacturers and suppliers, FDA, and NIST met for three days to discuss a bold vision for the future of protein therapeutic biomanufacturing (Erickson et al., 2021). One

element of the detailed plan that came out of that meeting was to survey subject matter experts for their assessment of the A-Mab case study, *e.g.*, project strengths, weaknesses, and gaps, and how follow-up case studies could be of benefit to the community. The survey results reinforced the comments above related to the impact of A-Mab as exemplified by the quotes below:

- "The most important contribution [of A-Mab] was to differentiate areas of consensus and agreement from areas of dynamic change and corporate platform practice."
- "We used the case study extensively when it was originally published. We use it far less today because our internal policies and procedures and subsequent experience make it less relevant. Creating it and using it was exceptionally important when it was written."
- "While not adopting all elements of A-Mab wholesale, it was foundational to how we updated our internal approaches to risk assessments and criticality."
- "A-Mab was utilized as a benchmarking reference to support similar approaches."
- "Each developer needs to examine what parts are useful and value added and consider implementing those parts."
- "We use all of A-Mab as a general training tool for new employees and referred to it through the creation of our original methodologies for late-stage process development and control strategy development."

A plan for N-mAb was designed with overall goals similar to those of A-Mab:

1. Create a case study to support teaching and learning for both industry and regulators around adoption of advanced manufacturing process technologies for mAbs. This case study will provide examples of the implementation of different process options and associated process analytical technology that can drive an integrated control strategy, including considerations related to process development and characterization, process validation, and deviation management consistent with current guidance in place. Note that ICH Q13 "Continuous Manufacturing of Drug Substances and Drug Products" was still in Draft state at the time this case study was prepared. Future updates to this document will provide more links to the updated guidance.
2. This N-mAb case study focuses on ICB for a monoclonal antibody; other complex protein therapeutics will be considered as part of a future update. Although implementation of continuous manufacturing for small molecules may be more advanced than for large molecules, there are important differences between these modalities, e.g., the ability to directly monitor product quality online via spectroscopic tools. Thus, it is not always beneficial to directly transfer concepts and technologies from small to large molecule production.
3. Enable effective approaches for achieving continual improvement across the process development and, more importantly, commercial arenas.
4. Provoke and challenge current thinking to stimulate discussion and advance new concepts

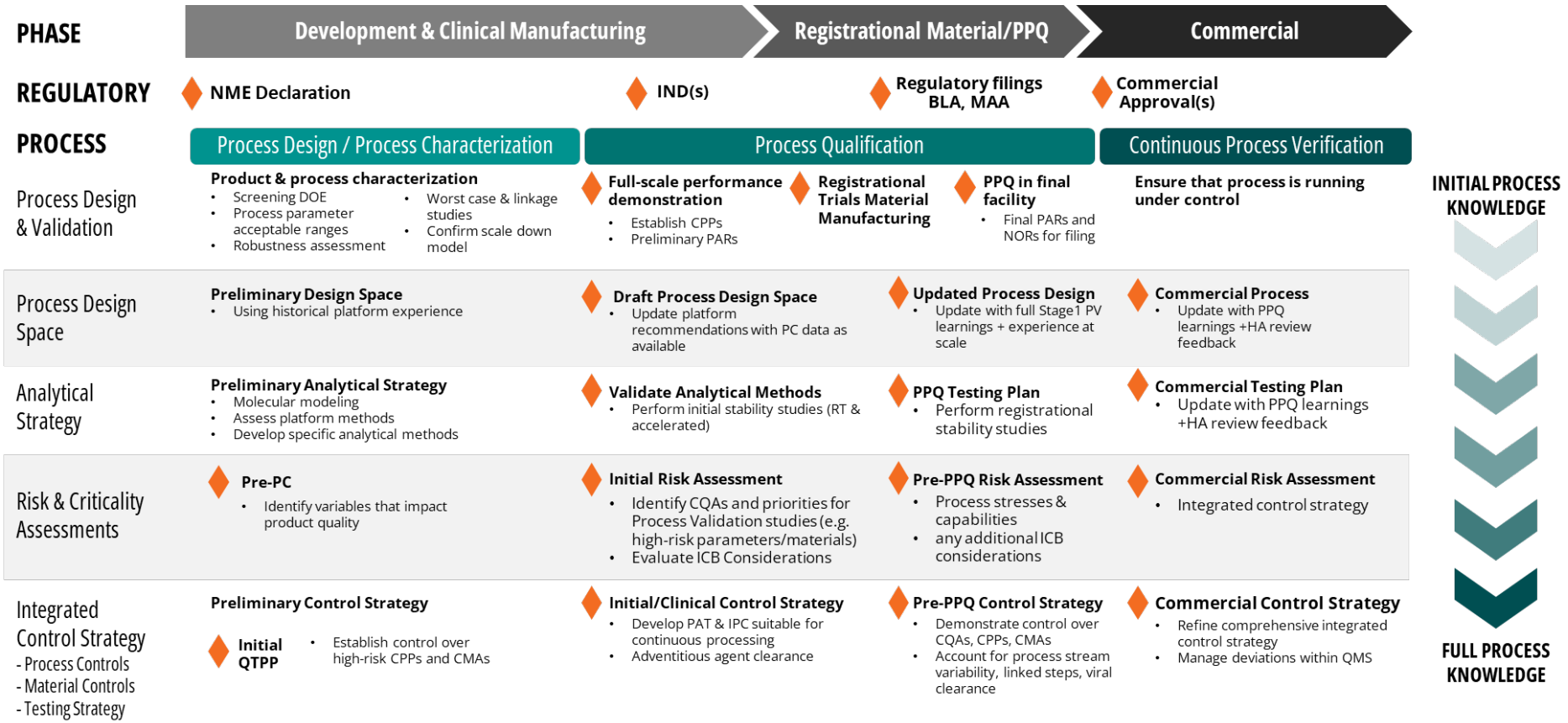


Figure 1.1. Biopharmaceutical development and manufacturing timeline. Overview of the elements of integrated control strategy development in the for a typical biopharmaceutical as they relate to the phases of development, regulatory submission milestones, and process development timeline. After commercial manufacturing, the process moves into the lifecycle management phase. As full process knowledge is accumulated, these learnings are integrated, testing and limits for IPC are refined, and responses to deviations are updated within the quality management system. Orange diamonds indicate key documentary milestones.

BLA	Biologics license application	HA	Health authority	NME	New molecular entity	PPQ	Process performance qualification
CMA	Critical material attribute	ICB	Integrated continuous bioprocess	NOR	Normal operating range	PV	Process validation
CPP	Critical process parameter	IPC	Integrated process control	PAR	Proven acceptable range	QTPP	Quality target product profile
CQA	Critical quality attribute	IND	Investigational new drug	PAT	Process analytical technology		
DOE	Design of experiments	MAA	Marketing authorization application	PC	Process characterization		

This document does not intend to:

- Suggest that continuous production is suitable for the manufacture of all pharmaceutical products.
- Provide guidance on issues related to the safe operation of a continuous process or continuous processing equipment. It is the responsibility of the user of this case study to establish appropriate health, safety, and environmental practices and determine the applicability of regulatory limitations prior to use.
- Recommend designs or operating regimes for continuous manufacturing. Any specific configurations discussed in this document are meant to serve as examples only.

The document is structured to provide details and guidance on key activities that occur during development and manufacturing, and that lead to an integrated control strategy based on continually updated process knowledge (**Figure 1.1**). Integrated process design requires process decisions among the possible upstream and downstream options (**Chapter 2**) and also includes process characterization (**Chapter 3**). Full-scale performance demonstration (**Chapter 4**) is an essential component of development for both the process and the control strategy. Adventitious agent control (**Chapter 5**) addresses the prevention, removal, and detection of viral and microbial contaminants. Process validation (**Chapter 6**) occurs in three main stages throughout development. Constructing an integrated control strategy (**Chapter 7**) requires integrating knowledge from early previous development stages through commercial manufacturing and may require the implementation of new analytical approaches with the goal of ensuring product quality. The last sections address the challenges of managing the quality aspects of the process in real time (**Chapter 8**) and look ahead to future directions for process, analytical, and control technologies (**Chapter 9**).

1.2 Description of prototype mAb and critical quality attribute (CQA) assessment

The concepts of QbD have been firmly ingrained in the business process if not the regulatory filings of most protein therapeutic process development organizations. Therefore, this document will refer readers to A-Mab for the general discussion around Target Product Profile (TPP), Quality Target Product Profile (QTPP), and considerations for determining the criticality of quality attributes. Finally, no discussion of A-Mab would be complete without acknowledging the many publications produced as a way to further clarify the concepts presented therein including (Alt et al., 2016; Flynn & Nyberg, 2014; Kelley, 2016; Kelley et al., 2016; Kepert et al., 2016; J. Xu et al., 2022) to name a few. According to Alt and colleagues, (2016), the TPP informs the generation of a QTPP, which will then drive identification of pCQAs followed by verification of the actual CQAs that impact product safety and efficacy. For simplicity, we can consider N-mAb to be an improved, more potent version of A-Mab so that most details in the TPP and QTPP may be carried over with the exception of dosing, which is reduced 5-fold as shown below in **Table 1.1** as a combined TPP and QTPP.

Table 1.1. Sample TPP and QTPP information for N-mAb.

GENERAL PROPERTIES	
Indication	N-mAb is a humanized IgG1 antibody intended as a treatment for indolent non-Hodgkin's Lymphoma (NHL) in an adult population only.
Safety	Only infusion- or injection-related side effects
Mechanism of Action (MOA)	The mechanism of action for N-mAb is through binding to a tumor cell surface antigen, Lymph-1, and stimulating B cell killing. Although N-mAb was designed so that the B cell killing is primarily through ADCC activity, involvement of CDC activity cannot be completely ruled out.
Relevant Post-Translational Modifications, Impurities, & Degradants (see details below)	Glycosylation/Galactosylation: pCQA – Efficacy Glycosylation/Fucosylation: pCQA – Efficacy (ADCC) Glycosylation/High Mannose: pCQA – Efficacy (PK/PD) Deamidation at Asn325: pCQA – Efficacy (ADCC) HMW species: pCQA - Safety (Immunogenicity) Host Cell Protein (HCP): pCQA - Safety (Immunogenicity)

	Must Have at Launch	Nice to Have for Life Cycle Extension
Route of Administration	IV administration at a weekly dose of 2 mg/kg	SC injection at a weekly dose of 150 mg
Dosage Form	Sterile liquid formulation in a single-use vial containing 1 mL	Sterile liquid formulation in a pre-filled single-use syringe containing 1 mL
Dosage Strength	75 mg/mL	150 mg/mL
Stability	2-year stability at 5 °C 14-day stability at 25 °C	3-year stability at 5 °C 30-day stability at 25 °C

ADCC Antibody-dependent cell-mediated cytotoxicity
CDC Complement-dependent cytotoxicity
MOA Mechanism of action

- Glycosylation/Galactosylation: pCQA – Efficacy (complement-dependent cytotoxicity, CDC) pCQA based on platform knowledge, to be confirmed during structure-function studies
- Glycosylation/Fucosylation: pCQA – Efficacy (antibody-dependent cell-mediated cytotoxicity, ADCC) pCQA based on platform knowledge, to be confirmed during S/F studies
- Glycosylation/High Mannose: pCQA – Efficacy (pharmacokinetics and pharmacodynamics, PK/PD) to be confirmed from clearance results on patient samples
- Deamidation at Asn325: A-Mab did not identify any deamidation sites as critical post-translational modifications (PTM). However deamidation was included as a preliminary CQA (pCQA) for N-mAb since it allows referencing a good example of the application of protein modeling for the identification of potential degradation "hot spots" as part of the initial risk analysis around QAs (Yan et al., 2018). For the purposes of this case study, we will consider deamidation at Asn 325 as a pCQA based on initial modeling, to be confirmed during structure-function studies as having an impact on ADCC activity.
- HMW species: pCQA - Safety (Immunogenicity based on platform knowledge)
- Host Cell Protein (HCP): pCQA - Safety (Immunogenicity based on platform knowledge and immunogenicity risk scoring tools)

Table 1.2. Overview of general classes of (p)CQAs and related controllability.

(p)CQA	Upstream Process (USP) Control/Changes	Downstream Process (DSP) Control/Changes
Glycosylation: Galactosylation, Fucosylation, Sialylation, etc.	• Control by cellular pathways: • Potential feedback control of feed, temp, pH • Feed forward control of duration possible	• Minimal to no control or change in DSP, with some exceptions, e.g., highly sialylated proteins
Glycosylation: High Mannose & A-glycosylated Variants	• Control by cellular pathways and cell viability: • Potential feedback control likely limited as these CQAs often indicate cell stress/nutrient limitation • Feed forward control of duration may be best control	• Minimal to no control or change in DSP
Amino Acid (AA) Sequence variants		
Other PTMs (oxidation, deamidation, glycation, amidation, etc.)	• Primarily control by chemical and/or enzymatic reactions • Feed forward control of duration possible	• Minimal to no control in DSP • DSP conditions may increase PTMs based on degradation pathway (pH, temp, light exposure, trace metals, etc.)
Aggregation & Fragmentation (HMWS & LMWS)	• Control by multiple pathways and influenced by protein structure/sequence • Feed forward control of duration possible	• Control by chromatography step(s) in DSP • DSP conditions can increase aggregation based on degradation pathway (pH, air/liquid surface, etc.)
Process-related Impurities: 1. Cell related (HCP, DNA) 2. Component of media, buffers, or ProA leachate	• Control by cellular pathways and cell damage or lysis • Control by known addition in USP process as media component	• Control by DSP chromatography step(s) and UF/DF • DSP may also introduce process-related impurities like residual ProA or buffer components as well as remove them

AA Amino acid
CQA Critical quality attribute
DSP Downstream process
HCP Host cell proteins
HMWS High molecular weight species
LMWS Low molecular weight species
ProA Protein A
PTM Post-translational modifications
USP Upstream process

The bioreactor is the source of almost all product-related impurities, but downstream unit operations can contribute to further product degradation pathways driven by particular sensitivities of a given molecule. Accelerating the flow of material through the process can result in a significant improvement in the control of product quality by reducing product hold times that could be deleterious. Reducing the dose for N-mAb also allows for the possibility of developing an extension

of this case study for the manufacture of a high concentration drug substance and possibly considering continuous aspects of drug product manufacturing in a later version.

Regarding the list of pCQAs identified above, these can be organized into some general classes of post-translational modifications and process-related impurities, which can be classified broadly as to their potential source and degree of control in both upstream and downstream processes, Table 1.2.

1.2.1 Overview of framework process

This section describes the overall framework or prototype for a connected, integrated, continuous bioprocess and borrows from the high level process description recently published (Coffman et al., 2021). Similar, but distinctly different approaches for fully continuous processing have also been described and demonstrated recently (Coolbaugh et al., 2021) and these approaches also inform this document. Along with an overall representative process, options will be presented for each step; however, the intent is not to cover every conceivable process option, but likely options based on certain practical or organizational limitations. The value in this approach is that the overall guidance can be applied to the needs of each organization.

An overall comparison of a simplified view of a prototype framework process compared with a batch process is shown in Figure 1.2. Additional considerations and options related to the detailed design of the framework process will be discussed throughout the case study.

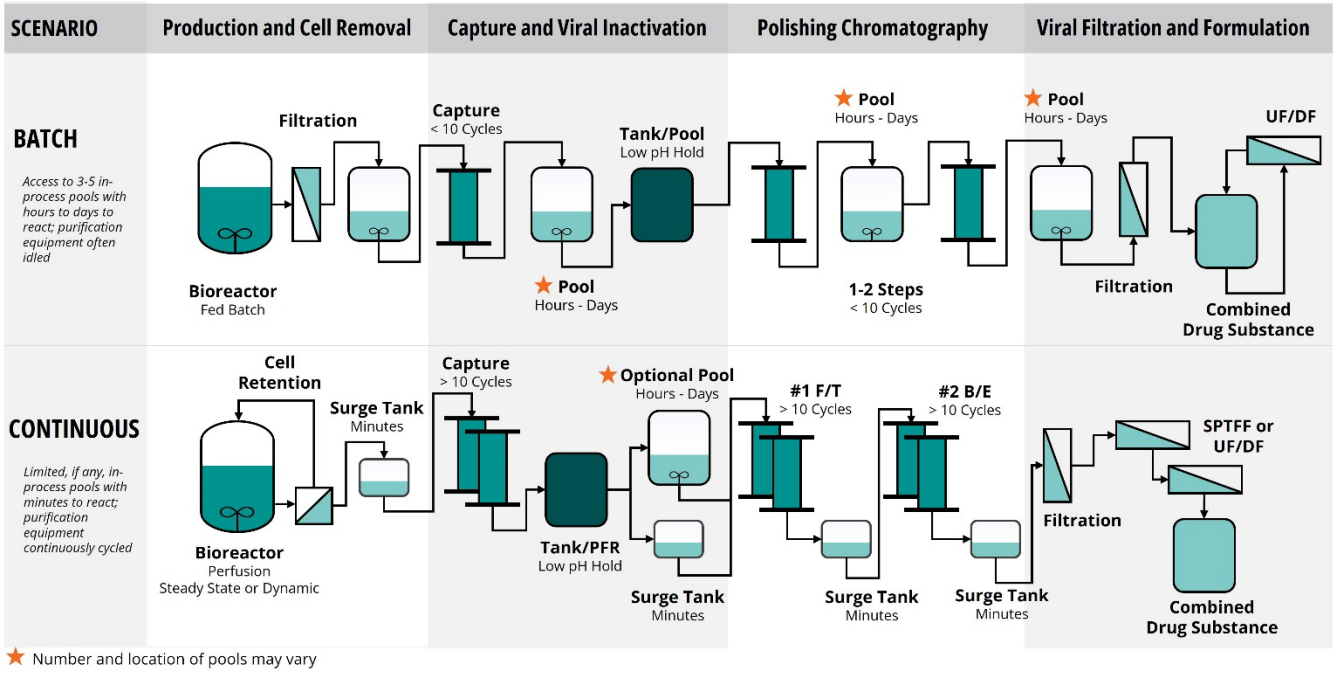


Figure 1.2. High level comparison of batch and continuous processes.

DP Drug product
DS Drug substance
PFR Plug-flow reactor
SPTFF Single pass tangential flow filtration
UF/DF Ultrafiltration/diafiltration

1.2.2 Decisions driving process options

Historically, a strong driver for adopting a perfusion mode of operation for the production bioreactor was to mitigate degradation pathways by reducing the residence time of product molecules in the bioreactor compared with fed-batch culture approaches (Bielser et al., 2018; Ryll et al., 2000; Schwarz et al., 2022; Walther, et al., 2019). While this case study is focused on mAbs, the improved control of product quality attributes seen with perfusion culture will likely translate to other new modalities of protein therapeutics (Gomez et al., 2020). Likewise, expanding the continuous operation to the downstream process and intensifying the overall process will also limit the impact of degradation pathways during various in-process hold steps by reducing hold times under conditions that are dictated by the purification process but that may be deleterious to stability. The overall objective of process design should always be to ensure process stability, robustness, and control. Furthermore, according to a recent report from the White House, the three pillars of a secure and robust supply chain are quality, diversification, and redundancy (The White House, 2021). An ICB approach can be used to simplify processes by using modular equipment and minimizing scale differences, and it can contribute to achieving these three pillars.

After the initial process design, the next areas of focus are typically elements including projected demand, cost of goods sold (COGS) or manufacturing cost targets, time and budgetary constraints for development activities, and facility capability at the projected manufacturing site(s). The overall economic analysis is critical (Yang, et al., 2020). However, it is also important to understand the trade-offs related to this decision when development costs are also considered (Farid, et al., 2020).

From a practical viewpoint, continuous manufacturing may not be desired for all molecules, including for some mAbs. Some molecules may require conventional manufacturing, especially if there is a requirement for extensive, non-platform approaches or if a very large projected demand requires the use of multiple large volume manufacturing sites that include both internal and external network sites.

Once a decision has been made to move forward with an ICB, it is critical to determine how a “batch” will be defined. The A-Mab case study did not include a definition for a batch in its Glossary, perhaps because it may have appeared obvious at the time that a batch equals the purification of one bioreactor volume, although some companies at that time were purifying partial volumes from one or more bioreactors at different times and sometimes at different sites by leveraging frozen post-capture pools. For this N-mAb case study, it is important to discuss the definition of a batch, which is given in the key terminology box, **Figure 1.3**.

KEY TERMINOLOGY: BATCH

It is possible to leverage the following definition of a batch from ICH Q7:

> *"A specific quantity of material produced in a process or series of processes so that it is expected to be homogeneous within specified limits. In the case of continuous production, a batch may correspond to a defined fraction of the production. The batch size can be defined either by a fixed quantity or by the amount produced in a fixed time interval."*

However, for this document, a slightly more detailed drug substance batch definition for N-mAb is preferred:

> *"**A specific quantity of purified product of interest having a unique identifier that enables traceability of raw materials, production bioreactor days, subsequent downstream unit operations, and a pre-defined, unique data set confirming adherence to in-process control limits and final product quality release specifications that enable disposition by quality systems and forward processing to drug product."***

Inherent in this definition is the concept that a batch is defined by a specific amount of material AND a pre-specified package of information related to the integrated control strategy that allows for assurance of product quality.

Note that the definition of a batch is not necessarily directly linked to the harvest duration of a single bioreactor. A single bioreactor could generate multiple downstream batches, or the harvest streams from multiple bioreactors could be combined into one or more downstream batches. For all these cases, it is important to explicitly and prospectively define how bioreactor harvests are to be divided or combined, as well as the expected range of downstream batches that will be manufactured as a result.

Figure 1.3. **Definition of Batch.**

Thus, for this case study, the definition of a batch as a defined quantity of material with an associated package of information is further clarified by including specific details related to pooling or dividing bioreactor harvests. For example, in the case of a single bioreactor, the duration of the harvest should be specified, and then a pre-defined quantity (full or partial volume of the target process output in increments of whole downstream process cycle mass increments) is further purified by one or more complete downstream polishing process cycles. Another example could be developed around the case where an intermediate hold vessel is used in the process. In this case, it is conceivable that all or a portion of the intermediate hold material would then be purified by one or more complete downstream polishing process cycles, for which the mass input to each downstream polishing cycle is determined by the target loading range and size of the first polishing

chromatography step, and all cycles would be pooled, mixed in a single vessel, and considered a single batch or lot.

Compared with a batch operation, an integrated, continuous operation requires a greater focus on the interfaces between unit operations and management of the overall process flow and timing. For example, the following concerns need to be considered in the planning of both bench-scale and full-scale process design:

- The variation of inlet and outlet flows for surge tanks, driven by upstream and downstream unit operations, should be matched to the working volume range of the surge tank (Thakur et al., 2021)
- The overall residence time of the process as well as the residence time distribution should be understood (Sencar et al., 2020)
- Productivity may be limited at scale by solution preparation and storage, as well as maximum or minimum pump flow rates on various skids, especially the one for Protein A capture chromatography.
- The transient behavior during startup or shutdown of the process as well as process pauses should be managed
- The impact of failure rates on batch definition and potential quality impact should be understood (Satzer et al., 2021)
- The integrated process design architecture and its impact on the control strategy and associated risk to benefit profile should be stated and clearly understood as to why it is acceptable. The description around risks and benefits may include decisions related to fully vs. partially continuous operation and strategies for pooling (cycles, multiple bioreactors, etc.).

A longer discussion of these concerns will be presented in **Section 4.3**

At the startup of the process, it may be desirable to postpone product collection until steady-state targets are achieved, e.g., surge tank volumes have reached their target levels and are being maintained. At the end of a production run, the last material through the process may need to be diverted to waste because controls designed for steady-state manufacturing may not support accurate control for this tail end of material, e.g., if a chasing buffer leads to significant product dilution. It is also important to consider the management of process perturbations. When the operation is restarted after a process perturbation, material may also need to be diverted to waste until steady-state operation has been achieved for a defined period of time that depends on the magnitude and duration of the process perturbation.

In an attempt to visualize the dynamic nature of the process, **Figure 1.4** was constructed by superimposing a visualization of the time course of a single 12 hour harvest cycle from the bioreactor as it moves through a fully continuous downstream process (Option 1 described in

Table 1.3). The black rectangles represent the center of this packet of material shown as either mass flow (left hand figure on bottom) or protein concentration (right hand figure on bottom). For the sake of easy visualization, axial dispersion throughout the process has not been shown but is a real consideration as described in (Sencar et al., 2020).

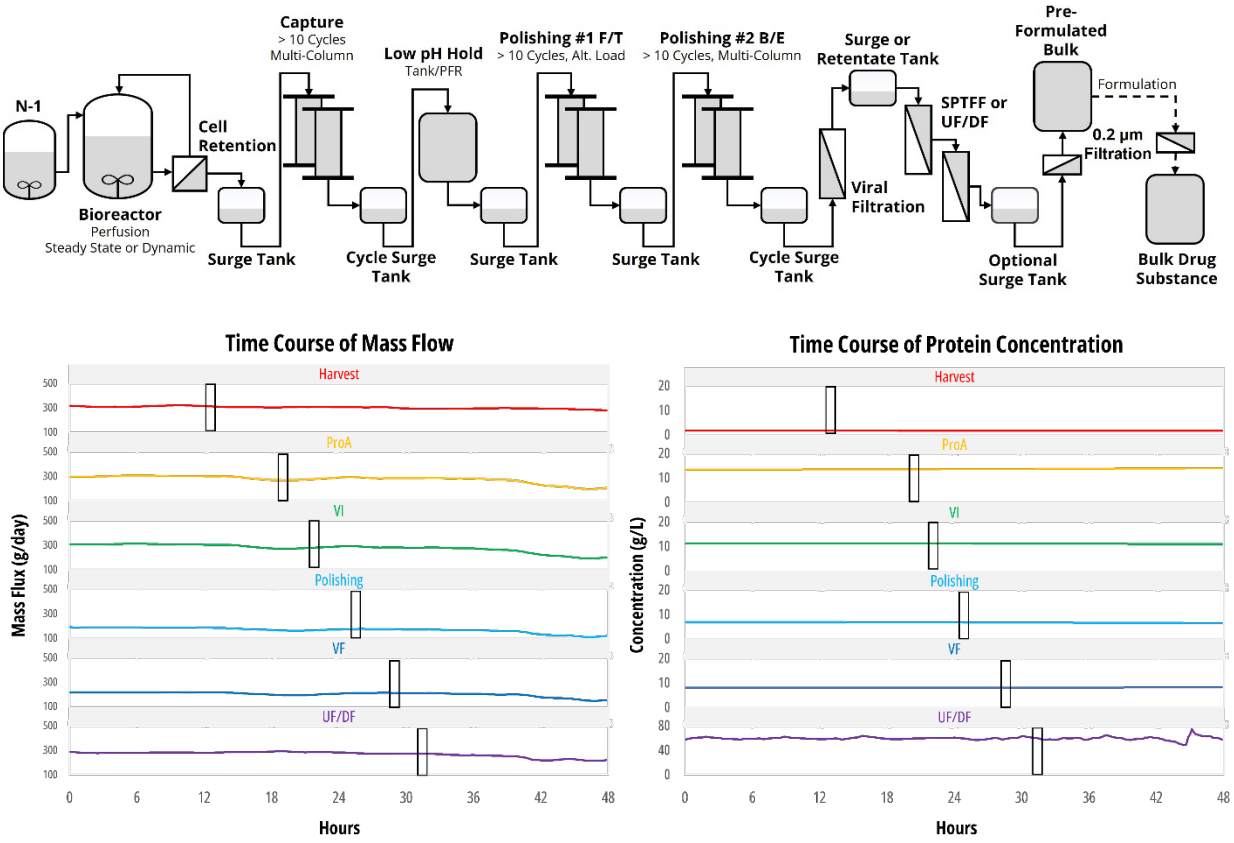


Figure 1.4. Example of process flow timeline and changes to product concentration.

B/E	Bind and elute
Chrom	Chromatography
F/T	Flowthrough
MCC	Multi-column chromatography
SPTFF	Single pass tangential flow filtration
UF/DF	Ultrafiltration/diafiltration

1.2.3 N-mAb process option details

As discussed above, several process options are possible for ICB. However, to make the discussion in this case study more manageable and still retain some diversity to drive further discussion, two major process options will be discussed, based on the presence or absence of an intermediate batch surge tank, along with several minor options. For example, in both major options, the production bioreactor will be run in a perfusion mode involving feed of nutrients into the bioreactor combined with a withdrawal of material for further downstream processing throughout most of the production period. However, the perfusion operation may be run either in a steady-state mode, wherein the inlet and outlet flow rates and other target process parameters (e.g., cell mass) are maintained at a constant value, or in a dynamic mode, wherein the flows in and out as well as the cell density profile will vary over time. The main difference will be in the cell density profile and in the concentration of product and impurities present in the feed to the capture step. Similarly, cell removal can be achieved by either tangential flow filtration (TFF) or alternating flow filtration (ATF) within either major option. The two major options for the eight stages of processing are detailed in

Table 1.3 and Figure 1.5. In Option 1, Stages 1 through 7, corresponding to cell culture through virus removal, are run as ICB, and Stage 8, corresponding to ultrafiltration and diafiltration, is run as an integrated periodic or cyclical batch process. In Option 2, Stages 1 through 4, corresponding to cell culture through virus inactivation, are run as ICB, followed by a batch pool tank, and Stages 5 through 8 are run as periodic batch processes where all or part of the batch is processed through the non-continuous unit operations via sequential, often alternating, batch operations until completion.

Table 1.3. Summary of major and minor process options to be discussed for N-mAb.

Process Stage	Option 1 ICB: Stages 1-7 Cycled Batch: Stage 8	Option 2 ICB: Stages 1-4 Periodic Batch: Stages 5-8	Other Options Discussed	Differences from Batch Operation and Other Comments
Stage 1 Preculture	N-1 batch or perfused N-1			
Stage 2a Production Bioreactor	Steady-state perfusion or dynamic perfusion			Potential for variations in product quality and titer, especially for dynamic perfusion
Stage 2b Cell Removal	TFF or ATF			Potential for product retention and fouling
Unit Op Connection	Surge tank			
Stage 3 Capture Chromatography	Continuous multi-column chromatography (MCC): one column always being fed		Dual-column: alternating loading of columns	Varying load material in terms of titer but still controlled within PAR/NOR limits. Feed may be paused between column loads for dual column
Unit Op Connection	Cycle surge tank			
Stage 4 Virus Inactivation	In-line viral inactivation in a continuous plug flow reactor		Alternating batch tank pH incubation	Continuous operation with occasional variations in protein concentration
Unit Op Connection	Surge tank	Batch pool tank		Option 2 batch pool tank represents batch attributes
Stage 5 F/T Polishing Chromatography	Dual-column alternating loading of columns Note that this is essentially the same as continuous MCC: one column always being fed since load duration >> all other step durations			Varying load material but controlled within PAR/NOR limits Feed may be paused between column loads for dual column
Unit Op Connection	Surge tank	Cycle Surge Tank	Size of surge tank	
Stage 6 B/E Polishing Chromatography	Continuous MCC: one column always being fed	Dual-column alternating loading of columns		Varying load material but controlled within PAR/MOR limits Feed may be paused between column loads for dual column
Unit Op Connection	Surge tank	Cycle Surge Tank	Size of surge tank	
Stage 7 Virus Filtration	Alternating batch set-up, pre- and post-integrity test		Continuous operation	Varying protein concentration in load material
Unit Op Connection	Surge tank in UF/DF	Surge tank		
Stage 8 Concentration & Diafiltration into Formulation Buffer	Two-tank batch UF/DF: alternating use of tanks as surge & TFF recycle		Continuous SP-UF1 w/countercurrent DF & SP-UF2 Two parallel batch UF/DF systems	Continuous operation, varying protein concentration Decisions around need for a surge tank vs use of two tanks integrated into the UF/DF skid will depend on unique volumes

Process Stage	Option 1 ICB: Stages 1-7 Cycled Batch: Stage 8	Option 2 ICB: Stages 1-4 Periodic Batch: Stages 5-8	Other Options Discussed	Differences from Batch Operation and Other Comments
			operated in an alternating mode	involved in a particular process design
Unit Op Connection	Batch pool tank or multiple bags		Surge tank or individual bags to be pooled later	Batch pool tank represents batch attributes; batch consolidation could occur immediately after UF/DF or as part of formulation step to create DS

ATF	Alternating flow filtration
B/E	Bind and elute
DF	Diafiltration
F/T	Flowthrough
MCC	Multi-column chromatography
N/A	Not applicable
NOR	Normal operating range
PAR	Proven acceptable range
SP-UF	Single pass ultrafiltration
TFF	Tangential flow filtration
UF/DF	Ultrafiltration/diafiltration

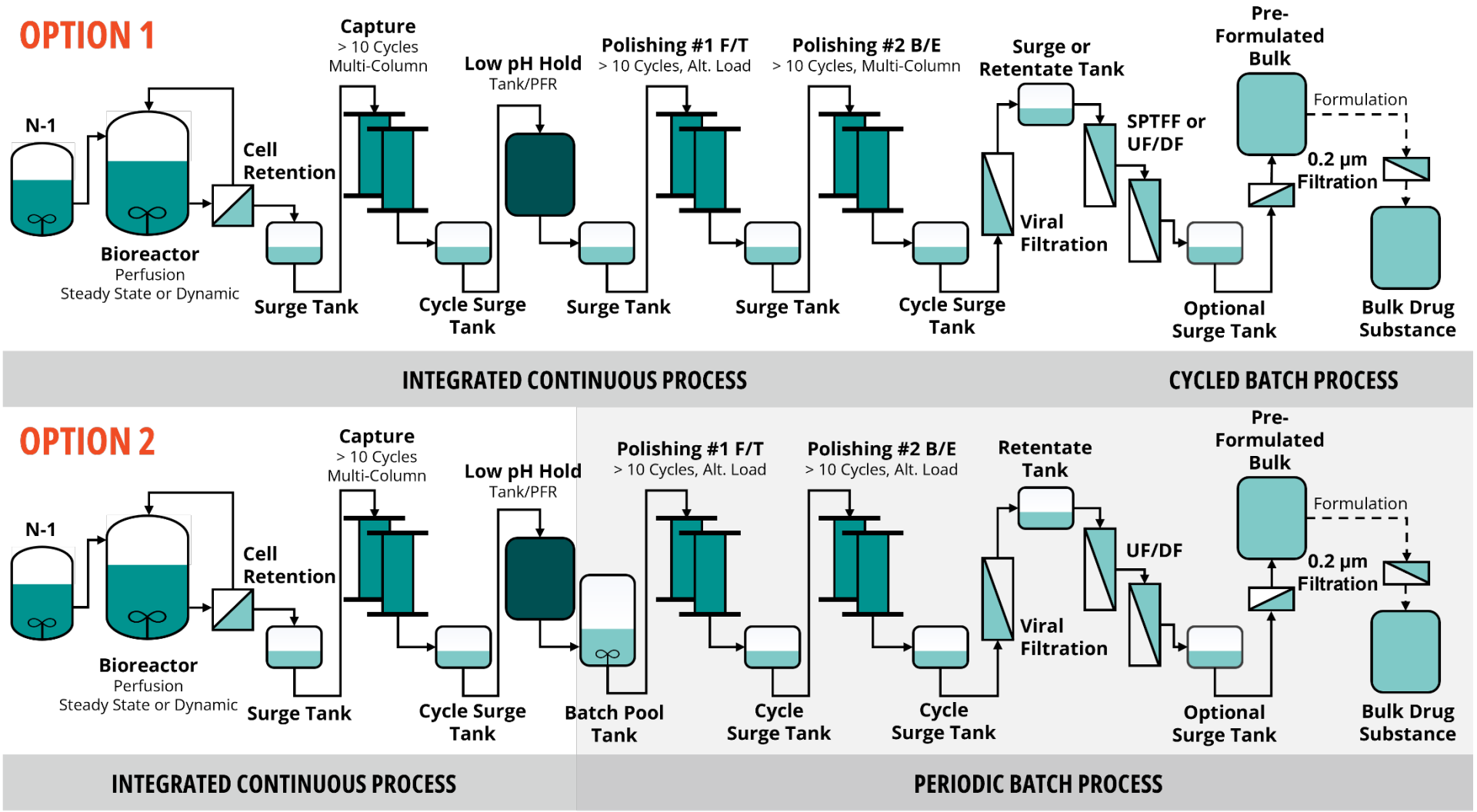


Figure 1.5. **Two major options for integrated continuous bioprocesses.**

KEY TERMINOLOGY: SURGE TANK

A **small surge tank** may be used to serve as a 'break tank' between two unit operations sized to accumulate sufficient volume to accommodate small fluctuations in product output from a preceding unit operation and/or product input to a subsequent unit operation. It is also useful as a way of managing pressure differences. This surge tank will typically have a residence time on the order of minutes to tens of minutes.

A **cycle surge tank** may be designed to accumulate one (or a few) sub-cycles exiting a unit operation. This will be most useful for a unit operation with a highly variable output, e.g., bind and elute chromatography, that would benefit from homogenization before application to subsequent unit operations that may be sensitive to such variability. A cycle surge tank would typically be designed according to the anticipated cyclic output volume from a unit operation with a corresponding variation in the residence time. the concept that a batch is defined by a specific amount of material AND a pre-specified package of information related to the integrated control strategy that allows for assurance of product quality.

A **batch pool tank** is designed to accumulate material equivalent to the mass anticipated for a single drug substance lot, thereby allowing for collection and homogenization at one or more points within an integrated and continuous process. Examples of applications of the batch pool tank concept include (a) collection of material after UF/DF, before final formulation and sterile filtration (Option 1) or (b) collection of virus-inactivated intermediates (Option 2). The batch pool concept may be useful if product quality is highly variable across all or a portion of the bioreactor and where homogenization may be beneficial for the performance of subsequent unit operations that may be more sensitive to variability than Protein A capture chromatography. In addition, a batch pool tank can enable more robust control or deviation management strategies. Batch pool tanks should be used selectively because, depending on the batch size and product concentration, they may require a vessel of significant footprint inconsistent with the principles of continuous manufacturing. In addition, a batch pool tank will likely have a sufficiently long residence time (>24 hours) that requires demonstration of chemical and microbial intermediate stability.

Figure 1.6. Definition of Surge Tank. Two specific types of surge tanks include cycle surge tanks and batch pool tanks.

Several surge tank configurations may be considered and are listed as options in Table 1.3 and Figure 1.5. In general, a surge tank (as defined in Figure 1.6) should be as small as possible to minimize dispersion of product across the integrated unit operations and therefore maximize the controllability and traceability of product fluid elements moving through the system. However, the size of the surge tank will also be dictated by potential variations in flow over the course of the process such that the tank volume falls between defined minimum and maximum operating limits to maintain sufficient mixing and reduce the potential for agitator-induced foaming. In some cases,

determination of these limits can require significant consideration (Sencar et al., 2020). Two different subclasses of surge vessels are also considered, including a cycle surge tank and a batch pool tank. Selection of a surge vessel depends on the needs of the integrated process. They are also compared with a batch hold tank, which is representative of the entire batch. A more detailed discussion around the design of surge tanks as part of the integrated design can be found in **Section 2.4.1**.

1.3 Overview of integrated control strategy elements and development

An integrated control strategy is foundational to the control of clinically relevant CQAs, derived from the TPP and QTPP, during manufacturing regardless of whether the process is run in a batch or continuous mode of operation. According to ICH Q10, a control strategy is a planned set of controls, derived from current product and process understanding, that assures process performance and product quality. On a more basic level, one could think of a control strategy as a set of controls or buffers that mitigate the impact of known variability in raw materials, process control, measurement variance, and plant operations on product quality and process consistency based on a deep understanding of the product and process. Development of an integrated control strategy requires a detailed risk analysis for each CQA and relevant process characterization studies followed by demonstration as part of process validation. A comprehensive and transparent control strategy clearly connecting the analytical and process controls will be more effective than independent controls over process parameters, analytical testing, and raw materials. An integrated control strategy will consider learnings from product and process characterization and will be designed such that the degree of control is commensurate with the level of risk to product safety or efficacy. Risk level is determined by assessing (1) how impactful a quality attribute is to safety and efficacy, (2) the ability of the process to robustly control an attribute, and (3) the effectiveness of the formulation and storage conditions in maintaining an attribute over the intended shelf life. Attributes at greater risk could require more direct controls of product quality (more control points, higher testing frequency, tighter limits) and/or more indirect controls through process parameters and material attributes. These ideas guide control strategy design for any kind of process. It should be understood that both the risk assessments and the resulting control strategies are living documents and that multiple versions will be generated over the project lifetime. These are indicated in **Figure 1.1**, and a more detailed view that focuses on three major versions of the control strategy (prior to the start of clinical manufacturing, prior to the start of registrational batch manufacturing or PPQ, and the commercial control strategy) is shown below in **Figure 1.7**.

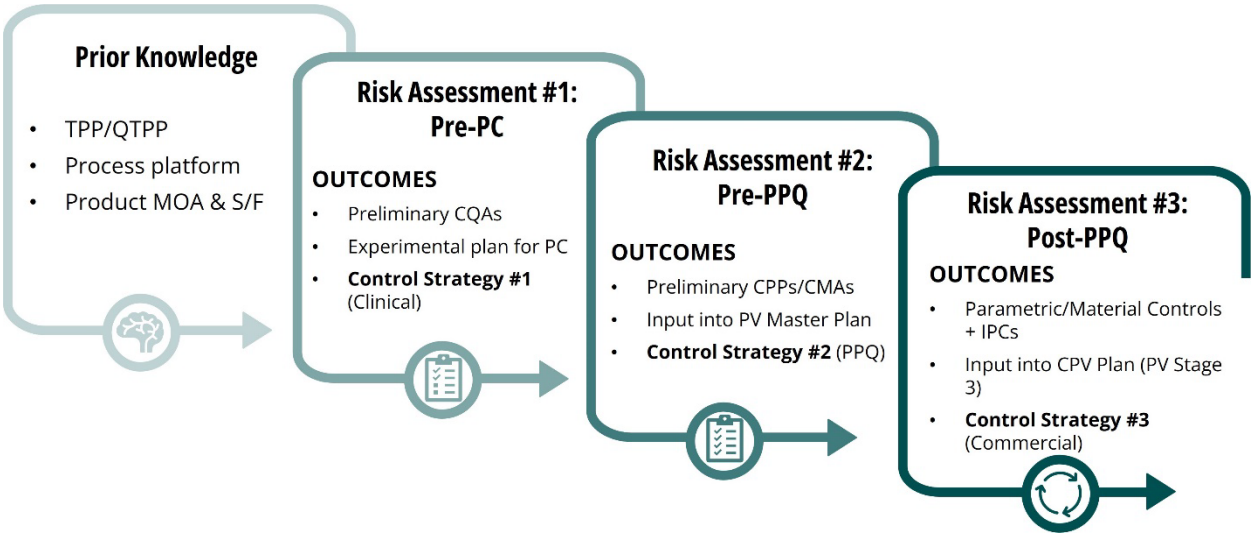


Figure 1.7. Detailed view of connection between risk assessments and control strategies.

CMA	Critical material attribute
CPV	Continued process verification
CPP	Critical process parameter
CQA	Critical quality attribute
IPC	In-process controls
MOA	Mechanism of action
PC	Process characterization
PPQ	Process performance qualification
PV	Process validation
QTPP	Quality target product profile
S/F	Structure-Function relationship

The foundation of any control strategy is a set of good manufacturing practices (GMP) and procedural controls, governed by quality management systems, which must be fully functional in the facilities used to manufacture the DS. Although a phase-appropriate approach to the implementation of GMPs and quality oversight may be used, this should be established prior to the start of clinical manufacturing. Elements of GMP and procedural controls should include:

- Facility details such as the building management system, especially regarding details around controls for temperature, humidity, and environmental controls including the environmental monitoring plan
- Utilities
- Process equipment, including installation qualification (IQ)/operational qualification (OQ)/performance qualification (PQ) as well as maintenance and calibration programs
- Staff qualification and training
- Batch instructions
- Sample plans and procedures for sample management
- Analytical methods, including controls for assay qualification, validation, and transfer
- Data and automation systems

An integrated control strategy should define elements of process control as well as control of product quality along with a description of how these elements work together. Thus, control of the materials and the process parameters combined with a series of in-process tests for verification of performance combined with release and stability testing of the DS and DP provide the core of the

control strategy. For the control of process and product, the integrated control strategy should include the following:

1. Controls should be established over the attributes of materials entering the process that ensure suitability for use, and those attributes that are critical to the control of quality attributes should be defined. Suitability is confirmed by testing with acceptance criteria, for example, release specifications and any confirmatory testing for raw materials and consumables to ensure they are suitable for use. Acceptance criteria for raw materials must be met before they are used to create culture media or buffers. Likewise, acceptance criteria for prepared culture media and buffers must be met before they are released for use in the DS manufacturing process.
2. Specified ranges should be established for process parameters, especially process parameters that represent a higher degree of criticality or risk to the process and that are directly controlled, e.g., temperature, pH, and flow rate. Proven acceptable ranges (PARs) for process parameters should be established and documented in accordance with the relative risk they represent. Monitoring of control over process parameters can be achieved using a combination of continuous data from in-line probes and routine testing of specific samples for process performance attributes as described below.
3. An in-process control (IPC) plan should be established to ensure that samples taken at specified points in the process meet pre-defined targets to verify that product quality (measurement of product quality attributes) and process parameters (measurement of controlled parameters) are controlled as intended and that process performance (measurement of process performance attributes) is as expected. IPCs may be associated with an action limit and/or an acceptance (rejection) limit and, in some cases, may be linked to a predefined instruction if a decision is necessary based on the result of a test.
4. Release specifications for DS and DP will ensure that the final product is of acceptable quality to meet the requirements of the QTPP.
5. Stability specifications for intermediates, including DS and DP, are based on stability-indicating product quality attributes.
6. Development of proven acceptable ranges for process parameters and material attributes are typically established during process characterization and Stage 1 process validation experiments, and "the degree of control over those attributes or parameters should be commensurate with their risk to the process and process output. In other words, a higher degree of control is appropriate for attributes or parameters that pose a higher risk." (FDA Guidance for Industry. Process Validation: General Principles and Practices, 2011). Then control of the process within these ranges is demonstrated and documented during process performance qualification (PPQ) or Stage 2 process validation. However, additional information gathered during non-routine assessments of the control strategy such as process and product characterization studies, process validation studies, comparability exercises, and product quality impact investigations related to process excursions should be used to refine the integrated control strategy over time.

The elements of an integrated control strategy described above are expected to be the same for both batch and integrated continuous bioprocesses (ICB). These elements are developed in parallel throughout the product life cycle based on studies, either planned or as part of an investigation, in

which the impact of various stresses on product quality is determined. Product and process understanding is built over time from the results of a number of activities executed by CMC teams (Figure 1.8) operating within the framework of an overall CMC process.

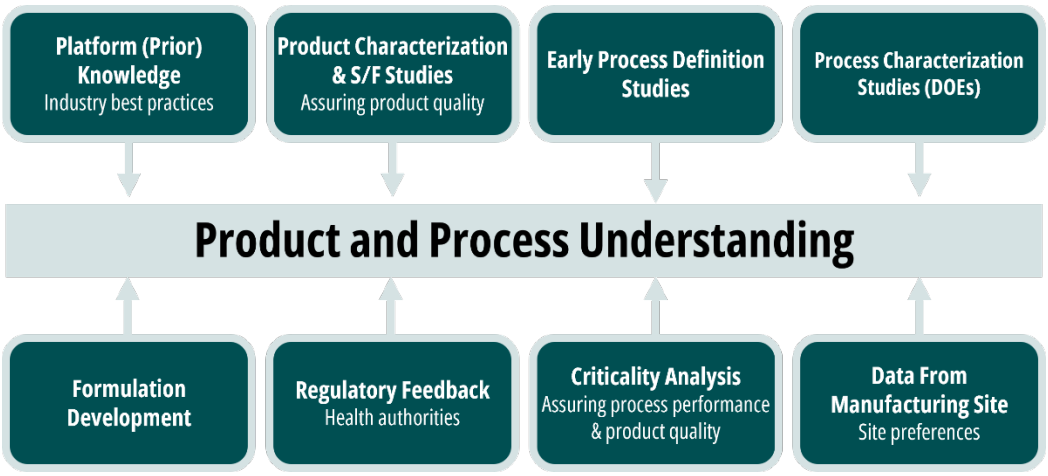


Figure 1.8. Inputs into product and process understanding.

Examples of process development and characterization studies for the overall framework design options described previously will be provided in detail in Chapter 3. The results of those studies will contribute to an updated version of the integrated control strategy put in place prior to PPQ as described in Chapter 6. After review of the results from PPQ along with responses to questions from Health Authority reviews of regulatory filings and manufacturing facilities, a comprehensive integrated control strategy can be established. This strategy also takes into account the practical and current technologies available to enable active process and product monitoring that are described in Section 7.2. Because of the limited availability of analytical technologies suitable for integration into a cGMP process, it is likely that a continuous process will offer fewer opportunities for IPC testing of intermediate pools with a composition that is representative of the whole batch. Therefore, the base case allows for the integration of process analytical technology (PAT) options, if available to enable rapid response and actions (see Chapter 8); however, it is not dependent on active monitoring and control of product quality. It is expected that future advances in PAT technologies will facilitate revised and updated approaches to active processing (see Chapter 9) that current process development and characterization efforts will take advantage of technologies available for development work that may not yet be appropriate for cGMP operations. It is also likely that soft sensors or multivariate models based on material or process performance attributes will become more important for assurance of control of continuous processes.

Note that the terms attribute(s) used above (e.g., quality, product, material) and parameter(s) (e.g., process, operating, and equipment) are typically categorized with respect to criticality using a risk-based approach that is often in general alignment with A-Mab but may differ across companies, and the authors recognize that different organizations often determine their own terminology and

categories with respect to criticality. With a lifecycle approach to process validation that employs risk-based decision making throughout that lifecycle, the perception of criticality as a continuum rather than a binary state, consistent with the FDA Guidance on Process Validation (FDA Guidance for Industry. Process Validation: General Principles and Practices, 2011) may be more useful. All attributes and parameters should be evaluated in terms of their roles in the process and their impact on the product or in-process material, and they should be re-evaluated as new information becomes available. The degree of control over those attributes or parameters should be commensurate with their risk to the process and process output. In other words, a higher degree of control is appropriate for attributes or parameters that pose a higher risk. The expectation is that each manufacturer will establish the meaning and intent of its terminology and categorization in an internally consistent manner.

Although not technically part of the integrated control strategy, establishing a robust electronic data system can enable many facets of process development, clinical manufacturing, process validation, deviation management, and the preparation of regulatory dossiers. Clear and current connections across work done at different scales by different groups will be extremely useful in establishing process knowledge and enabling real time decision making. An effective knowledge management system can also enable a more effective quality risk management system (Lipa et al., 2020). Existing systems used for managing and analyzing electronic data may need to be further adapted for use in an ICB context because of the larger amount of data generated in an ICB setting as well as the greater need to record and analyze time-dependent inputs (including raw material lot information) and outputs. Further discussion of knowledge-based system support for real time decision making will be presented in **Section 9.4**.

1.3.1 Control strategy framework

An initial version of Control Strategy #1 should be generated for the first full-scale development or clinical batches. A minimalist approach is shown in **Figure 1.7**. At this time, little definitive information may be available for the product and process, so this version of the control strategy is commonly developed by relying on platform experience. However, creating a structured control strategy at this point in time is useful in that it provides a framework for tracking minor updates to the control strategy as product and process knowledge increases. The more informed the initial control strategy is by early development work for the specific product, the lower the risk in terms of control of CQAs. The simplified table shown in **Table 1.4** is a useful way to track elements of the control strategy. The first column lists individual pCQAs that were developed in the initial criticality assessment. The second column lists the source of changes to the pCQA (typically the bioreactor for most post-translational modifications but downstream impact is certainly possible), the response of the pCQA in various stress and accelerated stability studies, whether the pCQA is stability-indicating based on real-time stability results, and which process steps allow for clearance or a reduction in the level of the pCQA. Note that much of the information in the second column is developed over time, often late in development, so platform experience as well as protein modeling work may be useful for understanding product variability early in clinical manufacturing. The third column lists process controls, both in terms of process parameters and material attributes that may impact variation in a given pCQA. The fourth column provides an understanding of how well controlled a pCQA is during the process. The fifth column lists various elements of an analytical testing strategy such as in-process controls (IPCs), release testing, and stability testing. Early in clinical manufacturing, release

and stability testing will play a large role in the control of product quality as process knowledge is being developed. The final column provides an assessment of the residual risk associated with the control of a pCQA.

Examples are provided in **Section 3.3** for risk assessment tables that enable the development of a process development and process characterization (PD/PC) plan. Further discussion in that chapter describes the work involved to update the control strategy for manufacture of registration batches (material made to supply pivotal clinical trials) and PPQ, which is discussed in **Section 4.4**. After additional product and process knowledge has been acquired during PPQ and through the activities leading to the approval of the initial regulatory filings, a further update to the control strategy for commercial manufacturing should be prepared as discussed in **Chapter 7**. Also, since the control of adventitious agents often represents a unique subset of the overall control strategy, it is presented as a separate discussion in **Chapter 5** including considerations

It is also important to note that the evolving versions of the control strategy are essential to assessing deviations and other aspects of managing the process in real time. A unique difference from A-Mab is that this N-mAb case study includes a discussion of the challenges involved in managing deviations in real time as necessitated by a continuous process, and these challenges are described in **Chapter 8**. That chapter will expand on how decision processes can be built to manage deviations in both CPPs and CQAs. High-level views of the points to consider as part of the decision pathways are shown in **Figure 8.1** for deviations related to CPPs. **Chapter 9** presents some future-looking perspectives on the impact of advanced analytical methods as well as the potential for real time decision making assisted by machine learning or artificial intelligence tools

Table 1.4. Control Strategy #1: Prior to start of clinical manufacturing.

pCQA	Potential Source of Changes to pCQA, Stress Response, and Clearance	Process Controls: Parametric & Material Attributes	Process Capability	Analytical Controls: Testing Strategy	Residual Risk
Glycosylation: Galactosylation	Source: Bioreactor Stress Response: TBD Stability Indicating: No Clearance: None	Bioreactor PPs: Platform: Temp, pH, DO, harvest day Bioreactor MAs: Platform: Trace Metals	Infer from platform & process stress studies	Recommend IPC testing for initial clinical runs DS Release Testing DS Stability Testing	Medium
Glycosylation: Fucosylation	Source: Bioreactor Stress Response: TBD Stability Indicating: No Clearance: None	Bioreactor PPs: Platform: Temp, pH, DO, harvest day Bioreactor MAs: Platform: Trace Metals		Recommend IPC testing for initial clinical runs DS Release Testing DS Stability Testing	Medium
Glycosylation: High Mannose	Source: Bioreactor Stress Response: TBD Stability Indicating: No Clearance: None	Bioreactor PPs: Platform: Temp, pH, DO, harvest day Bioreactor MAs: Platform: Trace metals		Recommend IPC testing for initial clinical runs DS Release Testing	Medium
Deamidation at Asn325	Source: Bioreactor, DSP Stress Response: pH, heat based on platform, actual results TBD Stability Indicating: TBD Clearance: None	Bioreactor PPs: Platform: Temp, pH, DO, harvest day DSP PPs: Platform: Intermediate hold time & temp		DS Release Testing DS Stability Testing	Low
HMWS	Source: Bioreactor, DSP Stress Response: pH, heat, shaking, light, metals, freeze/thaw based on platform, actual results TBD Stability Indicating: TBD Clearance: CEX = TBD	Bioreactor PPs: Platform: Temp, pH, DO, harvest day DSP PPs: Platform CEX: protein load, peak cutting DSP MAs: Platform CEX: elution buffer pH		Consider IPC testing for initial clinical runs DS Release Testing DS Stability Testing	Low
Host Cell Protein (HCP)	Source: Bioreactor Stress Response: None Stability Indicating: No Clearance: Chrom Step(s)	Bioreactor PPs: Platform: Temp, pH, harvest day DSP PPs: Platform: protein load, peak cutting		DS Release Testing	Low

CEX Cation exchange
Chrom Chromatography
DO Dissolved oxygen
DP Drug product
DS Drug substance
DSP Downstream process
IPC In-process control
HCP Host cell protein
HMWS High molecular weight species
MA Material attribute
pCQA Preliminary critical quality attribute
PP Process parameter
TBD To be determined

Chapter 2

Integrated Process Design: Process Option Decisions

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

2 Integrated Process Design: Process Option Decisions

2.1 Process design

2.1.1 Overall approach

Understanding critical aspects of both product quality and the impact of the process involves multiple interrelated activities that frequently occur in parallel over time. Thus, it is important to continually maintain close communication across the groups performing this work and to update any impacts to the control strategy being developed. Several activities provide valuable information to identify which product attributes must be controlled to assure product safety and efficacy. These activities include product characterization by means of assessment of structure/function relationships, potential hotspots for modification, formulation robustness, and stress stability. In other words, the product is evaluated in a variety of studies to determine the quality requirements for assurance of safety and efficacy. Note that some of these studies, for example, stress stability studies involving variations in pH, temperature, or light exposure, are important in elucidating key sensitivities related to risk assessment in the determination of criticality of a quality attribute (Halley et al., 2020). The sensitivities identified by such studies can often be linked to process-related stresses present during full-scale manufacturing such as hold times at a sub-optimal pH or light exposure, which will require mitigation in order to reduce process-related risk and improve process capability (Das et al., 2020).

Process characterization is a key activity to understand the performance robustness of the process design when it is subjected to expected sources of long-term variability. As part of this assessment, it is critical to understand the clear purpose of each unit operation in the process and how variability at a given step can impact the product. Sources of variability may be variations in material attributes or process parameters. Characterization will provide valuable information about what process controls will be required for the process to produce product of the required safety and efficacy. In other words, the design is evaluated during characterization to more fully determine what process controls will be required.

Throughout the product lifecycle, various studies can be initiated to discover, observe, correlate, or confirm information about the product and process. All studies should be planned and conducted according to sound scientific principles, appropriately documented, and internally approved in accordance with the established procedure appropriate for the stage of the lifecycle. (FDA Guidance for Industry. Process Validation: General Principles and Practices, 2011)

The general principles for the overall approach described above also apply in the context of ICB, but their specific application may vary according to the nature of the processing. As discussed in subsequent sections, the approaches typically applied in batch processing contexts can be readily adapted to enable development, characterization, and, ultimately, validation, of ICB processes. This adaptation will require specific discussion of (1) different development and characterization approaches that may be required for continuous modes of operation and (2) different control, characterization, and validation approaches required for integrated (connected) unit operations.

2.1.2 Development

The process framework options laid out in **Chapter 1** allow for a great number of potential permutations. Therefore, a subset of these potential configurations was selected to provide specific examples of integrated and continuous manufacturing processes and discuss the considerations related to their process design and characterization. The examples provided here are similar to the process details provided by (Coffman et al., 2021) with some differences included to provide some variation that one could expect.

For the N-mAb upstream process design, we will cover scenarios that include both dynamic perfusion and steady-state perfusion operation of the bioreactor to highlight the key similarities and differences related to the process development of such systems. For the downstream process design, we will consider the following:

a) Continuous capture using a multi-column system with loading control via a process analytical technology (PAT) tool like inline UV absorbance measurement of load and flowthrough streams
b) Virus inactivation using a plug-flow reactor
c) Two polishing chromatography steps with the first operated in flowthrough mode (e.g., anion exchange) and the second operated in bind-and-elute mode (e.g., cation exchange)
d) Direct flow virus filtration
e) Alternating batch ultrafiltration and diafiltration (continuous operation is discussed as an additional option)
f) All pH and conductivity adjustments are achieved via in-line conditioning

Specific to the integration of the unit operations, we consider that the downstream processing steps are integrated to *either* dynamic *or* 'steady-state' perfusion because the proposed development and characterization approach is generalizable to accommodate either production format. Sub-sections may refer to other scenarios or configurations as well, even if they are not the main focus.

2.2 Upstream process design

2.2.1 General considerations

The prototype upstream process discussed in this section is based on a perfusion bioreactor operation, whether operated in steady-state or dynamic mode (**Glossary**). Out of scope for this discussion is any intentional product retention or intensified batch process.

2.2.2 N-1 seed train

It is expected that the seed train for an intensified, continuous process will not differ significantly from the seed train currently employed for a batch process. For example, decisions related to whether each bioreactor run is directly linked to a single working cell bank (WCB) vial or if multiple production bioreactors can be initiated from a single WCB vial via splits in the seed train will be driven by factors such as cell line stability and operational cadence requirements, as they are now for batch manufacturing. The N-1 seed bioreactor may be run either as an independent batch with an optional feed or as a perfused culture. For the perfused culture, the initial batch culture is

followed by a defined retained cell perfusion phase. In either case, transfer to the production bioreactor occurs at a predetermined inoculation trigger.

2.2.3 Production bioreactor

2.2.3.1 Overview - dynamic vs. steady state

The N-stage perfusion production reactor can be run using two approaches: dynamic or steady state. In both scenarios, perfusion is initiated at the start of the batch to build cell mass to a significantly higher level than in traditional fed-batch culture. Protein is harvested continuously via perfusion through the cell retention device and processed downstream in a connected manner. As discussed in Section 1.2.3, key differences between the dynamic and steady-state process formats are the cell mass profile and the length of the batch. For dynamic perfusion, in which the perfusion rate varies over the duration of the culture, a number of options exist of which the HILVOP process described by (Gagnon et al., 2018) is one example. See Figure 2.1 for example cell density profiles for dynamic and steady-state processes. Significant differences will also be observed in the product titer profile between dynamic and steady state modes of perfusion operation as depicted in Figure 2.2. Additional assumptions of dynamic and steady-state perfusion processes are depicted in Table 2.1 for reference.

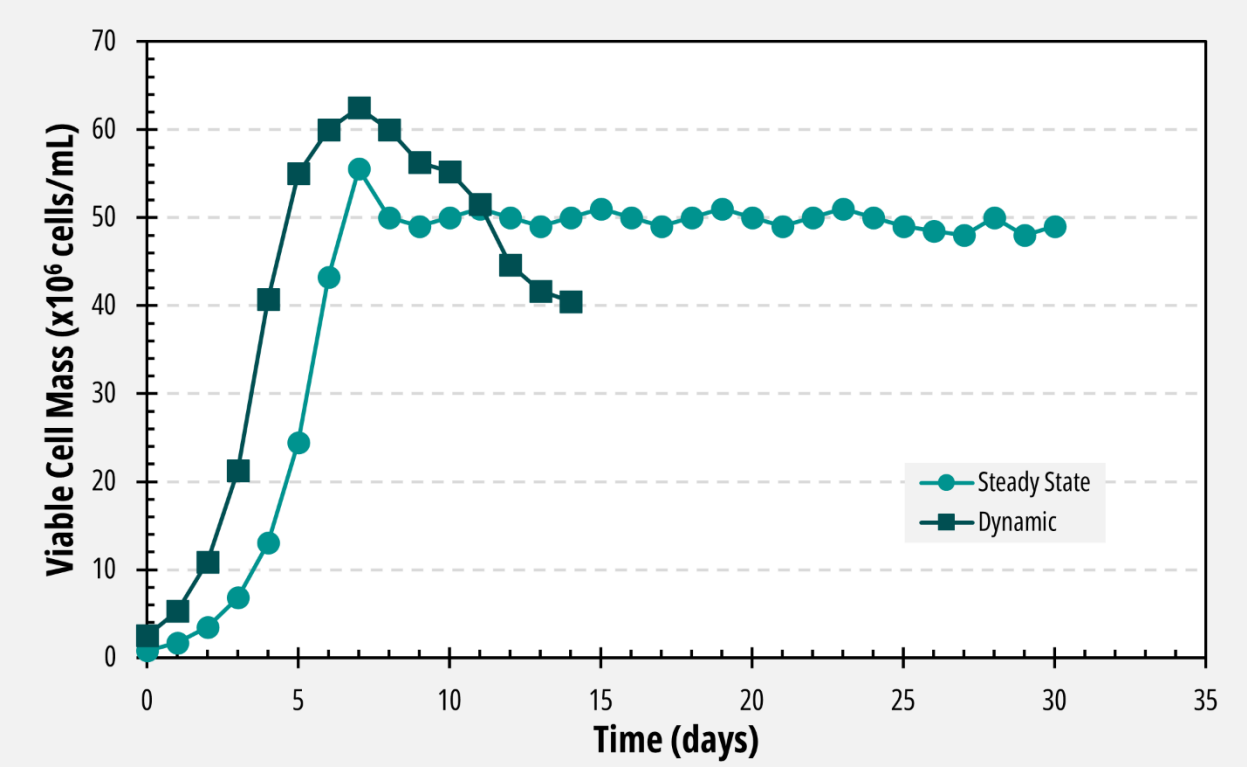


Figure 2.1. Viable cell density profile for dynamic and steady-state perfusion.

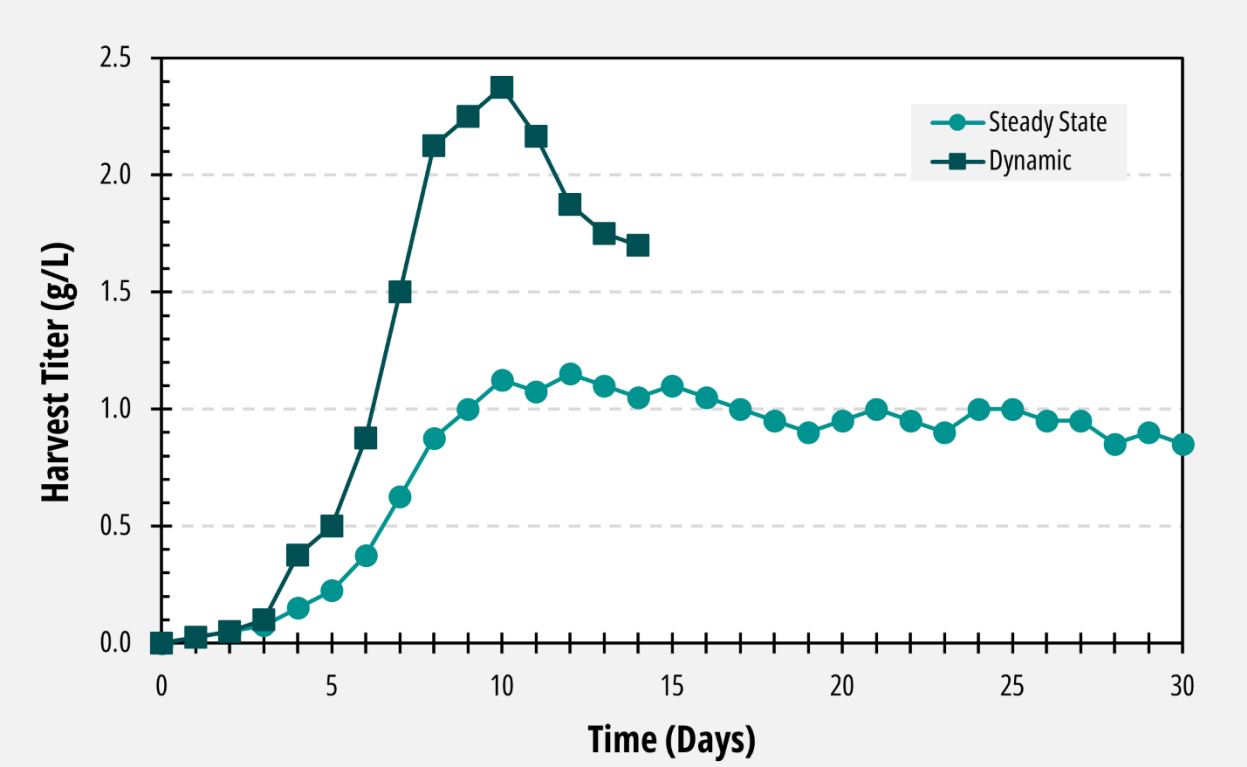


Figure 2.2. **Product titer profile example for dynamic and steady-state perfusion.**

Table 2.1. **Comparison of dynamic and steady-state perfusion processes.**

Parameter	Dynamic	Steady-State
Perfusion Rates (Vessel Volumes / day)	~1–2 VVD (range on both ends may be wider in some cases depending on design)	~1–2 VVD
Process Shift at Peak VCD	Yes, Temperature common	Yes, Temperature common
Media concentrates	Yes	Yes
Batch Duration	~10–15 days	~20–40 days
Cell Bleed	No	Yes
Harvest Phase	Triggered early in batch	Triggered upon transition to steady state
Titer During Harvest Period	Varies significantly (up to 1–1.5 orders of magnitude)	Consistent within +/- 10% on g/L basis
Product Quality During Harvest Period	May be generally consistent or vary significantly across the harvest duration depending on CQA Process development performed to minimize product quality drift	Generally consistent or some variation may occur across the harvest duration, e.g., charge isoforms may vary 10% Process development performed to minimize product quality drift

Note: Dynamic perfusion volumetric productivity (Qp): 0.5 to 4 g/L/day (Salm et al., 2017)

CQA	Critical quality attribute	VCD	Viable cell density
Qp	Volumetric productivity	VVD	Vessel volumes per day

2.2.3.2 Production bioreactor process development

High productivity in the upstream N-stage is a key focus area of development to meet product demands at a moderate scale of production while targeting a low cost of goods using an ICB operating model. Thus the assumption for N-mAb is a cell-specific productivity of 40 picograms (of product) per cell per day (pcd) which is similar to published values (Coffman et al., 2021; Mahal et al., 2021). A standard development approach may be applied to define the optimal setpoints of classical upstream operating parameters including pH, temperature, and percent dissolved oxygen (DO). A Design of Experiments (DOE) approach may be used to identify the optimal combination of these key parameters. Additionally, historical knowledge of a given host cell line as run within a fed-batch process optimized for growth can be leveraged for the growth phase of the perfusion culture.

2.2.3.3 Perfusion parameters

The design of a continuous perfusion bioreactor process should consider three phases of the culture: growth, transition, and production. During the growth phase, operating parameters such as perfusion rate are optimized for high cell growth to achieve the target peak cell density. Initiation of the transition phase may trigger changes in operations such as a reduction in temperature to slow the culture metabolism while maintaining sufficient growth rate to keep the cells in a healthy state of production over time.

The transition phase should be minimized so that steady-state perfusion is reached as soon as possible, and studies focused on the transition from growth to a productive steady-state phase are valuable to accelerate the shift to harvesting protein and to minimize the variation of the titer in the feed stream to the downstream steps.

While waste metabolite concentrations are less of a concern in perfusion mode than in batch mode because the medium in the bioreactor is constantly refreshed, it is important to maintain cell health so that cell mass can also be maintained over time. The nutrient levels in the perfusion media need to be optimized to support cell health and to work in concert with the perfusion rate and cell bleed rate (cell bleed only applicable to steady-state perfusion). As a starting point, existing fed-batch basal media and feed media can be combined and adapted to generate perfusion media that support the target cell mass at a reasonable cell-specific perfusion rate (CSPR). To fully optimize the perfusion media, the cellular uptake rates and production rates of metabolites are analyzed, and adjustments are made iteratively (Konstantinov et al., 2006; Wolf et al., 2019). Multiple supplemental feed solutions may be applied to address the unique needs of the growth phase versus the steady-state phase, or a single perfusion solution may be used at different CSPRs between the phases (Wolf et al., 2020). Additionally, a single comprehensive perfusion solution can be applied, or separate stock solutions may be additionally perfused or fed via daily bolus additions if needed. For example, a glucose stock solution may be maintained as a separate feed if required by the variation of glucose uptake rate (GUR). During development, separate stock solutions may be fed as building blocks to gain data for subsequent combination into an optimal single solution for operational simplicity.

The perfusion rate can be monitored or controlled by using either a CSPR or a vessel volume per day (VVD) rate. Upon studying the optimal CSPR in development, a fixed VVD can be applied operationally assuming a known predictable growth profile, especially for steady state perfusion. The CSPR can be linked to daily offline viable cell mass measurements or controlled using online

viable cell mass readings via a PAT tool such as biocapacitance (Mercier et al., 2016). The growth phase versus the steady-state phase of the culture will have different optimal CSPR values. Additionally, during the steady-state phase, a cell bleed is often employed as a method to maintain a fairly constant cell density and viability as well as protein titer harvest. Similar to CSPR, cell bleed can also be controlled via an online PAT tool such as biocapacitance. Biocapacitance, also called dielectric spectroscopy, can also be used to monitor the health of the cells in the bioreactor (Downey et al., 2017; Metze et al., 2020). The reactor volume is maintained constant by holding the sum of the perfused harvest volume and the cell bleed volume (leaving the system) equivalent to the volume of perfusion medium, including additions, entering the system.

2.2.3.4 Media concentrates

While a high VVD rate may yield optimal growth, operational feasibility needs to be considered, and thus it is important to find a balance between growth and required media amounts at higher VVD. During the production phase of the batch, a flowrate range of ~0.5-3 VVDs has been adopted to minimize the number of media solution preparations. Additionally, media concentrates can be used to further optimize the handling and overall process operability. Once the optimal perfusion basal medium is determined at a 1x concentration, additional development can be performed to assess the solubility of the medium components at the time of preparation and their stability over time at concentrations up to 10x. Some component forms may need adjustment to avoid exceeding saturation limits at ambient temperatures, and solution pH and other characteristics may need optimizing as well. Media concentrates should be monitored over time (e.g., 1 month at ambient sterile conditions) for degradation to aid in selecting a final concentration, component form, and pH. The final selected concentration should be tested in a cell culture experiment to confirm equivalent performance to the 1x prepared medium.

2.2.4 Cell retention filtration

Cell separation in a perfusion operation can be performed via alternating flow filtration (ATF) or tangential flow filtration (TFF) as discussed in **Section 1.2.3**. Both formats require considerations of filter sizing, fouling, shear rates, flux rates, and protein retention (sieving). Note that for batch operations, cell removal or harvest is a separate unit operation or process stage, but for ICB, it is integrated into the bioreactor operation.

The performance of the perfusion filter will typically decline over time and may require proactive filter change-outs through an elongated run of 30 days or more. As the filter starts to foul, protein retention is typically observed and quantified by determining % sieving value for the product. The change in protein retention profile should not significantly impact the volumetric productivity and subsequent harvest stream titer, but it could cause some variations in product quality or process impurity levels, and the downstream steps should have the flexibility to handle these variations. Filter performance is characterized at small scale in a scale-down predictive format to define the filter loading, flux capacity, anticipated sieving profile (mean bioreactor residence time), and subsequent area needed per batch (Pinto & Brower, 2020; Pinto et al., 2020; Walther et al., 2019). Accordingly, one may incorporate multiple planned filter change-outs dependent on the length of the batch. Note that for a well-characterized process, any filter change-outs should be planned events, for example necessitated by cell line characteristics, rather than unplanned or random events. In addition, filter change-outs should be designed to maintain the closed nature of the

process as much as possible, consistent with details presented in Section 5.2.2. If unexpected changeouts are encountered, a process performance that differs from anticipated flux and sieving profiles may trigger review within the Quality Management System, as described in Section 8.4.

2.2.5 Product quality variation

Throughout the development of a continuous process, it is important to obtain an understanding of the variation in process performance attributes over time and to generate daily product quality data. Note that post-translational modifications resulting from typical degradation pathways are often reduced in perfusion cultures compared with fed-batch processes due to the reduced residence time of the product within the bioreactor (Karst et al., 2018).The extent of data generated during process development and characterization is fundamental to establishing a control strategy that is aligned with continuous operations. Comprehensive process development studies will enable a control strategy that is based on process understanding. The control strategy is reduced to practice via adherence to parametric and material attribute controls, as well as monitoring of process performance attributes with minimal off-line sampling and testing for product quality (PQ) until integrated robust PAT options are available. In a steady-state process, over the duration of the harvest, the product quality parameters may drift, but minimal changes are observed. It is critical to maintain product quality in the upstream steps within a range consistent with downstream capabilities over the duration of the process. Attributes that are generally not impacted by downstream purification steps (e.g., glycosylation and charge isoforms) should fall within a known acceptable range to eliminate the need for a full batch pooling step after the viral inactivation stage as in Option 2 in Table 1.3. Instead, multiple batches could be prepared and tested for final release at the drug substance stage.

In the scenario where a product quality attribute varies significantly over culture time, beyond what the downstream process will clear or modulate to create a relatively consistent drug substance over time, then installation of a batch pool is recommended. The location of the batch pool (e.g., post viral inactivation, post final ultrafiltration and diafiltration (UF/DF)) within the downstream process should be determined based on downstream capabilities and on the control or decision points for the quality attribute in question. In this scenario, the ability to sample, test, and react in a timely manner must be taken into consideration as well as the need for batch pool stability studies. Rapid real time testing for PQ may be desirable or even necessary for certain molecules, but careful consideration must be given to the technology robustness, cGMP capabilities, and ability to automate such approaches into a continuous framework. This concept is further discussed in Section 8.2. To further illustrate scenarios involving product quality variation, an informed mock dataset has been generated based on consensus industry experiences. Specific examples drawn from that dataset are shown below to illustrate situations that could require decisions related to choices of process options.

Product charge isoforms encompass a large range of potential protein molecule modifications, often in multiple combinations. When assessing the consistency of charge species, a key determination is whether the modification is CQA-related or non-CQA-related and how it impacts potency, efficacy or safety. Additionally, the ability to obtain a comprehensive data set during development is critical to the control strategy that will be established to enable current good manufacturing practices (cGMP) operations. The examples below represent potential scenarios resulting from acquiring a thorough

dataset during development studies. Note that, in these examples, product quality variation typically represented by time course data over the duration of the harvest period, with generic time-based indicators of early, middle, and late, rather than specifically associating a profile with either a typical dynamic or steady state perfusion duration. This is for the sole purpose of creating an example and not to imply that either mode or perfusion operation is more or less susceptible to a given variation in product quality. In any applications of these concepts to practice, product quality variation should be thoroughly examined and understood for each product and cell line.

2.2.5.1 C-terminal lysine or proline amidation variation example

Basic species charge isoforms have sometimes been observed to drift upwards over the course of a perfusion culture. This observation is commonly attributable to either C-terminal lysine clipping over time as carboxypeptidase triggers the formation of additional basic species (Harris, 1995) or amidation of a proline residue near the C-terminus (Kaschak et al., 2011). The root cause for both of these examples is typically variation in the levels of certain trace metals such as zinc or copper. Given the non-CQA nature of C-terminal lysine (C-terminal lysine removal naturally occurs as well *in vivo*), or proline amidation the drift in basic species over the course of the batch is usually deemed acceptable (Brorson & Jia, 2014; Harris, 1995; H. Liu et al., 2016). Allowable ranges for non-CQA attributes can be set to a reasonably wider range because they do not have an impact on safety and efficacy and because the relatively small variation observed can still be considered normal process variation. Characterization that demonstrates elevated amounts of existing basic peaks and confirms that no new peaks appear over the batch time course further supports the acceptability of a small upward trend over time. Further process development or batch pooling is not required if such profiles are observed and documented with supportive rationale.

2.2.5.2 Deamidation variation example

The percentage of acidic charge isofoms is impacted by a variety of molecule attributes including deamidation. Assessing the CQA vs non-CQA nature of product quality attributes is key in determining the attribute ranges. For example, if the deamidation occurs on an amino acid in the FC gamma R3 binding domain of the antibody (such as ASN325), deamidation would be considered a CQA, and the consistency of this attribute over the continuous perfusion batch should be assessed more critically (in contrast to the previous scenario for C-terminal lysine). Two scenarios are presented in **Figure 2.3.** Variation of total product deamidation over the course of the culture. For the "Variable deamidation" profile, the % total deamidation drifts upwards over the course of the batch, and either further process development or the installation of a batch pool is recommended. However, the insertion of a batch pool to enable consistency of deamidation throughout the downstream process will create a host of additional activities such as establishing sampling, testing, action limits, and deviation responses for the pool as well as generating a detailed understanding of any continued deamidation that could occur during the hold in the batch pool tank. In contrast, the "Steady deamidation" profile illustrates an acceptable level of variation over the batch since a directional drift is not observed, and thus fully integrated continuous processing through downstream purification can be performed without a batch pool step.

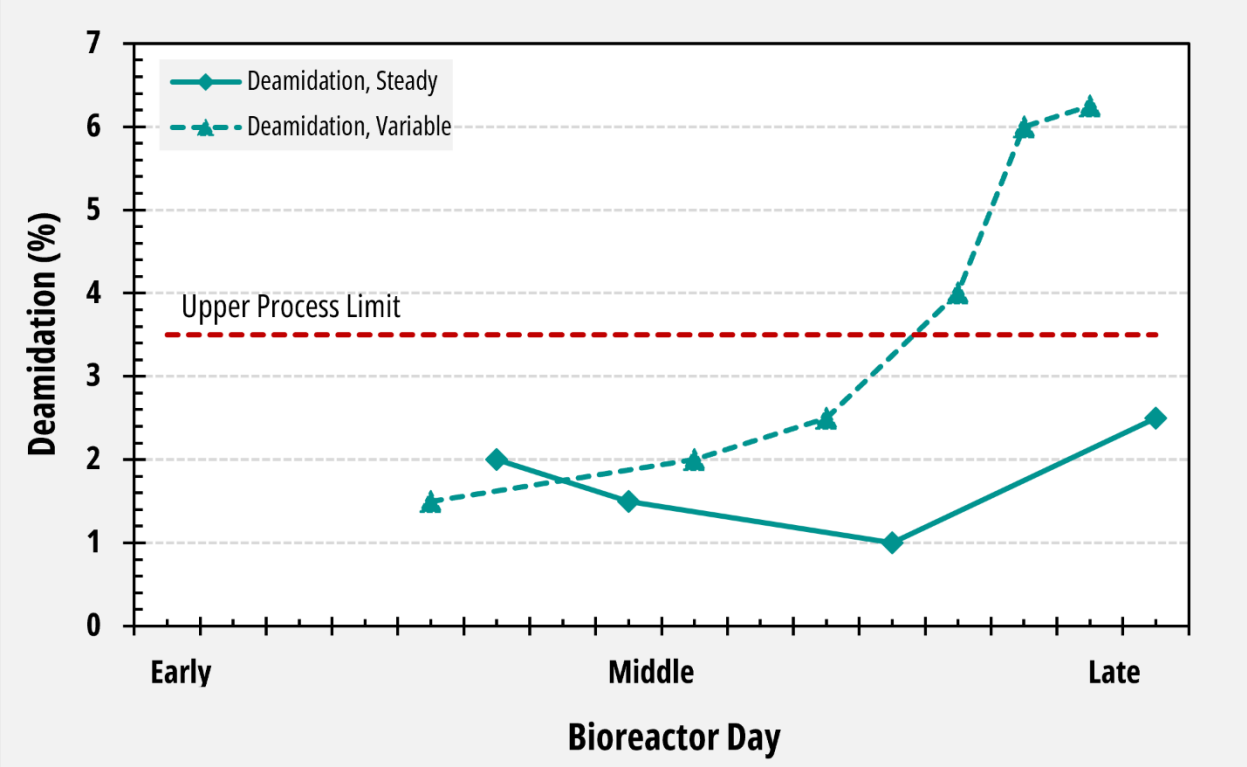


Figure 2.3. Variation of total product deamidation over the course of the culture.

2.2.5.3 Glycosylation variation example

Example data for glycosylation patterns is presented in Figure 2.4 (for G0F and G1F species) and Figure 2.5 (for G2F and High Mannose species) to illustrate two scenarios. In the first ("Steady"), glycosylation is deemed consistent enough to support connected integrated downstream processing over the course of the batch, and in the second ("Variable"), the glycosylation is too variable and would require a batch pool step as in the previous discussion on deamidation. (The variable example depicts increasing high mannose species over time. Since high mannose is considered potentially immunogenic and can also impact pharmacokinetics (PK), consistent levels should be maintained across drug substance batches. Thus, in the variable scenario below, either further upstream process development is required or a batch pool step needs to be inserted.

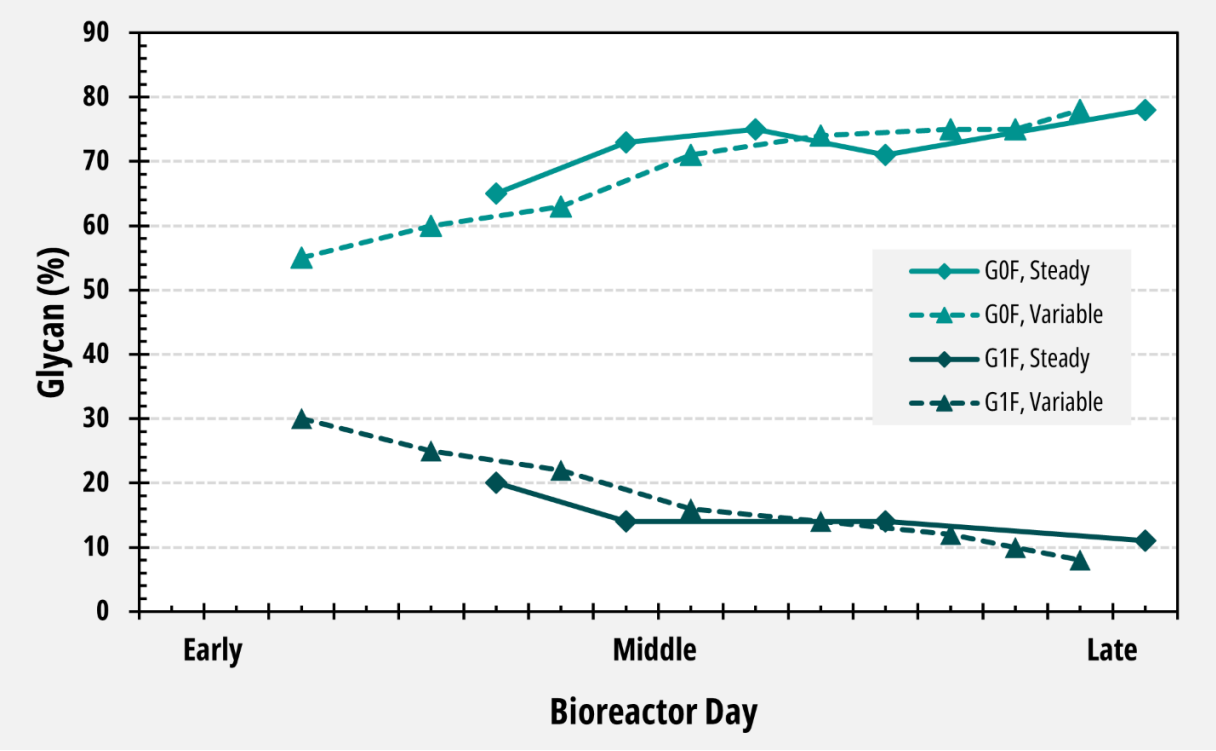


Figure 2.4. Variation in product glycosylation patterns for G0F and G1F in cultures with steady (solid lines) and variable (dashed lines) product quality. G0F and G1F are galactosylation variants.

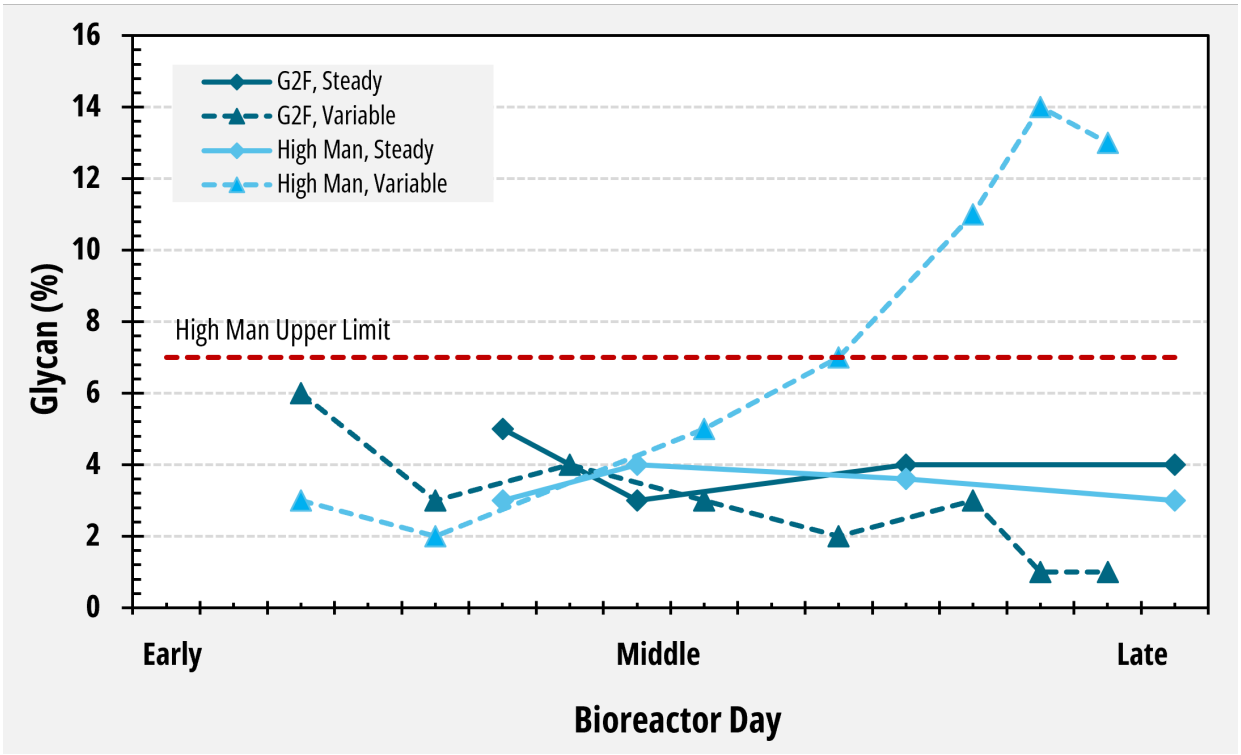


Figure 2.5. Variation in product glycosylation patterns for G2F and high mannose in cultures with steady (solid lines) and variable (dashed lines) product quality. G2F is a galactosylation variant and High Man is high mannose.

2.2.5.4 Impurity variation at upstream harvest example

Impurity clearance is a key function of the downstream process for batch or continuous process formats; however, the downstream process is designed to clear such impurities up to a specific amount. Thus, in order to implement fully integrated continuous processing, impurity levels at upstream harvest need to be maintained below the upper limit of downstream process clearance capability. Example data for both product-related impurities (e.g., HMWS or aggregates; **Figure 2.6**) and process impurities (e.g., host cell protein (HCP); **Figure 2.7**) illustrate both a dynamic perfusion and a steady-state perfusion upstream process. The upper limits for aggregates and host cell protein are included to illustrate the need to pool a batch or to actively adjust the downstream process to handle an excursion of impurities above this threshold.

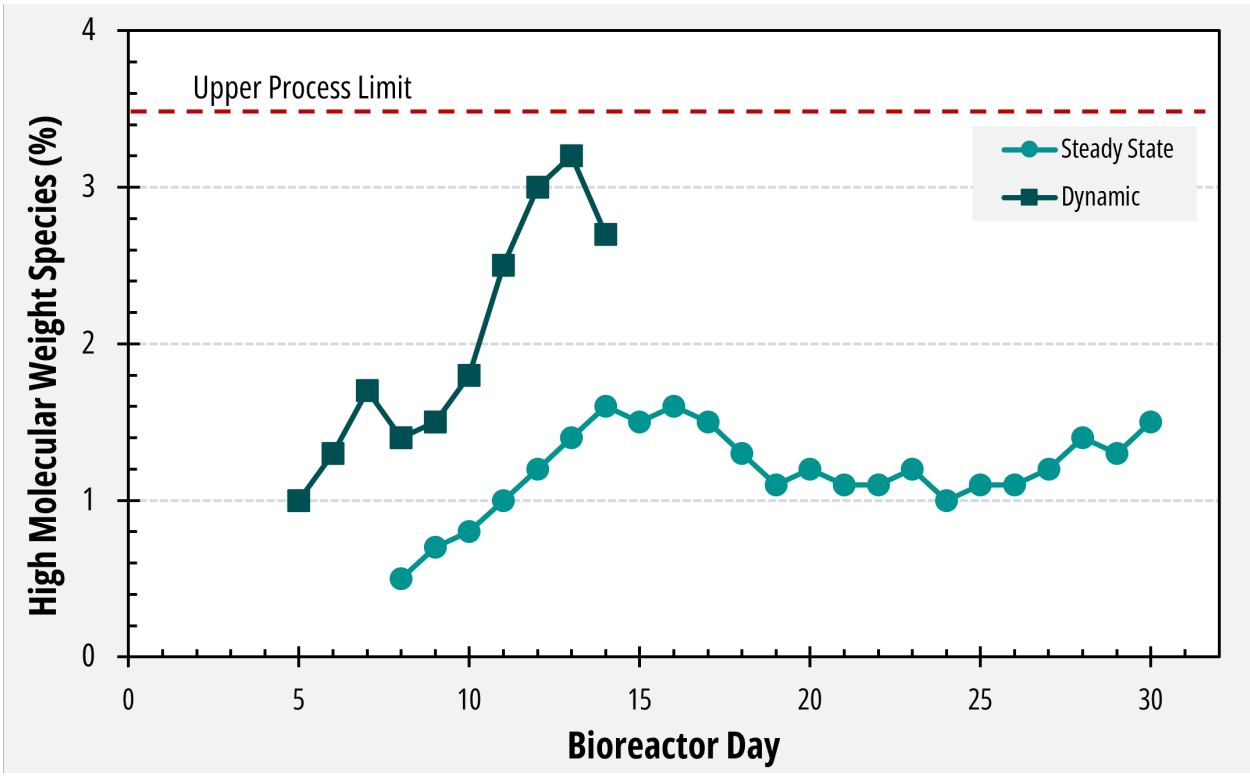


Figure 2.6. Variation in product high molecular weight species (HMWS) over the duration of the culture for steady state (circles) and dynamic (squares) perfusion processes**.**

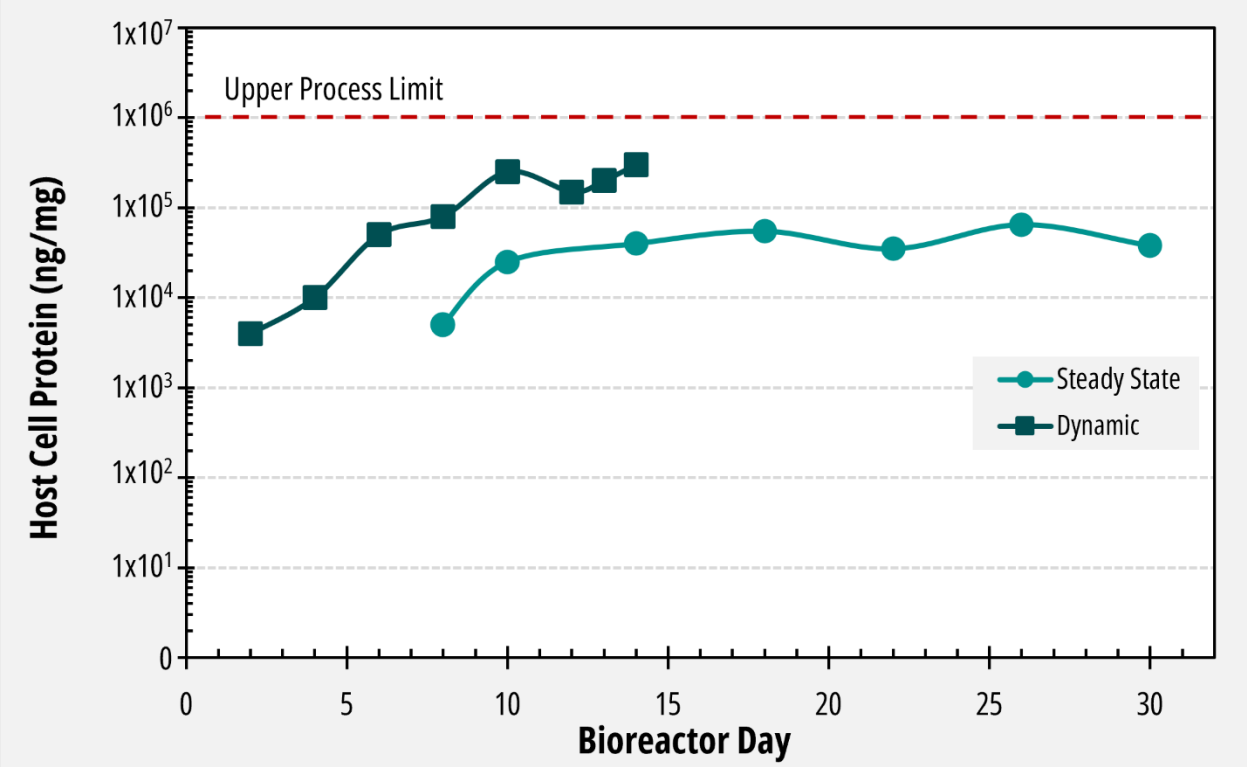


Figure 2.7. Variation in host cell protein over the duration of the culture for steady state (circles) and dynamic (squares) perfusion processes**.**

2.3 Downstream process design

2.3.1 Capture chromatography

Process development studies for the capture chromatography step, most often using Protein A-based resins, for an integrated process is similar to that performed for conventional, batch process development, with a few notable exceptions or adjustments. Accordingly, existing tools for process development, such as batch bench-top chromatography skids and high-throughput techniques such as RoboColumns (or equivalent), can be used to screen, assess, and select classical batch process parameters like elution pH, residence times, and wash composition. Some of the most notable exceptions that are specific to integrated and continuous processes are described below.

2.3.1.1 Design of column loading

To accommodate a continuous feed stream exiting the bioreactor, an integrated capture process typically uses multiple columns operating in parallel. A key process development activity is the selection of the number and size of columns to be used to capture the product, taking into consideration the binding capacity of the columns, the amount of time required for non-loading steps (wash, elute, strip, etc.), and the mass and volume flux emanating from the bioreactor. The specific selection of the number and geometry of columns has been described in the literature for a variety of multi-column chromatography configurations (Baur et al., 2016; Gillette et al., 2021; Godawat et al., 2015; Vogg et al., 2018).

A second area of focus specific to integrated capture is the development of the loading control strategy using an inline sensor and a methodology to either detect product breakthrough or estimate the mass loaded on a column as a function of time via advanced analytical tools such as Raman probes or by using a model-based soft sensor approach. It is critical to develop this loading control strategy as part of process development using representative harvest streams to ensure that the breakthrough profile due to the signal from the product, contaminating proteins, and small molecule impurities is reproducible from run to run and has the distinguishing features required for the specific control methodology. Similarly, the representative harvest material that is tested should cover the entire duration of the bioreactor process. This requirement is particularly important for dynamic perfusion, where the composition of the harvest stream varies widely over the course of the bioreactor process, but it may still be necessary for steady-state perfusion, because even subtle changes in harvest composition may still have an impact.

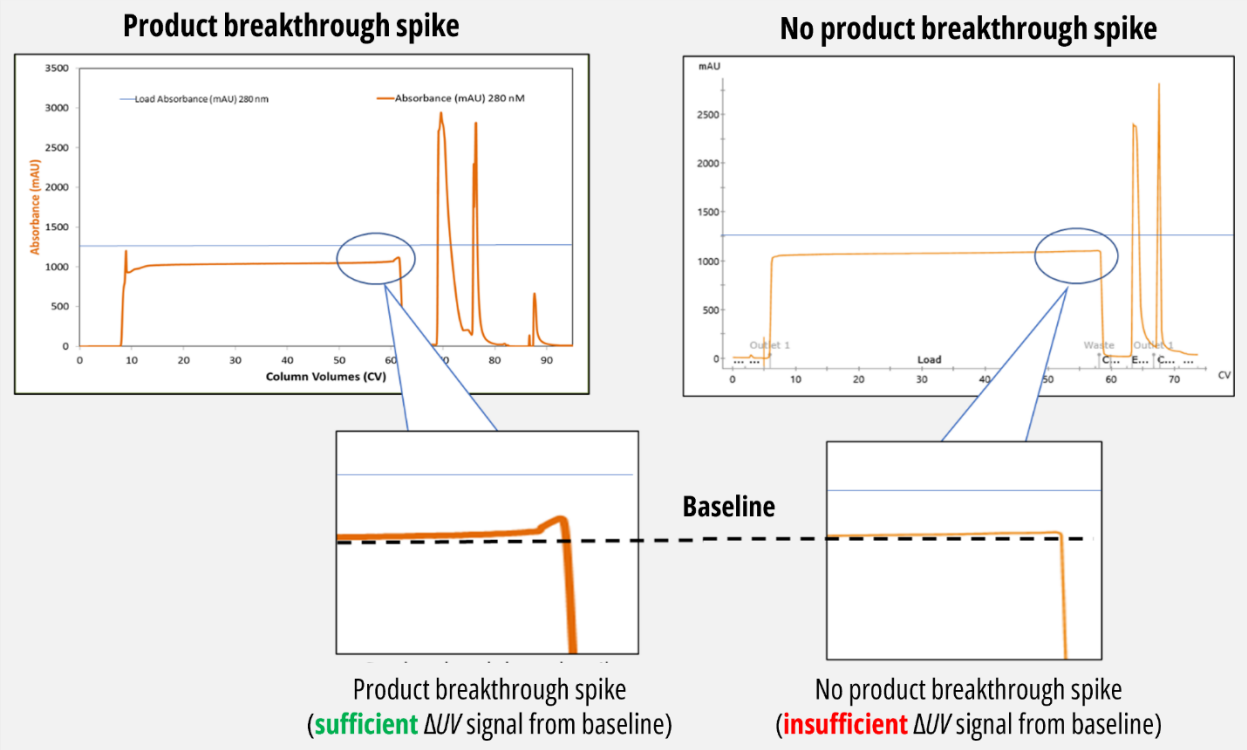


Figure 2.8. Examples of breakthrough curves leveraged for deltaUV-based load control. The orange line represents outlet UV, the blue line represents load UV, and the black dashed line indicates the load breakthrough baseline.

An example of the development of such a loading strategy using the dynamic loading strategy methodology (also known as "deltaUV") is provided below. This methodology was originally described by Cytiva for the Periodic Countercurrent Chromatography (PCC) system (Chmielowski et al., 2017). In this approach, online UV measurement is performed for both the load and flowthrough

streams to dynamically infer column loading by detection of product breakthrough. This approach is presented in visual form in **Figure 2.8**.

Several characteristic features of the loading and flowthrough UV signals are important to the development of a robust loading strategy. For the load UV, the UV signal should be relatively steady to support a robust deltaUV calculation. Specifically, the time constant for changes in feed stream composition from the bioreactor should be significantly greater than the duration of a single column loading step. Assuring that this requirement is met should be straightforward for steady-state perfusion, but it should be evaluated for dynamic perfusion to ensure that the load UV signal does not change too quickly at any point in the intended capture period.

For the breakthrough UV, two key features of the response signals are essential: (1) the difference between load and breakthrough UV should be sufficiently large to achieve sufficient signal-to-noise ratio, and (2) the baseline should be sufficiently flat to support robust calculation of the baseline breakthrough UV level. In **Figure 2.8**, the left panel and inset represent a breakthrough UV signal that is likely to meet these criteria and, therefore, enable robust loading control. Conversely, the right panel and inset represent a breakthrough UV signal that may not be sufficient. For both evaluations of the UV response, representative harvest material should be assessed across the entire bioreactor process duration, independent of bioreactor mode, to ensure that the loading strategy and associated process parameters are robust to any changes in shape of the relevant UV signals.

Additional methodologies for online load detection and control may also be considered. For example, load control can leverage the use of soft sensors, which can create empirical, statistical models for the load based on combined measurements of load, flowthrough, and eluate UV signals. Other PAT techniques, such as spectroscopic methods like Raman or Fourier transform infrared (FTIR) (Thakur et al., 2020), may also be employed. In all cases, these techniques should be implemented and developed through the course of process development to enable the determination of relevant process parameters to support the development of the control strategy.

2.3.1.2 Breakthrough or shared loading

In some configurations, multi-column capture may be designed such that the breakthrough of one column is loaded onto a second column to minimize product loss and maximize resin utilization. This capture strategy is typically termed breakthrough or shared loading. It results in columns that are "over-loaded" relative to more traditional approaches where a safety factor is applied (typically 10-20%) to avoid loading up to the dynamic binding capacity (DBC) of a column in bind and elute mode. For processes seeking to use such a strategy, the impact of loading columns above typical batch binding capacities must be considered during process development. For example, washes that may be designed to displace product-associated impurities may perform differently with respect to impurity clearance or process recovery in an "over-loaded" column relative to a standard batch comparator. The behavior of product and impurities on a multi-column capture step can likely be tested using typical batch approaches, such as RoboColumns or bench-scale batch chromatography.

A second consideration is the potential impact on product quality of shared or breakthrough loading because of differential exposure to or accumulation of product-degrading impurities. Specifically, it is possible that the composition of the mobile or stationary phase on the breakthrough ('second') column is not the same as that observed on the equilibrated, unloaded first column. Therefore, if

the intended configuration includes shared loading, it is beneficial to perform multi-column runs mimicking the final configuration periodically throughout process development and with a representative feed stream across the duration of the bioreactor process to confirm that there is no adverse impact on product quality (e.g., fragmentation, post-translational modifications (PTMs), etc.) due to shared / breakthrough loading. This may be most easily accomplished via periodic direct integration of the capture chromatography step with bioreactors during process development.

2.3.1.3 Column sizing

While it is generally true that column sizing is important for all chromatography applications, there are some unique considerations specific to capture process development for multi-column chromatography. As described for various multi-column configurations (Angelo et al., 2018; Gillette et al., 2021; Godawat et al., 2012; Godawat et al., 2015; Ötes et al., 2018), the total number of columns required in a capture system is dependent on the binding capacity and on the volume and mass flux received from the bioreactor. Typically, column bed heights are significantly shorter (~12 cm and less) for multi-column capture. Accordingly, it is important to approach development using columns, residence times, and linear velocities that map appropriately to the multi-column system available in the intended full-scale operation.

2.3.2 Virus inactivation

Process development approaches leveraged for batch chromatography may also be generally applied to virus inactivation in an integrated and continuous process for both low pH or solvent / detergent methods in either a two-tank or plug flow reactor configuration. Process development includes an assessment of the impact of pH (or solvent / detergent concentration) and temperature on product quality attributes as a function of exposure time. Batch approaches are applicable because engineering solutions have already been developed and implemented to ensure homogeneous exposure to the virus inactivating condition, for example by the design of a two-tank system to ensure complete emptying (Manser et al., 2019) or by validation of a tubular flow reactor through residence time distribution modeling and/or experimentation (Brown & Orozco, 2021; Brown et al., 2020; David et al., 2019; Gillespie et al., 2019; Martins et al., 2020; Orozco et al., 2017; Parker et al., 2018; Senčar et al., 2020)

2.3.2.1 Titration development

For integrated processing, particularly in the case of inline titration to acidic and then neutral pH, it is important to develop the titration as part of routine process development. Titration development can be performed by straightforward experiments that determine the volumetric amount of titrant required to achieve the target pH levels at the beginning and end of the incubation (note that no specific development is expected for viral inactivation by solvent / detergent because a strict volumetric addition is assumed). One key assumption for this case study is that the low pH will not be achieved by "titrating against the peak" eluting from the capture chromatography step. Instead, homogenization of one of more eluates will be performed in a surge vessel or homogenizing tubular flow reactor. Without such a simplifying approach, significant titration development activities are likely required. A typical titration development experiment is depicted in **Figure 2.9**. In this example, the top portion of **Figure 2.9** shows a scheme without a cycle surge vessel, and in which the protein concentration, pH, and conductivity exiting the Protein A step vary significantly as a function of time. This configuration creates difficulties in titrant scheme development and process control.

Conversely, homogenization using a cycle surge vessel of one or more Protein A eluates before passing the material on to the titration, as shown in the lower portion of Figure 2.9, minimizes the variability. In this case, only modest changes in solution properties during a periodic Protein A elution are observed because it is desirable to maintain a constant flow rate out of the cycle surge vessel.

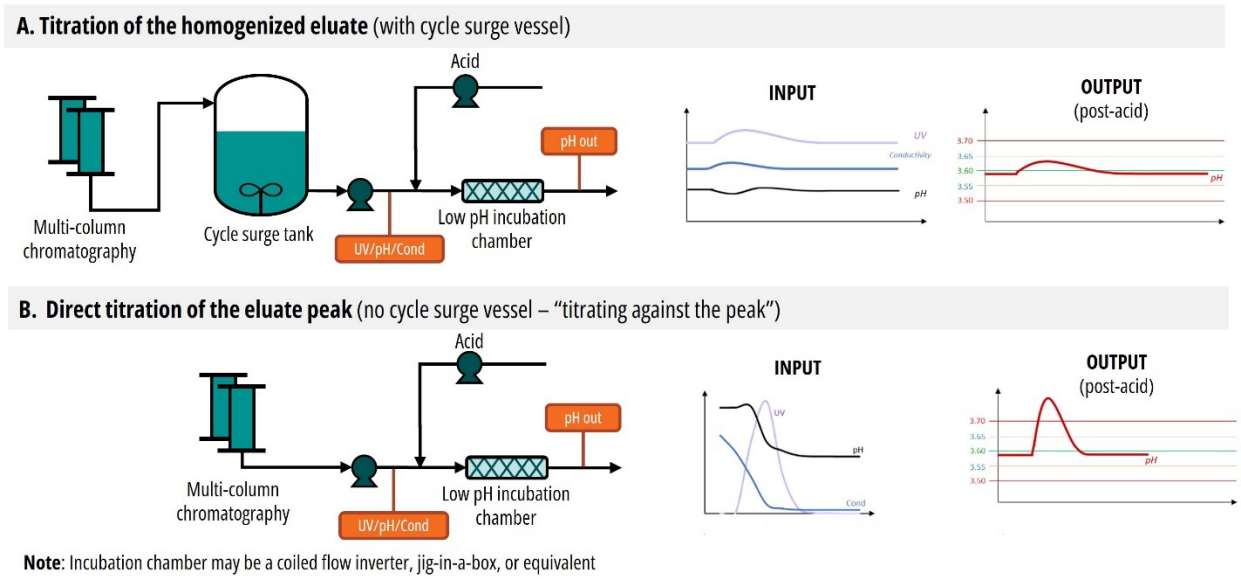


Figure 2.9. Comparison of simulated input variability and output control of pH for virus inactivation with and without cycle homogenization (titrating against the peak).

MCC Multi-column chromatography

In such an experiment, the concentration of target protein in the Protein A eluate is varied, either by simulation by dilution with elution buffer or, ideally, by creation of eluate pools from representative portions across the bioreactor campaign for both dynamic and steady-state perfusion modes. This may be more important for dynamic perfusion, where changes in composition may impact the titrant requirements to achieve target pH. It may also be valuable to determine titrant addition requirements for a range of pH conditions if a product is less stable at low pH and/or to support determination of the operational design space for the subsequent polishing step. Model-based approaches may also be useful in predicting titration profiles (Thakur et al., 2021).

2.3.3 Polishing chromatography

It is expected that approaches used for the development of batch processes will be applicable for polishing steps within integrated and continuous processes independent of operating mode (flowthrough or bind-and-elute). Typical setups such as RoboColumns, bench chromatography, and other related equipment and development techniques could be used to determine classical set-points for parameters like pH, conductivity, and loading parameters. As discussed in the Capture Chromatography Column Sizing section (Section 2.3.1.3), considerations related to column sizing, residence time, and linear velocities should be incorporated early in development. These considerations may be particularly important for polishing relative to capture steps because of the

significantly lower mass and volume fluxes applied to polishing steps in an integrated setting relative to batch operations. An additional limiting assumption that is critical to simplifying the development of polishing steps is the presence of a relatively homogeneous feed stream with minimal change in composition over the entirety of a column cycle. During process development, the feed to the polishing step should be sampled across the bioreactor duration and used to identify and assess the robustness of process parameters to ensure sufficient impurity clearance across the entire expected bioreactor design space. Different product- or process-related impurities may be more or less prevalent at different points across the campaign (especially for the case of dynamic perfusion), and this may require trade-offs favoring purity over yield.

2.3.4 Virus filtration

Virus filtration in a continuous process is most likely to be run under constant flow conditions, rather than using the constant-pressure control typically employed for batch processing. There is not much current evidence to suggest that membrane capacity will be significantly different (either higher or lower) between the two modes of operation (Bohonak et al., 2021). Therefore, membrane area sizing during initial process development can be most easily performed using a traditional pressure-driven determination of membrane capacity. However, process capability under constant (low) flux for a long duration should be confirmed at small scale prior to scaleup and prior to validation of viral clearance (Johnson et al., 2022).

As this step occurs substantially downstream and comes after two bind and elute (B/E) chromatography operations, much of the variability in product quality and mass flow over time due to differences in steady state versus dynamic perfusion is expected to be mitigated. Additionally, collection of an elution pool from B/E polishing chromatography column #2 into a cycle surge vessel prior to viral filtration limits the input range of concentration to this step. Sizing of this surge vessel either for a short residence time or a full elution cycle will dictate the concentration range over which the virus filter capacity will need to be evaluated, with the highest concentration likely to be worst-case for capacity. An increase in feed concentration will also result in a higher membrane pressure drop across the virus filter at the same flux. In addition to evaluating capacity, it would therefore be useful to consider the maximum required driving force, particularly if the surge vessel is sized for minimal residence time and/or eliminated to allow direct flow of the elution peak from the polishing chromatography to the virus filtration (David et al., 2019; Shirataki et al., 2021).

In many batch processes, a prefilter is employed to increase the capacity of the virus filter by reducing the level of multimers and larger aggregates in the protein stream. While the requirement for a prefilter may be reduced in a continuous process by more direct coupling of the virus filter to a cation exchange chromatography step, in which aggregates are removed, process development should still include an evaluation of the need for a prefilter and its selection along with the primary virus filter sizing. The potential for changes in the levels of multimers and aggregates over time should be a consideration during surge vessel sizing, and prefilter sizing and selection should accommodate worst-case process estimates.

One significant difference between process development of a virus filtration step for a continuous versus a batch process is the need to consider scaleup and implementation strategy in order to determine the operating flux. The standard assumption is that the virus filter will be changed out

periodically throughout the course of a batch. From the initial pressure-driven determination of membrane capacity (kg/m^2) and knowledge of production-scale mass flow (kg/day), the virus filter area will be selected based on how long it will be run, and this will have a direct impact on the process flux of the installed area. It may be useful to perform development work over a range of fluxes to provide some flexibility around scaling options. Some considerations for changeout frequency would be 1) to use the lowest reasonable process flux that results in good process performance and achieves viral clearance, 2) to use the longest duration that can be validated at spiking lab as well as the lowest flow rate that is practical on the spiking lab equipment, and 3) to understand or evaluate device holdup volume and impact on buffer fill/flush operations and residence time distribution at very low flux. Alternative configurations can also be considered, for example, installation of manifolded parallel devices that could be opened sequentially to reduce changeout while also increasing flux.

Both changeout strategy and surge vessel sizing will impact the likelihood and/or frequency of process pauses that could occur during virus filter loading. Sizing a virus filter to accommodate the mass in a single elution cycle from the previous chromatography step would reduce the need for planned pauses between elution cycles, but this approach may not be practical from a labor standpoint. Sizing the cycle surge vessel to allow collection of more than one elution cycle, and then running the virus filter slowly enough that the surge is not depleted until the filter capacity is reached would similarly minimize the need for planned pauses, but it would increase residence time in the operation. Discontinuities in both the upstream flow as well as the flow to the subsequent UF/DF operation need to be considered as potential sources of process pauses in the viral filtration. Unplanned pauses should be accounted for in the same way as for batch processing, although multiple pauses are far more likely to occur in a continuous process than during a single batch. Since process pauses during the loading of the virus filtration have the potential to reduce viral clearance levels, they should be included as part of validation testing.

Beyond load capacity, load duration, and the validated viral removal window, a virus filtration operation needs to demonstrate pre- and post-use filter integrity to ensure validated performance. It is important to devise methods to flush and test the filter devices prior to putting them online with the product stream and prior to removing them from use. In the case of a system with parallel lines switching back and forth into operation, these steps can likely be performed while a device is in the offline mode without impacting the loading duration of the online filter. However, if a single-train setup is envisioned, the surge vessel sizing and the overall process flux to balance mass flow will need to accommodate the time required to turn around filter setups (buffer flush to chase out product and post-use integrity of the online filter, installation of a new filter followed by flush and pre-use integrity testing). Methods for tracking and segregating product in the case of failure of a post-use filter integrity test should be built into the manufacturing control strategy. Finally, the cyclic product dilution resulting from the buffer used to push product out of a fully loaded filter and the buffer displaced out of a newly installed filter will need to be mitigated in the subsequent concentration (UF) step.

As briefly alluded to above, a critical consideration for virus filtration is the regulatory requirement that process-scale operating parameters remain within the window validated to effectively remove virus based on small-scale spiking studies. Therefore, process development needs to be linked with

scaleup strategy to clearly identify and evaluate process impact for parameter ranges (filter flux, protein concentration, aggregate level, load duration, prefilter selection, among potential others) that are likely to be encountered during manufacturing runs and define the extent of testing required as part of the reduced-scale validation studies. The ability to measure the log reduction value (LRV) at small scale over the desired window of conditions should be confirmed so as not to place further limitations on scaleup options.

2.3.5 Ultrafiltration and diafiltration (UF/DF)

Key objectives for the final concentration and buffer exchange (ultrafiltration and diafiltration, UF/DF) are to deliver product at the correct conditions (concentration of protein and buffer excipients) for formulation and to avoid impacting product quality in a negative way (e.g., no increase in aggregate level). For cycled batch UF/DF (Option 1 in

Table 1.3), two additional process options will be considered. The primary option, which is most straightforward and largely similar to a typical batch process, is a cycling system. The second option, which is more complex and less proven in industry to date, is a fully single-pass assembly that accomplishes concentration followed by buffer exchange via serial concentration/dilution using buffer added either co-currently or counter-currently (Coolbaugh et al., 2021). For periodic batch UF/DF (Option 2 in **Table 1.3**), a standard TFF system would be used.

2.3.5.1 Cycling tank UF/DF

A cycling system can be implemented in two ways: (1) a single tangential-flow filtration (TFF) membrane assembly is connected to two recycle vessels that cycle back and forth between filling and the UF/DF operation (**Figure 2.10**) or (2) two fully parallel systems (recycle vessel and membrane assembly) are cycled (**Figure 2.11**). The first system has the benefit of lower equipment requirements and higher membrane utilization. In both configurations, the dual recycle vessels eliminate the need for a separate surge tank between the viral filtration (VF) and UF/DF steps. However, for the single membrane assembly, the flexibility to accommodate potential process upsets or unplanned extended membrane cleaning between cycles is reduced if there is no additional tank to collect the VF output while the membrane is offline. A system design that allows for periodic cleaning, as well as synchronized sizing between the VF and UF/DF to align UF/DF cycle volumes with VF changeout, can significantly mitigate these risks.

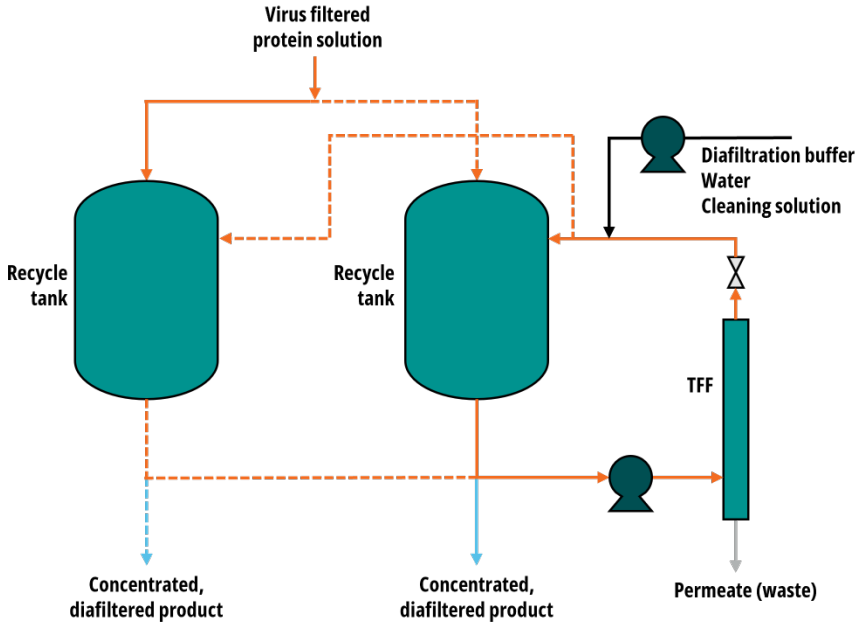


Figure 2.10. Continuous UF/DF using a cycling system: Configuration Option 1 using one TFF system connected to two recycle vessels. One vessel fills with virus-filtered product while the membrane assembly is on-line to the second recycle vessel to perform UF/DF, product recovery, and periodic cleaning. When the TFF assembly is finished processing the fluid in the second vessel, it switches online to the first vessel to perform UF/DF while the second vessel begins filling with virus-filtered product.

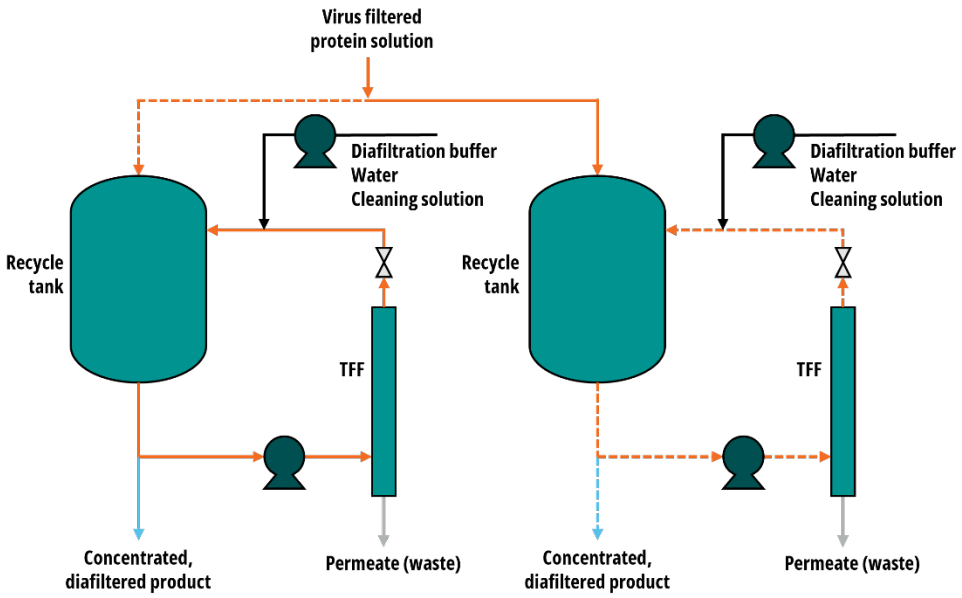


Figure 2.11. Continuous UF/DF using a cycling system: Configuration Option 2 using two parallel TFF systems. The recycle vessel on one system fills with virus-filtered product while the second system performs UF/DF, product recovery, and cleaning. The systems cycle back and forth between filling and processing.

For either configuration of a cycling UF/DF system, process development requirements will be largely similar to those of a batch process. Beyond selection of membrane material and molecular weight cutoff, setpoints should be established for crossflow, transmembrane pressure (TMP), concentration at which diafiltration is performed, and target number of diavolumes. These data provide an understanding of the interplay between membrane area, cycle time, and cycle load volume or load mass, which will be required to select a cycling strategy for scaleup. Early understanding of whether volumetric exclusion or Donnan effect will impact buffer exchange at the target protein concentration for DF and maintenance of final excipient concentration and solution pH during final concentration is critical to defining an appropriate process design but is no different for continuous versus batch processing.

As noted in the Virus Filtration section, **Section 2.3.4**, a buffer chase at the end of filter load will result in some dilution of the product that is delivered to the UF/DF operation. The impact of incoming product concentration variability on flux and process time will need to be characterized and incorporated into scaleup calculations. If the UF/DF cycles are expected to have a wide range of initial product concentration due to large chase volumes, small recycle tank sizes, or other implementation choices, the use of a fixed ratio of load mass per membrane area could be considered as a scaling basis. This would constrain the impact of concentration variability to the initial concentration step only, while diafiltration and any final concentration would be consistent with each cycle. Cycle time could then be designed to accommodate the lowest incoming concentration, as this would require the longest initial concentration time. However, if the range of incoming concentration will be small, cycle loading based on constant feed volume may provide a simpler control strategy. To simplify and streamline the overall process train, UF/DF cycle time selection should be made in conjunction with cycling, changeout, and surge vessel sizing decisions on the previous unit operations. Beyond aligning average volumetric and mass flow through the UF/DF step with the rest of the process train, the following three additional aspects of cycle size selection should be considered to reduce process risk: product recovery time, membrane cleaning time, and minimum working volume

In addition to the time required for concentration and diafiltration, product recovery time will need to be included in each cycle. Estimates for recovery time will depend on how many steps are involved (tank drain, buffer displacement from membranes and lines, etc.), and whether the operation is fully automated or a mix of auto and manual procedures. A good estimate may be difficult to obtain at the process development scale, and institutional or platform knowledge will be helpful. The product recovery strategy will also result in some amount of product dilution with buffer to achieve optimal yield. Any requirement for overconcentration during the final UF step should be evaluated during process development to ensure suitability of the process setpoints (crossflow, TMP, etc.), to verify that product quality is not impacted, and to enable accurate definition of process time for cycle sizing.

Membrane cleaning time will most likely be required at least periodically to maintain flux performance and to reduce risk of bioburden if the process assembly is not fully closed. The number of cycles that can be performed between cleaning should be established during process development, although this can be challenging due to the high product mass requirements for testing. Flexibility to accommodate frequent cleanings will mitigate risks during scaleup. The total

number of process cycles on a given set of membranes and the criteria for continued use can be established in the same way as for traditional batch processing.

Finally, minimum working volumes of TFF systems do not scale completely linearly with membrane area since piping/tubing and pump sizes are discrete, so if a high final product concentration needs to be achieved, resulting in a low final product volume, then reasonable estimates of working volume for several proposed cycle sizes should be evaluated. Overconcentration to allow for dilution during product recovery must be included in the final volume calculation.

2.3.5.2 Single-pass UF/DF

A fully single-pass UF/DF operation has the advantages of being truly continuous and requiring minimal hardware. However, robust scaleup and optimal process control is still being established as the technology has not been as widely utilized. While the process development requirements for single-pass UF/DF should not be significantly different between batch and continuous processing applications, some of the key considerations are discussed here. Membrane path length (also expressed as number of membrane device sections in series) and crossflow setpoint are the primary drivers of overall conversion factor (product concentration factor), so they should be the focus of process development. Retentate pressure setpoint is somewhat less critical than in recirculating TFF applications but, for final UF/DF operations, it needs to be set high enough to overcome protein osmotic pressure at the end of the channel in order to avoid significant Starling flow; this should be established during process development as well (Lutz et al., 2015). Additional path length or lower crossflow will result in a safety factor that provides some amount of overconcentration to ensure the final protein concentration target is robustly achieved upon scaleup. As in the recirculation tank configuration, the impact of incoming product concentration variability on conversion will need to be characterized and incorporated into the selection of membrane path length and crossflow setpoint.

Diafiltration in single-pass TFF mode can be accomplished via sequential product concentration and re-dilution with buffer over multiple sections of membrane (**Figure 2.12**). The choice exists to introduce buffer either co-currently or counter-currently to the product flow (Zydney, 2015). Alternative approaches to single-pass diafiltration have also been recently demonstrated within a single membrane cassette (Tan et al., 2021). While countercurrent operation will dramatically reduce buffer usage, it may require additional membrane sections to achieve buffer exchange targets, and it comes with an overall higher implementation challenge. Development of the diafiltration process will require characterization of a reasonable concentration factor target for each membrane section prior to re-dilution and confirmation that the target is robustly maintained over extended operation. In addition, as the product is exchanged from the starting excipient matrix into the diafiltration buffer, there is a high likelihood that the mass transfer coefficient will change, which will impact flux and concentration factor at each section; this will need to be characterized during process development and subsequently managed in the process control strategy.

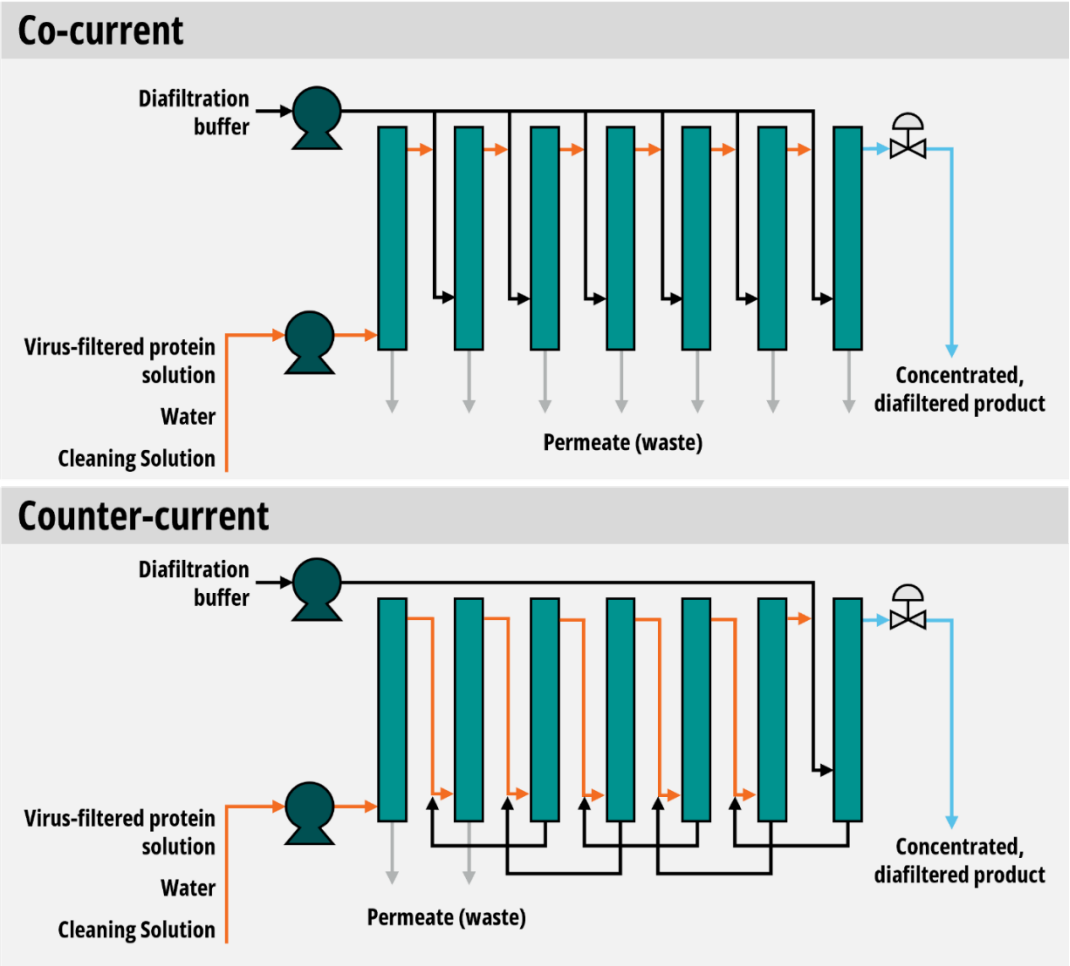


Figure 2.12 Continuous UF/DF using a fully single-pass system. In the co-current layout, membranes are configured in series to perform concentration (no buffer addition) and diafiltration (with buffer addition). Periodic cleaning can be performed if incoming feed is collected in a surge vessel prior to the TFF system. Buffer can be introduced either co-current (top) or counter-current (bottom) to the product flow. In counter-current operation, clean buffer is directed to the last diafiltration membrane section while permeate from each section is used as buffer for the preceding section. While this does increase operational complexity, it enables a significant decrease in buffer use as compared to the co-current configuration

As in standard TFF, high final product concentration targets increase the likelihood that volumetric exclusion and/or Donnan effect will impact buffer exchange efficiency and lead to an offset in expected final buffer excipient concentrations and pH. Since the membrane area requirement in single-pass diafiltration is reduced if high concentrations can be achieved in each sequential membrane section, an optimal design for area may result in more interference due to Donnan effect during DF. And the offset will increase during any final concentration that occurs after DF. Therefore, it will be important to evaluate small molecule clearance (from prior buffers in the process train), final excipient concentrations, and final pH early in the process development cycle of single-pass UF/DF to select a design that accomplishes the required exchange and meets the final buffer specifications. Mitigation may be required, either by diafiltration with an offset buffer or by supplementation of excipients after product recovery.

While the truly continuous nature of single-pass UF/DF means that the product stream from the upstream steps can flow directly into this step, a surge tank will be required both as a pressure break to allow for a feed pump to drive the TFF system as well as to enable temporary product collection during process upsets or during membrane cleaning. Maximum membrane loading prior to cleaning can be assessed during process development by characterizing process performance over time, specifically maintenance of flux, final product concentration, and buffer exchange targets. For process-scale implementation, control of bioburden should also be considered when determining membrane cleaning frequency, particularly if the system and membrane assembly are not fully closed.

2.4 Process integration development

For an integrated and continuous process, additional consideration is required to develop the approach for the integration of the various unit operations. Two key areas are unique and of particular importance for this purpose: surge tank design and process flow or residence time considerations. These are discussed in the following sections.

2.4.1 Surge tank design

The use and design of surge tanks (Thakur et al., 2021; Zijlstra et al., 2017) between the unit ops should be considered for their impact on process performance and product quality. This approach contrasts with that for a typical fed batch approach, where the facility fit considerations are typically part of a technology transfer activity and less of a feature that can be proactively considered during process development. The opportunity to consider integration design stems from the intensification afforded by continuous processing and the increased flexibility in surge vessel design enabled by the relatively smaller footprint and availability of a range of single-use vessels. Additionally, integration design may be more relevant for an integrated and continuous process than for a batch process. This difference is due to the need or desire to accommodate more heterogeneity (see **Section 2.2.5**), whether from variability in product titer exiting a dynamic perfusion reactor or from sequential processing of material with differing levels of process-related impurities (HCP, DNA, etc.) or product variants (glycosylation, deamidation, etc.).

A number of surge tank configurations as described in **Section 1.2.3** may be considered. In general, surge tank size should be minimized wherever possible to minimize dispersion of product across the integrated unit operations, and thereby maximize the controllability and traceability of product fluid elements moving through the system.

2.4.2 Process flow and residence time considerations

Upon selecting the integration architecture, that is the sequence and sizing of unit operations and surge vessels, for the integrated continuous processing options described in the previous sections, it is important to understand and characterize the mass flow characteristics of the integrated system at scale. This allows for the estimation of progression of material through the process. In **Figure 2.13**, the mock data depict both the velocity of total mass through each unit operation (measured as mass flux in grams / day) as well as the instantaneous protein concentration within each unit operation as a function of time. Understanding the overall flow characteristics of the integrated

process system and its variance can then be used to establish a rough relationship between sampling, testing, and how far forward in the process a decision or action could be taken. The rectangles indicate the progression of a segment of material exiting the bioreactor as harvest and progressing through the integrated process. The spacing of the rectangles along the X-axis is based on an understanding of the residence time within each section. While this simple depiction may prove useful for general characterization of the system, an understanding of tail distributions and their procession through the process may also be useful in some limited cases, such as for viral safety considerations.

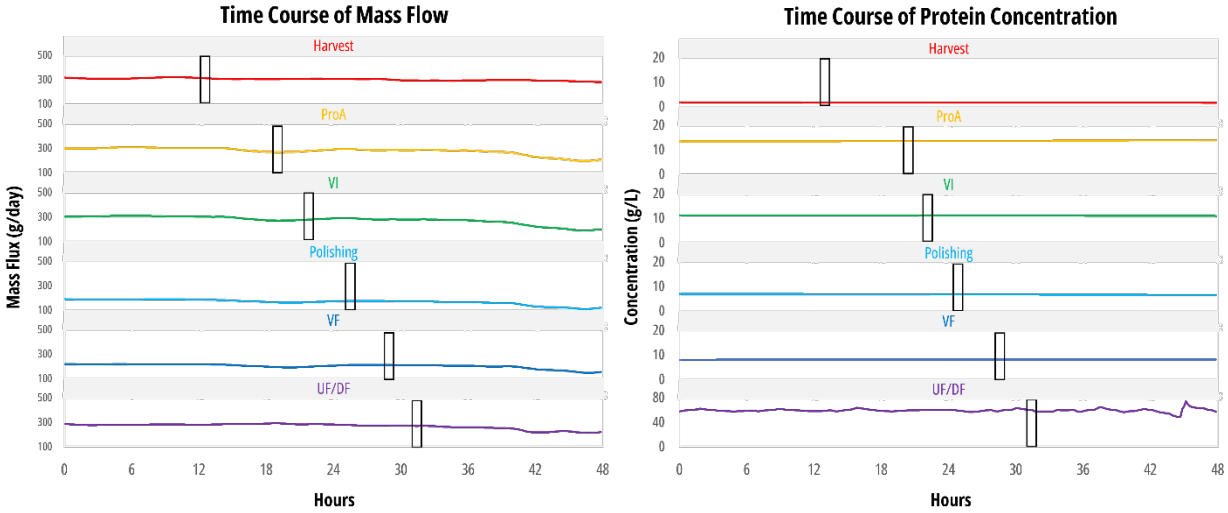


Figure 2.13. Mass flux and concentration as a function of time in an integrated continuous system. The black rectangles indicate the progression of a slice of material exiting the reactor and proceeding through UF/DF (Detail from **Figure 1.4**).

As part of understanding the various features of the residence time distribution of an integrated system, it may be helpful to perform residence time modeling through the course of routine process development (Sencar et al., 2020). Residence time modeling can be performed *in silico* through a variety of tools, including mechanistic modeling of unit operations and surge vessels, as well as Monte Carlo simulations of perturbations. Tracer experiments can also be performed, either at the bench scale, to support the development of mechanistic models, or at the pilot scale (which may also be the final commercial scale) to obtain directly relevant data to characterize the residence time distribution across the integrated process. These experiments can be performed to deliberately evaluate the introduction of different types of disturbances, such as step changes and pulse injections, in the integrated system, and will allow relevant IPCs to be established for the system. These IPCs would provide an alert if mass flow changes approach a point where the volume of any of the surge tanks could reach a high or low limit. An understanding of the variance across the system could also be useful in understanding overall process output as a function of percent capacity utilization, which Kingman had shown to be a very nonlinear relationship at high utilization rates for traditional manufacturing systems (Kingman, 1961). Note that applying the concepts of the

Kingman formula specifically to an ICB has the potential to generate a better understanding of the impact of variance at specific unit operations on the overall process.

2.4.3 Process time (disruption) characterization

During routine manufacturing, there may be need to pause or delay forward processing, for example, as a result of an observed deviation. A disruption in forward processing of material needs to be considered as part of characterization studies for both batch and continuous manufacturing. However, the approaches necessary to study product stability for a continuous process may differ significantly from those typically employed for a batch process.

During batch manufacturing, multiple product pool tanks or process intermediate collection vessels that can accommodate all of the product from a given step exist as part of normal operation. Each of these tanks generates a homogenous solution with predictable property attributes, such as protein concentration, solution matrix, product pH, and product conductivity. The biochemical stability studies executed to support maintaining the product within these process intermediate collection vessels typically need to use representative material and mimic the product contact material of the vessel as well as the surface-to-volume ratio if possible. Process intermediates are stored at either room temperature or chilled (if there is the capability to chill the material at the manufacturing site), and samples are pooled and tested for product quality over a defined time period. The worst-case room temperature for these studies is typically defined based on the operating limits of the manufacturing plant temperature controls and is typically chosen to be the high end of the temperature control range. Note that these studies may parallel certain accelerated stability studies performed as part of analytical development, and there is often much to be gained in terms of product knowledge by consolidating information about degradation pathways as early as possible. Additional studies may be required to ensure adequate microbial control at full scale during the allowable process intermediate hold times.

For a continuous process, the surge tanks and/or product pool tanks employed during continuous manufacturing may not be sufficiently large to collect all of the material that is actively being processed by each unit operation. As a result, a pause in the process would result in some material in the surge tanks and some material still at various phases of each unit operation. An assessment of the impact to product quality from pausing the process in this manner would require collecting stability data for the numerous potential conditions in which the material may be stored.

Using Protein A chromatography as an example, the stability of the product under the following conditions should be determined to define allowable pause durations:

- During loading, in which the load material is exposed to the resin
- During application of wash buffer while the product is bound to the column
- During application of the elution buffer

Each condition is likely to impact product quality differently, and one strategy for addressing this difference could be to limit pauses to accommodate the least stable of these conditions. Another approach could be to identify the most stable phase and to manage pauses so that material is processed to this phase in the process before pausing. For simplicity, we can assume that the Protein A step is being operated with three columns, each loaded in sequence and therefore at

different stages (load, wash, or elute) of the process at any given time. A pause could be managed so that material actively being eluted from one column is collected, the column that is in the wash phase would be paused immediately, and the column that is in the load phase would be forward processed to allow the pause to occur under what is typically the more stable conditions of the wash phase. As with the batch process, demonstration of microbial control over the pause would also be required. See **Section 8.5.1.4** for specific examples of applying characterization data to support deviation pause timing and duration.

2.5 Scale-down model qualification

2.5.1 Selection of appropriate scale-down model

When executing process characterization studies to assess the impacts of process parameters on product quality and process performance, the most important aspect of the model selected is that it should be predictive of the commercial scale facility. A good understanding of the scaling principles for each unit operation is required to determine which parameters must be consistent between the scale-down model (SDM) and the commercial scale. For example, scaling principles for chromatography are well understood, and keeping column bed height and linear velocity constant between the two scales is sufficient to construct a small-scale model that will be predictive of scales where column diameter and column volume are larger.

In traditional manufacturing processes, a batch approach is taken in the development and qualification of the SDM. While the process could be viewed as several linked unit operations, the operation of these unit operations may be quite different in practice from that of a batch manufacturing process, and the continuous manufacturing process therefore requires an SDM capable of elucidating the impact of these differences. For example, in a typical batch process, the load material for a chromatography step is a homogenous pool that is the product of the previous step. In contrast, for continuous processes, the load material may not be homogenous, depending upon the existence or the residence time of any surge tanks upstream of the column. This means that parameters such as protein concentration, pH, conductivity, or impurity levels could vary over the course of the load of a single cycle or across multiple cycles run over a larger timescale. This variation is typically not a concern for upstream unit operations, which are generally initiated from a single inoculation event for each bioreactor stage, including the production bioreactor, even though it is subsequently operated in continuous mode. However, in these cases, an understanding of the variability in the harvest output stream is necessary to assess how well the SDM represents the commercial-scale process.

The design of either a steady-state or dynamic production bioreactor model must account for the unique operating conditions needed to support a high cell density culture so that the biological environment and the resulting stresses on cellular performance can effectively represent the commercial-scale experience. In all cases, it will be necessary to operate the production bioreactor model consistently in perfusion mode for the full duration intended for the commercial-scale process. For the downstream process, four options exist for developing an SDM capable of overcoming the challenges that are specific to continuous processes:

1. Batch – The SDM for a continuous process can utilize a traditional batch model, exploring each unit operation independently. For each step, this would require an assessment of the variability in feed material that would be produced by the previous step followed by the performance of studies to screen these ranges. A linkage assessment can be conducted after generating characterization data to ensure that all ranges are supported by a data package that provides the connection between the SDM and full-scale operation. In some instances, worst-case linkage studies may be needed to verify that a step can accommodate the predicted output of the previous step.

2. Partially Connected – A partially connected SDM is one in which two or more steps are operated in sequence continuously. This model can generate data on the impact of a process parameter on the performance of subsequent steps. This model also has the advantage of allowing the investigation of interactions between parameters over multiple steps.

3. Pilot Continuous Model – Because the scale of continuous manufacturing is typically closer to what is commonly used for pilot scale operation as opposed to the large-scale fed-batch operations, it may be possible to conduct some or all of the characterization studies at a pilot scale representative of the commercial manufacturing scale. With this set-up, the actual continuous process can be studied, allowing the assessment of parameter impacts over the rest of the process. This model would require more material, but it would ensure that every step is challenged with the variable material generated from the prior step, as opposed to the more complicated screening required with the batch model. This pilot-scale model could be useful in performing linkage studies after characterization studies have been executed with the batch model.

4. Mechanistic Model – A useful option for SDM definition is to develop mechanistic models for some or all of the unit operations. For example, a model describing the performance of a chromatography step can be developed through modeling transport around and within the resin beads as well as protein ligand interactions. This model may require additional studies to assess parameters of the resin and to verify the model predictions. This method requires a good understanding of the physiochemical interactions occurring within the column. For bioreactor models, an understanding of the impact of various stresses (e.g., mechanical and interfacial shear and variations in nutrients or dissolved CO_2) is important to developing a useful SDM (Ahuja et al., 2015; Sieblist et al., 2016). Also, looking at the entire process as an integrated system, some form of residence time modeling or testing, which can determine the expected distribution of material affected by a process condition or deviation, is important to consider. Such studies can be executed in commercial-scale equipment to directly inform control strategies in response to operational deviations. Note that model-based approaches are useful not only for establishing SDM details but also as a part of site-to-site transfers, as well as for troubleshooting unexpected process performance.

As digital twins are generated based on hybrid models built on mechanistic and empirical aspects, the execution of process characterization studies can become more efficient (Roush et al., 2020). Establishing a verified digital twin model and confirming with process data is the first step. Once the verified digital twin exists, a subset of wet lab experiments (versus traditional PC studies) can be coupled with the digital twin model to provide a fully characterized process. A digital twin for the bioreactor is particularly useful in developing a better understanding of how the operational design

space changes over the duration of production with respect to particular process sensitivities such as shear, low or high pCO_2, etc. (Appl et al., 2020; Li et al., 2019).

In addition to the options presented above, an opportunity exits with the development of PAT to incorporate this technology within a small-scale model to generate a larger, richer data set. For example, measuring aggregate levels eluted from a column as a function of time while loading process parameters are varied would be one way to assess any interaction between parameters of these linked steps and determine the magnitude of their impact on this CQA. This understanding could allow control of this CQA through a modified collection strategy based on loading parameters or a modified elution strategy to clear more of this impurity. Also, monitoring inline product concentration in the production bioreactor harvest stream could allow for real time adjustment of the capture column operations. Further, implementing PAT at both commercial scale and small scale could help to overcome some of the complications associated with the verification of the SDM, which are discussed below.

2.5.2 Verification/qualification of the scale-down model

An important component of a robust process characterization package that supports the process parameter ranges and classifications is the verification of the SDM to demonstrate its ability to predict the performance of the commercial-scale process. For batch manufacturing processes, this is typical accomplished either by using independent process runs at the two scales, or through execution of "satellite runs", which are scaled-down runs that use the same starting material as corresponding commercial-scale lots. In both instances, the product quality and process performance outputs of a step are compared across scales.

2.5.2.1 Bioreactor

For upstream unit operations, the continuous process typically starts with the initiation of the production bioreactor using a typical bolus inoculation from a previous batch or intensified N-1 stage. In this respect, the starting material for the production bioreactor can typically be well-controlled in terms of its starting cell density and media composition. However, the outlet harvest stream may vary over time in terms of product concentration and/or product quality attributes related to the decline of cellular and filtration performance within the bioreactor. A list of typical process performance and quality attributes useful for a comparison across scales is provided in Table 2.2.

Table 2.2. Typical upstream SDM qualification process performance and quality attributes.

Upstream Performance or Quality Attribute	Frequency of Data Points
Viable cell density	Daily
Viability	Daily
Growth rate	Daily
pCO2	Daily
Metabolic components (e.g., glucose, lactate, ammonia, amino acids, etc.)	Daily
Permeate product concentration	Based on timeframe for partial batch harvest loading
Permeate product quality attributes	Based on timeframe for partial batch harvest loading

As a result, an understanding and comparison of the entire profile of product concentration and quality attributes over the entire harvest timescale of the process is necessary to ensure alignment across scales. Depending on the control scheme of the continuous process, the data may be obtained from various methods, as described below.

2.5.2.2 Partial pooling for loading of capture chromatography

If harvest permeate is collected in a surge tank before partial batch loading onto one or more capture columns (numbered as 1 through n), then an understanding of the product concentration and quality attribute profile variation from partial batch to full batch is necessary. Further, the profiles in Partial Batch #1 through Partial Batch #n for a continuous scale down model batch should be shown to be representative of the partial batches obtained at the commercial scale. The level of granularity to be studied should be determined according to process development experience and likely depends on the variability observed or anticipated. To conduct such a comparison, modeling and definition of the expected time periods corresponding to each partial batch should be established such that the collection, sampling, and testing of the pool for each time period is needed to determine the profile. The product concentration and quality attribute profile from partial batches can then be compared as output parameters from each scale.

2.5.2.3 Continuous inline loading of capture chromatography

If harvest permeate is continuously loaded from a surge tank onto one or more capture columns, then the load to each capture column may be changing over time. This change is based on the dynamic profile in the surge tank as fresh harvest permeate is captured from the production bioreactor while the well-mixed contents are continuously loaded onto one or more columns. An understanding of the time-based product concentration and quality attribute profile over the course of loading onto each capture column, which may also vary from column to column over the course of the extended continuous production batch, will be necessary for comparison between scales. To conduct such a comparison, a clear understanding of the residence time distribution modeling of the surge tank may be applied along with slip stream sample analysis of the surge tank outlet stream at various time points for each column load. The dynamic product concentration and quality attribute profile for each column loaded can then be compared as output parameters from each scale. The SDM also represents a good opportunity to evaluate any models for predicting the step yield or purity at this stage which could be useful in manufacturing (Walch et al., 2019).

2.5.2.4 Strategy for viral clearance validation and small-scale model development

As for batch processes, viral inactivation in continuous processes must be validated with a representative SDM. The designed small-scale model will need to assure that the critical process parameters (e.g., temperature, pH, and incubation time) are represented. For chromatographic or filtration unit operations, the SDM selected for validation of the removal of other impurities should suffice for viral clearance. However, a continuous viral inactivation step represents certain challenges in terms of selecting an appropriate SDM. Based on scientific understanding, it is justifiable to perform the validation study using standard bench-top batch methodologies instead of performing the study in a continuous mode using a scale-down incubation chamber. The effects of temperature, pH, and incubation time can be studied directly. Coupled with the residence time model described in **Section 5.3.2.3**, which can be used to determine the earliest and latest particle to

exit the incubation chamber, suitable control limits can be identified to ensure robust, continuous viral inactivation at manufacturing scale.

Alternatively, if the process conditions are within the established design space for a modular claim (a viral clearance claim leveraging prior knowledge, i.e., without product-specific data), no actual study may be needed. For example, ASTM E2888-12 (2019) outlines the parameters that ensure five log-reduction value (LRV) of murine retrovirus, mediated by low pH. The process parameters outlined in the document include hold temperature (≥ 15 °C), hold time (≥ 30 min), hold pH (≤ 3.6), buffer matrix (citrate, acetate, and glycine), sodium chloride (NaCl) concentration (≤ 500 mM), and protein concentration (≤ 25 g/L). The modular claim can be leveraged for continuous manufacturing if the engineering control of process conditions can be demonstrated. In either option, with or without an actual viral validation study, the continuous incubation chamber used for full-scale cGMP manufacturing needs to demonstrate sufficient engineering controls of pH, temperature, and incubation duration through residence time distribution. More detailed descriptions of viral clearance or inactivation validation studies at reduced scale are presented in **Section 3.7**.

2.5.2.5 General considerations

Data analysis comparing continuous production bioreactor stages across scales will show dynamic, time-based components regardless of the exact operational mode for the harvest and capture column loading. As previously discussed in the A-Mab White Paper (CMC Biotech Working Group, 2009), the dynamic nature of upstream fed-batch processes can be evaluated and compared between scales by applying multivariate analysis (MVA). Due to the further time-based complication of production bioreactor performance indicators and profiles in continuous operations, the application of MVA, and in particular principal component analysis, can enable comparison of not only discrete time point parameters, but also the dynamic profile of culture growth, viability, and metabolic states (Banton et al., 2020; Tsang et al., 2014).

Due to the high productivity of continuous processing, the number of commercial-scale batches required for clinical supply and process and performance qualification (PPQ) prior to the submission of license applications may be limited. To address this limitation, the large-scale data set may be extended to comprise both cGMP batches and representative pilot batches to reach the typical number of batches required for MVA. Therefore, the generation of a continuous pilot-scale model that can be shown to be representative of the commercial-scale model is recommended to effectively qualify the bench-scale model.

With an understanding of the entire profile of product concentration and quality attributes over the entire harvest timescale of the process, material can be sampled at defined points to assess the impact to downstream performance over the range of the varying attributes. Sufficient material can be sampled from the pilot, clinical, or commercial manufacturing site that the predictability of the upstream SDM can be confirmed, and the remaining material can be forward processed to qualify the capture step. Note that the intensification of the bioreactor makes it more likely that near-final- or final-scale bioreactor equipment will be available earlier in development and in non-commercial cGMP or non-cGMP manufacturing settings. This would require taking samples from the product of the capture step that corresponds to the same or the worst-case loading profile used in the SDM studies. The use of PAT at both commercial and small scale could minimize some of these

complicating factors by allowing the monitoring of product quality and process performance in real time, which would not require sampling material at precise time points to compare across scales. An alternative to sampling material from the commercial scale to use for qualification studies at small scale would be to leverage a pilot-scale facility that is close to or equivalent in scale to the continuous manufacturing facility. Under this scenario, critical process parameters at the pilot-scale facility can be varied over their acceptable ranges to show process capability and to confirm the predictive multivariate models developed using the SDM.

Chapter 3

Integrated Process Design: Process Characterization

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

3 Integrated Process Design: Process Characterization

3.1 Overview of process characterization

Process characterization activities are key to evaluating, improving, and demonstrating that the process design is robust and that it will meet quality and performance requirements when challenged with measurement variance or variability in process controls and plant operations (process parameters), materials (material attributes). These challenges are designed to represent the normal and expected sources of long-term variability that the process will encounter. Process characterization studies provide the foundation for establishing requirements for control of process parameters (univariate proven acceptable ranges (PARs) or a multivariate Design Space) and specifications for materials, which are both essential elements of an integrated control strategy as detailed in Section 1.3.

The first stage of process validation results in the definition of a process, including its control strategy. It incorporates product and process design activities conducted during development to gain a deep understanding of the product and manufacturing process. Product characterization includes structure/function assessment, sometimes called structure-activity relationship (SAR) assessment, hotspot analysis, formulation robustness assessment, and various studies demonstrating product stability. This characterization data is used together with the results of non-clinical and clinical studies to establish the product quality requirements that must be met by the process. The process must also satisfy practical and economic requirements: it must yield sufficient product to meet the needs of patients and do so at a reasonable cost.

3.2 Step descriptions

Two main options for the process were laid out in Table 1.3 and Figure 1.5. In this section, these options are used to aid in the discussion of key aspects of process characterization (PC), such as the pre-PC risk assessment (identified as Risk Assessment #1 in Figure 1.7), characterization studies, and subsequent post-PC parameter classification. Option 1 connects the process in a fully integrated continuous manner from the production bioreactor through the virus filtration step, at which point the process moves into cycled batch operation for the final ultrafiltration/diafiltration (UF/DF) step. A fully integrated continuous scenario such as Option 1 adds complexity because of prior step interactions, and those are considered in the following pre-PC risk assessment. Option 2 connects the process in a fully integrated continuous manner from the production bioreactor step through the viral inactivation step, after which a batch pool tank is used to consolidate all material constituting a batch, and then the remainder of the process is run as a periodic batch process. Both options can be run via an upstream steady-state perfusion format or a dynamic perfusion format, but dynamic perfusion could create more complexity at the Protein A capture step because of varying titer and impurities in the harvest stream over time. For dynamic perfusion, a batch pool step could be added after the virus inactivation (VI) step if needed to mitigate variability in product quality. Including a batch pool tank in the process will drive additional work in PC. Note that a pool step is included for the final drug substance step in both options. Table 3.1 shows a summary of these considerations, which have been added to provide a more specific version of Table 1.3 for this section.

Table 3.1. Process characterization considerations related to choice of process options.

Process Stage	Option 1 ICB: Stages 1–7 Cycled Batch: Stage 8	Option 2 ICB: Stages 1–4 Periodic Batch: Stages 5–8	Considerations Impacting Process Characterization
Stage 1 Preculture	N-1 batch or perfused N-1		
Stage 2a Production Bioreactor	Steady-state perfusion or dynamic perfusion		Degree of control over variance in deamidation, high mannose species and HMW species in perfusion bioreactor drive decision around need for a Batch pool tank post-VI (Stage 4)
Stage 2b Cell Removal	TFF or ATF		Potential for product retention and fouling
Unit Op Connection	Surge tank		
Stage 3 Capture Chromatography	Continuous multi-column chromatography (MCC): one column always being fed		Degree of variance in load material in terms of titer and impurities will drive effort required to define PAR/NOR limits as well as low pH titration design
Unit Op Connection	Cycle surge tank		
Stage 4 Virus Inactivation	In-line viral inactivation in a continuous plug flow reactor		
Unit Op Connection	Surge tank	Batch pool tank	Including a batch pool tank in Option 2 will drive additional work for intermediate hold stability studies in terms of product quality (small scale) and microbial hold studies (at scale)
Stage 5 F/T Polishing Chromatography	Dual-column alternating loading of columns. Note that this is essentially the same as continuous MCC: one column always being fed since load duration >> all other step durations		
Unit Op Connection	Cycle Surge tank		
Stage 6 B/E Polishing Chromatography	Continuous MCC: one column always being fed	Dual-column alternating loading of columns	
Unit Op Connection	Surge tank		
Stage 7 Virus Filtration	Alternating batch set-up, pre- and post-integrity test		
Unit Op Connection	Surge tank in UF/DF	Surge tank	
Stage 8 Concentration & Formulation	Two-tank batch UF/DF: alternating use of tanks as surge & TFF recycle		Continuous operation, varying protein concentration Decisions around need for a surge tank vs use of two tanks integrated into the UF/DF skid will depend on unique volumes involved in a particular process design
Unit Op Connection	Batch pool tank or multiple bags		

ATF Alternating flow filtration
B/E Bind and elute
DF Diafiltration
DSP Downstream process
F/T Flowthrough
HCP Host cell protein
HMW High molecular weight
ICB Integrated continuous bioprocess

MCC	Multi-column chromatography
NOR	Normal operating range
PAR	Proven acceptable range
PQ	Performance qualification
TFF	Tangential flow filtration
UF/DF	Ultrafiltration/Diafiltration
VI	Virus inactivation

3.3 Pre-PC risk assessment

3.3.1 Description of risk assessment tool

The Pre-PC Risk Assessment is a science- and risk-based approach to identify variables and process/unit operations that may have an impact on product quality. The parameters are ranked into different risk categories that will advise which variables will require further studies to understand the process and define a space design that maintains product quality.

There are different risk assessment strategies advised by the Risk Management guideline ICH Q9 (2005), including Design Failure Mode and Effects Analysis (FMEA) and Ishikawa diagrams (Hakemeyer et al., 2016). As noted in **Section 1.2**, several publications have provided details on the particular approach used by a given organization (Alt et al., 2016; Flynn & Nyberg, 2014; Kelley, 2016; Kelley et al., 2016; Kepert et al., 2016; Xu et al., 2022). It would be difficult at this point to select one approach over the other; therefore the risk assessment approach for N-mAb is in alignment with the risk assessment tool used for A-Mab (CMC Biotech Working Group, 2009). Two ranking scores are assigned to every parameter at each process stage. The first ranking score is assigned to evaluate the main effect of the process parameter on the CQAs and process performance attributes (PA). The second ranking score is assigned based on the potential for interactions with other parameters or with outputs from preceding steps. The second ranking score is a new concept introduced here for integrated processes because of the potential for interactions, not only between process parameters from an individual step, but also with outputs from the preceding step. These outputs may vary due to normal heterogeneity exiting a step (i.e., product quality changes over time in the bioreactor) or due to the surge tank architecture between the unit operations (i.e., direct integration vs. a cycle homogenization vessel). The Severity score is calculated by multiplying the two ranking scores for each parameter. The score matrix is then used to determine if the parameter should be considered for additional studies required to define the design space. The risk assessment tool should be generalizable across most, if not all, integrated framework architecture, including Options 1 and 2. For Option 2, an elevated interaction score is less likely because of the batch pool tank inherent to the Option 2 framework.

Note that our approach for scoring in the risk assessment was the same as that used for A-Mab, and to clarify the scores and their meaning, the following tables have been included for scoring impact assessments (**Table 3.2**), severity calculation matrix (**Table 3.3**), and severity classifications (**Table 3.3**) as presented in the A-Mab document.

Table 3.2. **Impact assessment of attributes; Main effect ranking.**

Impact Description	Impact Definition*	Main Effect Ranking based on Impact on Attributes	
		Critical Quality Attribute	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

Adapted from A-Mab (CMC Biotech Working Group, 2009)

Table 3.3. **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

Adapted from A-Mab (CMC Biotech Working Group, 2009)

Table 3.4. **Severity classification.**

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

Adapted from A-Mab (CMC Biotech Working Group, 2009)

The pre-process characterization risk assessment for the cell culture step is shown in Table 3.5. The pre-process characterization risk assessment for the purification steps is shown in Table 3.6. For the downstream steps, the potential impact of variability in outputs from preceding steps is a key theme throughout the risk assessment. Importantly, many aspects of the risk assessment, including the parameters included, widths of the parameter ranges considered, and the outcome of the impact scoring, are highly dependent on the framework of the integrated process, especially the way in which the steps are integrated by different types of surge vessels (Figure 1.6).

Table 3.5. Pre-PC risk assessment scoring rubric – cell culture. The Severity (Max) color corresponds to the values in Table 3.3.

Phase	Parameter	Assessment Parameter Range	Main Effect (CQA)	Main Effect (PA)	Highest Main Effect Score	Interaction (CQA)	Interaction (PA)	Preceding Step Interaction	Highest Interaction Score	Severity (Max)
Dynamic or Steady-State Perfusion	Growth Phase pH	+/- 0.1 outside OR	8	8	8	4	4	4	4	32
	Growth Phase Temperature (°C)	+/- 1.5 °C	8	8	8	4	4	2	4	32
	Growth Phase Seed Density (x10^6 vc/mL)	+/- 30%	2	4	4	3	4	4	4	16
	Growth Phase Perfusion Rate (CSPR, nL/cell/day)	+/- 20%	1	4	4	1	4	1	4	16
	Production Phase pH	+/- 0.1 outside OR	8	8	8	4	4	4	4	32
	Production Phase Temperature (°C)	+/- 1.5 °C	8	8	8	4	4	2	4	32
	Production Phase Perfusion Rate (VVD)	+/- 20%	8	8	8	4	4	1	4	32
	Production Phase Shift Timing (day) [A]	+/- 1 day	4	4	4	4	4	4	4	16
	Dissolved Oxygen (%sat)	30-100%	2	4	4	2	2	1	2	8
	Retentate Rate (ATF or TFF; L/min)	+/- 20%	1	4	4	2	2	1	2	8
	Antifoam Addition Amount	+/- 2x of daily target	2	2	2	2	2	1	2	4
	Perfusion Growth Media Composition	+/- 10% of target	1	2	2	2	2	1	2	4
	Perfusion Media Composition after Shift	+/- 10% of target	2	2	2	2	2	1	2	4
	Additional Feed Amount	+/- 10% of target	1	2	2	2	2	1	2	4
Steady-State Perfusion Only	Production Phase VCD/Biocap Target (x10^6 vc/mL)	+/- 30%	2	4	4	4	1	4	4	16
	Secondary Production Phase VCD/Biocap Target (x10^6 vc/mL)	+/- 30%	2	4	4	4	1	4	4	16
	Timing of Secondary Production Phase VCD/Biocap Target (day)	+/- 1 day	4	4	4	4	4	4	4	16

ATF	Alternating flow filtration	OR	Operating range	vc	Viable cells
CQA	Critical quality attribute	PA	Process performance attribute	VCD	Viable cell density
CSPR	Cell-specific perfusion rate	TFF	Tangential flow filtration	VVD	Vessel volumes/day

Table 3.6. Pre-PC risk assessment scoring rubric – purification. Note: The Severity (Max) color corresponds to the values in Table 3.3.

Phase	Parameter		Assessment Parameter Range	Main Effect (CQA)	Main Effect (PA)	Highest Main Effect Score	Interaction (CQA)	Interaction (PA)	Preceding Step Interaction	Highest Interaction Score	Severity (Max)
Capture Chromatography (ProA)	Bed Height		8-12 cm	1	1	1	1	1	2	2	2
	Temperature		15-25 °C	4	4	4	1	1	2	2	8
	Loading/Wash Residence Time		4-8 min	1	4	4	1	4	4	4	16
	Loading	Dynamic Perfusion	5-60 g/L	4	4	4	4	4	4	4	16
		Steady-State Perfusion	40-60 g/L	1	1	1	1	4	4	4	4
	End Loading Breakthrough Trigger		2-6 %	1	2	2	1	1	2	2	4
	Residence Time (All Other Steps)		3-6 min	1	2	2	1	1	2	2	4
	Elution pH		3.3-3.7 units	8	8	8	4	1	4	4	32
	Elution Conductivity		2-5 mS/cm	4	4	4	2	1	2	2	8
	Elution Start/End Collection UV		100-250 mAU	1	2	2	1	1	1	1	2
Virus Inactivation	Temperature		15-25 °C	1	1	1	2	1	1	2	2
	Low pH Target		3.3-3.7 units	8	1	8	4	1	1	4	32
	Incubation Duration		55-75 min	4	1	4	4	1	8	8	32
	Protein Concentration	No BT Load Control	3-20 g/L	4	4	4	4	1	1	4	16
		BT Load Control	12-16 g/L	1	1	1	1	1	8	8	8
	Post-Incubation pH	No BT Load Control	6.5-7.5 units	1	1	1	1	1	1	1	1
		BT Load Control	6.5-7.5 units	1	1	1	1	1	1	1	1
	Titrant Properties		various	1	1	1	1	1	1	1	1
Polishing Chromatography 1 (AEX F/T)	Bed Height		16-20 cm	1	1	1	1	1	1	1	1
	Temperature		15-25 °C	1	1	1	1	1	1	1	1
	Residence Time (Load)[1]		4-10 min	1	1	1	1	1	4	4	4
	Residence Time (All Other Steps)		2-5 min	1	1	1	1	1	1	1	1
	Loading	No BT Load Control	100-250 g/L	4	4	4	4	4	4	4	16
		BT Load Control	200-250 g/L	1	1	1	1	1	1	1	1
	Load pH		6.5-7.5 units	8	2	8	4	2	1	4	32
	Load Conductivity		4-8 mS/cm	4	2	4	2	1	1	2	8
	Start/End Collection UV		100-200 mAU	1	1	1	1	1	1	1	1
	Wash Conditions		various	1	1	1	1	1	1	1	1

Phase	Parameter		Assessment Parameter Range	Main Effect (CQA)	Main Effect (PA)	Highest Main Effect Score	Interaction (CQA)	Interaction (PA)	Preceding Step Interaction	Highest Interaction Score	Severity (Max)
Polishing Chromatography 2 (CEX B/E)	Bed Height		8-12 cm	1	1	1	1	1	1	1	1
	Temperature		15-25 °C	1	1	1	1	1	1	1	1
	Residence Time (Load)[1]		4-10 min	1	1	1	1	1	4	4	4
	Residence Time (All Other Steps)		2-5 min	1	1	1	1	1	1	1	1
	Loading	No BT Load Control	20-50 g/L	4	2	4	1	2	4	4	16
		BT Load Control	40-50 g/L	1	2	2	1	2	2	2	4
	Load pH		6.5-7.5 units	4	2	4	4	2	4	4	16
	Load Conductivity		4-8 mS/cm	4	2	4	2	1	2	2	8
	Wash pH		7.2-7.6 units	4	1	4	4	1	4	4	16
	Wash Conductivity		3-5 mS/cm	1	1	1	4	1	4	4	4
	Elution pH		7.5-8.0 units	4	1	4	4	1	4	4	16
	Elution Conductivity		10-14 mS/cm	1	1	1	1	1	1	1	1
	Elution Start/End Collection UV		100-200 mAU	1	1	1	1	1	1	1	1
Virus Filtration	Operating Pressure [2]		psi	1	1	1	1	1	1	1	1
	Mass Loading [2]		g/m^2	1	4	4	1	1	2	2	8
	Volumetric Loading [2]		L/m^2	8	4	8	1	1	4	4	32
	Volumetric Flux [2]		LMH	1	4	4	2	1	4	4	16
	Protein Concentration	CEX Homogenized	20-25 g/L	1	2	2	2	1	2	2	4
		CEX Variable	5-35 g/L	8	4	8	2	8	8	8	64
	Other Load Properties	CEX Homogenized	various	1	1	1	1	1	1	1	1
		CEX Variable	various	1	1	1	1	8	8	8	8
UF/DF & Form	Various		various	Scoring Same as Batch (Assuming batch UF/DF and homogenization in retentate vessel before processing)							N/A

[1] to maintain volume balance for dynamic perfusion option

[2] Filter dependent

AEX Anion exchange
B/E Bind and elute
BT Breakthrough
CEX Cation exchange
CQA Critical quality attribute
Ctrl Control
F/T Flowthrough
Form Formulation
PA Process performance attribute
ProA Protein A
UFDF Ultrafiltration / Diafiltration

Some of these dependencies are observable beginning with the capture chromatography step. For example, the loading range (g of protein/L of resin) assessed for integration to a dynamic perfusion step is considerably wider than that for the steady-state perfusion. This is due to the relatively higher variability in product mass output from the dynamic format as well as the assumption that, for the early days of the bioreactor harvest period, the column will not be loaded to its full capacity to minimize duration of the loading step, whether from the perspective of bioburden control or column stability. The loading residence time is another parameter that has to be considered differently for integrated processing. This is due to the constraint that all volume exiting the reactor is passed through the capture step and that, in some cases, the perfusion rate may be varied deliberately as part of the bioreactor process. Accordingly, the range of potential loading residence times to consider is wider than for a non-integrated process. However, the impact of this parameter is low due to the low flow rates generally required for continuous processing. The load breakthrough cutoff trigger is an additional parameter unique to continuous processing. This parameter is most likely to impact process performance (recovery) but should be reasonably well controlled to a narrow range by automation.

For virus inactivation, the impact of direct integration with the capture step is evident because of the high score assigned to protein concentration for the preceding step interaction, which is due to the difficulty of accurately hitting the target low pH. In this case study, for both Options 1 and 2, the integration is assumed to be mediated by a cycle surge vessel that homogenizes the compositional variability of the Protein A eluate peak. However, in a case where an inline adjustment is performed without the homogenization, the need to 'titrate against the peak' would lead to a more challenging process control problem and, correspondingly, the pre-PC risk assessment would likely have more parameters to consider and higher impact scores for many of the parameters. In the case where the capture step loading has been controlled to a consistent level, the range considered for protein concentration is narrow, and the virus inactivation risk assessment is nominally the same as for batch processing. In situations where there is variability in the Protein A capture column loading (whether due to lack of breakthrough load control or low titer output from early harvest in dynamic perfusion) and corresponding variability in the eluate, the potential impact on protein concentration for the pH adjustment steps results in a higher impact scoring and greater likelihood that this parameter should be studied as part of process characterization.

For the polishing chromatography steps, the pre-PC risk assessments for both options begin to resemble more closely those typically encountered for batch processing. Again, especially for Option 1, the impact of variability in loading on Protein A propagates down the process until the bind-and-elute cation exchange step. This leads to a wider range of protein loading to the column to be considered for the pre-PC risk assessment. The polishing chromatography is likely less sensitive to differences in protein loading as compared to the titration of the Protein A eluate to low pH for virus inactivation. This is reflected in the relatively lower impact scoring for the parameters impacted by variability in loading on Protein A, whether due to lack of breakthrough control or significant variability in mass output from the bioreactor. The impact of this variability could be mitigated by inclusion of a PAT tool to control protein loading on the polishing chromatography steps; however, this was not considered for the primary frameworks in this case study. The remaining parameters for polishing chromatography would be scored the same for a batch process as for an integrated process.

For virus filtration, two integrated cases are simulated in the risk-based impact assessment (RBIA) pre-PC assessment to further illustrate the impact of choosing different surge vessel configurations. In one case, a cycle surge tank is incorporated to homogenize one or more cycles eluting from the cation exchange (CEX) step. In this case, the risk assessment output for the viral filtration (VF) step should resemble that typically observed for a batch process, even though the VF step is integrated. In the case where eluate variability is allowed to enter the virus filtration step, for example where only a small (~5 min residence time) surge vessel is included, the range of protein concentration to consider for the assessment is much wider, and the corresponding risk that this parameter may impact product quality or process performance is greater. Additionally, while it is not typical to consider impact due to variability in matrix composition for batch processes, an integrated process without at least a cycle surge tank may require characterization of the impact of variable pH or conductivity on virus filter performance. As discussed in the process development section, the pressures and fluxes encountered in the VF step of an integrated and continuous process are much lower than for batch processes. Therefore, depending on the virus filter selected, additional care may be necessary when evaluating the potential impact of pressure and flux.

Finally, in this case study, the primary framework option has focused on cyclic batch operation of UF/DF and formulation. However, in the case of continuous UF/DF by single pass TFF (SPTFF), process parameters unique to the specific configuration and mode of operation (co-current, countercurrent, multi-stage, single pass, etc.) should be carefully defined and evaluated, perhaps with a greater level of potential uncertainty and impact assigned than for a typical batch process due to the relative newness of the single-pass technology applied to integrated continuous processing.

As is the case for a batch process, the outputs of the RBIA pre-PC risk assessment may be used to inform the designation of parameters as preliminary critical process parameters (pCPPs), where appropriate. In general, parameters scored as 'red' in the risk assessment table would be pCPPs, while those categorized as 'yellow' could either be considered pCPPs or declassified based on platform historical knowledge or SME judgment.

3.4 Upstream process characterization study design and execution

Upstream process characterization of continuous perfusion processes leverages the classical approaches as applied to fed-batch cultures while also incorporating additional unique aspects resulting from continuously harvesting product for many days. The overall approach can be categorized into four activities as described in **Table 3.7**.

3.4.1 Screening studies

Screening studies would largely follow a traditional fed-batch strategy with the intention to determine the parameters with the most significant effects on process and product quality parameters and those likely to interact for evaluation in the full characterization studies. These studies may be designed as fractional factorial studies to limit the number of experiments necessary to study an expanded set of controlled parameters. Such a screening study would identify parameters with little or no process impact, which can then be eliminated from a full response surface model (RSM) design of experiments (DOE) study. They may also be executed as one-factor-

at-a-time (OFAT) studies, as is often the case during development, to establish controlled parameter ranges for evaluation in the RSM/DOE studies that are likely to produce statistical effects for analysis while not resulting in complete process failure.

A summary of parameters tested based on the risk assessment discussed above and typical ranges evaluated for dynamic or steady state perfusion processes is presented in Table 3.8 and Table 3.9.

Application of prior knowledge may support removing screening studies once multiple program experience has been gained within a company's platform continuous process. Additionally, studies to support range screening may have occurred within the development of the specific program and thus would not be required prior to moving into formal process characterization studies.

Table 3.7. Upstream process characterization activities and considerations.

Activity [1]	Considerations
1. Screening studies	• May be performed during development • Application of prior knowledge may support removing screening studies once experience has been gained with multiple programs within a company's platform continuous process.
2. Process characterization studies	• Critical parameters listed in Table 3.5 to be studied • Growth phase and steady-state phase (or production phase) to be studied separately • Growth phase studies may utilize a truncated batch
3. Model confirmation and worst-case conditions confirmation	• Model confirmation studies of controlled parameter conditions at edge of anticipated action limits • Separate studies combining both growth and steady-state phase conditions for worst-case combination evaluation • Select subset of conditions to link to downstream in worst-case linkage studies (See Section 3.6)
4. Worst-case condition recovery evaluation	• Optional study block where controlled parameters at or outside action limits are run for a period of time, and then the reactor conditions are brought back to the centerpoint. Batch recovery phenomena are observed. • Optional studies to support deviations and batch control strategy

[1] The activities discussed refer to N-stage continuous perfusion. Inoculum expansion characterization would follow traditional fed-batch culture approaches and is thus deemed out of scope for the N-mAb case study. For additional guidance on inoculum expansion characterization, see the A-Mab case study publication.

Table 3.8. Summary of upstream controlled parameters studied in process characterization.

Upstream Controlled Parameter	Dynamic or Steady-State Perfusion		
	Set Point	Operating Range	Characterization Range
Growth Phase pH	7.1	7.0–7.2	6.9–7.3
Growth Phase Temperature (°C)	36	35.5–36.5	34.5–37.5
Growth Phase Seed Density (x10^6 vc/mL)	2.0	1.8–2.2	1.4–2.6
Growth Phase Perfusion Rate (CSPR, nL/cell/day)	0.05	± 5% (0.0475–0.0525)	± 20% (0.04–0.06)
Production Phase pH	7.1	7.0–7.2	6.9–7.3
Production Phase Temperature (°C) [1]	33	32.5–33.5	31.5–34.5
Production Phase Perfusion Rate (vvd) [1, 2]	2	± 5% (1.9–2.1)	± 20% (1.6–2.4)
Production Phase Shift Timing (day) [1]	6	5.75–6.25	5–7
Dissolved Oxygen (%sat)	50	45–55	20–80
Retentate Rate (ATF or TFF) (L/min)	Target	± 5%	± 20%

[1] May have multiple shift timing parameters if various operational parameters (i.e., temperature, perfusion rate, media composition, etc.) are shifted on different days.
[2] Production phase perfusion rate may be controlled via CSPR for dynamic perfusion.

Table 3.9. Additional upstream steady-state perfusion parameters studied in process characterization.

Upstream Controlled Parameter	Steady-State Perfusion		
	Set Point	Operating Range	Characterization Range
Production Phase VCD/Biocap Target [1] (x10^6 vc/mL)	100	90–110	75–125
Secondary Production Phase VCD/Biocap Target [1] (x10^6 vc/mL)	Target	± 10%	± 25%
Timing of Secondary Production Phase VCD/Biocap Target (day)	Target	± 0.25	± 1

[1] These parameters are included based on perfusion operations being controlled to a specific cell density or biocapacitance target with variable cell bleed to achieve that target. As a result, the cell bleed rate or cumulative value is evaluated as a process output parameter according to **Table 3.11**. If cell bleed rate or total is controlled instead to allow a variable cell density or biocapacitance process output, then that controlled parameter should be studied instead.

ATF	Alternating flow filtration	TFF	Tangential flow filtration
Biocap	Biocapacitance	vc	Viable cells
CSPR	Cell-specific perfusion rate	VCD	Viable cell density

3.4.2 N-Stage process characterization studies

Based on the pre-PC risk assessment summarized above, the controlled parameters to be studied during process characterization are summarized in Table 3.10.

Table 3.10. Summary of upstream controlled parameters studied in process characterization.

Upstream Controlled Parameter	Study Type for Characterization
Growth Phase pH	DOE RSM
Growth Phase Temperature	DOE RSM
Growth Phase Seed Density	DOE RSM
Growth Phase Perfusion Rate	OFAT
Production Phase pH	DOE RSM
Production Phase Temperature	DOE RSM
Production Phase VCD/Biocap Target [1] (Steady-state process only)	DOE RSM (Steady-state process only)
Production Phase Perfusion Rate	OFAT
Production Phase Shift Timing [2]	OFAT
Dissolved Oxygen	OFAT
Retentate Exchange Rate (ATF or TFF)	OFAT
Secondary Production Phase VCD/Biocap Target [1] (Steady-state process only)	OFAT, possible factorial with timing of later shifts
Timing of Secondary Production Phase VCD/Biocap Target [2] (Steady-state process only)	OFAT, possible factorial with later shift targets

[1] These parameters are included for the control of perfusion operations to a specific cell density or biocapacitance target using variable cell bleed to achieve that target. As a result, the cell bleed rate or cumulative value is evaluated as a process output parameter according to Table 3.11. However, if cell bleed rate or total is controlled instead to allow a variable cell density or biocapacitance process output, then that controlled parameter should be studied instead.
[2] May have multiple shift timing parameters if various operational parameters (e.g., temperature, perfusion rate, media composition) are shifted on different days.

ATF	Alternating flow filtration
Biocap	Biocapacitance
DOE	Design of experiments
OFAT	One factor at a time
RSM	Response surface model
TFF	Tangential flow filtration
VCD	Viable cell density

Three controlled parameters typically included in fed-batch RSM/DOE studies were also identified for similar evaluation in continuous production bioreactor unit operations: pH, temperature, and seed density. Because the process impact of these parameters can extend from the growth phase to the production phase, a more meaningful response understanding may be achieved by decoupling the growth phase from the production phase during characterization for either dynamic or steady-state perfusion. For example, if parameters are varied according to an RSM/DOE design

during the growth phase, the culture may respond poorly to those parameters during the production phase, and substantial impact to the quality of the process and product could occur. In contrast, this impact would not be observed if the parameters were controlled using an RSM/DOE design only during the production phase where the culture was healthy at the shift from growth to production phase. As a result, to understand more clearly the culture responses in both phases, an RSM/DOE design including pH, temperature, and seed density parameters is executed for the growth phase, and a separate but analogous RSM/DOE design of pH, temperature, and steady state VCD/biocap target is executed for the production phase. Conditions evaluated in an RSM for the growth phase may be carried forward into the production phase at center point so that only the growth phase impacts are assessed for the recovery response in the production phase. Conversely, RSM conditions for the production phase would be carried forward from center point growth phase cultures so that the pre-shift culture health and state does not influence the response of the production phase to the RSM condition. The RSM design should be established to model all main effect, quadratic, and two-way interactions of these three parameters, and should remain aligned to well-established approaches for fed-batch processes. For reference, a general designation of the growth and production phases for both dynamic and steady-state processes is presented in **Figure 3.1**.

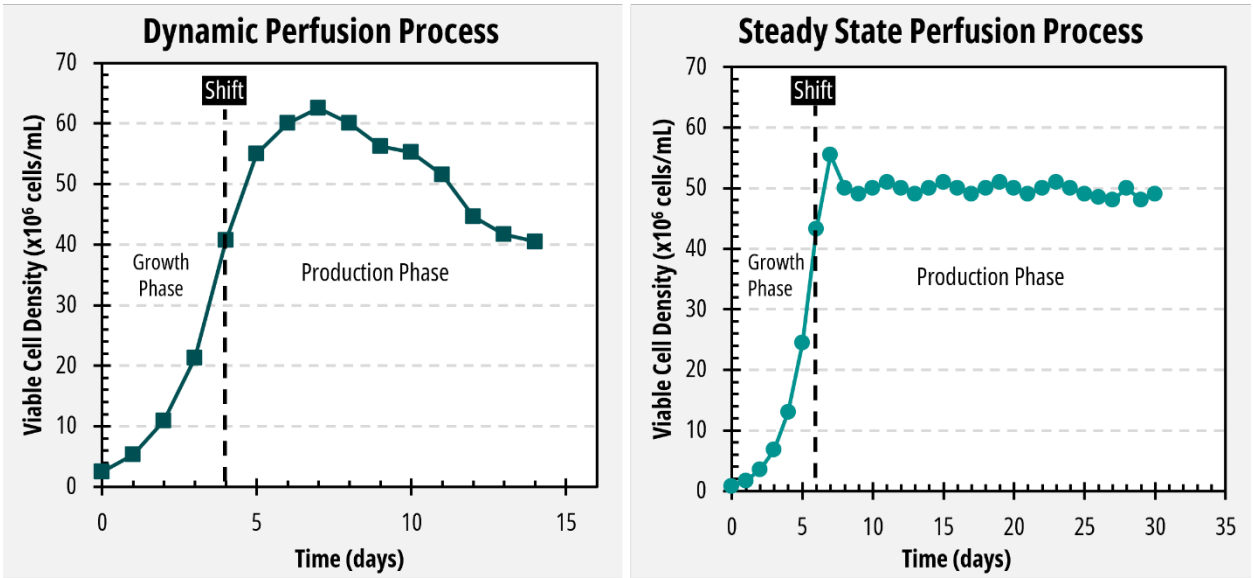


Figure 3.1. Comparison of growth and production phases in dynamic and steady state processes.

In addition to the parameters typically warranting further characterization in traditional fed-batch processes, **Table 3.10** also includes the parameters related to continuous processing (e.g., perfusion and retentate rates, dissolved oxygen, and timing of the shift to production phase). Several of these parameters that require characterization may be studied in a one-off manner (one factor at a time, OFAT) rather than by increasing the complexity and size of the RSM design. Such parameters should have a low likelihood of interactions based on prior development data and are therefore not a necessary or valuable addition to RSM and other DOE approaches. Typically, these parameters may

be evaluated during robustness or screening studies to a sufficient extent to support the design space and control strategy for each one. In general, the growth and production phase perfusion rates, retentate rate, dissolved oxygen level, and the timing of any parameters shifted for the production phase are evaluated in one-off studies. The production phase shift is often accompanied by a change in the perfusion medium composition, though this is typically not studied in characterization studies beyond the normal robustness and refinement experiments conducted during development.

Because continuous processes are harvesting product for an extended duration, process characterization experiments should be run for the time necessary to ensure full determination of the effect of experimental conditions on process performance and product quality. These experiments may include determination of appropriate time point analysis to ensure that the characterization package generated will support the intended downstream processing scheme and process control strategy. This analysis of time dependency may be done for daily results or for results of fixed blocks of days aligning to downstream pooling and batch definition strategies. However, such approaches will generate several RSM models for each performance or product quality parameter, compounding the breadth of analysis needed. Therefore, initial studies evaluating limited time points tested throughout the steady-state harvest phase may allow an initial evaluation of the time dependency of various process and product quality output parameters. Daily retains may be obtained and archived to lighten the initial analytical load until a time-dependent impact can be assessed. If time dependency is observed for RSM conditions, an analysis of a full time-course data set by advanced statistical approaches may be necessary to enable a broader understanding of impacts over the harvest duration, and these approaches are discussed in **Section 3.4.3** below.

During the characterization studies on controlled parameters, the output process performance attributes to be evaluated (**Table 3.11**) should generally align with the parameters and their timeframe for evaluation as previously outlined in the scale-down model section above. Additional process attributes that were not explicitly targeted for comparison between scales can be added as needed, particularly if they represent additional indicators of process performance and consistency, such as metabolic markers or consumption rates.

Table 3.11. Summary of upstream output attributes evaluated in process characterization.

Upstream Output Attribute	Process Phase	Attribute Type/Purpose
Process Performance Attributes (PA)		
Viable Cell Density	All	Cell growth/health
Viability	All	Cell growth/health
Metabolites	All	Cell growth, metabolism
Cell Growth Rate	Growth phase	Cell growth
Cell Bleed Rate/Total	Production phase	Cell growth
Bioreactor, Permeate Titer	Production phase	Productivity, membrane sieving
Product Quality Attributes		
Glycosylation – High Mannose	Production phase	*
HMWS	Production phase	*

* Glycosylation and HMWS shown as examples only; product quality attributes are molecule-specific and should be determined by risk assessments and additional SAR analytical work.

HMWS High molecular weight species SAR Structure-activity relationship

3.4.3 Analysis of data

Since the aspect of time dependency is different between the growth and production phases, differing approaches to data analysis may be necessary for studies conducted for each phase.

3.4.3.1 Growth phase statistical analysis

Growth phase conditions to be studied are referenced in **Table 3.10**. The output from the growth phase is the steady-state profile for steady-state perfusion or the peak cell mass for dynamic perfusion. Thus, a single output day or value that represents the culture production phase following the growth phase can be used to predict the impact of growth parameters and set growth phase parameter ranges. This approach can be managed via a similar DOE or OFAT statistical manner as applied to traditional fed-batch cultures.

3.4.3.2 Production phase statistical analysis

Time is a key factor in the analysis of production phase process characterization studies because harvesting takes place over many days. Traditional fed-batch cultures can be studied using a single harvest day as the output when assessing input parameter impact on key outputs such as titer and product quality attributes. Culture duration is generally studied within classical fed-batch process characterization and is performed by selecting two additional culture days to study (for a total of three timepoints) such as a day before and a day later than the target harvest day. These three timepoints support a low, middle, and high condition within process characterization studies.

In contrast, continuous perfusion processes are intended to harvest constantly over a given duration, and thus key output parameters such as titer and product quality are being assessed for impact over the full harvest duration. Addressing the question as to whether a practically significant

change in process outputs is observed over the harvest duration, alternate statistical analysis methods could be evaluated. Table 3.12 suggests approaches that could be applied to the production phase of continuous processes. Note that the Bayesian approach described in the last row is equally applicable to time dependent parameters and to non-time dependent parameters, and also has an advantage for non-linear responses, such as the high Mannose example, over frequentist approaches. The last bullet point in that row could potentially be expanded to cover the development of process models which could be used to continuously control the process output if parameters identified as significant are measured in real-time, and the model has adequate prediction properties.

Table 3.12. Approaches to production phase analysis.

Method	Considerations and Approach
Time Dependency Assessment	• Assess input parameters for time dependency • Apply univariate control charts • Evaluate to assess time dependency (i.e., ANOVA of time as a significant effect on parameter or set variance criteria)
Standard DOE/RSM Model approach for non-time-dependent parameters	• Apply to non-time-dependent parameters • Define criteria for acceptable output parameter variation based on downstream capabilities • Select approximately 3–5 days during the production phase to generate individual DOE/RSM prediction models and compare outcomes across timepoints • Perform ANOVA evaluation of time as a significant effect on outcomes across days
Mixed Model Repeated Measurement for time-dependent parameters	• Apply to time-dependent parameters or use to further justify the lack of time dependence • Apply to fixed parameter OFAT or DOE studies • Apply covariance of time factor to model
Bayesian Hierarchical Model	• Model linear and non-linear relationships • Estimate time along with other control parameters as random variables • Evaluate posterior distribution of time and fixed control parameters for significance • Estimate probability of specific outcomes using posterior predictive distribution

DOE Design of experiments
OFAT One factor at a time
RSM Response surface model

3.4.3.3 Production phase statistical analysis: G2F scenario

To illustrate differences in time-independent and time-dependent processes, examples of potential impacts of pH on G2F glycosylation profiles in both scenarios are presented and discussed below.

In Figure 3.2, no apparent time dependence is observed within the normal analytical and process variability for the center point pH condition. In addition, although the low and high pH conditions

evaluated shifted the glycosylation profile from the center point, the effect was consistent and demonstrated no time dependency.

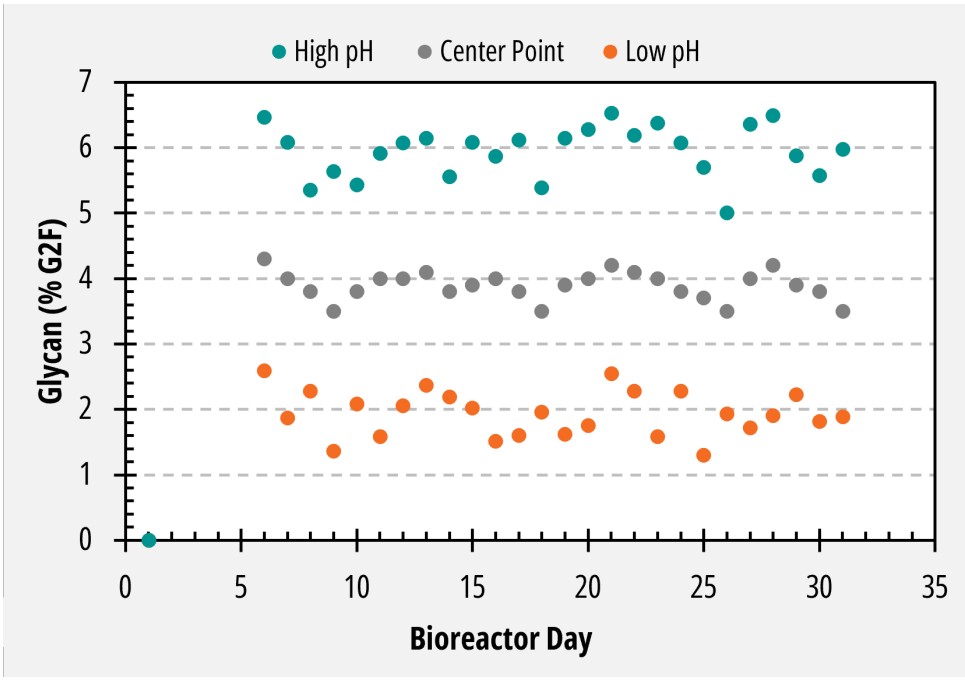
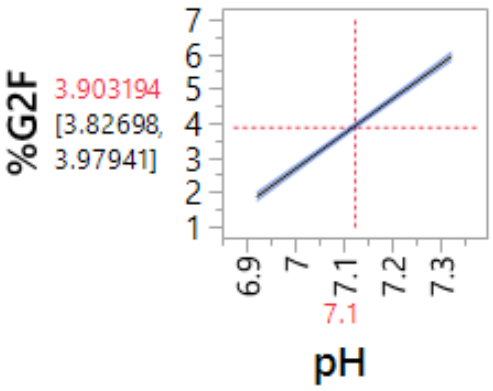


Figure 3.2. Time course characterization results of %G2F as a function of pH with no time-dependent impacts.

Assessment of time dependency can be carried out in several ways. The first approach may involve simply comparing the daily results against some limit of acceptable variability for the process. This limit could be based on analytical variability, normal process variability, acceptable downstream process limits, or a combination of these. If more statistical rigor is desired, a second approach could be to include the time parameter (i.e., culture day) as an anticipated effect in the DOE model analysis to determine if it has a statistically significant effect. In the case of the data shown in Figure 3.2, including culture day along with pH in a fixed effects least squares model indicates that, while the pH effect is statistically significant, the culture day parameter does not have a statistically significant impact (p-value greater than 0.05) within the variability observed (Figure 3.3).



Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-68.05	1.674	-40.67	<.0001
pH	10.13	0.235	43.07	<.0001
Culture Day	0.003	0.006	0.51	0.6110

Figure 3.3. Statistical model of %G2f response as a function of pH in a time-independent process.

In contrast, Figure 3.4 shows an example with a slight drift in the %G2F profile over the production phase at the center point pH condition. Further, when tested at the low and high pH conditions, different time dependencies are observed. At low pH, the glycan profile is consistent and does not even exhibit the drift observed at the center point condition. However, at high pH, the drift in the glycan profile is exacerbated beyond that observed at the center point condition. Therefore, not only did the low and high pH conditions shift the glycosylation profile from the center point, the magnitude of the effect was dynamic over time.

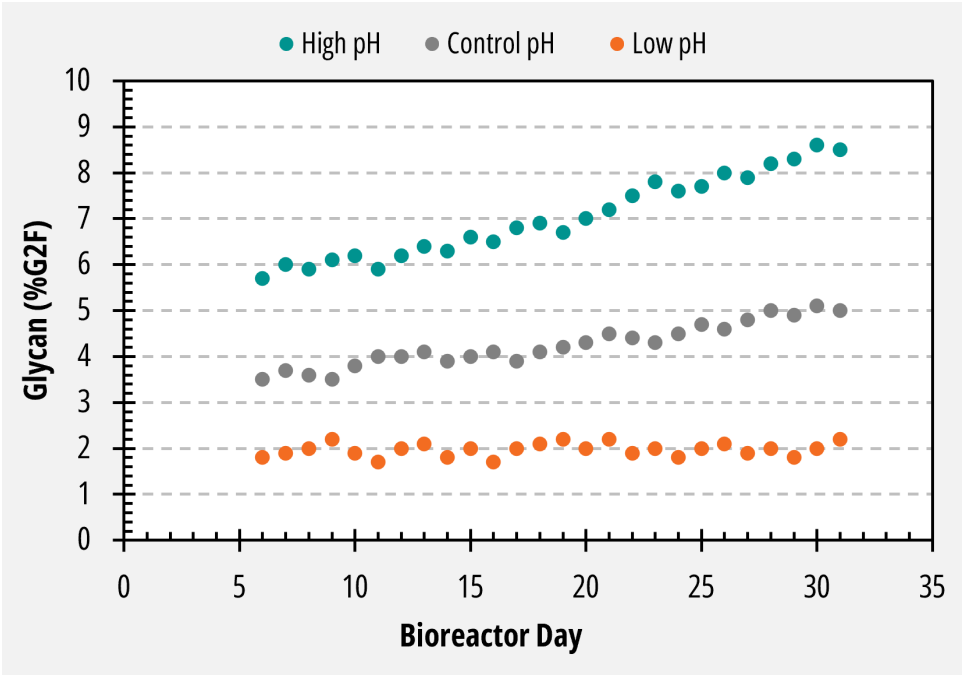


Figure 3.4. Time course characterization results of %G2F as a function of pH and time.

As stated above, the significance of apparent time dependency in the data set can be assessed against an allowable range of variability based on analytical variability, normal process variability, acceptable downstream process limits, or a combination of these.

A third approach to analyzing and modeling time dependency would be to generate separate models of the pH effect on the glycan profile for various timeframes across the production phase. For example, modeling the pH impact on %G2F over a limited set of time points as repeated measures depicted in **Figure 3.5** will generate a different pH effect model for each segment of time over the production phase. The dynamic change in the glycan response at higher pH can be observed by the shifting slopes of the model prediction from the early to later time frames. Although this will provide information around the impact of pH on the glycan at various portions of the production phase, the separate models do not provide a cohesive model to predict the pH impact at any given day when the time dependency is impacting the effect even within the smaller timeframes.

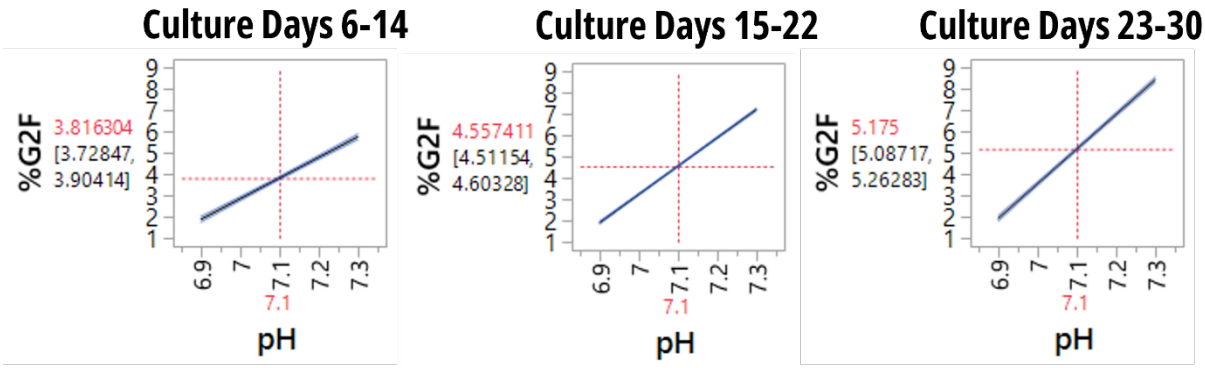
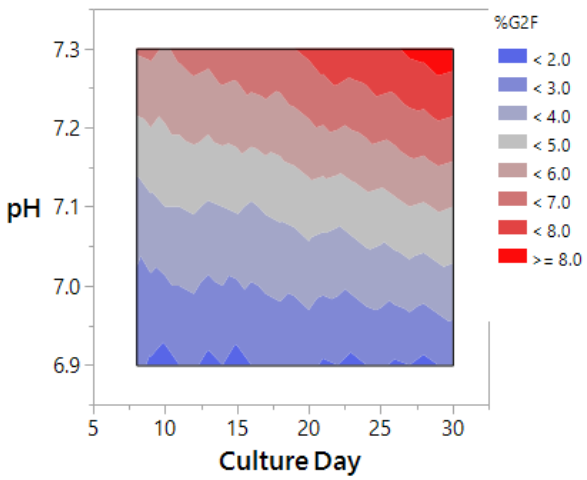


Figure 3.5. pH effect models show dynamic changes to pH prediction profiles for %G2F as a function of culture day.

Alternatively, the time parameter (i.e., culture day) could be included as an anticipated effect in the DOE model analysis to determine if it has a statistically significant effect. In the case of the data shown in **Figure 3.4**, including culture day along with pH in a fixed effects least squares model with repeated measures over multiple production phase days indicates a statistically significant effect from both pH and culture day, as well as an interaction between the two parameters (**Figure 3.6**).



Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-88.75	0.990	-89.66	<.0001
pH	12.97	0.139	93.24	<.0001
Culture Day	0.062	0.0034	18.04	<.0001
pH*Culture Day	0.299	0.0210	14.26	<.0001

Figure 3.6. Two-parameter model of %G2F response as a function of pH and culture day.

The mixed model repeated measurement approach discussed above can be applied to understand process output ranges. These models may also be used to find combinations of operational parameter combinations to enable acceptable operating ranges as well as action limits to ensure acceptable performance with confidence. This approach will inform timing and sampling needs to support the control strategy.

3.4.4 Alternative Bayesian statistical approach

An alternate statistical option that incorporates time course data and assesses its significance is a Bayesian approach. Unlike the frequentist approach employed for the more traditional RSM/DOE modeling shown above, which estimates the model parameters as unknown constants, the Bayesian approach treats all the model parameters as random variables. As a result, the posterior distributions of these model parameters are generated based on the large quantity of samples generated from the Markov Chain Monte Carlo (MCMC) model fitting process. These distributions of model parameters can then be used to make predictions of the response variable, which is also in the form of distribution, thereby incorporating the appropriate statistical uncertainty in the model. This approach can also provide direct answers to questions regarding probability of success, such as the likelihood of meeting acceptance criteria for specific conditions. To illustrate differences in Bayesian approaches for models with unknown or known response dynamics, examples of potential impacts of pH on G2F glycosylation profiles and culture duration on high mannose levels are presented and discussed below.

3.4.4.1 Bayesian Statistical Approach: G2F Scenario with Non-Informative Priors

To illustrate this difference in model analysis and application to response estimation, the data for G2F variation shown in **Figure 3.2** was analyzed using Bayesian mixed effect modeling with non-informative priors (i.e., purely data-driven modeling). **Figure 3.7** contains the posterior distribution

of the model parameters for the model with the best performance comparison. The location of the distribution with respect to zero indicates the level of significance of the model.

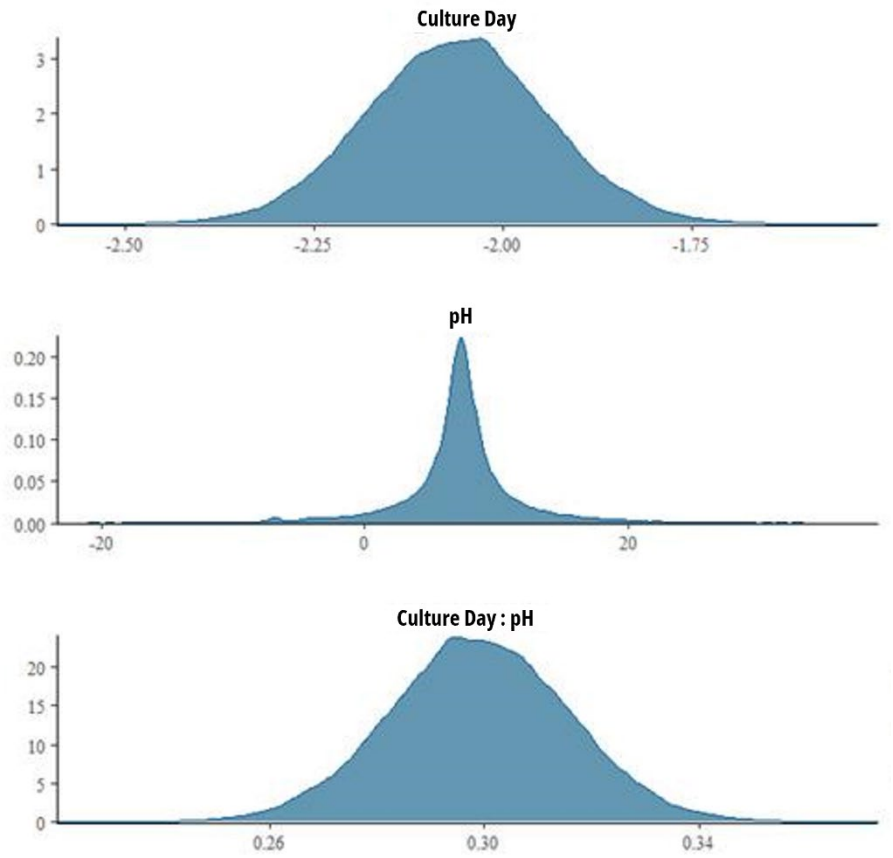


Figure 3.7. Posterior distributions of Bayesian model parameters for the %G2F response.

Based on the posterior distributions, we can make statistical inference for these model parameters as shown in **Table 3.13**, where the last two columns show the two-sided 95% credible intervals. Because the value of zero is not contained within the 95% credible interval of the distribution for culture day or the interaction of culture day and pH together, the model suggests these are significant. In the case of the distribution for pH, which does encompass zero, this suggests that there is a small probability that this model parameter may have a negligible effect. Because the majority of the distribution is to the right of zero, there is minimal chance that the pH parameter is not significant.

Table 3.13. Statistical estimates and 95% credible intervals[1] for the Bayesian posterior distributions of model parameters.

Model Parameters	Estimate	Est. Error	Q2.5	Q97.5
b_Culture_Day	-2.061	0.120	-2.296	-1.824
b_pH	7.203	4.667	-4.021	17.596
b_Culture_Day:pH	0.299	0.017	0.266	0.332

[1]The 95% credible interval for the parameter estimate is defined as the range between the 2.5% quantile (Q2.5) and 97.5% quantile (Q97.5).

Based on the posterior distributions of the model parameters described above, the posterior predictive distribution of the %G2F can be generated at different levels of pH and as a function of culture day. This is illustrated in Figure 3.8 and Figure 3.9, and the statistical summary parameters are provided in Table 3.14. The effect of culture day is clearly illustrated by the shifted predictive distributions of higher predicted %G2F responses at later culture days in both figures. In addition, the stronger influence of pH at later culture days can be observed in the larger shift to higher %G2F predictive means at later culture days with pH 7.3 relative to pH 7.1.

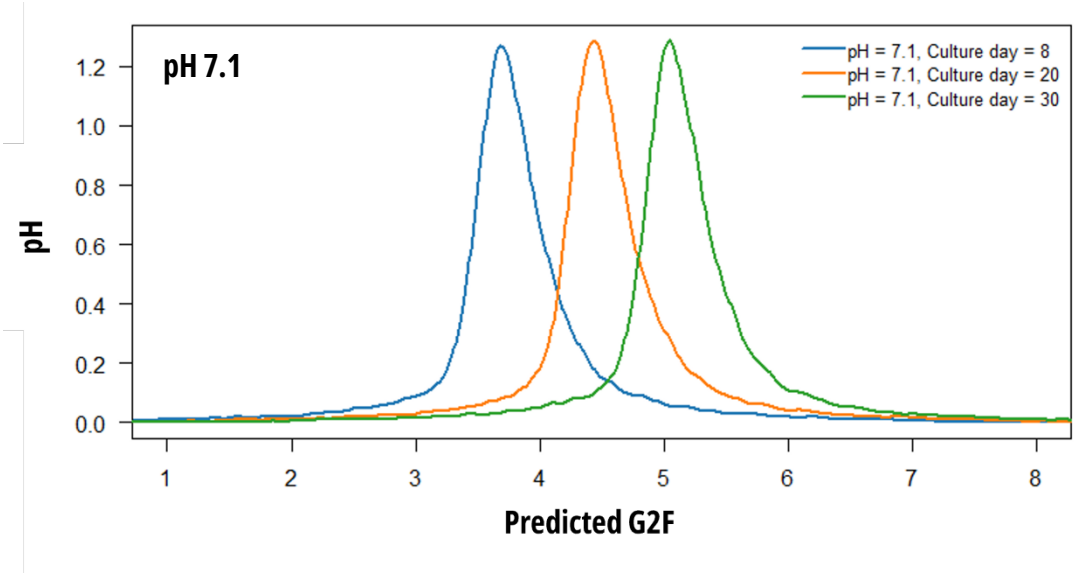


Figure 3.8. Bayesian posterior predictive distributions for the %G2F response as a function of culture day at pH 7.1.

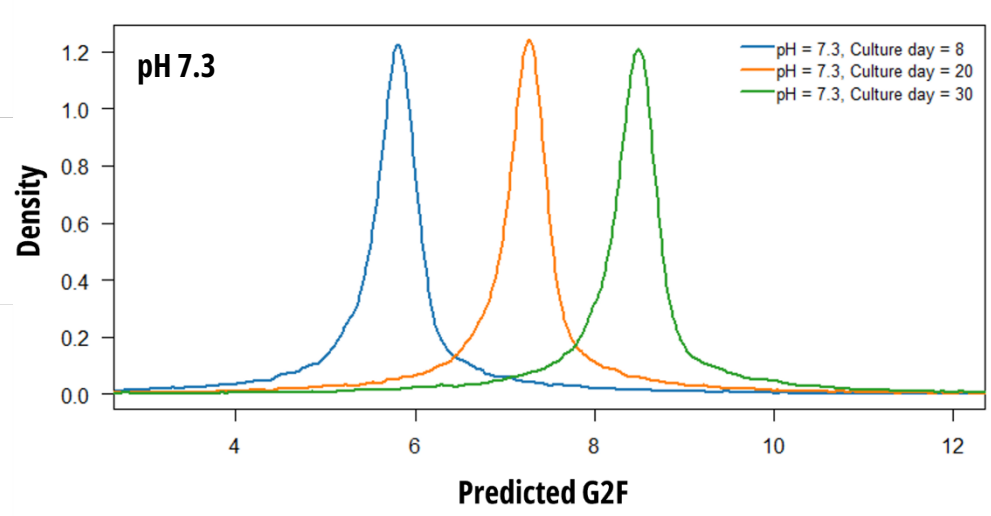


Figure 3.9. Bayesian posterior predictive distributions for the %G2F response as a function of culture day at pH 7.3.

Table 3.14. Statistical means and 95% credible intervals[1] for the Bayesian posterior predictive distributions of %G2F as a function of pH and culture day.

Summary Statistics of Posterior Distribution of %G2F at pH 7.1			
Culture Day	**8**	**20**	**30**
Mean	3.82	4.56	5.18
Q2.5	2.09	2.84	3.47
Q97.5	5.71	6.42	7.06
Summary Statistics of Posterior Distribution of %G2F at pH 7.3			
Culture Day	**8**	**20**	**30**
Mean	5.74	7.20	8.41
Q2.5	2.98	4.48	5.66
Q97.5	8.34	9.79	11.01

[1]The 95% credible interval for the parameter estimate is defined as the range between the 2.5% quantile (Q2.5) and 97.5% quantile (Q97.5).

The Bayesian posterior predictive distributions can be used to set process action limits. The distribution generates additional confidence that the G2F in the case study example above will not exceed 11% if the process runs at pH 7.3 through the batch duration. As an example, if it was deemed critical that G2F should not exceed 9%, an action limit could possibly be implemented to set an upper pH range and culture day combination to prevent this scenario.

Like the mixed model repeated measurement approach, the Bayesian approach can be applied to understand process output ranges, identify combinations of acceptable operational parameter ranges to ensure acceptable performance with confidence, and inform timing and sampling needs

to support the control strategy. These and other statistical approaches may evolve to establish an eventual best practice of fully characterizing and understanding the time complexity element of continuous perfusion processing.

Based on the example data, a linear mixed effects model was developed using Bayesian approach with non-informative priors. The statistical inference from Bayesian modeling is based on the posterior distribution, hence the process characterization will be based on the posterior predictive distribution of the response G2F. The use of the posterior predictive distribution in a Bayesian framework results in a different interpretation of a parameter/response relationship compared to a frequentist model. In a frequentist approach, point estimates of parameter effects provide the *average* expected response at a specified condition. In contrast, the posterior predictive distribution in a Bayesian approach reflects the probability of an outcome. **Figure 3.10** displays the relationship of pH and culture day on G2F with contours for the 95th percentile of the posterior predictive distribution of G2F across the range of pH and culture days. For any pH and culture day pairing, 95% of G2F results are expected to be no more than the associated contour value.

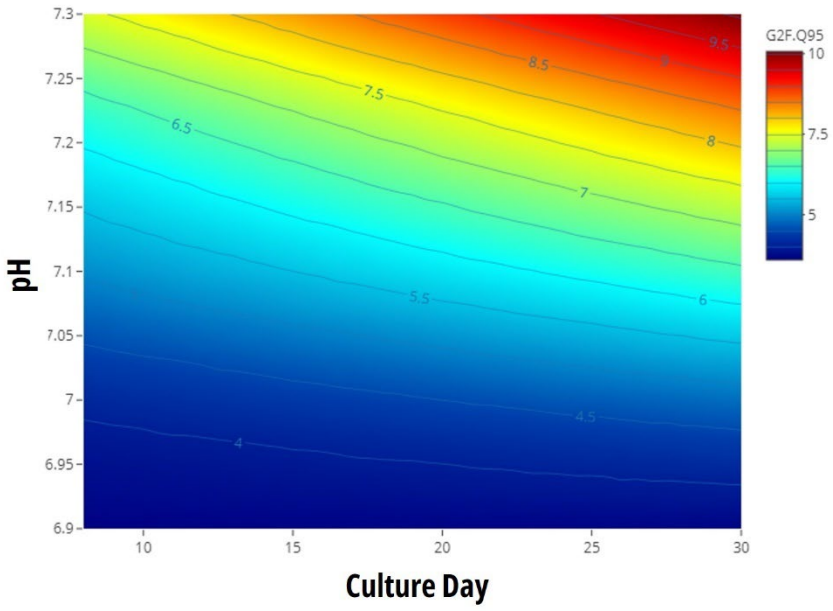


Figure 3.10. Contour plot of the 95th percentile of the posterior predictive distribution of G2F.

These plots can be used for visualization and computation to determine acceptable operating conditions. For instance, the blue region in **Figure 3.11** shows the conditions of pH and culture day for the region in which the 95th percentile of the posterior predictive distribution of G2F does not exceed the upper specification limit of 9%.

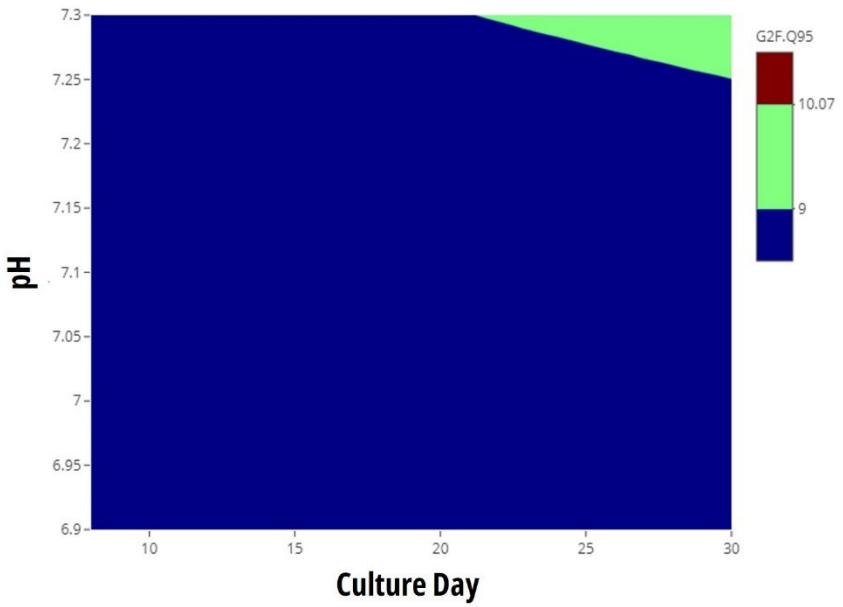
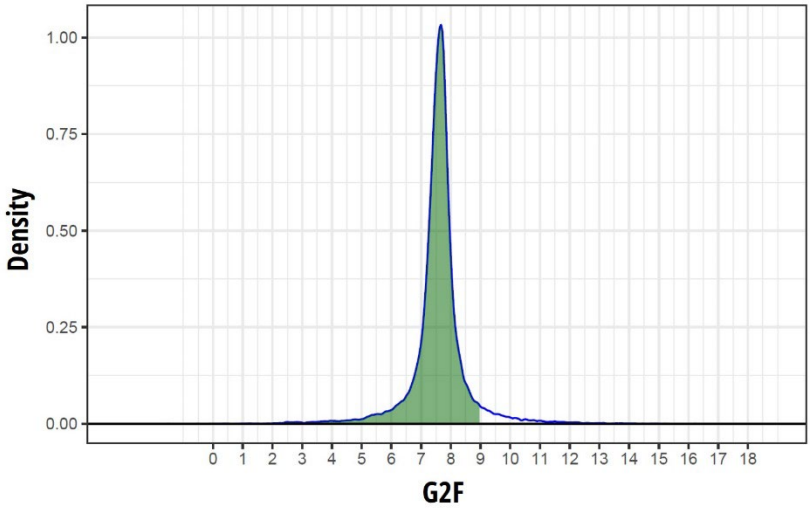


Figure 3.11. Conditions of pH and culture day for the region in which the 95th percentile of the posterior predictive distribution of G2F does not exceed the upper specification limit.

The computation results indicate that, if pH is set to be less than 7.26, then the probability of G2F exceeding 9% on day 30 is less than 1- 0.95=0.05.

Figure 3.12 shows the probability density curve of the posterior predictive distribution of G2F for pH=7.25 and culture day=30, as well as the summary statistics of this distribution.



Mean	7.60
Median	7.62
Mode	7.67
Q99	8.98

Figure 3.12. Probability density curve of the posterior predictive distribution of G2F for pH=7.25 and culture day=30. Green shaded area reflects 95% probability that G2F is no greater than 9% at this condition.

The percentile can be varied depending on the desirable assurance. Figure 3.13 is the contour plot of the 99th percentile of the posterior predictive distribution of G2F based on all the combinations of pH levels and culture days.

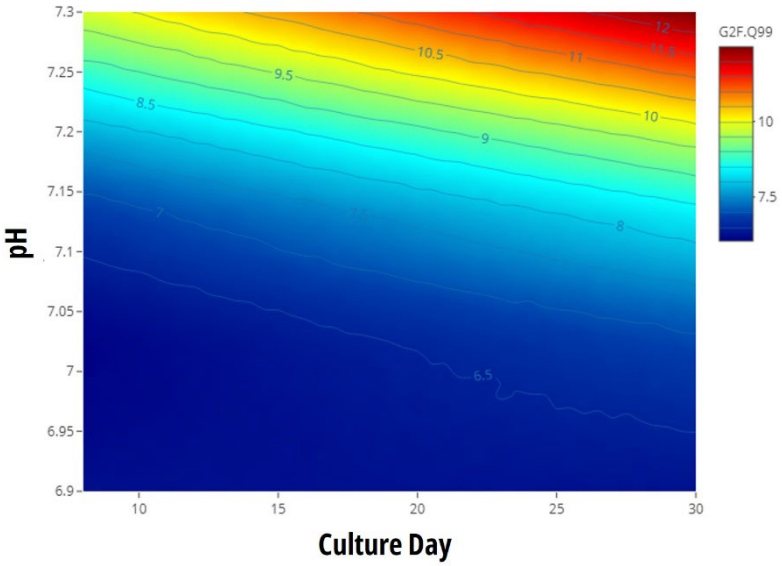


Figure 3.13. Contour plot of the 99th percentile of the posterior predictive distribution of G2F.

The blue region in Figure 3.14 shows the conditions of pH and culture day where the 99th percentile of the posterior predictive distribution of G2F does not exceed the upper specification limit of 9%.

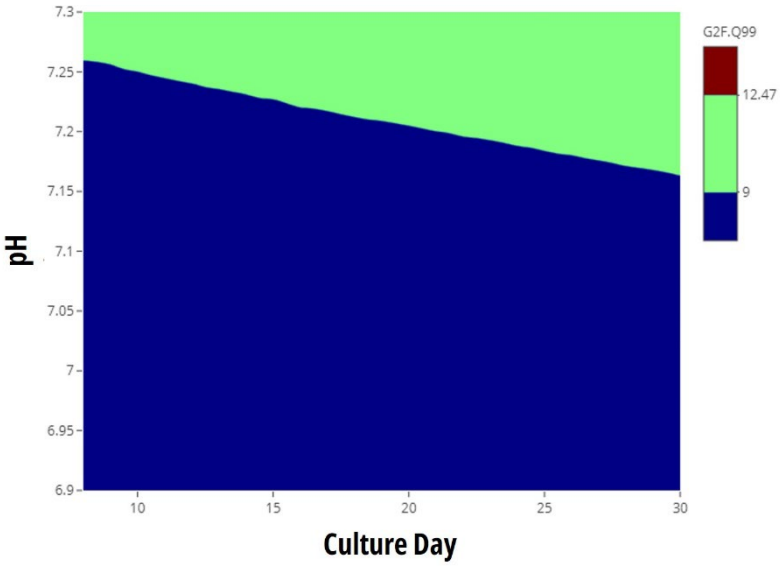
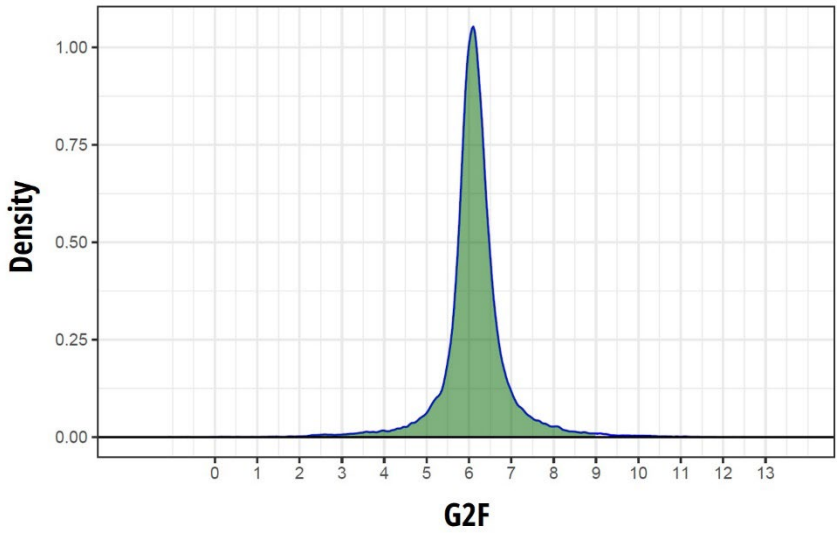


Figure 3.14. Conditions of pH and culture day where the 99th percentile of the posterior predictive distribution of G2F does not exceed the upper specification limit.

It is clear that the region that assures at least 99% probability that G2F will not exceed 9% is smaller than the region associated with 95% probability. Specifically, the pH must be less than 7.17 to assure that the probability of G2F exceeding 9% on day 30 is less than 1- 0.99=0.01.

Figure 3.15 shows the probability density curve of the posterior predictive distribution of G2F for pH=7.16 and culture day=30, as well as the summary statistics of this distribution.



Mean	6.14
Median	6.12
Mode	6.10
Q99	8.86

Figure 3.15. Probability density curve of the posterior predictive distribution of G2F for pH=7.16 and culture day=30. The green shaded area reflects 99% probability that G2F is no greater than 9% at this condition.

3.4.4.2 Bayesian Statistical Approach: High Mannose Scenario with Informative Priors

Nutrient levels can impact cell health, which is also known to impact levels of high mannose species in some situations. In an earlier case, we observed a significant increase in high mannose species over the course of the perfusion batch, in this specific example it was for the dynamic perfusion option whereas high mannose species were well controlled in a steady state perfusion study. In order to study the hypothesis that nutrient levels impact the levels of high mannose species, a process characterization study would be performed by varying the target perfusion rate. The figure below illustrates the mock results of the study and the impact of perfusion rate on high mannose species and cell health (percent viability) in this dynamic perfusion example.

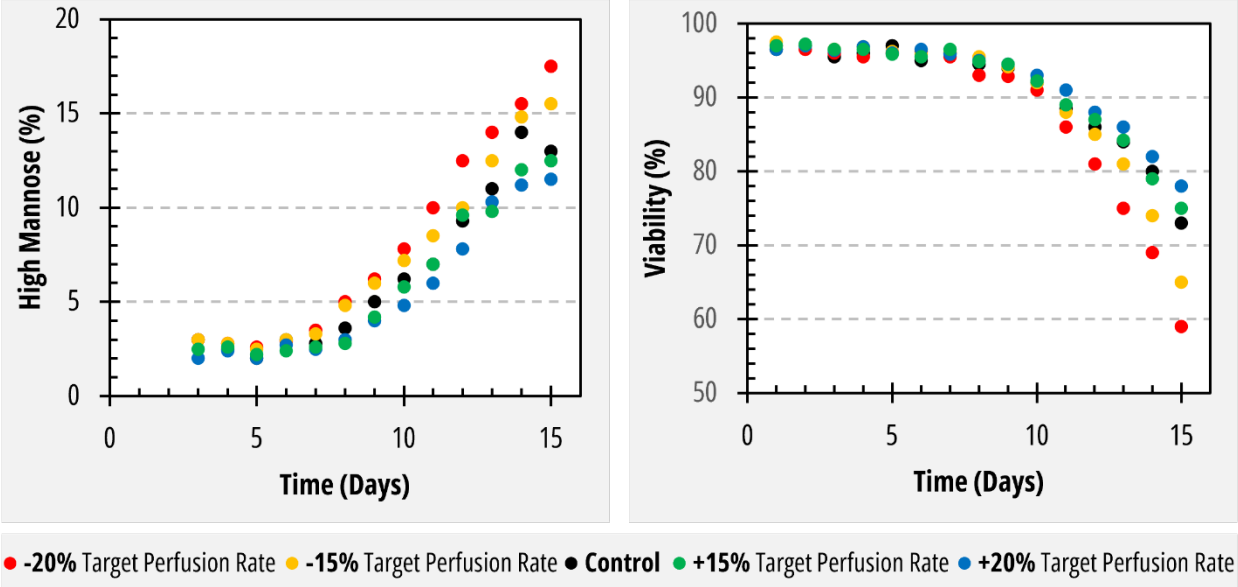


Figure 3.16. High mannose trajectory (left) and viability (right) in the bioreactor for variable product quality.

The high mannose graph, in Figure 3.16. High mannose trajectory (left) and viability (right) in the bioreactor, shows that high mannose has a non-linear response for the different levels of perfusion rate. If there's no established function from first principle or prior knowledge to describe this non-linear response, then it needs to be identified based on the current data set using a statistical software package, such as JMP. Using JMP, it was found that the Logistic 4P Rodbard model has a better fit to the data for all the levels of perfusion rate, and hence the Logistic 4P Rodbard function was used to represent the underlying growth curve function as shown in Equation 1.

Equation 1. The Logistic 4P Rodbard function

$$\text{High\_mannose} = c + \frac{(d-c)}{\left(1+\left(\frac{\text{Culture\_Day}}{b}\right)^{a}\right)}$$

Where the model constants refer to the following curve characteristics:

a =Growth Rate
b = Inflection Point
c = Lower Asymptote
d = Upper Asymptote

Table 3.15 provides a summary of the fitted model for the overall growth curve, i.e., without respect to the levels of perfusion rate. The normal distribution of the parameter estimates will be used to derive priors for the Bayesian modeling where the effect of the perfusion rate can be evaluated.

Table 3.15. Parameter estimates for Bayesian model of high mannose.

Parameter	Estimate	Std Error	Lower 95%	Upper 95%
a_intercept	-5.10	1.03	-7.12	-3.08
b_intercept	11.26	0.90	9.49	13.04
c_intercept	2.51	0.30	1.93	3.09
d_intercept	18.14	2.84	12.57	23.71

The flexibility of a Bayesian approach is well-suited to model the non-linear impact of the perfusion rate on high mannose species (**Figure 3.16.** High mannose trajectory (left) and viability (right) in the bioreactor). The relationship can be examined through the comparison of a series of hierarchical logistic 4P Rodbard models, in which each of the four parameters above is assessed individually. Each assessment is performed by replacing the respective parameter with a linear function of the percentage change of target perfusion rate. The comparison results show that the model utilizing parameter **d** as a linear function of perfusion rate has the best fit to the data, and hence it is deemed as the final model for further inference or prediction use. As a result, parameter **d** in the model above is estimated by both an intercept and a slope relating the percent change of perfusion rate (**d**= d_intercept + d_Percent_Change_of_Target_Perfusion_Rate * Δ% Target Perfusion Rate). The adjusted parameters, fit to the original dataset now accounting for the linear relation of d to perfusion rate, are provided in **Table 3.16**.

Table 3.16. Adjusted parameters for the Bayesian model of high mannose.

Parameter	High Mannose	
	Estimates	CI (95%)
a_intercept	-4.85	-5.56 – -4.23
b_intercept	11.39	10.77 - 12.16
c_intercept	2.44	2.20 - 2.68
d_intercept	18.63	16.74 - 12.01
d_Percentage_Change_of_Target_Perfusion_Rate	-0.18	-0.22 - -0.15
Observations	65	
R^2 Bayes	0.984	

Figure 3.17 shows the posterior distribution of the model parameters for the model with the best performance comparison. Since none of the posterior distributions encompass zero, this indicates that all parameters are statistically significant within the model.

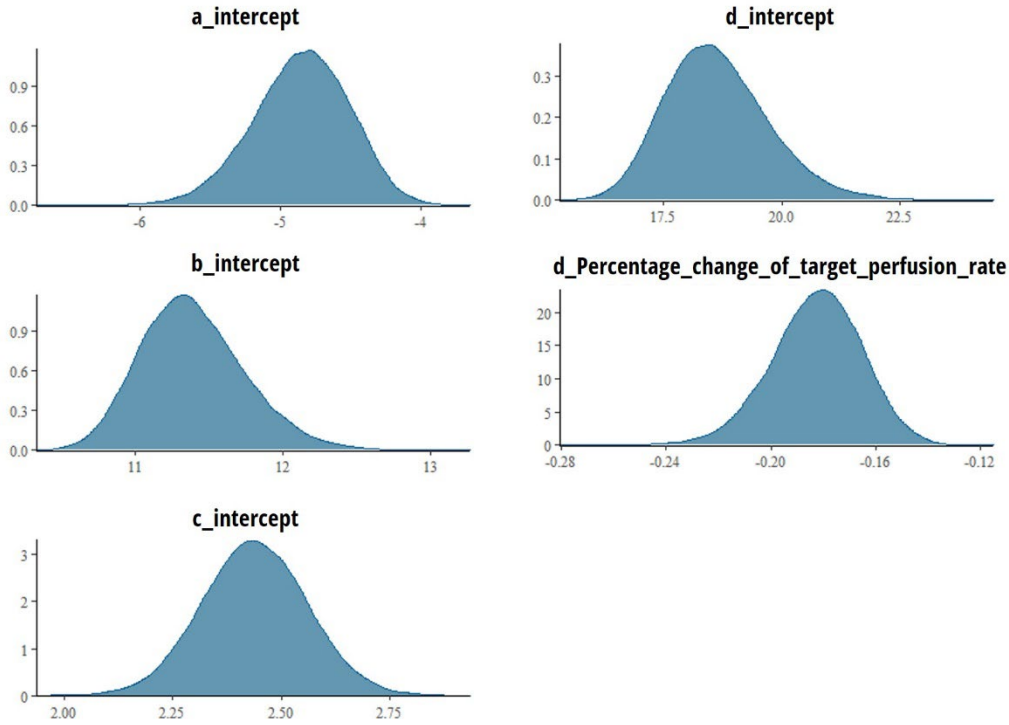


Figure 3.17. Posterior distribution of the model parameters for the Bayesian model for high mannose.

Based on the above posterior distributions of the model parameters, the posterior predictive distribution of the % High Mannose can be generated at different levels of percent change of target perfusion rate and as a function of culture day. This is illustrated in Figure 3.18. The effect of percent change of target perfusion rate is illustrated by the progressively higher predicted %High Mannose responses as the perfusion rate decreases from +20% of target to -20% of target perfusion rate. A related response was observed for the process performance attribute of viability (Figure 3.16) in that viability decreased with a decrease in perfusion rate. These two observations are consistent with each other and suggest that the target perfusion rate, and the associated acceptable range, in this example may not be fully optimal for control of the high mannose species.

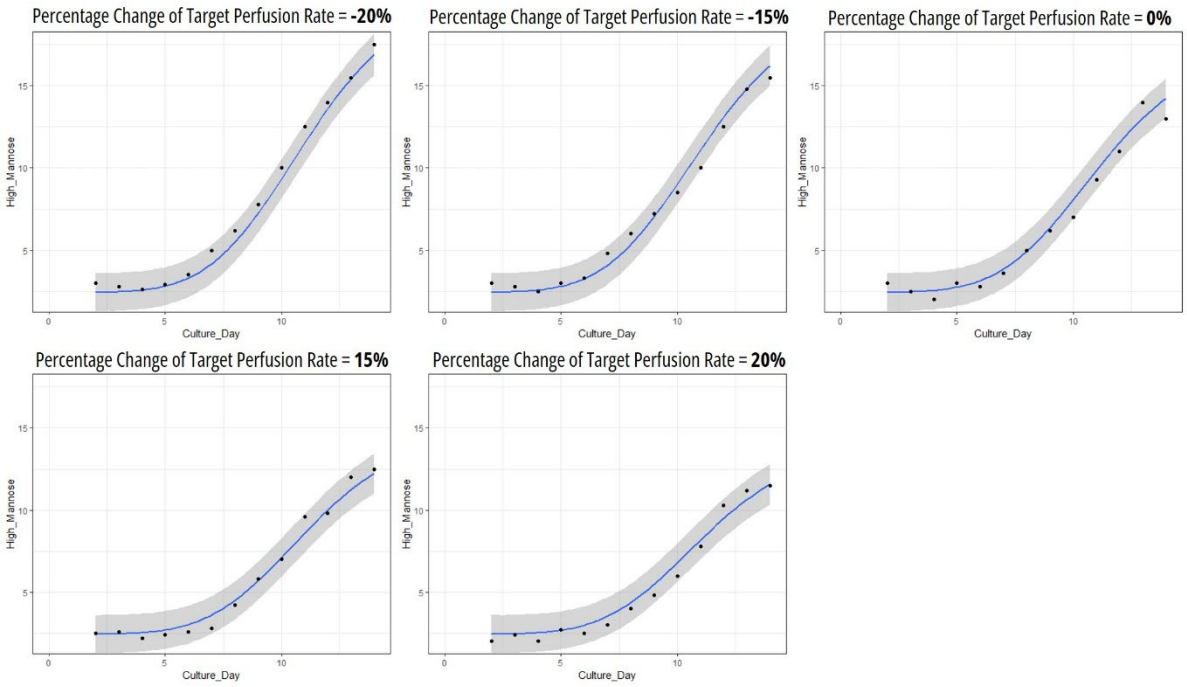


Figure 3.18. Impact of allowed variation of target perfusion rate and culture day on high mannose.

The final model can be used for development of the control strategy. For example, it could be used to determine with 95% probability that a percentage change in the target perfusion rate of -5.73% or more is required to ensure that the high mannose on Culture Day 14 is no more than 16%. The posterior predictive distribution in **Figure 3.19** displays the probability density plot of high mannose for this % change of target perfusion rate after 14 days culture.

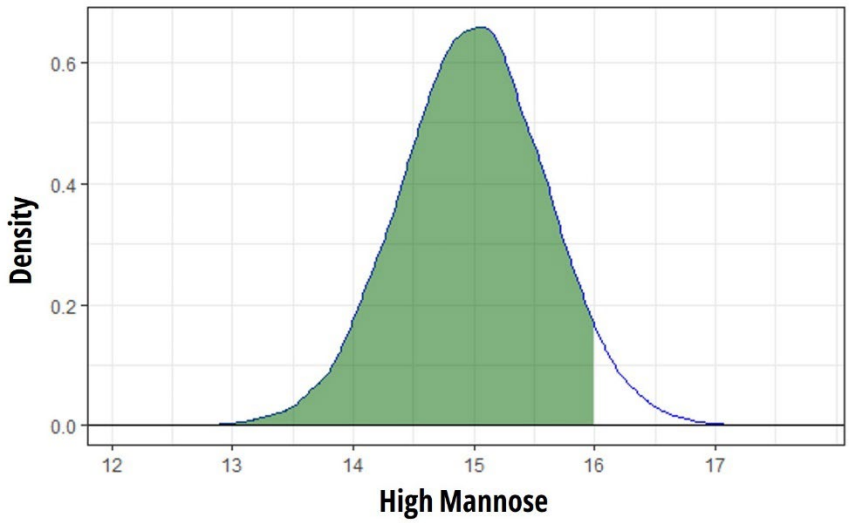


Figure 3.19. Bayesian probability density plot of high mannose on culture day 14. The green shaded area displays the probability of High Mannose ≤16 (95%).

3.4.5 Model confirmation and worst-case conditions evaluation

The expectation to complete RSM model confirmation is aligned with traditional fed-batch approaches. Generally, combinations of controlled parameters that are predicted to generate process or product quality effects on the edge of action limits for critical parameters are targeted to ensure sufficient predictive power of the RSM, as well as the appropriate setting of controlled parameter ranges to ensure that action limits are not exceeded.

In addition to this traditional expectation, the added complexity of the separated growth and steady-state RSMs may also be addressed through similar studies. Though separated in the RSMs to ensure that phase-specific responses are not impacted by the condition of the other phase, certain conditions may be of interest to study for their overall impact through the full production stage. For example, some RSM-controlled parameter conditions can result in substantial impacts on critical product quality attributes that are within action limits when applied to either phase alone. These conditions may further be studied to determine if there is an additive effect if the non-center point condition was in effect throughout both growth and steady-state phases. This type of study could be of particular interest for certain controlled parameters such as pH or temperature that would likely have an offset for the full batch duration due to calibration errors.

3.4.5.1 Worst-case condition recovery evaluation

Additional studies to be considered during process characterization are worst-case recovery studies. As stated above, conditions will be identified from the RSM that cause substantial impact to key or critical output parameters when applied for the full growth or steady-state phases. Though necessary to demonstrate an understanding of the design space and justification of the control strategy for continuous processing, the long process harvest durations also mean that controlled parameters may transiently exceed operational ranges but are unlikely to remain unmitigated for the entire batch duration. Therefore, the impact of transient excursions of worst-case controlled parameter combined conditions over several days, and the subsequent recovery profile, may provide valuable process understanding that can be built into a system for addressing real-time process excursions and establishing the required response strategy for harvest management. This is discussed in more detail in **Section 8.4.6**.

3.4.5.2 Sampling and analytical characterization testing

In order to properly characterize the impact on the continuous harvest stream, daily sampling and analytical characterization of product quality attributes is recommended. By studying the key outputs of titer and product quality daily during characterization, the process becomes well-understood, and sampling and testing frequency can therefore be minimized once the process is running in the clinical and commercial setting. A daily frequency of measured outputs is valuable to support a more thorough statistical analysis of the characterization responses. In addition, a daily frequency of measured outputs is informative to justifying lower frequency sampling in a commercial setting.

3.5 Downstream process characterization study design and execution

For illustrative purposes, the downstream process characterization section of N-mAb will focus on downstream integrated with a dynamic perfusion bioreactor and without breakthrough load control. Downstream process characterization of continuous processes leverages the classical approaches as applied to batch unit operations while also incorporating additional unique aspects resulting from continuously or connected downstream unit operations. The overall approach can be categorized into four activities as described in Table 3.17

Table 3.17. Downstream process characterization.

Activity	Considerations
1. Screening studies	• May be performed during development • Most likely can apply prior knowledge from pool-based process for unit operations in periodic stage* • May support removing screening studies once multiple program experience has been gained within a company's platform continuous process • Additional parameter studies to address output parameters from current step are inputs for subsequent steps
2. Process characterization studies	• Critical parameters listed in Table 3.3 and Table 3.4 to be studied
3. Model-based worst-case or linkage studies	• Model-based linkage studies • Model-based simulation at worst-case conditions
4. Experimental linkage studies using discrete or connected downstream unit operations	• Select subset of conditions to link to upstream (e.g., age of HCCF) in worst-case linkage studies • Optional study block where linkage studies using integrated and connected downstream unit operations to confirm the results from model-based simulation studies. As discussed in Section 3.3.1 (RBIA), leveraging breakthrough load control and, to a lesser extent, a steady-state perfusion bioreactor process simplifies process characterization by decreasing the width of ranges and number of parameters to be studied

* For additional guidance on prior knowledge from pool-based process characterization, see the A-Mab case study publication (CMC Biotech Working Group Emeryville, 2009).

HCCF Harvested cell culture fluid

3.5.1 Screening studies

Screening studies would largely follow a traditional pool-based strategy with the intention to determine the parameters within each downstream unit operation with the most significant effects on process and product quality parameters for evaluation in the full characterization studies. These studies may be designed as fractional factorial studies to limit the number of experiments necessary to study an expanded set of controlled parameters. Such a screening study would identify parameters with little or no process impact, which can then be eliminated from a full RSM DOE study. They may also be executed as OFAT studies to eliminate the parameters with no impact on product quality.

A summary of parameters tested based on the risk assessment discussed above and typical ranges evaluated for continuous downstream processes is presented in **Table 3.18**.

Due to the unique aspect of continuous downstream unit operations for the connected steps, additional parameters that may impact the process conditions in the preceding or subsequent steps are summarized in **Table 3.19** with the intent of gaining insight into the following considerations:

- The variability of material attributes in feed stock may impact the process performance, such as the age of the harvested cell culture fluid (HCCF)
- Unexpected pauses or loss of cycles could impact the overall process performance
- When adding a new step for the purpose of connecting downstream unit operations, such as in-line titration, the input parameters (e.g., pH and conductivity) are controlled indirectly as the output parameters from previous steps (e.g., titrant to product ratio). This needs to be documented as part of the overall risk assessment and resulting control strategy.

In the pool-based process, pH and conductivity can be controlled by multiple titrant additions followed by pH and conductivity measurements to verify that target conditions are met. In the continuous and connected operation, if pH and conductivity cannot be controlled directly for the process step that requires in-line conditioning, then it is necessary to rely on the control of flow rates to ensure that the target titrant-to-product ratio is delivered for either acidic or basic titrant. In some cases, the control can be enhanced by application of inline sensors and corresponding control algorithms to supplement flow control. The goal of the screening study is to understand how the titration ratio impacts pH and conductivity.

Table 3.18. Summary of downstream controlled parameters studied in process characterization.

Unit Ops	Downstream Controlled Parameters	Unit	Set Point	Operating Range	Characterization Range
Capture Chromatography (ProA)	Temperature	°C	20	15–25	15–25
	Loading/Wash Residence Time	min	6	5–7	4–8
	Loading (from Dynamic Perfusion)	g/L	50	10–55	5–60
	Elution pH	Units	3.5	3.4–3.6	3.3–3.7
	Elution conductivity	mS/cm	3.5	3–4	2–5
Virus Inactivation	low pH Target	units	3.5	3.4–3.6	3.3–3.7
	Incubation Duration	min	65	60–70	55–75
	Protein Concentration (no BT load ctrl) [1]	g/L	N/A	5–18	3–20
	Post-Incubation pH (no BT load ctrl)	units	7.0	6.8–7.2	6.5–7.5
Polishing Chromatography 1 (AEX-FT)	Loading (no ProA BT load ctrl)	g/L	200	120–220	100–250
	Load pH	units	7.0	6.8–7.2	6.5–7.5
	Load Conductivity	mS/cm	6	5–7	4–8
Polishing Chromatography 2 (CEX-B/E)	Loading (no ProA BT load ctrl)	g/L	35	25–45	20–50
	Load pH	units	7.0	6.8–7.2	6.5–7.5
	Load Conductivity	mS/cm	6	5–7	4–8
	Wash pH	units	7.4	7.3–7.5	7.2–7.6
	Elution pH	units	7.7	7.6–7.9	7.5–8.0

[1] The ranges evaluated would be narrower with BT load control

AEX Anion exchange
B/E Bind and Elute
BT Breakthrough
CEX Cation Exchange
FT Flowthrough
N/A Not applicable
ProA Protein A

Table 3.19. Additional controlled parameters to be studied in process characterization.

Downstream Controlled Parameter	Characterization Range	Purpose of Studies
Age of culture	Early, middle, and late days of culture	Assess the impact of material attributes in feed stock
Technical pause	Pause up to 30 min	Support deviation control strategy
Loss of cycles	Loss of 20% at beginning, 20% evenly distributed and 20% in the end	Support deviation control strategy
Acidic titrant-to-product ratio	For in-line titration +/-10%	Verify that process parameters are controlled by previous steps
Basic titrant-to-product ratio	For in-line titration +/-10%	Verify that process parameters are controlled by previous steps

3.5.2 Results of screening studies and process characterization

3.5.2.1 Capture chromatography (ProA)

The screening studies identified two parameters that showed no to minimal impact on the process performance and product quality, and these parameters are therefore excluded from further characterization study (Table 3.20).

Table 3.20. Summary of results for capture chromatography (Protein A) in screening study.

Controlled Parameters	Study Type for Screening Studies	Outcome of Studies
Temperature	OFAT	No impact on product quality observed
Technical pause duration (total time with no flow) during ProA loading as a result of perfusion pause	OFAT	No impact on product quality observed after total pause duration of up to 30 minutes

OFAT One factor at a time
ProA Protein A

Table 3.21. Summary of controlled parameters studied for process characterization of the ProA step.

Controlled Parameter	Study Type for Characterization
Age of HCCF	DOE RSM or special OFAT, see ***Table 3.18***
Loading/wash residence time	DOE RSM
Loading	DOE RSM followed by linkage study if needed
Elution pH	DOE RSM followed by linkage study if needed
Elution conductivity	DOE RSM
Loss of ProA cycles	Linkage study

DOE	Design of experiments	ProA	Protein A
HCCF	Harvested cell culture fluid	RSM	Response surface model
OFAT	One factor at a time		

The remaining controlled parameters, summarized in Table 3.21, are subsequently studied by a discrete scale-down model following the strategy described in Section 2.5.1

The linkage study for both ProA load (from dynamic perfusion, which represents the greatest variability) and elution pH is designed to address the output parameters from ProA that may have a potential impact on the input parameters of the subsequent step, viral inactivation. More specifically, if the ProA load from the dynamic perfusion cell culture (Figure 3.20) is based on continuous fixed volume loading, the load density is not actively controlled but can be monitored as a process output. Both high and low load may impact the product concentration in the Protein A pool and could subsequently impact the pH condition at the next step (continuous viral inactivation) if pH conditioning is performed by fixed ratio titration. A linkage study may be performed based on the results if necessary.

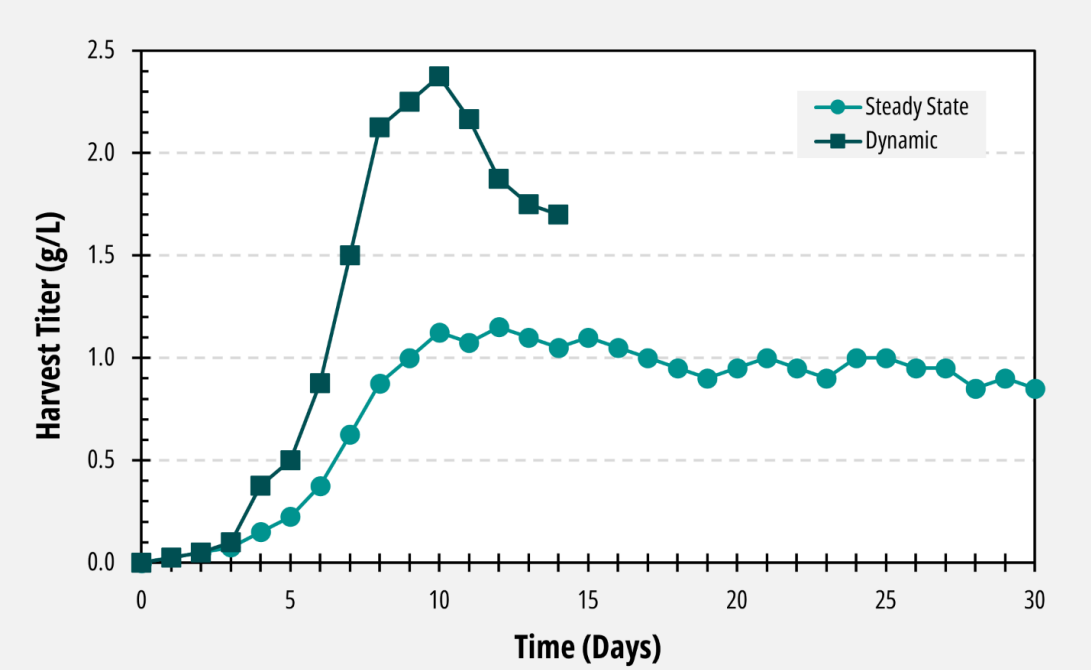


Figure 3.20. Harvest titer as a function of time for dynamic (squares) and steady-state perfusion process (circles).

As with the high or low column load scenario described above, low or high elution pH may lead to a high variability of pH in the Protein A elution pool and could potentially impact the pH of the next step (continuous viral inactivation). The pH conditions are monitored as a process output. The failure mode and effect along with an experimental design to study the age of HCCF are summarized in Table 3.22. A linkage study may be performed based on the results.

Table 3.22. Failure mode and effect and experimental design to study the age of HCCF.

Parameter	Failure Mode and Effect	Experiment Design
Age of HCCF (Dynamic perfusion process only)	**Day 1**: lowest titer and possible highest ratio of impurity (i.e., HCP) to product **Day 8**: highest cell density and highest titer, thus possible highest Protein A load and highest product concentration in Protein A eluate, which may cause high levels of HMWS and impact product concentration, pH, and conductivity **Day 10**: lowest cell viability may cause highest level of impurities	**Design 1**: the age of HCCF is one of the inputs in DOE study **Design 2**: perform the rest of DOE or OFAT three times using the load material from D1, D8, and D10,

DOE Design of experiments
HCCF Harvested cell culture fluid
HCP Host cell protein
HMWS High molecular weight species
OFAT One factor at a time

Development studies have demonstrated that the product quality in harvest samples varies only slightly (e.g., 0.5–1.5% HMWS) for steady-state perfusion, and more significantly (e.g., 1.0–3.2% HMWS) for dynamic perfusion over the course of bioreactor duration as shown in

Figure 3.21.

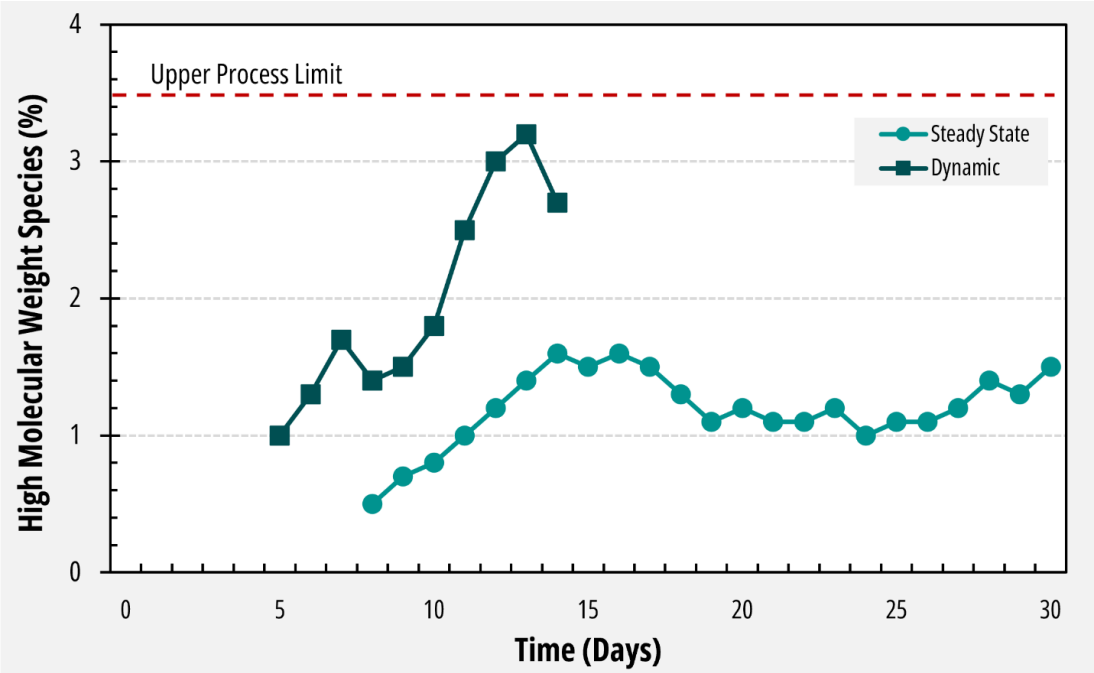


Figure 3.21. High molecular weight species (HMWS) as a function of time for dynamic (squares) and steady-state (circles) perfusion process.

Experimental Design 1 (**Table 3.22**) is used to study the age of HCCF by considering the age of HCCF as one of the input parameters in the multivariate DOE study design. Load material was obtained from Day 1, 8, and 10 of a 100 L or 500 L scale run (or a representative scale). If HCCFs from multiple bioreactors are combined prior to loading onto the Protein A column, the load material for PC studies should be representative of multiple bioreactors, and sufficient justification should be provided for this approach. Product quality outputs of the process characterization experiments were HMWS and HCP. In addition, DNA and Protein A levels were monitored throughout the process characterization studies.

Statistical analysis by commonly used software, such as JMP, was performed after the DOE study. Statistically significant effects with $p < 0.05$ were detected, and a predictive model was developed for step yield, product concentration, pH of Protein A pool, conductivity of Protein A pool, HMWS, and HCP. The multivariate experimental design revealed the following parameter impacts on step performance and product quality:

- % HMWS is impacted by the age of HCCF. A linkage study is performed (see **Section 3.6.1**)
- HCP levels are impacted by protein load but not by the age of HCCF
- % HMWS is impacted by protein load and elution pH. A significant interaction between protein load and elution pH is identified.
- Product concentrations are impacted by protein load. A linkage study is performed (see **Section 3.6.3**)
- The pH of the Protein A pool is impacted by elution pH. A linkage study is performed (see **Section 3.6.3**)
- The following parameters do not have statistically significant impacts on the step performance and product quality. No further study is needed.
    - Yield
    - Conductivity of Protein A pool
    - Residence time or column bed height

In addition to the results of the multivariate study given above, the univariate studies of technical pause at both worst-case and center point conditions showed no significant impact on performance or product quality across the range tested.

3.5.2.2 Virus inactivation step

Based on the results of screening studies, the parameters shown in **Table 3.23** have shown no to minimal impact on process performance and product quality, and they are therefore excluded from further characterization study.

Table 3.23. Summary of results for viral inactivation step in screening study.

Controlled Parameters	Study Type for Screening Studies	Outcome of Studies
Protein concentration (no BT load ctrl)	OFAT	No impact on product quality observed
Post-incubation pH (no BT load ctrl)	OFAT	No impact on product quality observed
Acidic titrant-to-product ratio +/- 10%	OFAT to study pH as an output of titration	pH after the first in-line titration prior to continuous virus inactivation is well within characterization range (pH 3.3–pH 3.7); thus, no additional study is needed
Basic titrant-to-product ratio +/- 10%	OFAT to study pH as an output of titration	pH after the second in-line titration after continuous virus inactivation is well within characterization range (pH 6.5–7.5); thus, no additional study is needed

BT Breakthrough OFAT One factor at a time

The second in-line conditioning step will likely involve a titration by basic solution and salt. The pH variability is impacted by the first in-line conditioning step. The screening study uses the load at center point condition and performs a linkage study to address the variability of the load. The controlled parameters to be studied during process characterization are summarized in **Table 3.24**.

Table 3.24. Summary of controlled parameters studied for viral inactivation step in process characterization.

Controlled Parameter	Study Type for Characterization
Low pH target	Worst case
Incubation duration	Worst case
Loss of cycles	Linkage
pH prior to 2nd in-line condition	Linkage

3.5.2.3 Polishing Chromatography 1

Based on the results of screening studies, the parameters in **Table 3.25** have shown no to minimal impact on the process performance and product quality, and they are therefore excluded from further characterization study. The in-line conditioning step will likely involve a titration by acidic solution and dilution by water for injection (WFI). The addition process for the titrant needs to be carefully designed, and the pumps used to deliver the flow rate need to be in the well-calibrated dynamic range.

Table 3.25. Summary of results for polishing chromatography 1 step screening study.

Controlled Parameter	Study Type for Screening Studies	Outcome of Studies
Total duration of Technical Pause up to 30 minutes	OFAT	No impact on product quality
Acidic and WFI titrant-to-product ratio +/- 10%	OFAT to study pH and conductivity as output parameters of in-line condition step	Both pH and conductivity after in-line titration are well within characterization range (pH 6.5–7.5, conductivity 6–8 mS/cm); thus, no additional study is needed

OFAT One factor at a time
WFI Water for injection

After screening studies, the controlled parameters to be studied during process characterization are summarized in Table 3.26.

Table 3.26. Summary of controlled parameters studied for polishing chromatography 1 step in process characterization.

Controlled Parameter	Study Type for Characterization
Loading (no ProA BT load ctrl)	DOE RSM
Load pH	DOE RSM
Load conductivity	DOE RSM
Loss of cycles	Linkage
pH prior to in-line conditioning	Linkage
Conductivity prior to in-line conditioning	Linkage

BT Breakthrough
DOE Design of experiments
RSM Response surface model

3.5.2.4 Polishing Chromatography 2

Based on the results of screening studies, the parameters listed in Table 3.27 have shown no to minimal impact on the process performance and product quality, and they are therefore excluded from further characterization study.

Table 3.27. Summary of results for polishing chromatography 2 step in screening study.

Controlled Parameter	Study Type for Screening Studies	Outcome of Studies
Loading (no ProA BT load ctrl)	OFAT	No impact on product quality observed
Total duration of Technical Pause up to 2 hours	OFAT	No impact on product quality observed
Acidic and salt titrant-to-product ratio +/- 10%	OFAT to study pH and conductivity as output parameters of in-line conditioning step	Both pH and conductivity after in-line titration are well within characterization range (pH 6.5–7.5, conductivity 4–8 mS/cm); thus, no additional study is needed

BT Breakthrough
OFAT One factor at a time
ProA Protein A

The in-line conditioning step will likely involve a titration by the acidic solution and a salt addition as needed. The variability of pH and conductivity in the load is impacted by the preceding step. The screening study can use the load at center point conditions, and a linkage study can be performed to assess the potential impact due to the variability of the load. The controlled parameters to be studied during process characterization are summarized in Table 3.28

Table 3.28. Summary of controlled parameters Studied for polishing chromatography 2 step in process characterization.

Controlled Parameter	Study Type for Characterization
Load pH	DOE RSM
Load conductivity	DOE RSM
Wash pH	DOE RSM
Elution pH	DOE RSM
Loss of cycles	Linkage
pH prior to in-line condition	Linkage
Conductivity prior to in-line condition	Linkage

DOE Design of experiments
RSM Response surface model

3.5.2.5 Analysis of DOE RSM data for non-ProA steps

DOE RSM studies for non-ProA steps are performed for each unit operation separately using a batch mode scale-down model as described in Section 2.5.1. The controlled parameters that are studied are well aligned with those used in the A-Mab case study. The methodology of statistical data analysis and the results from DOE RSM studies are also expected to be the same or similar as in A-Mab.

3.6 Worst-case linkage studies

Batch processes are defined by a series of batch unit operations, which each process the entire batch of material in a specific order. In contrast, processes designed with unit operations that are integrated without a batch pool vessel between each unit operation and have multiple unit operations run continuously introduce certain special considerations. After all DOE RSM studies are completed, the following linkage studies are performed, either by process model or experimentally.

3.6.1 Linkage Study 1 to address the impact of the age of HCCF

As described in Section 3.5.2.1, results from DOE RSM studies show that the age of HCCF impacts % HMWS in the ProA pool. The worst-case HCCF sample (Day 14 +/-1 day) with %HMWS at 3.2% is processed by viral inactivation, Polishing Chromatography 1 and Polishing Chromatography 2 under relevant worst-case conditions, such as the lowest pH during viral inactivation. The result confirms that %HMWS in the product of Polishing Chromatography 2 is at an acceptable level. This approach is similar to that traditionally used for a pool-based process, and it can be conducted by a discrete scale down model and one step at a time.

3.6.2 Model-based Linkage Study 2 to address the loss of cycles

Because the product quality varies as a function of cell culture duration, the loss of product cycles can ultimately impact the product quality in the combined pool, but the impact is predictable. Nonetheless, an evaluation of any lost cycles should be performed as part of the Quality Management System (see Section 8.5). Failure mode assessment is performed to evaluate how final product quality attributes, such as % HMWS, which varies over the bioreactor duration as shown in Figure 3.21 might be affected by the loss of cycles during a run. The cycles can be discarded at the beginning or the end of a run, or the losses can be evenly distributed across the run. The total quantities discarded represent up to 20% of the total.

Table 3.29 shows the predicted % HMWS under the worst-case conditions, which are that 20% of the total is discarded, either all at the beginning or at the end, or that the losses are evenly distributed. The results from Study 1, which showed that HWMS levels as high as 3.2% could be adequately removed by the downstream process under worst case conditions, indicate that loss of 20% of the batch due to cycle discards would be acceptable.

Table 3.29. Predicted % high molecular weight species (HMWS) as a result of material loss due to operations or other issues.

Loss of Cycles	% High Molecular Weight Species	
	Steady-State	Dynamic
Range	0.5–1.6%	1.0–3.2%
0%	1.2%	2.1%
20% all at the beginning	1.3%	2.5%
20% all at the end	1.2%	1.8%
20% evenly distributed	1.2%	2.0%

3.6.3 Model-based Linkage Study 3 to address outputs from a previous step as inputs for the next steps

Based on the results from the studies in Section 3.5.2.1, both protein concentration and elution pH will impact the pH in the ProA pool. Under the worst-case conditions, the pH in the ProA pool is in the range of 3.4 – 3.6. The pH adjustment is done via in-line conditioning; therefore, the pH in the acidified pool is passively controlled by the acidic titrant-to-product ratio. Under the worst-case conditions, the anticipated pH in the acidified pool is in the range of 3.3–3.7, which is still within the range studied and determined to be acceptable during process characterization. The impact on the subsequent two steps is assessed by following a similar strategy, as illustrated in Figure 3.22. Model-based linkage study.

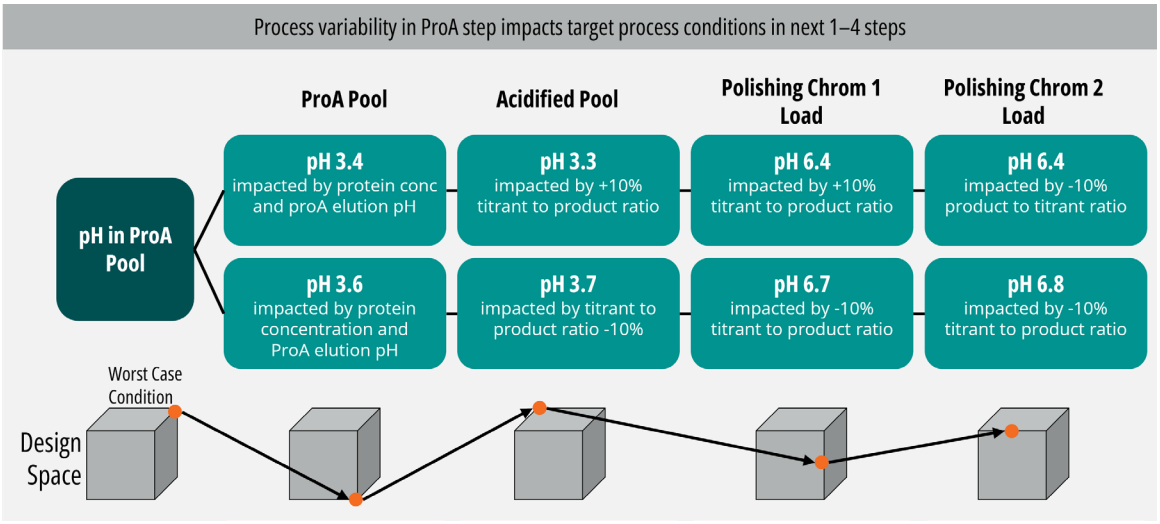


Figure 3.22. Model-based linkage study.

3.6.4 Experiment-based linkage study

As illustrated in Sections 3.6.2 and 3.6.3, the results from the model prediction have been confirmed, and they showed acceptable performance in all cases. The experiment with the connected downstream unit operations is not necessary, because the results from the model can be confirmed by running a full-scale connected system while deliberately running the process at the worst-case conditions.

3.6.5 Creating integrated data structures to support clinical manufacturing and PPQ

It is important to establish a solid understanding of the relationship between variation in process parameters or material attributes and product quality attributes as it evolves. It is often useful to begin building a process information database along the lines of Table 3.30. Note that this represents a summary of some examples of controlled parameters and material attributes from Table 3.8 and Table 3.18. Initial results from PC studies can then be used to supplement platform knowledge in the selection of target ranges for initial clinical manufacturing runs. Additional data

from PC studies along with data from clinical and validation runs can then be added to create a useful reference for managing decisions in the commercial process.

Table 3.30. Summary table for process parameters and material attributes.

Process Parameter or Material Attribute	Experience Range in PD/PC	Proposed Range in Clinical Mfg
Bioreactor		
Growth Phase pH	6.9–7.3	7.0–7.2
Growth Phase Temperature (^{0}C)	34.5–37.5	35.5–36.5
Growth Phase Perfusion Rate (CSPR, nL/cell/day)	0.04–0.06	0.045–0.055
Production Phase Shift Timing (day) [A]	5–7	5.5–6.5
Production Phase pH	6.9–7.3	7.0–7.2
Production Phase Temperature (^{0}C) [A]	31.5–34.5	32.5–33.5
Production Phase Perfusion Rate (vvd) [A]	1.6–2.4	1.9–2.3
Dissolved Oxygen (%sat)	20–80	40–60
Loading/Wash Residence Time (min)	4–8	5–7
Loading (from Dynamic Perfusion)	5–60	10–55
Elution Buffer pH [B]	3.3–3.7	3.4–3.6
Elution Buffer Conductivity (mS/cm) [B]	2–5	3–4
Viral Inactivation		
low pH Target [C]	3.3–3.7	3.4–3.6
Incubation Duration (mins) [C]	55–75	60–70
Protein Conc (no BT load ctrl) (g/L)	3–20	5–18
Post-Incubation pH (no BT load ctrl)	6.5–7.5	6.8–7.2
Loading (no ProA BT load ctrl) (g/L)	100–250	120–220
Load pH	6.5–7.5	6.8–7.2
Load Conductivity (mS/cm)	4–8	5–7
Loading (no ProA BT load ctrl) (g/L)	20–50	25–45
Load pH	6.5–7.5	6.8–7.2
Load Conductivity (mS/cm)	4–8	5–7
Wash pH [B]	7.2–7.6	7.3–7.5
Elution pH [B]	7.5–8.0	7.5–8.0

[A] Both temperature setpoint and perfusion rate change with this shift timing parameter
[B] Controlled as a material attribute of the buffer solution
[C] ASTM E2888-12 Standard Practice for Process for Inactivation of Rodent Retrovirus by pH (2019)

AEX Anion exchange
B/E Bind and elute
CEX Cation exchange
CSPR Cell-specific perfusion rate
FT Flowthrough
HMWS High molecular weight species
PAR Proven acceptable range
PC Process characterization
PD Process development
PPQ Process performance qualification

3.7 Viral clearance validation at small scale

3.7.1 Viral clearance during continuous capture

The continuous capture step is anticipated to enable rapid cycling with smaller Protein A columns, resulting in cost reduction and increased productivity, among other potential benefits. However, if the continuous capture is claimed as a viral clearance step, the increased cycling frequency may result in a significant increase in the number of critical control parameters that must be monitored. If sufficient overall viral clearance can be demonstrated with the remaining downstream process, then manufacturers may choose not to claim viral clearance for the continuous capture step.

If viral clearance must be claimed for the capture step, small-scale continuous capture systems may present challenges if they are utilized as a scale-down model for virus clearance studies. Continuous capture systems require a more complex flow path to enable multiple column operations, and they also include additional column phases, such as startup, steady-state, and shutdown, during which column operations may differ. Additionally, the appropriate small-scale system may not be available if an external contract research organization is used to perform the viral clearance study especially when considering the wide variety of continuous capture systems. Two approaches have been described in the literature to address these concerns, as summarized below.

As most continuous capture systems leverage two columns in series in the load phase, a modified flow path with a batch chromatography system may be utilized to mimic the load phase from the continuous capture system. Viral clearance studies were performed with both the continuous capture system and the surrogate model. Equivalent virus removal was achieved with both systems for both enveloped and non-enveloped viruses. Additionally, it was demonstrated that resin age and overloading of the columns did not have a significant impact on virus removal (Angelo et al., 2019, Angelo et al., 2021).

The observations from these studies support a potentially simpler approach, in which a single-column batch chromatography was demonstrated to provide similar viral clearance to a continuous capture system in a direct comparison. This work evaluated how the modification of chromatographic parameters, including the linear velocity and resin capacity utilization, could impact virus clearance as a result of moving from a single column to a multi-column operation. A DOE approach was applied to two model monoclonal antibodies, and two bacteriophages were used as mammalian virus surrogates. Under the conditions tested in the DOE, single-column and multi-column modes yielded similar log reduction values (LRV). The parameters identified as impactful for viral clearance in single-column mode were predictive of multi-column modes. Thus, these results support the hypothesis that the viral clearance capabilities of a multi-column continuous Protein A system may be evaluated using an appropriately scaled-down single column and associated equipment (Chiang et al., 2019).

3.7.2 Viral inactivation

Within a batch process, viral inactivation may be achieved in two ways: by exposure to detergent, typically introduced after the harvest unit operation, or exposure to low pH, typically immediately after the capture step. Virus inactivation may be performed in hold tanks, and multiple hold tanks may be used to minimize disruptions in the process flow. Operationally, each hold tank is similar to batch processing, and therefore batch scale-down models can be used. Alternatively, a continuous

incubation chamber may be used. Continuous incubation chambers pose unique engineering challenges, which are discussed in more detail below.

3.7.3 Viral clearance during polishing chromatography

The N-mAb downstream process typically consists of one or two polishing chromatography steps, which are either operated in bind/elute mode or flowthrough mode. If no prior unit operation can influence the polishing step dynamically, the overall viral validation approach to the polishing step is identical to that for a batch process.

In contrast, if the polishing step can be dynamically influenced by its prior unit operation, the viral validation approach can become more operationally challenging. In this scenario, product concentration and conductivity/pH both change during loading onto the polishing step. To mimic this configuration during the viral clearance study, two bench-scale unit operations with a surge vessel in between and an inline virus spiking configuration may be needed. This set-up greatly challenges and complicates the viral clearance study.

Alternatively, a discrete scale-down model of this polishing step can be operated as a stand-alone step for the viral clearance study. In this model, the homogenous load to the polishing step represents the worst-case condition possibly produced by the prior step. This approach is feasible only if the worst-case conditions can be identified by bracketing experiments covering the range of expected dynamic conditions. This approach does not require simultaneous operation of the prior unit operation or inline virus spiking in a viral clearance lab. The worst-case condition can be identified in advance by prior knowledge or by running an additional discrete viral clearance study. When multiple columns are used within a polishing step, each column can be treated as if a single column is employed because the flowthrough from the first column is not loaded onto the second.

3.7.4 Viral clearance by nanofiltration

The integration design for the N-mAb framework architectures has been explicitly chosen to mitigate potential impacts on viral filtration due to feed stream heterogeneity, such as that due to elution from bind and elute chromatography, via the inclusion of cycle surge tanks. Accordingly, traditional approaches to viral clearance validation for viral filtration should apply directly to integrated processing. Adaptations to traditional study design to accommodate for longer pause durations and/or lower volumetric fluxes may be incorporated. In the absence of a cycle surge tank, likely impact due to feed stream heterogeneity will need to be studied and, if necessary, mitigated. Some of the resulting considerations are described herein. A continuous process is expected to result in a virus filter inlet stream with well-controlled but variable product concentration, buffer composition, and flow rate or pressure, especially if the preceding step is operated in bind and elute mode. This variability in the feed stream is a challenge to simulate during viral clearance studies. To overcome this challenge, the validation of the virus filter may be performed while it is directly connected to a bench-scale chromatography system using an in-line virus injection method, thus enabling the most representative feed conditions for the virus filter. However, it is expected to be more practical or feasible to validate the virus filter as a stand-alone step. Studies with non-infectious virus-like particles may help elucidate the worst-case conditions for virus retention to be evaluated during a stand-alone virus filtration validation study. Feed concentrations from 6 to 50 g/L have been demonstrated to impact the flux and capacity of the Viresolve Pro (Millipore-Sigma), while still

delivering consistent virus removal across this range of product concentrations (Bohonak et al., 2021), and similar performance results have been obtained for the Planova 20N and BioEX (Asahi) nanofilters (Lute et al., 2020). Furthermore, the extended duration of the virus filtration step in continuous mode cannot be easily reproduced in a viral clearance lab for two main reasons: the lack of stability of some viruses used for spiking and the formation of non-representative foulants over time in the spiked starting material, which may result in filter fouling that is not typical of manufacturing. To mitigate these concerns, Bohonak et al., (2021) and Lute et al., (2020) have outlined strategies for viral validation studies lasting up to 4 days with the introduction of freshly spiked load material every 12 or 24 hours or with the in-line injection of virus-spiked buffer. However, this mode of operation requires a more complex testing strategy to demonstrate that the virus filter is challenged with a consistent viral titer throughout the trial. The minute virus of mice (MVM) may be studied as a single worst-case condition due to the small size of the virus, and the same log reduction value (LRV) may be applied to other model viruses when determining the overall clearance capability of the downstream process (Gefroh et al., 2014). The stability of MVM compared to other model viruses may allow for a single spike of virus, resulting in a simpler scale-down model for continuous virus filtration; however, this possibility would need to be evaluated on a case-by case basis.

Chapter 4

At-Scale Performance Demonstration

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

4 At-Scale Performance Demonstration

4.1 Overview

Operation of the process at the proposed full scale may be considered as an integrated part of process development, and certain sensitivities are easier to observe at full scale. Some benefits of moving to full scale could include:

- A decrease in the number of process performance qualification (PPQ) runs if sufficient experience at full scale is obtained for the same process. Experience may be obtained through full-scale campaigns in either the clinical GMP or non-GMP (pilot) settings
- More effective demonstration of integration, automation, process analytical technology (PAT) control elements, and responses to perturbations or disturbances
- The need for a fully integrated bench-scale model is reduced

Critical supporting information for a performance demonstration includes similar (or the same) batch records as will be used for manufacturing, preliminary failure mode and effects analysis (pFMEA) risk assessments, and any other documentation identified by individual manufacturing organizations. Scenarios may include running the performance demonstration at full scale in the same facility as for manufacturing or in a different facility, as well as under cGMP or non-cGMP conditions in either facility. Several categories of data and studies should be considered:

- Intermediate hold time evaluations if applicable, especially related to microbial clean hold times for any hold tanks with an expected hold duration of longer than 24 hours
- Preparation of end-of-production cell bank(s) (EOPCB) for evaluation in support of the limit of *in vitro* cell age for production (LIVCA); characterization of the EOPCB is typically performed as part of Stage 2 PV, but it may be performed earlier as well to provide additional process-related data
- Testing for quantitation of retroviral-like particles (RVLPs) for endogenous virus load including at different harvest timepoints across the bioreactor duration, where applicable
- Assessing process, control, and equipment similarity as part of a facility fit risk assessment including engineering analyses, rationale, data as needed) to assure functional equivalence
- In the future, it is anticipated that plug and play capabilities for self-identifying and qualifying instruments and probes will also be enabled by continuous processing

4.2 Decisions on process options and scenarios

For the purposes of continued illustration of the case study, a more limited set of process options is considered in this section. A steady-state perfusion mode of operation for the bioreactor was chosen as preferable for the following reasons.

The results from the bioreactor scale-down model (SDM) in both dynamic and steady-state perfusion modes of operation indicated that for high mannose species were not well controlled over time in the dynamic perfusion studies (**Figure 3.16**). Aggregation was reasonably well controlled in the steady state perfusion SDM. Total deamidation was also well controlled in the steady state perfusion

SDM and resembled the less variable profile observed in Figure 2.3. Detailed peptide mapping analysis revealed that more than 90% of the total deamidation was due to deamidation at Asn325. Additional results from stress and accelerated stability studies indicated that Asn325 deamidation was 10-fold lower at pH 5 than at pH 7 (0.02%/day at pH 5 vs 0.2%/day at pH 7), so limiting hold times downstream at pH 7 is beneficial to the overall control. For example, even if the hold time at pH 7 after either virus inactivation (VI) or post UF/DF was 10 days, the expected increase in Asn325 deamidation would be approximately 2%. Also, due to a lower retention time of the product molecule in the bioreactor and during harvest compared with a typical fed-batch process, the observed Asn325 deamidation level after Protein A was ~0.5%. Therefore, avoiding an in-process hold allowed the anticipated level of Asn325 deamidation to be less than 1% in the drug substance (DS), and this was confirmed during early clinical runs. With a 1:1 impact on bioactivity (1% Asn325 deamidation = 1% loss in antibody-dependent cell-mediated cytotoxicity (ADCC) activity), the analytical testing plan for Asn325 was that release testing would be continued through the PPQ runs for verification of control and then discontinued because it could be considered as not clinically relevant. High mannose species potentially could have been controlled through the use of a batch pool tank after the viral inactivation step or at the final drug substance pooling step but uncertainty about the exact nature of potential nutrient limitation in this scenario, and thus risk around control over time, and the potential for increased levels of deamidated product-related impurities swung the decision to a process with the characteristics of Option 1 previously described in Table 1.3.

The bioreactor SDM performance results were confirmed during early clinical manufacturing. the performance of initial clinical runs was similar to what had been observed at reduced scale in terms of magnitude and overall pattern of variance (Figure 4.1).

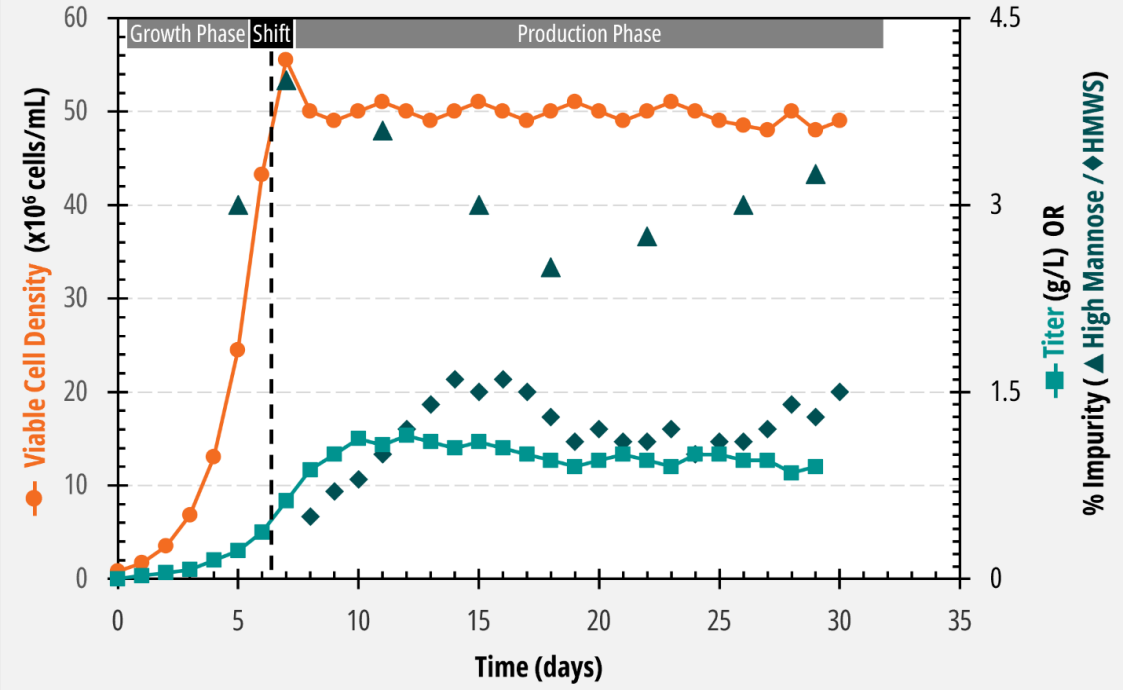


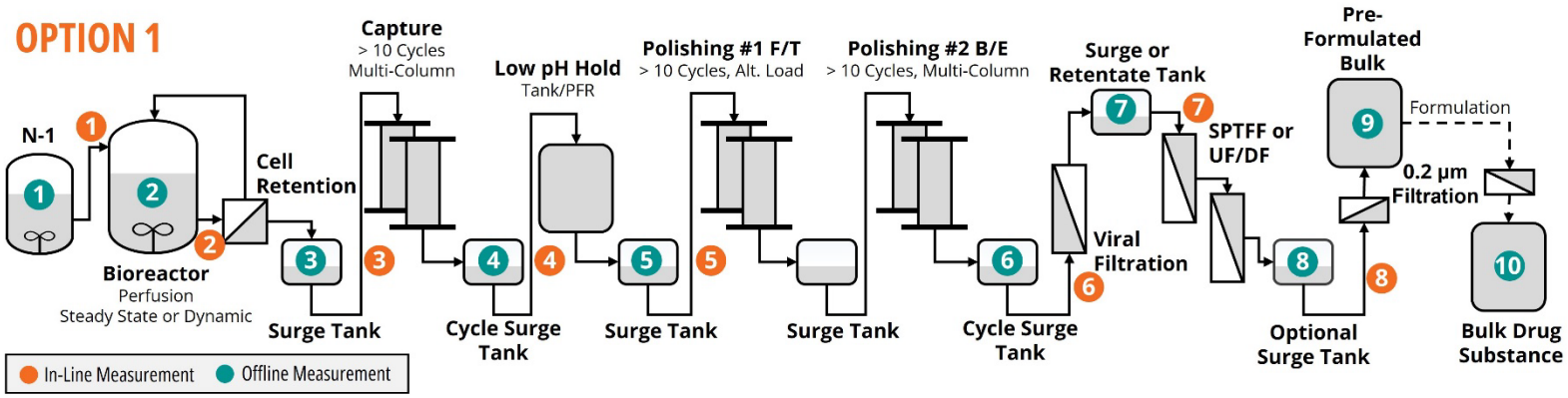
Figure 4.1. Example data from initial clinical run at full scale.

As for the details of the downstream process decisions related to unit operation sizing and cycling operation within Option 1, one could design the sizing approach according to (at least) two approaches:

1. Execute a more traditional batch philosophy in a more automated way, leveraging PAT and other techniques for enhanced robustness in the control strategy. This will lead to a more traditional approach, but will also lead to larger intermediate vessels, larger columns, and therefore fewer cycles on a column per run. This is also closer to the Option 2 downstream periodic cycling operation described earlier (**Table 1.3**).
2. Leverage the availability of PAT tools for load control (like deltaUV) to ensure consistency in column loading, particularly for the capture step, in the case of fluctuations in bioreactor titer over time. This then becomes a mixed sizing and scheduling problem driven by volume and mass fluxes allowing one to be more efficient in surge vessel and column sizing. Minimization of surge vessels will lead to a consistent, low mass and volume flux between unit operations, although it may be necessary to implement less traditional flowrate regimes and/or column bed heights to maintain continuous flow, particularly for polishing chromatography.

The discussion in **Chapters 2** and **3** has provided operational details in support of these types of decisions. A variation on the second option above for the downstream design could be to allow the ProA column size float, product to product, according to mass and/or volume flux and scheduling considerations (Godawat et al., 2012; Löfgren et al., 2021), utilize BT load control for the ProA column to optimize the process to that point and then employ a more generic sizing approach for the AEX and CEX columns because the volume reduction post-ProA and lower cost of non-affinity resins translates to a more straightforward (and less optimized or customized) design at that point. This has the added benefit of being generalizable across our two options presented in this N-mAb case study and could be more suitable for a plant designed to handle a range of products and bioreactor productivities as opposed to a bespoke design for a single product. The downstream process design for this case study will follow this latter approach to generate a detailed view of how the control strategy for manufacturing registrational material and then PPQ would develop.

Additional data to support these decisions were generated from additional small-scale studies as well as from full-scale operation in both non-cGMP and cGMP clinical manufacturing runs using a sampling plan as shown in **Figure 4.2**. At this point in the evaluation of the process at scale, it is important to collect sufficient data around the various unit operations over time to ensure that the expected performance predicted from work with SDM has been realized as well as to establish a reasonable set of acceptance criteria for Stage 2 PV or PPQ. It is also important to collect additional data to support microbial control by the process, especially for systems with complex automation or valving. Note that, in **Figure 4.2**, the color of the sample points reflects either an in-line measurement (orange) or a sample point for offline testing of either process performance or quality attributes (teal). Note that the column labeled "Elapsed Time" refers roughly to the procession of a specific aliquot of material as it traverses the process and is based on the details in **Figure 2.13**. The overall elapsed time is obviously process-specific, but this column has been included as a reminder that the sampling, testing, and possible reaction to a result all occur in real time as the process continues.



Sample Point	Process Step	Elapsed Time (h)	Capacitance	Inline A280	Fluid Mass Flow	pH	Conductivity	VCD/Viability	Metabolites	Titer/A280	Glycosylation	Deamidation	HMWS	HCP	Initial Risk (of variance)	Sampling Frequency: Initial Runs
			Performance Attribute							Quality Attribute						
1	N-1		•					•							Low	Last day
2	Bioreactor		•					•	•						High	Every 2d after inoculation
			•							•	•	•	•			Every 4d after start of perfusion
3	Post-ATF/TFF Permeate Surge Tank	0			•					•					Low	1. Prior to 1st capture cycle 2. Once every 24h 3. Prior to last capture cycle
4	Post-ProA Surge Tank	8		•	•	•	•			•		•	•		Medium	1. Prior to 1st capture cycle 2. Twice weekly, after cycle homogenization 3. Prior to last capture cycle
5	Post-VI Surge Tank	12		•	•	•	•			•		•	•		Low	1. After 1st cycle 2. After last cycle
6	Post-Polishing Chrom Pre-VF Surge Tank	14		•	•	•	•			•		•	•		Low	1. After 1st cycle 2. Sample from the surge tank corresponding to each lot of planned DS 3. After last cycle
7	Post-VF, Pre-UFDF Surge	16		•	•	•	•			•					Low	Every 24h
8	Post-UFDF, Pre-0.2 µm	18		•	•	•	•			•					Low	Every 24 h
9	Post-UFDF, Post-0.2 µm filter	18								•					Low	Representative sample of every bag filled
10	Bulk DS									•	•	•	•	•	Low	Representative sample of every bag filled

Figure 4.2. **Analytical sampling and testing plan for initial clinical runs through PPQ.**

4.3 Operational challenges – general considerations

Initial runs at the intended manufacturing scale can provide important information about certain elements of process performance that are difficult to assess through the use of scale-down processes. These elements include the following:

- Management of start-up, shut-down, and response to any transient changes, especially in the production bioreactor. This should include but not necessarily be limited to a comparison of the transient behaviors of process performance attributes (e.g., VCD) as well as product quality attributes (e.g., glycosylation).
- Assessment of the performance of overall mass flow management of the continuous system as a whole as it relates to process flowrates, surge tank sizing, and the potential for variance associated with certain unit operations. It is difficult to fully assess this aspect of the integrated system at small scale due the large impact of sample volumes on the overall mass flow.
- In all cases, it is essential that the primary in-line process sensors are robust, reliable, and accurate over the expected run time of the process and the likely range of the parameters to be measured. Specific considerations, as detailed in ASTM-E2968-14 (2016), should be given to:
    - The long-term effects of fouling and buildup of product on a sensor
    - The effects of changes in environmental conditions over the life of the process and the sensor
    - The requirements for cleaning, recalibration, or maintenance in order to maintain sensor performance over the expected duration of the process
    - The impact on the process and quality control strategy of short periods of planned or unplanned maintenance of a sensor
    - The impact on the process of the complete failure of the sensor
    - The requirement for duplicate or redundant sensors
    - The potential to use information from alternative sources of data (that is, surrogate measurements) to enable the operation of the process in the event of failure or maintenance of the sensor
    - The strategy for reconciling potentially different values from duplicate, redundant sensors or alternative sources of data
    - The strategy for maintenance of any models used to predict CQAs.

Early experience operating at-scale for an integrated downstream process also enables development of enhanced understanding of potential sensitivities in process performance and product quality due to residence time broadening inherent to larger volume systems. This may include longer exposure to more extreme conditions (i.e., low pH, high concentration) than a corresponding small-scale system or more representative data in terms of potential impact of stresses more typically encountered at larger scales of manufacturing (shear, air-liquid interface, etc.).

The capability to operate the bioreactor at or near the commercial scale may also enable the testing, whether intentional or otherwise, of potential scale-related impacts related to inclusion of cell retention devices in the process. While fouling models may be developed at the bench scale, due to

the relative newness of cell retention devices for intensified cell culture, early large-scale experience will likely be highly beneficial to support process development and establishment of an SDM. At-scale experience also enables testing of change-out strategies and associated impacts on the culture health due to operational requirements, durations with minimal or no flux through the ATF or TFF, and robustness of the control strategy of the integrated downstream in the face of such a disruption.

Operating at scale will also challenge some of the assumptions made when establishing certain ranges evaluated during the PC studies. For example, a total process pause limit of 30 minutes during the ProA capture step was evaluated during PC based on previous experience. However, during one of the clinical manufacturing runs, two process pauses of 20 and 25 minutes each, for a total of 45 minutes, were experienced during one cycle of operation. The material from this cycle was segregated and used in a laboratory study to evaluate the impact of a longer total process pause time. Acceptable results were obtained in the protocol-driven study and documented as part of the investigation of this special cause variation. This data was used to document a proposal to expand the allowable total pause time to 45 minutes, and the full extent of this pause would be validated during Stage 2 PV as a means for updating the commercial control strategy.

An additional benefit of operating at scale is the opportunity to gain experience with different lots of raw materials and identify any special cause variation (an unexpected variation that results from unusual occurrences) caused by minor variations in raw material attributes. Note that this may truly be special cause variation in that the impact is observed in only one or a few products, or it may be a signal for common cause variation (expected variation in a process), which impacts most of the portfolio of products if it is found to be a signal for poor control over a raw material.

For example, one could imagine variation in the trace metal content of cell culture media impacting the glycosylation profile of the product and resulting in an out of trend (OOT) investigation (Loebrich et al., 2019; Markert et al., 2020). After a very accelerated root cause analysis, the specific metals responsible for the OOT were identified as copper and manganese, and it was determined that these components could be controlled by release testing of the cell culture media lots. Therefore, an additional control on the material attributes of copper and manganese levels was established for the rest of clinical manufacturing and for PPQ.

Similarly, the use of multiple CEX resin lots could identify a sensitivity not observed during the SDM runs performed as part of PC. In this case, the use of a new lot of resin resulted in reduced ability to clear HMWS at the CEX step and another OOT investigation. One could imagine that the relevant attributes of the resin were found to be difficult to adequately control as a release test on incoming material, but additional lab work identified a reduced pH range for elution that proved to be robust across multiple resin lots tested. Thus, in this case, the material attributes for the resin were not changed, but the material attributes for the CEX elution buffer were tightened to allow for consistent performance. Note that this is not a preferred way of establishing ranges, but in some cases, it can become a pragmatic option.

4.4 Updates to the control strategy prior to registrational batches and PPQ

The integrated control strategy should consider learnings from product and process characterization and is designed such that the degree of control is commensurate with the level of risk to product

safety or efficacy based on known risks of variability. It is important to understand that the control strategy is not static but rather evolves over time to account from continued learnings over the product lifecycle as depicted in Figure 4.3. Risk level is determined by assessing (1) how impactful a quality attribute is to safety and efficacy, (2) the ability of the process to robustly control an attribute, and (3) the effectiveness of the formulation and storage conditions in maintaining an attribute over the intended shelf life. Attributes at greater risk could require more direct controls of product quality (more control points, higher testing frequency, tighter limits) or more indirect controls through process parameter and material attributes. The following additional considerations should be addressed in preparation for the manufacture of material for registrational trials and PPQ:

- Characterization data should be interpreted with respect to the need for controls (materials, parameters, in-process controls (IPCs)
- Special controls for startup and shutdown. Develop a strategy for managing material that does not meet requirements during startup and shut down which could include actions taken several steps downstream.
- Interpret what is known about product stream variability and tolerance for variability into a system of routine controls. Establish how these controls would result in actions if IPC or CPP limits were exceeded. This strategy could include actions taken several steps downstream.

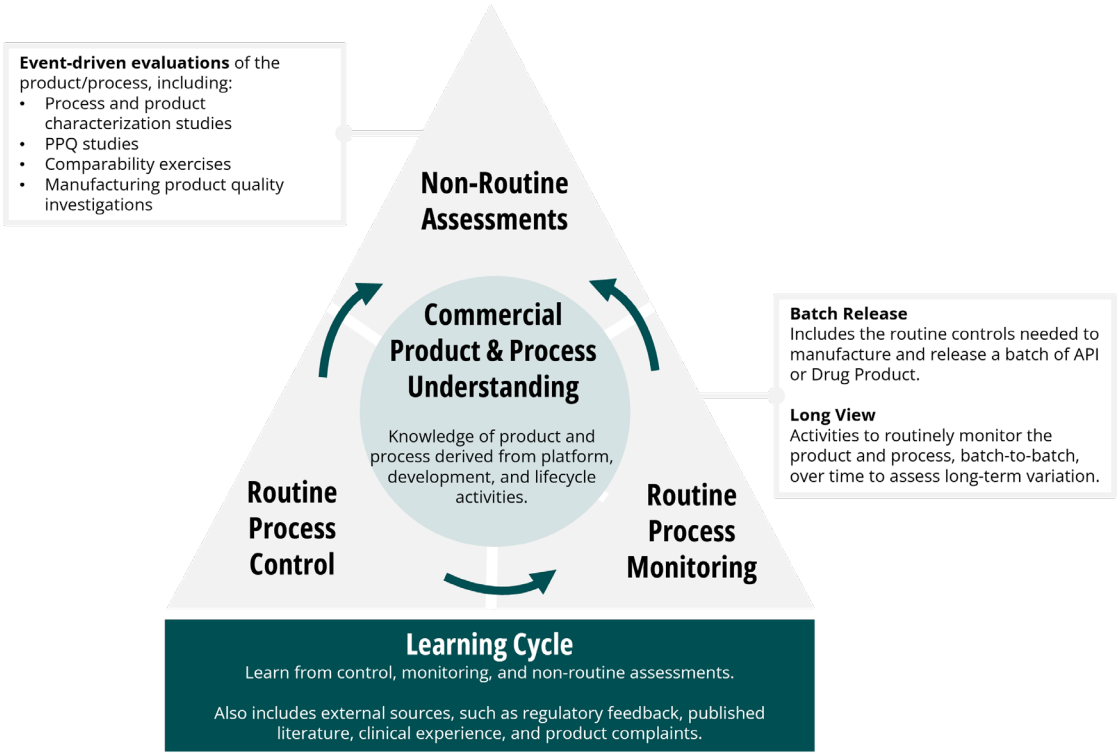


Figure 4.3. Development of a control strategy over time based on continued learning cycles.

Table 4.1 identifies examples of controls over certain parameters and material attributes that allow for improved process capability and hence reduced risk to product quality, and it represents an

update to Control Strategy #1 outlined in **Table 1.4** Also, the results from the preliminary set of accelerated, stress, and real-time stability studies have been included, and these contribute to the definition of the overall integrated control strategy as noted above in **Section 4.2**. Therefore, the proposed process definition going forward will include a steady-state perfusion process without an intermediate batch pool tank after the viral inactivation step.

Table 4.1. Control Strategy #2: Updated prior to registrational batches and PPQ.

CQA	Potential Source, Stress Response* and Clearance	Process Controls: Parametric Controls & Material Attributes	Process Capability	Analytical Controls: Testing Strategy including IPCs	Residual Risk & Justification
Glycosylation: Galactosylation	Source: Bioreactor Stress Response: None Stability Indicating: No Clearance: None	Bioreactor PPs: Temp, pH, DO, harvest day Bioreactor MAs: Trace metals	High	DS Release Testing	**Low** – Risk is reduced after PC studies demonstrating control ranges
Glycosylation: Fucosylation	Source: Bioreactor Stress Response: None Stability Indicating: No Clearance: None	Bioreactor PPs: Temp, pH, DO, harvest day Bioreactor MAs: Trace metals	Medium	DS Release Testing	**Low** – Risk is reduced after PC studies demonstrating control ranges
Glycosylation: High Mannose	Source: Bioreactor Stress Response: None Stability Indicating: No Clearance: None	Bioreactor PPs: Temp, pH, DO, harvest day Bioreactor MAs: Trace metals	*Low in Dynamic Perfusion*	*Recommend batch surge tank with IPC Testing for initial clinical runs* *DS Release Testing*	**Low** – Steady State Perfusion chosen as preferred operating mode for improved control of high mannose species
			High in SS Perfusion	DS Release Testing	
Deamidation at Asn325	Source: Bioreactor, DSP Stress Response: pH, heat Stability Indicating: Yes Clearance: None	Bioreactor PPs: Temp, pH, DO, harvest day DSP PPs: DS/Intermediate hold time & temp	High	DS Release Testing DS Stability Testing (through PPQ then review recommendations for continued testing as part of stability)	**Low** – Risk is maintained low by controlling exposure time during process to conditions promoting degradation
High Molecular Weight Species (HMWS)	Source: Bioreactor, DSP Stress Response: pH, heat, shaking, light, metals, freeze/thaw	Bioreactor PPs: Temp, pH, DO, harvest day DSP PPs: VI: pH & time	*Medium in Dynamic Perfusion*	*DS Release Testing* *DS Stability Testing*	**Low** – Steady State Perfusion chosen as preferred operating mode which demonstrated

CQA	Potential Source, Stress Response* and Clearance	Process Controls: Parametric Controls & Material Attributes	Process Capability	Analytical Controls: Testing Strategy including IPCs	Residual Risk & Justification
	Stability Indicating: Yes Clearance: CEX = 3-fold	CEX: protein load DSP CMAs: CEX elution buffer pH Trace metal leaching Procedural Controls: Protect process intermediates from light when risk of exposure is high Ensure vortexing and foaming in surge tanks is minimized	High in SS Perfusion	Consider control of CEX load based on IPC for HMWS post-VI to maximize yield DS Release Testing DS Stability Testing	greater control in bioreactor during PC studies
Host Cell Protein (HCP)	Source: Bioreactor Stress Response: None Stability Indicating: No Clearance: Chrom Step(s)	Bioreactor PPs: Temp, pH, harvest day DSP PPs: protein load, peak cutting	High in SS Perfusion	DS Release Testing	**Low** – Steady State Perfusion chosen as preferred operating mode which demonstrated greater control in bioreactor during PC studies

CEX	Cation exchange
Chrom	Chromatography
CMA	Critical material attribute
DO	Dissolved oxygen
DS	Drug substance
DSP	Downstream process
HCP	Host cell protein
HMWS	High molecular weight species
IPC	In-process control
MA	Material attribute
PP	Process parameter
PPQ	Process performance qualification
SS	Steady state
VI	Virus inactivation

Table 4.2. Summary table for process parameters and material attributes.

Process Parameter or Material Attribute	Experience Range in PD/PC	Control Range for Clinical Mfg	Proposed Range for PPQ
Bioreactor			
Growth Phase pH	6.9–7.3	7.0–7.2	7.0–7.2
Growth Phase Temperature (°C)	34.5–37.5	35.5–36.5	35.5–36.5
Growth Phase Perfusion Rate (CSPR, nL/cell/day)	0.040–0.060	0.045–0.055	0.0475–0.0525
Production Phase Shift Timing (day) [1]	5–7	5.50–6.50	5.75–6.25
Production Phase pH	6.9–7.3	7.0–7.2	7.0–7.2
Production Phase Temperature (°C) [1]	31.5–34.5	32.5–33.5	32.5–33.5
Production Phase Perfusion Rate (vvd) [1]	1.6–2.4	1.9–2.3	1.9–2.1
Dissolved Oxygen (%sat)	20–80	40–60	45–55
Trace metal (Mn, Cu) conc in cell culture media	Not Tested	Cu^{++}: 0.2*–2 mM [2] Mn^{++}: 0.2*–2 mM [2]	Cu^{++}: 1.5–2.5 mM [2] Mn^{++}: 1.5–2.5mM [2]
ProA Capture Chromatography			
Loading/Wash Residence Time (min)	4–8	5–7	5–7
Loading (BT load control) (g/L-resin)	5–60	45–55	45–55
Elution Buffer pH [3]	3.3–3.7	3.4–3.6	3.4–3.6
Elution Buffer Conductivity (mS/cm) [3]	2–5	3–4	3–4
Process Pause (min)	15–30	<30	<45 [4]
Viral Inactivation			
Low pH Target	3.3–3.7	3.4–3.6	3.4–3.6
Incubation Duration (mins)	55–75	60–70	60–70
Protein Conc (ProA BT load ctrl) (g/L)	3–20	15–18	15–18
Post-Incubation pH (ProA BT load ctrl)	6.5–7.5	6.8–7.2	6.8–7.2
Polishing Chromatography 1 (AEX – F/T)			
Loading (ProA BT load ctrl) (g/L-resin)	100–250	180–220	180–220
Load pH	6.5–7.5	6.8–7.2	6.8–7.2
Load Conductivity (mS/cm)	4–8	5–7	5–7
Polishing Chromatography 2 (CEX – B/E)			
Loading (ProA BT load ctrl) (g/L-resin)	20–50	35–45	35–45
Load pH	6.5–7.5	6.8–7.2	6.8–7.2
Load Conductivity (mS/cm)	4–8	5–7	5–7
Wash pH [2]	7.2–7.6	7.3–7.5	7.3–7.5
Elution pH [2]	7.5–8.0	7.5–8.0 [5]	7.5–7.6 [5]

[1] Both temperature setpoint and perfusion rate change with this shift timing parameter
[2] Learnings from G2F/trace metal OOT Investigation
[3] Controlled as a material attribute of the buffer solution
[4] Learnings from CPP excursion investigation for process pause
[5] Learnings from HMWS OOT linked to pH and resin lot

AEX Anion exchange
B/E Bind and elute
BT Breakthrough
OOT Out of trend
PAR Proven acceptable range
PC Process characterization

CEX	Cation exchange	PD	Process development
CSPR	Cell-specific perfusion rate	PPQ	Process performance qualification
F/T	Flowthrough		

For illustrative purposes, the process characterization case studies in **Chapter 3** examined the configuration where no breakthrough load control was applied. Correspondingly, great variability in input parameters was covered during process characterization. In practice, and with some additional experience at scale, it is likely that breakthrough (BT) load control, such as delta UV or an equivalent soft sensor, would be leveraged to decrease the system complexity. Therefore, the process summary information table, started as **Table 3.30** summarizing the outcomes from process development characterization, should be updated to focus on the simpler case (with breakthrough load control) to reflect the actual control ranges used in clinical manufacturing as well as to propose control ranges for the manufacture of clinical supplies to support registrational trials and PPQ runs as shown in **Table 4.2**.

Chapter 5

Adventitious Agent Control

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

5 Adventitious Agent Control

5.1 Overview of adventitious agent control for continuous manufacturing processes

Ensuring that biopharmaceuticals are free of adventitious microbial or viral contamination is critical to the safety of these products.

> *"Microbiological control is a regulatory requirement and one that can be defined as the continued interaction of science and applied technology with products, processes, materials, equipment, and personnel entering the manufacturing areas. In the Code of Federal Regulations (CFR) Title 21 Parts 210 and 211, control of microbial contamination is addressed in several subparts, including Subpart C–Buildings and Facilities, section 211.42, and more specifically, in Subpart F–Production and Process Controls, section 211.13. A good microbiological control program starts with understanding the risks for microbial contamination of the manufacturing process and identification of possible types of contaminants. The results obtained from such risk assessment can be used during facility and equipment designs as well as when establishing equipment and personnel flow patterns. Once possible sources of contamination have been identified, control and preventative measures can be implemented and qualified/validated." (Clontz, 2009)*

Control of potential viral contamination is equally important, and the broad requirements are described in ICH Q5A (R1, 1999). Implementation of viral safety and microbial control strategies involves multi-pronged, complementary approaches (Clontz, 2009, Shiratori & Kiss, 2017). Similar to the control strategy developed for other product quality attributes, the adventitious agent control strategy should represent a buffer against known variations that could impact product quality based on an understanding of those risks. There are three key elements to adventitious agent risk mitigation that apply to both batch and continuous manufacturing (**Figure 5.1**). First, prevention of virus or bioburden entering the production process, including raw material selection and testing, as well as implementing barrier technologies; second, removal of potential viral or microbial contaminants from the process; and third, detection of viral or microbial contaminants during processing. These elements should be assessed from the very beginning of development throughout implementation at scale.

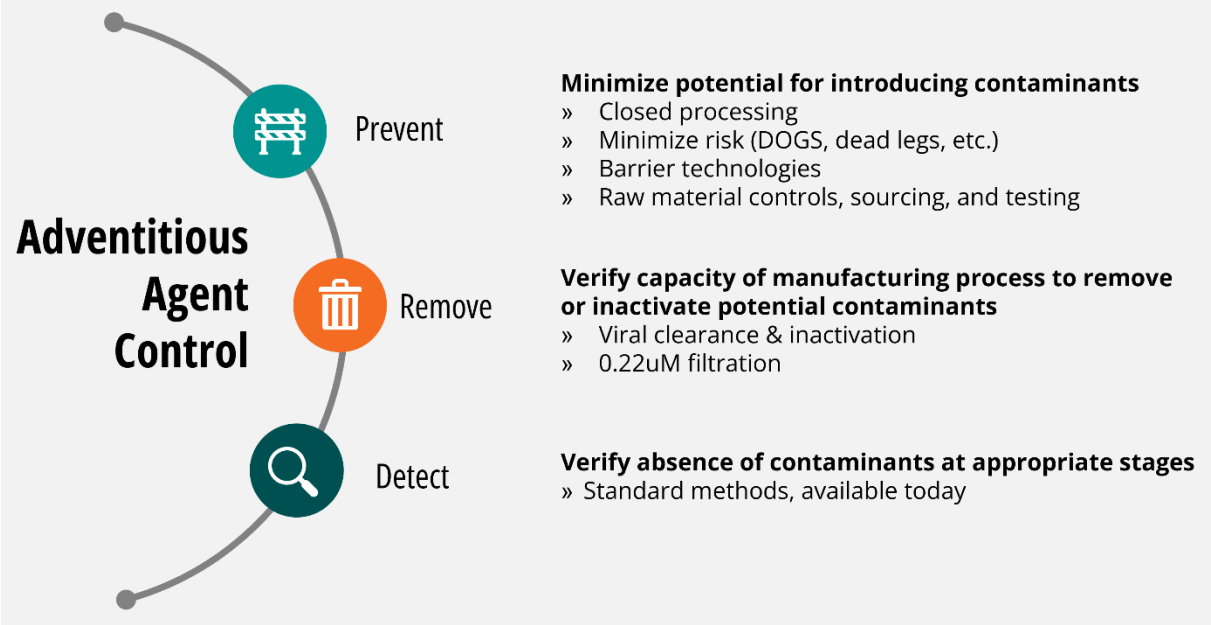


Figure 5.1. A complementary approach ensures microbial and viral safety of biological products.

Compared to batch processing, continuous manufacturing requires unique considerations with respect to adventitious agent control. One obvious consideration is that the timespan of continuous upstream operations is anticipated to be 2-8 weeks or longer in the production bioreactor, whereas current batch operations are typically completed within 2-3 weeks. In both cases, the rich growth environment for microorganisms and the presence of cells in the upstream process provide an environment conducive to microbial and viral replication and expansion. Thus, similar controls and testing strategy as for batch upstream operations must be utilized to avoid contamination, regardless of the timespan of operations, and these could even be increased based on a risk analysis.

Additional unique considerations relate to the equipment and the process design and these should be reflected in the studies establishing fit for use as part of the Stage 2 PV design (Section 6.3.1). The use of pre-sterilized single-use bioreactors for continuous manufacturing eliminates the need for clean-in-place and/or steam-in-place (CIP/SIP) of equipment, which is typical for batch processes. Continuous downstream operations are expected to last a short period of time for each cycle, but the total duration for which adventitious agent control must be maintained will be linked to the bioreactor cadence and harvest duration. Continuous downstream processes rely on surge tanks between interconnected unit operations, thus placing greater importance than for batch processes on the closed processing methodologies needed to sustain operations for the extended duration. To demonstrate a state of microbial control for interconnected unit operations, growth-promoting media may be processed through the manufacturing-scale production equipment for a duration longer than the intended continuous manufacturing process. While batch processes allow for the sampling and testing of discrete product pools before advancing to the next unit operation, continuous manufacturing relies more heavily on surge vessels instead of pool tanks, and therefore,

more frequent sampling of process intermediates may be required. The frequency of testing will be driven by a risk assessment and may be determined by processing time or volume, and it may also take into consideration the turnaround time of a test method relative to the time available to make a decision based on test results. The detailed considerations for a continuous manufacturing adventitious agent control strategy described below will focus on appropriate facility and manufacturing controls, in addition to confirmatory sampling and testing of process samples.

5.2 Prevention

5.2.1 Control of raw materials

Extensive characterization and testing of raw materials is essential to reduce the risk of a potential contaminant entering a continuous process. Any animal-derived raw materials, if present, can be tested for viral contamination via *in vitro* and virus-specific polymerase chain reaction (PCR) assays. Screening of raw materials using molecular tools like next-generation sequencing (NGS) may be able to identify any contaminating organisms in the raw material. A variety of other culture-based, *in vivo,* and molecular methods can also be performed to ensure the absence of adventitious viral contaminants within their limits of detection. Raw materials testing is performed before manufacturing is initiated, at a time when rapid results are less critical.

5.2.2 Control of facility and equipment by closed processing

Facility- and equipment-related risk mitigation factors could include but not be limited to minimizing process elements frequently associated with microbial ingress such as diaphragms, O-rings, gaskets, and seals along with reducing the potential for microbial growth within equipment skids by eliminating static fluid points (e.g., dead legs). The use of closed systems mitigates the risk of contamination by adventitious agents, reduces the amount of human intervention and manipulation, and protects operators. According to the International Society for Pharmaceutical Engineering (ISPE) (ISPE Baseline Guide Vol 6: Biopharmaceutical Manufacturing Facilities, 2013), closed processing is "a process condition when the product, materials, critical components or container/closure surfaces are contained and separated from the immediate process environment within closed/sealed process equipment. A process step (or system) in which the product and product contact surfaces are not exposed to the immediate room environment." In the context of biopharmaceutical manufacturing, we must expand our definition of closed processing slightly to allow for the addition of materials, while avoiding exposure to the room environment. Because this addition occurs via aseptic connections or sterilizing-grade filters, this mode of processing may still be considered "fully closed." Alternatively, a "functionally closed" system may be required if the system is opened to the environment while making connections or installing filters or columns as long as it can be returned to a closed state via in situ sanitization or sterilization procedures (Johnson et al., 2011, Estape et al., 2017). Closed processing is anticipated to be a critical part of the microbial control strategy for continuous manufacturing because multiple, interconnected unit operations are run for potentially 2–8 weeks. It is not practical to routinely disrupt the process flow to sanitize the process equipment, and therefore it is critical to establish and maintain a closed system throughout the duration of a batch. Process closure considerations related to process fluids (e.g., cell bleed, Protein A chromatography flowthrough) exiting the integrated system to waste

should also be considered. By utilizing closed production systems, adventitious agent contamination risks are mitigated, and product safety is maintained.

In addition to ensuring product safety, closed processing with primarily single-use components may result in economic and environmental benefits. For both fully and functionally closed systems for drug substance manufacturing, the environmental controls could justify classification of the process area as a controlled non-classified environment (CNC) with support from a risk assessment in the quality management system. This would imply that three requirements are met: (1) the components of the system operated in closed mode provide control of the safety assurance level, (2) the highest risks are identified, and (3) a mitigation approach has been developed to address these risks. Additionally, closed processing may result in reduced requirements for testing, documentation, and validation.

5.2.3 Barrier methods for control of viral ingress

Another means of reducing the risk of viral contamination from raw materials is the use of a barrier method to treat the cell culture medium and any supplemental components before they enter the bioreactor. Several methods exist, ranging from high temperature short time (HTST) heating to UVC irradiation to virus barrier filters. Some methods, such as HTST and UVC, must be carefully evaluated to ensure that they do not damage any components of the medium. These methods may be used in fed-batch processes, but they are anticipated to be more critical for continuous manufacturing processes, which may be operated for longer periods of time. Note that HTST can be very effective in reducing the risk of microbial as well as viral contamination, especially for very small microorganisms as detailed by a case study from Genentech (Chen et al., 2012).

5.2.4 Filtration

While closed processing should ensure a high degree of protection from the ingress of adventitious agents, the use of intermediate bioburden control by filtration will remain an option to be considered in continuous manufacturing, especially in the case of Process Option 2, which includes an intermediate batch hold tank (**Figure 1.5** or

Table 1.3). For process intermediates, a risk assessment with respect to bioburden contamination should be performed to determine if 0.2 μm filtration is required. Processing times from harvest to drug substance may be less than 24 hours in the absence of intermediate pool tanks; however, surge tanks between unit operations may be utilized for up to 8 weeks of processing. If a pooling step is included after the last connected step, it is advisable to include 0.2 μm filtration prior to the pool vessel. If particulate removal is needed, an appropriate filtration train should be considered. However, the presence of any intermediate filters may impact the ability to maintain a closed system, especially if routine filter changeouts are required, and intermediate filters may impose other process characterization activities such as stability assessments of the intermediate pool if that pool has a significant hold time as previously discussed.

5.2.5 Considerations for upstream continuous operations

All equipment and components used in the upstream operations are gamma-irradiated or autoclaved. Single-use components, such as single use bioreactor (SUB) bags, are also used. Sampling processes during cGMP operations use sample manifolds or welders. Gas flow into the bioreactor is provided through a gamma-irradiated 0.1 μm filter. Dissolved oxygen (DO) and pH are

continuously measured and are considered leading indicators of potential microbial contamination, especially if incorporated into a multivariate model for the bioreactor along with gas flow rates, agitation, and other related measures.

5.2.6 Considerations for downstream continuous operations

In general, it is preferred to leverage gamma irradiated, pre-assembled single-use tubing assemblies wherever possible. For prepacked chromatography columns, several microbial control strategies may be considered. Pre-packed columns leveraging an alcohol- or caustic-based storage solution can be utilized to achieve sanitization before introduction of these columns into an integrated process. Alternatively, chromatography columns can be pre-sterilized using gamma irradiation and a protective solution in order to minimize deleterious effects from the sterilization process (Varner et al., 2021). This approach affords a greater level of microbiological safety, but it also requires potentially significant supply chain considerations.

Results from microbial challenge studies together with risk assessments should be used to identify the most appropriate microbiological contamination control strategy for each specific integrated system. If a system comprises pre-sterilized components and sterile connections, it may be possible to operate for a long duration without intermittent sanitization (Coolbaugh et al., 2021). Alternatively, if the level of control is deemed insufficient based on risk assessment or development data, periodic cleaning of all or selected parts of the integrated system may be implemented. An example strategy for such an approach could include sanitization of the entire single-use flow path with a 0.5 N NaOH solution, including all instrumentation, chromatography columns, and flow chambers, is for a minimum of 15 minutes between every periodic cycle

5.3 Removal

5.3.1 Background

Despite careful testing of raw materials, cell banks, and in-process intermediates along with operation in a closed or functionally closed system, there is still a risk that an adventitious agent could enter the manufacturing process. Therefore, the second pillar of the adventitious agent safety strategy is the removal of viral or microbial contaminants from the process. For microbial contaminants, this is typically achieved by robust cleaning protocols along with judicious use of 0.2 μm filters in the process flow. For viruses, ICH Q5A (R1, 1999) states that manufacturers of biologics products should demonstrate the viral clearance and inactivation capacity of any purification process used to produce monoclonal antibodies and recombinant proteins for human therapeutic use. The overall viral clearance efficiency of the purification process should be demonstrated using model viruses representing process-specific virus risks. The results should ensure that there is excess capacity for viral clearance of retrovirus-like particles that may be present in the unprocessed bulk. This guideline applies to both fed-batch and continuous manufacturing processes.

The capacity of the manufacturing process to remove or inactivate enveloped and non-enveloped viruses is assessed in viral clearance studies as described in relevant sections within **Section 3.7**. These studies involve scaling down individual process steps and performing the following activities for each step: spiking virus into the process intermediate, performing the process step, and measuring the virus remaining after the process step. Similar to a platform fed-batch process, the N-mAb purification process for continuous manufacturing includes two dedicated viral clearance

steps: virus inactivation and viral filtration. Significant viral clearance can also be achieved by chromatography steps, including anion exchange. The challenges unique to operating viral clearance at scale for continuous processing as well as proposed solutions to those challenges are discussed in this section.

5.3.2 Control strategy for viral inactivation and filtration

5.3.2.1 Viral inactivation

The continuous incubation chamber used for full-scale cGMP manufacturing needs to demonstrate sufficient engineering controls of pH, temperature, and incubation duration through residence time distribution. In the continuous viral inactivation operation, the system remains functionally closed; therefore, in-process material undergoing viral inactivation will not be sampled and tested off-line during each run. The following approaches are recommended to assure pH and incubation time targets are met in order to establish a robust control strategy.

Within the continuous viral inactivation flow path, the first pH probe is located between the post-acidification static mixer and the incubation chamber. This pH probe may be used within a feedback loop to adjust the amount of acid added to the system to achieve the target inactivation pH. If the target inactivation pH is not achieved, material can be directed to waste before entering the incubation chamber. This control strategy mitigates the risk of commingling the material that was exposed to inadequate viral inactivation conditions with the larger batch. A final pH probe is included immediately after the post-neutralization static mixer. If the target neutralization pH is not achieved, material can similarly be diverted to drain because the product is not within the acceptable processing limits for the subsequent unit operation. Although these diversion valves may be used as part of normal processing, a deviation may still be triggered if excessive amounts of material are diverted to drain in order to identify the root cause of the excursion. Excessive diversion of material could lead to disruptions in subsequent unit operations.

An optional pH probe may be included immediately after the incubation chamber. This probe provides increased confidence that the target inactivation pH was achieved and sustained throughout the incubation chamber. It may also be utilized to detect and mitigate drift of the first pH probe. Manufacturers may perform a risk assessment to determine whether to include this pH probe in the design of their continuous inactivation flow path.

As with batch processes, in-line pH probes for continuous processes need to be calibrated using pH standard solutions prior to use, but they must also be verified periodically during processing, either by demonstrating that a selected process buffer is confirmed to be within an acceptable range or by collecting a sample of the product stream to verify the pH with an off-line probe. This off-line confirmation may trigger a single-point calibration of the in-line pH probes, thus mitigating the risk of pH probe drift and eliminating the need for the optional pH probe described above.

Since the flow path of the incubation chamber is fixed, the incubation time would be directly impacted by the flow rate of material entering the incubation chamber. In-line flowmeters should be used to ensure that the flow rate is within an acceptable range, while accounting for the impact on the residence time distribution within the incubation chamber.

5.3.2.2 *Design of incubation chamber*

The design of an incubation chamber will be discussed in the context of low pH viral inactivation, but similar design considerations apply to inactivation with detergent (Section 5.3.2.4). An example incubation chamber is shown in Figure 5.2, where a pump transfers the feed from the post-Protein A step, which is either in a cycle surge or batch pool tank, while another pump transfers the acidic titrant for in-line pH adjustment. A static mixer combines the acid and the feed to achieve the target pH, e.g., 3.5 (acceptable range pH ≤ 3.6). The target pH can be achieved and confirmed by two different approaches. The system may leverage a feedback control strategy to adjust the flow rate of the acidic titrant pump to ensure that the target pH is reached. Alternatively, the system may be operated at a pre-determined volumetric ratio of product to acid, such that the pH target is achieved without dynamic control. Development work should be performed through rigorous offline screening of worst-case chemical conditions (including product concentration, pH, and buffer composition) to determine the appropriate volumetric ratio while accounting for flow meter variability. In both approaches, the pH is confirmed to be within the targeted range using an in-line pH probe located at the inlet of the incubation chamber. If the target condition is not met, the product stream will be diverted to drain and discarded. The distance between the divert valve and the pH probe should allow for sufficient response time of the pH probe to ensure that material which is out of the desired pH range does not pass the diversion point before an action can be taken. Several approaches have been developed to date to ensure that adequate residence time is attained within the incubation chamber (Brown & Orozco, 2021; Brown et al., 2020; Gillespie et al., 2019; Martins et al., 2020; Orozco et al., 2017; Parker et al., 2018; Senčar et al., 2020).

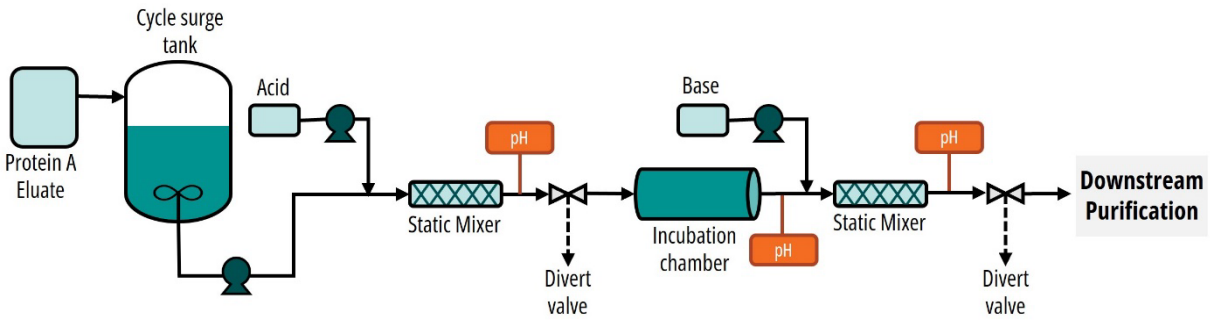


Figure 5.2. Diagram of continuous viral inactivation chamber.

5.3.2.3 *Characterization of residence time and control of the duration of viral inactivation*

To implement a continuous viral inactivation process, a thorough understanding of the design considerations that impact the residence time distribution in the incubation chamber must be demonstrated, while also accounting for other process parameters, such as temperature or protein concentration, that may impact the effectiveness of viral inactivation. The volume of the incubation chamber and the flow rate of the acidified product stream are selected such that the product is incubated in the incubation chamber for the target duration (e.g., ≥ 30 minutes) while also taking into consideration the maximum time spent at low pH before degradation of the product occurs. In

addition, the effects of axial dispersion on residence time distribution must be considered because they impact the incubation time. The Reynolds and Dean numbers can be utilized to model the residence time distribution and optimize the design of the incubation chamber. Coiled tubing with bends and turns is often included in the incubation chamber design to induce Dean vortices, which promote radial mixing and increase efficiency relative to straight tubing, resulting in a narrower residence time distribution. A pulse injection study may be utilized to confirm the residence time distribution and support scale-up and scale-down of the continuous incubation chamber. For example, (Brown et al., 2020) illustrated a scenario where the pulse injection had an average incubation time of 78.8 minutes, while only 0.00003% of the pulse had exited the incubation chamber after 61.2 minutes and 99.865% of the pulse had exited the incubation chamber within 88.7 minutes. To scale up the continuous incubation chamber, the internal diameter or length of the tubular reactor can be increased. However, the characteristic residence time distribution needs to be well-maintained. A residence time distribution curve needs to be generated by a pulse injection method to qualify the device prior to use. Alternatively, a pH measurement at the outlet of the incubation chamber may be used to obviate the need to pre-qualify an individual device if the risk of insufficiency is deemed sufficiently low. A dataset showing how different design parameters impact the process needs to be generated to demonstrate that scaling has no significant impact on the operations.

5.3.2.4 Considerations for detergent-based viral inactivation

If the product has poor stability at low pH, detergent-based virus inactivation may be utilized, typically after the cell removal step and prior to the capture chromatography step. When detergents are present at concentrations greater than or equal to the critical micelle concentration, they inactivate enveloped viruses by disrupting the outer lipid membrane (Conley et al., 2017). Within a continuous manufacturing process, the detergent for viral inactivation could be continuously added, using similar equipment to that used for the low pH viral inactivation step (Martins et al., 2019). However, unlike the established probes used to monitor pH and flowrate, there are currently no sensors available for the continuous monitoring of the detergent concentration at the beginning and end of the incubation time. Manufacturers may seek to use in-line flowmeters to monitor the continuous addition percentage of the detergent stock solution, while also determining the sampling frequency for off-line confirmation of detergent concentrations. Excursions in the ratio between the in-line flowmeters for the product and detergent stock solution would trigger a deviation event leading to the diversion of product to a waste vessel until the process returns to a state of control. The identification of a suitable sensor technology to continuously monitor detergent concentration would be expected to result in more robust control of a continuous detergent viral inactivation step.

5.3.2.5 Virus nanofiltration: small virus retentive filtration

As with batch processes, virus filtration for continuous bioprocesses is a crucial downstream processing operation. It provides a second, orthogonal viral reduction step based on size exclusion by a filter, and it must be carefully designed to ensure patient safety. Two general strategies can be considered for operating the virus filtration step within the continuous downstream process: periodic batch mode or continuous mode. The preferred strategy identified for both major process options (

Table 1.3) is to pool the product stream from the previous step for a fixed duration prior to operating the virus filtration step in batch mode. This approach also works for sub-batches of drug substance from a single continuous steady-state perfusion run or runs. This strategy would allow for the translation of the batch virus filtration operations to a continuous downstream process. Along with its ease of adaptation at manufacturing scale, this strategy also enables the use of existing virus filter scale-down models for process characterization and viral clearance, as defined in the A-Mab case study. For this strategy, the added hold time prior to virus filtration needs to be assessed for its impact on the viral filter capacity in addition to assessing product stability during that hold time. Any increase in aggregation during the hold step may necessitate the implementation of a robust prefiltration step to decrease the impact of aggregates on the virus filter performance.

The alternate strategy identified in

Table 1.3 is to perform the virus filtration step in a truly continuous mode with the virus filter directly connected to the previous and subsequent unit operations using surge vessels as appropriate. Continuous operation of the virus filter may reduce the manufacturing footprint of the step by eliminating or decreasing the size of intermediate hold vessels. However, a continuous operation may add to the complexity of the virus filtration step because a system with parallel virus filters may need to be considered to allow a seamless transition between virus filters as a result of capacity limitations or unplanned process disruptions. During the operation of the filtration step, there are several key differences between batch and continuous virus filtration process parameters. The unit operations in batch mode typically last for four to six hours, while continuous processes can be performed for days and require the periodic changeout of the virus filter, the timing of which may align with the sub-batching strategy. In all cases, operating pressures are much lower during continuous virus filtration and may require additional consideration when selecting and sizing an adsorptive pre-filter for the removal of potential aggregates that might lead to fouling of the virus filter. Batch systems may be open with manual or semi-automated control, while continuous processes are expected to be closed and highly automated, resulting in greater complexity. The feed stream for a batch process is homogeneous, but in continuous virus filtration, any variability in protein concentration, pH, and conductivity from the elution peaks of the previous chromatography step will challenge the virus filter. Small surge vessels or in-line dilution may be used to dampen these variations in fluid properties and enable a more consistent flow rate to the virus filter. In batch mode, the virus filter integrity test may be performed before the product is advanced to the subsequent unit operation, enabling reprocessing in the rare occurrence that the post-use integrity test fails. However, continuous connected processing eliminates the opportunity for reprocessing when virus filtrate is passed directly to the subsequent ultrafiltration and diafiltration steps prior to completion of the post-use integrity test. This mode of operation requires careful tracking of the product processed with each virus filter assembly to ensure that the adjacent sub-lots are not compromised if a post-integrity test fails. This complexity places even greater importance on the pre-use integrity testing and careful use of the virus filter during operation to minimize the risk of post-use integrity test failures.

5.4 Detection

5.4.1 Background

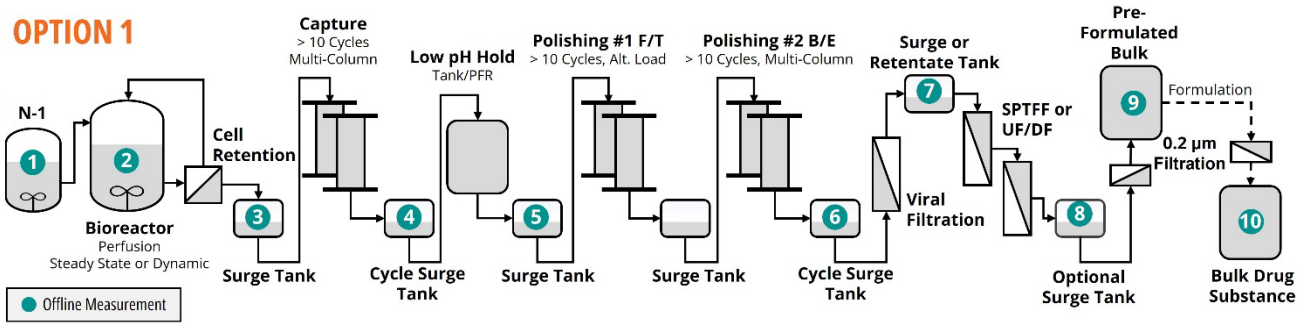
The third key piece to adventitious agent risk mitigation is the ability to detect a contamination in a production process. The importance of detection of adventitious agents is twofold: the straightforward benefit of identifying potential contaminants and a more subtle benefit in that adventitious contamination can also be seen as a signal flare that other undetected contaminants of the process, not necessarily of a microbial or viral nature, may be present. Viable in-line or at-line tests that would identify a potential contaminating microbe or virus in real time are not yet available, so the primary detection control for viruses involves testing of a sample of the unprocessed bulk and in-process testing at various points in the process. Low levels of virus that may have escaped detection during the screening of the raw materials may be amplified in a bioreactor if the production cells are susceptible to infection. Additionally, Chinese hamster ovary (CHO) and other rodent cell lines contain genetic sequences for endogenous retroviral-like particles (RVLP) that may be expressed during production of recombinant proteins. These endogenous viruses are quantitated in the bulk harvest during in-process testing. ICH Q5A (R1, 1999) outlines strategies for testing of unprocessed bulk that apply to both fed-batch and perfusion processes, including the flexibility to use a cell-free harvest sample when cell-containing samples are not readily available. With advances in single-use components to enable robust sampling of the perfusion bioreactor, in addition to the typical inclusion of a cell bleed stream, it is anticipated that a cell-containing sample will be practical in most scenarios. It is recommended that a worst-case sample be taken from the perfusion bioreactor (cells and culture media) at the end of perfusion. At that point, the cell count will have been at its maximum for the longest duration, maximizing the potential production of virus or virus-like particles. Alternatively, manufacturers may choose to sample periodically throughout the perfusion bioreactor duration (e.g., beginning, middle, end) during clinical manufacturing to confirm the worst-case sampling time, as well as to mitigate material risk related to downstream pooling strategies. Sampling and testing before planned cell retention filter changeouts may also be appropriate

5.4.2 Testing strategy for adventitious agent detection

The testing panel on this sample should be similar or identical to what is typically planned for a sample from a fed-batch bioreactor prior to further processing (harvest), i.e., *in vitro* testing, transmission electron microscopy, and virus-specific PCR tests. The assays performed detect a broad range of known or potential unknown viruses. Rapid testing is particularly important for continuous processing, especially for the scenario in which drug substance sub-lots need to be released while the production run is still ongoing. Viral safety testing typically has one of the longest turnaround times of all the tests required for release. The industry has made good progress in shortening these turnaround times with real-time PCR assays existing for both mycoplasma and adventitious virus as an alternative to the existing cell culture-based methods, reducing the time from weeks to days.

For the detection of microbial contamination, other than the unprocessed bulk sample from upstream, the ICB system should contain appropriate sampling locations based on quality risk assessments to enable detection of inadvertent contamination, while also avoiding any unnecessary contamination risk introduced through the sampling procedure. One might consider an analogy between the sampling and testing of an ICB with the approach to media fill testing for aseptic

processing (PIC/S Recommendation on the Validation of Aseptic Processes, 2011). An example bioburden testing strategy is shown in **Figure 5.3** as applied to Process Option #1 (**Table 1.3**) The sampling locations and frequency may be adjusted based on improved product and process understanding. When multiple unit operations are interconnected as a closed system with surge tanks, it may be justifiable to reduce the sampling locations. This table is similar in structure to **Figure 4.2**, which provided details on a sampling and testing plan for performance and quality attributes. Also note that as in **Figure 4.2** the column labeled "Elapsed Time" refers roughly to the procession of a specific aliquot of material as it traverses the process and is based on the details in **Figure 2.13**. The overall elapsed time is obviously process-specific, but this column has been included as a reminder that the sampling, testing, and possible reaction to a result all occur in real time as the process continues.



Sample Point	Process Step	Elapsed Time (h)	Bioburden/Endotoxin	AVA / Myco	Contamination Risk	Sampling Frequency	Rationale for Sampling Point
1	N-1		•		High	Last Day	Meets expectation to test during expansion
2	Bioreactor		•		High	Every 2d after start of perfusion	Meets expectation for End of Production (EOP) / unprocessed bulk harvest sample
			•	•		Last Day	
3	Post-ATF/TFF Permeate Surge Tank	0	•		Medium	1. Prior to 1st capture cycle 2. Once every 24h 3. Prior to last capture cycle	Meets expectation to test cell-free product stream prior to loading first column. Samples may be collected at end of each pooling interval, pending batching strategy.
4	Post-ProA Surge Tank	8	•		Medium	1. Prior to 1st capture cycle 2. Once every 24h 3. Prior to last capture cycle	Confirm closed processing
5	Post-VI Surge Tank	12	•		Low	1. After 1st cycle 2. After last cycle	Confirm closed processing
6	Post-Polishing Chrom, Pre-VF Surge Tank	14	•		Low	1. After 1st cycle 2. After last cycle	Confirm closed processing
7	Post-VF Surge	16	•		Low	Every 24h	Confirm closed processing
8	Post-UFDF, Pre-0.2 µm filter	18	•		Low	Beginning and end of every bag filled	Meets expectation to test prior to bioburden reduction filter
9	Post-UFDF, Post-0.2 µm filter	18	•		Low	Beginning and end of every bag filled	Meets expectation to test during product holds longer than 24h
10	Bulk DS		•		Low	Beginning and end of every bag filled	Meets expectation to test drug substance

Figure 5.3. Example of microbial testing strategy for an integrated continuous bioprocess.

ATF	Alternating flow filtration	EOP	End of production	TFF	Tangential flow filtration
AVA	Adventitious agent	FT	Flowthrough	UF/DF	Ultrafiltration/diafiltration
B/E	Bind and elute	Myco	Mycoplasma	VF	Viral filtration
Chrom	Chromatography	ProA	Protein A		

Sampling frequency could be reduced as more experience with the process as run in a given facility is gained and risks become better understood, for example, as part of a CPV program. If the downstream process is operated in a semi-continuous or periodic batch mode, additional samples may be collected at a frequency aligned with the selected batch pooling interval. A sample for bioburden and endotoxin may be collected from any pooling vessel before advancing to the next unit operation. An example of points to consider as part of the decision process for evaluating the results of bioburden testing is shown in Figure 5.4. Sampling of the process may be increased as part of a corrective and preventive action (CAPA) plan following an investigation. Using today's technology, a delay of several days is possible between sampling and results on quantitation of any bioburden present and possibly up to an additional week if identification of contaminating species is required. This could easily result in the loss of a significant portion of a batch depending on the sampling frequency. Implementation of rapid methods for bioburden quantitation could allow for a faster diversion of contaminated material. Rapid technologies should be evaluated to match continuous process timelines, while considering the time difference between the sampling point and the decision point. However, it is likely that rapid microbial methods will provide only quantitative results, and identification of the contaminating species may still take longer than the timing dictated by process dynamics for effective diversion of a contaminated aliquot of a batch.

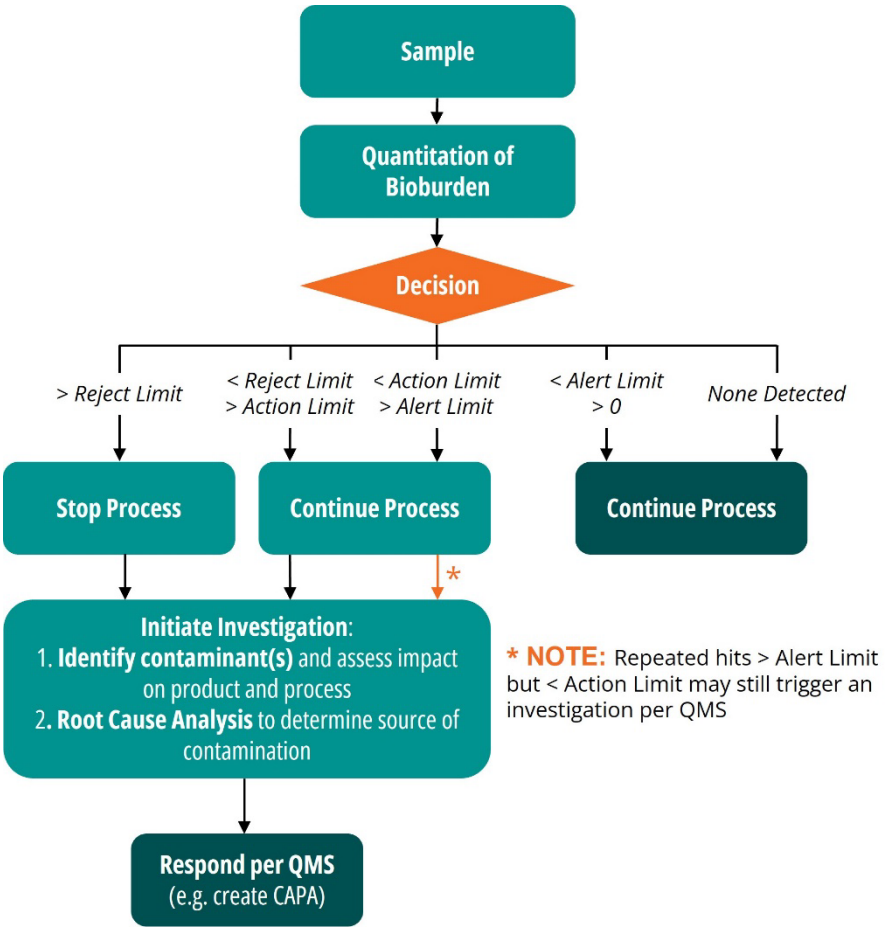


Figure 5.4. Considerations for bioburden deviations in an ICB framework.

Chapter 6

Process Validation:

Documenting and Demonstrating the Process at Scale

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

6 Process Validation: Documenting and Demonstrating the Process at Scale

6.1 Overall approach to process validation

Process validation activities are those which, over the product lifecycle, demonstrate the adequacy of the process design and control strategy for the manufacture of product to the required level of quality with adequate process performance. The philosophy of process validation as a lifecycle concept (FDA Guidance for Industry. Process Validation: General Principles and Practices, 2011, EMA Guideline on Process Validation for the Manufacture of Biotechnology-Derived Active Substances and Data to be Provided in the Regulatory Submission, 2016) applies equally well to manufacturing processes of any design, e.g., conventional batch processes as well as integrated, continuous processes. Regardless of the type of process, good process validation practice is built upon several foundational process design and control principles:

- Quality, safety, and efficacy are designed or built into the product.
- Quality cannot be adequately assured merely by in-process and finished-product inspection or testing.
- Each step of a manufacturing process is controlled to assure that the finished product meets all quality attributes including specifications.

The lifecycle approach to process validation used by most manufacturers commonly considers process validation activities as occurring in three stages, as described by FDA in their 2011 Guidance (FDA Guidance for Industry. Process Validation: General Principles and Practices, 2011):

1. Process Design: The commercial manufacturing process is defined based on knowledge gained through development and scale-up activities.

2. Process Qualification: The process design is evaluated to determine if it is capable of reproducible commercial manufacture. This stage has two elements: (1) design of the facility and qualification of the equipment and utilities based on demands of the process and (2) process performance qualification (PPQ).

3. Continued Process Verification (CPV): Ongoing assurance is gained during routine production that the process remains in a state of control.

The discussion of process validation will present general considerations for process validation studies for continuous and integrated manufacturing processes and illustrate how these could be applied through a set of specific examples based on earlier chapters.

6.2 Process Validation Stage 1: Process Design

The first stage of process validation results in the definition of a process, including its control strategy. It incorporates product and process design activities conducted during development to

gain a deep understanding of the product and the manufacturing process. Process Validation Stage 1 activities for selected steps of the N-mAb process are discussed in Chapter 3.

At the end of the process design stage of process validation, a complete, updated process description is available, including its control strategy, which includes the following elements described in Section 1.3:

- The specifications for incoming materials
- The required limits for process parameters
- The required limits for in-process tests
- Release specifications for the product, substance, or intermediate (as applicable)
- Stability specification for the product, substance, or intermediate (as applicable)
- The requirements of related cGMP and procedural controls (e.g., facility, utilities, equipment, training, batch instructions)

The definition of the intended commercial manufacturing process and control strategy may evolve over time, and modifications can be iterative between laboratory development studies and manufacturing activities for clinical supplies. It is good practice to document the process description in development reports or technology transfer documents, or both, prior to the onset of Process Validation Stage 2 activities. Note that the final control strategy licensed for commercial production may sometimes vary from that used when entering Process Validation Stage 2 as a result of feedback from health authorities that occurs during the review of the regulatory license application and/or during manufacturing site inspections as well as accumulated knowledge from additional manufacturing experience, including product investigations.

6.2.1 Considerations for process characterization

Batch processes are defined by a series of batch unit operations connected in a specific order. A defining characteristic of batch processes is that the entire batch of material is pooled and homogenized before and after each unit operation. In contrast, continuous and integrated processes are designed with unit operations that are integrated without a batch pool vessel between each unit operation, and some or all unit operations are run continuously. As a result, this design introduces certain special considerations for the process design and validation.

Linked unit operations (e.g., two linked chromatographic steps) can routinely propagate time-dependent variability in product stream quality (e.g., impurity content) or conditions (e.g., pH) from the first unit operation to the next, much more so than batch processes that completely homogenize the product stream between unit operations. Therefore, the product stream variability of the output of a sending unit operation as well as the robustness of a receiving unit operation should be evaluated as part of process characterization (Section 3.6), and the unit operations on the receiving end of a linkage should be designed to be robust to the normal and expected variability of the inlet stream. This variability would be largest for unit operations not connected through any surge vessel and would decrease as the size of the surge vessel increased (e.g., from small surge to cycle surge to batch pool). Process characterization activities may be designed to employ suitably accurate scaled-down models of the unit operations and surge vessels so that robustness can be evaluated at a reduced scale exactly as it would be experienced in the commercial environment. However, commercial-scale mixing vessels may be a challenge to accurately model at laboratory

scale. Alternatively, characterization studies may be designed to demonstrate that unit operations perform successfully under the "worst case" inlet stream conditions (Section 3.6).

Examples of continuous unit operations were discussed in Section 1.2.1 and may include continuous perfusion production bioreactors, multi-column chromatography (MCC), multi-unit filtration schemes, continuous inline process stream adjustments, or the use of a plug flow reactor for viral inactivation (VI). Note that some examples are inherently continuous technologies (e.g., in-line adjustments, plug flow reactor VI), and others are inherently batch operations where continuous flow is achieved using multiplicity and automation (e.g., MCC, multi-filter). Production by continuous perfusion is often considered to be a continuous unit operation, but it is inherently a batch operation of long duration due to the complexity and dynamics of cell culture.

Process characterization of continuous unit operations should use suitably accurate reduced-scale models as described in Section 2.5. For situations where the manufacturing unit operation is inherently batch (e.g., chromatography), data obtained using laboratory batch models can be readily leveraged if no continuous laboratory model system is available or suitable. For situations where the manufacturing operation is inherently continuous (e.g., plug flow reactor VI), an approach employing batch data can still be developed and justified if there is sufficient understanding of the key engineering variables of the unit operation (e.g., viral inactivation time, plug flow residence time distribution, pH control features, and temperature control requirements).

6.2.2 Virus clearance considerations

The viral safety aspects of a continuous process are specifically covered in Section 3.7. As part of the evaluation of the viral clearance, the small-scale experimental design must fulfill the same requirements as for batch processing, such as for the number and types of viruses, number of replicates, testing methodology, and experimental controls. The difficulty will reside in the definition of the "worst case conditions" and in demonstrating that the scale-down model is representative, as discussed above. The synchronization of the virus spiking, sampling points/pools, and definition of clearance (over a single cycle or multiple cycles) will also have to be carefully designed and justified with respect to full-scale operation.

6.2.3 Scale-down model considerations

As mentioned earlier, the driving principles of each unit operation remain valid, regardless of whether the operating mode is batch or continuous. For example, the performance of an ion exchange step will be dictated by the same process parameters, including the pH, conductivity, and load amount, for either operating mode. In addition, some "continuous" step designs, such as for chromatography, actually involve the synchronized cycling of multiple devices (e.g., columns), each operated in batch mode. Therefore, demonstrating that a small-scale model is representative (i.e., qualifying the model) could be performed in batch mode.

The execution of small-scale studies (e.g., for process characterization) is constrained by the timing / synchronization factors listed earlier. Operating in "batch mode" during these studies may be perfectly adequate in many cases if it is correctly justified. Ensuring that small-scale studies are representative of manufacturing conditions may constitute more of a challenge for certain steps, such as the following:

- Chromatography for multi-column, simulated moving bed chromatography operations involving "overloading" or "side fraction" recycling – i.e., the load flowthrough from one cycle is applied to the column used in the following cycle, or the side fractions of the eluate of a cycle are recycled in the load of the following cycles: In these cases, showing that the studies are representative may require the execution of at least two cycles at small scale because the "simple" overloading of a single column with an homogenous starting material may not constitute a representative model of a load with **i)** material coming from the flowthrough of the preceding cycle, completed by **ii)** material from the previous unit operation, for which the composition/properties may vary in time, and **iii)** the total amount of overload applied, which may vary slightly from one cycle to the next depending on the synchronization of i) and ii).
- Mixing operations in a surge tank used to contain the collected chromatography eluate as the peak exits the column: After elution, the material is retained in the surge tank for a variable duration that is a function of the total retention volume of the surge tank and the volumetric flow rate of the feed material. The retention time in the surge vessel along with the mixing characteristics of the vessel and the viscosity of the liquid will then determine how well the contents have been homogenized to a consistent composition. Also note that surge tanks should have defined minimum and maximum qualified volumes based on acceptable mixing performance (maximum volume) and prevention of agitation-based foaming (minimum volume). The residence time distribution obtained in manufacturing-scale equipment may be difficult to reproduce exactly in a small-scale model.
- Clarification / (nano)filtration / single-pass ultrafiltration/diafiltration (UF/DF) steps: Questions to be addressed include the following: How sensitive are these steps to variations in feed stream quality and composition? How will this sensitivity affect their throughput and retentive performances? Will a "bracketing approach" in batch mode be sufficient, or will the transitions observed in real processing between the tested extremes have an influence of their own?

As a result, the justification of the scale-down models used for process characterization and process validation studies as being representative is critically important for the acceptability of the results obtained during these studies as well as the acceptability of the ranges established using these scale-down models.

6.3 Process Validation Stage 2: Process Qualification

The second stage of process validation demonstrates that a process (including all elements of its control strategy) can perform effectively in a commercial manufacturing environment. Activities in this stage initially focus on the suitability and readiness of the manufacturing facility, process utilities, and process equipment. These activities must generally be completed prior to the initiation of subsequent studies to evaluate process performance (including the PPQ study).

The facility, utilities, and equipment for any process (batch or continuous/integrated) must be suitably designed and built, and utilities and equipment must be installed in compliance with design

specifications. Requirements of the facility, utilities, and process equipment are defined during Stage 1 of process validation (process design), and, accordingly, qualification of each system should be performed to ensure and document that it is fit for purpose based on the demands of the process (i.e., the intended equipment operating ranges and capacity requirements) as well as expected start-ups, interventions, and stoppages.

The PPQ study provides evidence that the process design and control strategy can perform effectively and as intended in the commercial manufacturing setting. It provides valuable information demonstrating the success of the process beyond the development environment (laboratory, pilot plant) in that it incorporates the actual facility, utilities, equipment, personnel, and procedures intended for commercial production. The design of the PPQ study, including the number of batches and manufacturing conditions (e.g., materials, batch size, and equipment settings), should be based on an understanding of how the process is run and should be relevant to its routine operations. Fewer unique study conditions are generally required if the process design data (from Stage 1 of process validation: process characterization) support an understanding of the impacts of variability in materials and process parameters, and if the effects of scale are well-understood and are acceptable. Generally, a PPQ study incorporates the collection and evaluation of a more comprehensive set of data throughout the process that are in addition to the data that are routinely collected and evaluated as part of the routine control strategy. These data are collected to confirm that product quality meets expectations throughout the process and are also often used to evaluate product quality uniformity at process points where uniformity is expected. The PPQ study should be run under a formal protocol and approved by the Quality unit.

6.3.1 Considerations for the qualification of facilities, utilities, and equipment

Processes with linked (integrated) unit operations may have some process equipment configurations that require special consideration as part of the equipment qualification activities of process qualification.

Surge vessels: Surge vessels (small, cycle, or batch pool) may be placed between unit operations to provide an element of physical disconnection and allow for small processing rate differences between linked consecutive steps. They may also provide an opportunity for partial (small, cycle) or full (batch pool) homogenization of material between steps. Mixing qualification activities for surge vessels should consider the minimum and maximum working volume of the vessels during routine production driven by considerations for effective mixing, avoiding conditions which could lead to foaming, etc. Additionally, automation actions taken if the minimum or maximum pool levels are reached must be included as part of equipment qualification studies.

In-line adjustments: Processes with linked unit operations may have the capability for adjustments (e.g., product concentration, pH, conductivity) to a product stream, avoiding the need for a batch pool vessel and homogenization. This adjustment may in fact be treated as separate unit operation. The associated equipment typically includes a pre-adjustment sensor, a pumping system for adding the diluent or titrant, an in-line mixer, a post-adjustment sensor, and an associated automation system for process control. An automated diverting valve may also be part of the system to divert a product stream that does not fall within the intended range after adjustment. The process equipment qualification plan should address the individual equipment components of the in-line

adjustment operation. The equipment should be challenged over the full expected range of the required adjustment and over the expected range of the flow rate. Automated actions taken to divert a non-conforming product stream should also be included as part of the equipment qualification studies.

Microbial control: Maintaining a process under aseptic conditions and detection of microbial contamination can be a challenge over long production periods. Design and use of a closed system and minimization of manual interventions can help reduce contamination risk, along with suitably developed cleaning and sanitization procedures (***Section 5.2.2***). Aseptic process simulations using either product or a growth-promoting surrogate product stream should be considered during equipment qualification to demonstrate the adequacy of the equipment design, cleaning and sanitization procedures, and processing procedures over a time period long enough to provide adequate assurance of microbial control over the intended production period. The equipment qualification studies should also consider start-up and shut-down dynamics and the possibility of process pauses that may be part of normal operation.

6.3.2 Considerations for the PPQ study design

Processes with linked (integrated) unit operations may require special considerations when designing the PPQ studies. An initial consideration may be connected to the definition of a batch itself. As indicated in the batch definition (***Figure 1.3***) a single upstream bioreactor run could, by definition, generate multiple final drug substance (DS) batches. If this option has been included as part of the batch definition, the range of batches generated for each upstream batch should be defined, and it would be useful to cover that range as part of the PPQ plan. While the PPQ may be primarily run under "target" conditions, the PPQ study design may also include appropriate challenges to demonstrate the response of the process to the types of variability that will likely be observed over normal operation. Some examples of conditions to challenge during PPQ include:

- Start-up and shutdown of a normal production batch
- Process pauses
- Higher and lower flow rates and flow rate perturbations

Data should be collected to demonstrate that the process and associated automation respond appropriately and either provide robust control and/or divert non-conforming product. Additionally, data should be collected from product streams between unit operations to demonstrate that the product exiting one connected unit operation and entering the next one is suitable and within the expected and required range of variability (e.g., for product concentration, pH, conductivity) based on process characterization studies performed during development. Data should also be collected from points between unit operations periodically throughout the production to demonstrate adequate microbial control throughout the production period.

Processes designed to incorporate continuous unit operations or unit operations that are linked without batch pool vessels (commonly used in fully batch processes) present special considerations for the design of studies that are part of Process Validation Stage 2 (process qualification). Note that the qualifications of facility, utilities, process equipment, and process performance may each have special considerations, and these are discussed below. Typically, a small alignment of the throughputs of successive process steps may result in residence time distribution variability that will

have to be characterized. In addition, the synchronization of successive operations may require that some unit operations be operated differently than in batch processing. For example, filtration or nanofiltration steps will likely be run under flow control mode, in which back-pressure increases with the processed volume (filter fouling), rather than under the constant pressure mode typically used in batch processing, in which the flow decreases with increasing processed volume. These conditions should typically be considered as part of the process design (Section 2.3.4) and characterization (Section 3.7.4), but verification of ranges during earlier manufacturing runs at scale will help inform the validation strategy.

As discussed previously, unit operations may be linked directly or linked using a surge vessel (small surge, cycle surge, or batch pool). In-line adjustments with in-line mixing (e.g., pH, product dilution) may also be required between unit operations. The process capabilities associated with surge vessels and in-line adjustments require additional equipment qualification activities. Additionally, data should be collected as part of PPQ to evaluate process stream variability between linked unit operations and provide assurance that any observed variability is expected based on development studies and is acceptable.

6.3.2.1 Example 1: PPQ design considerations for steady-state continuous perfusion integrated with continuous capture

In an integrated operation, the permeate from the production bioreactor cell retention device can be loaded continuously or semi-continuously onto the capture column. Synchronization between steps, operational changes, changes in raw material lots, etc. with the definition of a drug substance (DS) batch needs to be established. For example, the change of a lot of culture medium during a perfusion run may potentially have an impact on culture performance, i.e., it could affect cell metabolism/growth, productivity, and product quality as was identified in the example given in Section 4.4. This type of change in raw material (RM) lot or temporary deviations in operating parameters may also have delayed effects on the quality of the product, such as a change in glycosylation detectable only hours/days after the RM change or the occurrence of the temporary deviation. It is important to maintain a precise traceability of any changes in the batch history taking into consideration synchronization of product batches, raw material lot changes, resulting modification of cell culture behavior, and retention time distribution throughout the process. These details should be added to a process data summary like the example given in ***Table 4.2*** and will provide even greater utility. However, these changes in product quality are unlikely to impact the performance of the Protein A capture step directly. A more likely source of variability could arise from variation in product titer as the culture progresses. This variation will have a direct impact on the efficiency of the capture onto the Protein A, and it requires decisions concerning processing strategies that will affect not only the capture step but also the subsequent steps. The ”deltaUV BT load” approach to controlling Protein A column loading described in Section 2.3.1.1 is intended to help mitigate this concern, but it would be useful to collect data during PPQ to confirm that the control demonstrated in the scale-down model studies is effective at scale.

6.3.2.2 Example 2: PPQ design considerations for linked capture and continuous low-pH VI steps

The control of the linked capture and low-pH inactivation steps will be dictated both by the characterization of the inherent variability of some the continuous process parameters and by the manufacturing strategy selected by the developer. One challenge here is to ensure that the surge

tank sizing and process flowrate control ensure that titration in the low pH step remains relatively consistent and "titrating against the peak" as described in Section 2.3.2.1 is avoided. The characteristics of the capture elution stream are expected to remain relatively reproducible across purification cycles. Any variation in the cadence of the capture step output may affect other purification steps further downstream. For the low pH inactivation, it may affect the efficiency of the in-line mixing during acidification, as well as the retention time within the reactor / surge tank. Consequently, the level of exposure of an eventual virus contaminant to inactivating conditions may also be impacted.

The description above is provided as an illustration of how the normal variations of a process performance attribute (i.e., product titer) and the associated critical process parameters (i.e., the column load or titration volume) along a continuous manufacturing run will require the developer to select amongst different process design strategies. Each of these strategies will have a direct impact on three factors:

1. The scope of process characterization studies to be performed
2. The type of control strategy that will need to be implemented (e.g., basic or with PATs)
3. The final process performance in terms of robustness, cost of goods sold (COGS), etc.

The same type of balancing act will have to be repeated for other critical process parameters and material attributes exhibiting a variability inherent to the continuous process, such as the flow rate of the perfusion reactor effluent, the quality of the product, and the purity of loaded material.

6.4 Process validation stage 3: Continued process verification (CPV)

The third stage of process validation, Continued Process Verification (CPV), monitors the performance of the process and associated control strategy, as designed and implemented in the commercial manufacturing environment, to determine whether it remains in a state of control. If variations from a state of control are observed, they are typically investigated with the intent of understanding and reducing process and product variability. CPV is a program rather than a single study, and although it may be updated based on process and product understanding, it continues for the lifetime of the process and product.

The CPV program should by design include within its scope important process input and output variables, informed by Process Validation Stages 1 and 2. Typical input variables that are monitored within a CPV program include Critical and Key (if this classification is used) process parameters and selected attributes of input materials. Typical output variables included in a CPV program are selected in-process measurements of process conditions (e.g., pH or temperature), measurements of in-process product quality (e.g., purity), and measures of process performance (e.g., step yield). These data are mostly data that are routinely collected as part of the commercial process control strategy. The design of a CPV plan may also complement these data with data from additional/ supplemental samples and measurements that are not formally part of the process control strategy but serve to provide insight into the control of the process (e.g., daily measurements of cell viability or cellular metabolites in a production bioreactor).

It is important to design a CPV program with an objective means to evaluate the data collected. The principles and tools of statistical process control (e.g., control charts) are most often employed, and it is generally expected that personnel knowledgeable in process statistics assist in the design of the program for data review as well as participate in the normal data review process. It is important to establish a rational frequency for periodic review of the data (e.g., after each campaign, after a selected number of batches are produced, or on a time-based interval). Likewise, it is important to preestablish the guidelines that would trigger an investigative action (e.g., selected Western Electric or Shewhart rules to be used in conjunction with control charts).

CPV programs are typically governed by a company's Quality unit, and it is expected that the program evolves as process and product understanding mature. It is typical for the data in-scope of the CPV program (i.e., the input and output variables) to be adjusted over time (decreased or increased) based on an assessment of risk along with their control limits. Similarly, it is typical for the frequency of data collection and data analysis to vary (decreased or increased) with increasing experience and assessment of risk. Business pressures commonly exist that drive the CPV program in the direction of a reduced effort; that is, there is pressure to reduce CPV to the leanest possible level. The scope of data evaluated and the frequency of review may be reduced based on limited experience, and if taken too far, the value of CPV may suffer as important sources of process and product variability that occur over longer intervals are not evaluated and are missed (e.g., raw materials, personnel, equipment wear).

6.4.1 Considerations for CPV program design for continuous and integrated processes

The basic principles of CPV program development for batch processes also apply to the development of CPV programs for processes with continuous unit operations and integrated unit operations; however, there may be some differences in how the principles are practically applied. Three specific examples are discussed here.

6.4.2 CPV data from continuous unit operations and traceability

Traditional batch unit operations (e.g., a batch production bioreactor or batch chromatographic step) are run by cycling (one or more times) a unit operation, typically with product homogenization before and after. For a given batch of drug substance or drug product, this facilitates CPV data collection associated with a specific unit operation cycle, and as part of the CPV program, the state of control of a unit operation can be assessed through trending and evaluating data from each cycle.

Continuous unit operations, in contrast, do not operate cyclically and may provide data on a continual basis. The state of control of continuous unit operations that operate to a single mean target value (e.g., continuous in-line adjustment to a target pH) can be assessed using control charts with a single set of statistically derived control limits. Continuous operations (or batch unit operations of long duration such as perfusion production) with predictable but dynamically varying means can make use of a "3SD Tunnel". The "3SD Tunnel" employs a control chart with control limits of three standard deviations together with a dynamically varying process mean (BPOG, 2014, Continued Process Verification).

The CPV program should evaluate unit operations that approximate continuous behavior through cycling and multiplicity (e.g., MCC or multiple-unit filtration) through separate monitoring of each

similar but physically distinct unit (e.g., each filter unit or each chromatographic column). This will permit the detection and correction of unwanted variability associated within the unit operation.

Traditional batch processes typically feature single unit operations performed a single time (or for a predefined number of sub-cycles) with the in-process material pooled and homogenized before and after each unit operation. Further, it is typically very straightforward to associate data of specific batches of drug substance or drug product to data of specific cycles of each upstream unit operation. This facilitates the review and interpretation of related data across unit operations for specific material batches. In contrast, processes with highly integrated unit operations present a traceability challenge for the interpretation of CPV data across process steps, like the challenge for the interpretation of process data (and process excursion data) in support of routine batch release. CPV, like batch release, requires that the program be based on an established understanding of material flow, including residence time, throughout the full process.

Chapter 7

An Integrated Control Strategy for Commercial Manufacturing

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7 An Integrated Control Strategy for Commercial Manufacturing

7.1 Updates to the integrated control strategy after process performance qualification (PPQ)

Control Strategy #2, which was established prior to registrational and PPQ batches (Table 4.1), should be updated with learnings from post-PPQ experience, responses to questions from dossier submissions, and facility audits to form the basis for Control Strategy #3 for commercial manufacturing. This approach follows the progression shown in Figure 1.7, which relates continued risk assessments and increased process and product knowledge with evolution of the control strategy. This updated version is shown in Table 7.1, and an update to the list of examples of parametric and material attribute controls from the previous version (Table 4.2) is shown in Table 7.2.

Table 7.1. Control Strategy #3: Commercial control strategy updated after registrational batches and PPQ.

CQA	Potential Source, Stress Response and Clearance	Process Controls: Parametric Controls & Material Attributes	Process Capability	Analytical Controls: Testing Strategy including IPCs	Residual Risk
Glycosylation: Galactosylation	Source: Bioreactor Stress Response: None Stability Indicating: No Clearance: None	Bioreactor PPs: Temp, pH, DO, harvest day Bioreactor MAs: Trace metals (Cu & Mn)	High	DS Release Testing	Low
Glycosylation: Fucosylation	Source: Bioreactor Stress Response: None Stability Indicating: No Clearance: None	Bioreactor PPs: Temp, pH, DO, harvest day Bioreactor MAs: Trace metals (Cu & Mn)	Medium	DS Release Testing	Low
Glycosylation: High Mannose	Source: Bioreactor Stress Response: None Stability Indicating: No Clearance: None	Bioreactor PPs: Temp, pH, DO, harvest day Bioreactor MAs: Trace metals (Cu & Mn)	High	DS Release Testing	Low
Deamidation at Asn325	Source: Bioreactor, DSP Stress Response: pH, heat Stability Indicating: Yes Clearance: None	Bioreactor PPs: Temp, pH, DO, harvest day DSP PPs: DS/Intermediate hold time & temp	High	Discontinue Release and Stability Testing – Deamidation at ASN325 was determined to be not clinically relevant	Low

CQA	Potential Source, Stress Response and Clearance	Process Controls: Parametric Controls & Material Attributes	Process Capability	Analytical Controls: Testing Strategy including IPCs	Residual Risk
High Molecular Weight Species (HMWS)	Source: Bioreactor, DSP Stress Response: pH, heat, shaking, light, metals, freeze/thaw Stability Indicating: Yes Clearance: CEX = 3-fold	Bioreactor PPs: Temp, pH, DO, harvest day DSP PPs: VI: pH & time CEX: protein load DSP CMAs: CEX elution buffer pH Trace metal leaching Procedural Controls: Protect process intermediates from light when risk of exposure is high; Minimize vortexing and foaming in surge tanks	High	Consider control of CEX load based on IPC for HMWS post-VI to maximize yield DS Release Testing DS Stability Testing	Low
Host Cell Protein (HCP)	Source: Bioreactor Stress Response: None Stability Indicating: No Clearance: Chrom Step(s)	Bioreactor PPs: Temp, pH, harvest day DSP PPs: protein load, peak cutting	High	DS Release Testing	Low

CEX	Cation exchange
Chrom	Chromatography
CMA	Critical material attribute
DO	Dissolved oxygen
DS	Drug substance
DSP	Downstream process
HCP	Host cell protein
HMWS	High molecular weight species
IPC	In-process control
MA	Material attribute
PP	Process parameter
PPQ	Process performance qualification
VI	Virus inactivation

Table 7.2. **Summary table for process parameters and material attributes.**

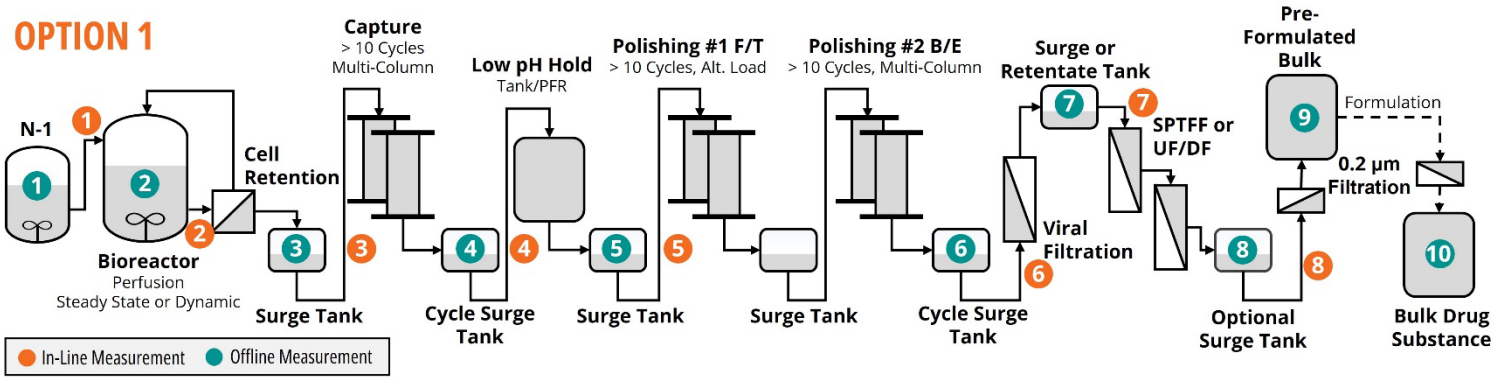
Process Parameter or Material Attribute	Experience Range in PD/PC	Control Range in Clinical Mfg	Control Range in PPQ	Proposed Commercial Target & PAR	Justification of Proposed Commercial PAR
Bioreactor					
Growth Phase pH	6.9–7.3	7.0–7.2	7.0–7.2	7.1 (7.0–7.2)	PV Stages 1&2
Growth Phase Temperature (^{0}C)	34.5–37.5	35.5–36.5	35.5–36.5	36.0 (35.5–36.5)	PV Stages 1&2
Growth Phase Perfusion Rate (CSPR, nL/cell/day)	0.04–0.06	0.045–0.055	0.047–0.053	0.050 ± 5% (0.047–0.053)	PV Stages 1&2
Production Phase Shift Timing (day) [A]	5–7	5.5–6.5	5.75–6.25	6.00 (5.75–6.25)	PV Stages 1&2
Production Phase pH	6.9–7.3	7.0–7.2	7.0–7.2	7.1 (7.0–7.2)	PV Stages 1&2
Production Phase Temperature (^{0}C) [A]	31.5–34.5	32.5–33.5	32.5–33.5	33.0 (32.5–33.5)	PV Stages 1&2
Production Phase Perfusion Rate (vvd) [A]	1.6–2.4	1.9–2.3	1.9–2.1	2.0 ± 5% (1.9–2.1)	PV Stages 1&2
Dissolved Oxygen (%sat)	20–80	40–60	45–55	50 (45–55)	PV Stages 1&2
Trace metal (Cu, Mn) conc in cell culture media	Not Tested	Cu: 0.2*–2 mM Mn: 0.2*–2 mM *OOT batch	Cu: 1.5–2.5 mM Mn: 1.5–2.5 mM	Cu: 1.5–2.5 mM Mn: 1.5–2.5 mM	Learnings from OOT Investigation
ProA Capture Chromatography					
Loading/Wash Residence Time (min)	4–8	5–7	5–7	6 (5–7)	PV Stages 1&2
Loading (BT load control) (g/L-resin)	5–60	45–55	45–55	50 (45–55)	PV Stages 1&2
Elution Buffer pH [B]	3.3–3.7	3.4–3.6	3.4–3.6	3.5 (3.4–3.6)	PV Stages 1&2
Elution Buffer Conductivity (mS/cm) [B]	2–5	3–4	3–4	3.5 (3.0–4.0)	PV Stages 1&2
Process Pause (min)	15–30	<30	<30	<45	PV Stages 1&2 Learnings from CPP excursion investigation
Viral Inactivation					
Low pH Target	3.3–3.7	3.4–3.6	3.4–3.6	3.5 (3.4–3.6)	PV Stages 1&2
Incubation Duration (mins)	55–75	60–70	60–70	65 (60–70)	PV Stages 1&2
Protein Conc (ProA BT load ctrl) (g/L)	3–20	15–18	15–18	15–18	PV Stages 1&2

Process Parameter or Material Attribute	Experience Range in PD/PC	Control Range in Clinical Mfg	Control Range in PPQ	Proposed Commercial Target & PAR	Justification of Proposed Commercial PAR
Post-Incubation pH (ProA BT load ctrl)	6.5–7.5	6.8–7.2	6.8–7.2	7.0 (6.8–7.2)	PV Stages 1&2
Polishing Chromatography 1 (AEX – F/T)					
Loading (ProA BT load ctrl) (g/L-resin)	100–250	180–220	180–220	200 (180–220)	PV Stages 1&2
Load pH	6.5–7.5	6.8–7.2	6.8–7.2	7.0 (6.8–7.2)	PV Stages 1&2
Load Conductivity (mS/cm)	4–8	5–7	5–7	6 (5–7)	PV Stages 1&2
Polishing Chromatography 2 (CEX – B/E)					
Loading (ProA BT load ctrl) (g/L-resin)	20–50	35–45	35–45	40 (35–45)	PV Stages 1&2
Load pH	6.5–7.5	6.8–7.2	6.8–7.2	7.0 (6.8–7.2)	PV Stages 1&2
Load Conductivity (mS/cm)	4–8	5–7	5–7	6 (5–7)	PV Stages 1&2
Wash pH [B]	7.2–7.6	7.3–7.5	7.3–7.5	7.4 (7.3–7.5)	PV Stages 1&2
Elution pH [B]	7.5–8.0	7.5–8.0 [C]	7.50–7.60	7.55 (7.50–7.60)	PV Stages 1&2 and Learnings from OOT Investigation

[A] Both temperature setpoint and perfusion rate change with this shift timing parameter
[B] Controlled as a material attribute of the buffer solution
[C] Variation in HMWS clearance linked to pH and resin lot

AEX	Anion exchange
B/E	Bind and elute
BT	Breakthrough
CEX	Cation exchange
CSPR	Cell-specific perfusion rate
Cu	Copper
HMWS	High molecular weight species
F/T	Flowthrough
OOT	Out of trend
PAR	Proven acceptable range
PC	Process characterization
PD	Process development
PPQ	Process performance qualification

In addition, the analytical sampling plan from clinical and PPQ runs (**Figure 4.2**) was updated for commercial manufacturing as shown in **Figure 7.1**. As in previous versions of the sampling plan, note that the column labeled "Elapsed Time" refers roughly to the procession of a specific aliquot of material as it traverses the process and is based on the details in **Figure 2.13**. The overall elapsed time is obviously process-specific, but this column has been included as a reminder that the sampling, testing, and possible reaction to a result all occur in real time as the process continues.



Sample Point	Process Step	Elapsed Time (h)	Capacitance	Inline A280	Fluid Mass Flow	pH	Conductivity	VCD/Viability	Metabolites	Titer/A280	Glycosylation	Deamidation	HMWS	HCP	Initial Risk (of variance)	Sampling Frequency: Initial Runs
		Performance Attribute								Quality Attribute						
1	N-1		•					•							Low	Last day
2	Bioreactor		•					•	•						Medium	Every 2d after inoculation
			•							•						Every 4d after start of perfusion
3	Post-ATF/TFF Permeate Surge Tank	0			•					•					Low	1. Prior to 1st capture cycle 2. Once every 24h 3. Prior to last capture cycle
4	Post-ProA Surge Tank	8		•	•	•	•			•					Low	1. Prior to 1st capture cycle 2. Weekly, after cycle homogenization 3. Prior to last capture cycle
5	Post-VI Surge Tank	12		•	•	•	•			•					Low	1. After 1st cycle 2. After last cycle
6	Post-Polishing Chrom Pre-VF Surge Tank	14		•	•	•	•			•					Low	1. After 1st cycle 2. Sample from the surge tank corresponding to each lot of planned DS 3. After last cycle
7	Post-VF, Pre-UFDF Surge	16		•	•	•	•			•					Low	Every 24h
8	Post-UFDF, Pre-0.2 µm filter	18		•	•	•	•			•					Low	Every 24 h
9	Post-UFDF, Post-0.2 µm	18								•					Low	Representative sample of every bag filled
10	Bulk DS									•	•	•	•	•	Low	Representative sample of every bag filled

Figure 7.1. **Analytical sampling and testing plan for commercial manufacturing.**

7.2 Analytical approaches to support an integrated continuous control strategy

The goal of the integrated control strategy is to ensure that critical quality attributes are controlled within the target ranges that maintain product safety and efficacy, and an analytical testing strategy is a critical component of this strategy. In addition, an effective continuous process control strategy demands rapid detection and reaction to a perturbation to ensure consistent final product quality. In continuous manufacturing, process steps are tightly interconnected, and the material generated in one step is directly processed to the following steps. A perturbation on one unit operation therefore has the potential to rapidly "propagate" through multiple downstream unit operations, with a potential impact on the final material produced.

Control of product quality can be achieved by direct monitoring of the quality attributes during manufacturing. Such monitoring could be quite specific for a given product and process. For example, as explored in the case study in this document for control of high mannose glycan species, one could imagine that a rapid, attribute-specific method such as lectin-based HPLC for rapid analysis of high mannose species described by (Kim & Albarghouthi, 2022) would be quite useful. However, such bespoke methods do not have general utility, unless control of a specific attribute is a consistent challenge. It may be more useful to explore the application of LC-MS-based multi-attribute methods (MAM), around which much has been published recently, for rapid assessment of CQAs as part of either final testing of DS or DP (Camperi et al., Goyon et al., 2021; Mouchahoir et al., 2021; Rogers et al., 2017; Rogstad et al., 2019), as an enhancement to stability testing (Vallejo et al., 2021), or as a component or real time process analysis (Apostol et al., 2021; Dykstra et al., 2021; Jakes et al., 2021; Y. Liu et al., 2020). The most desirable approach involves direct monitoring of the CQAs throughout the process using methods that are directly correlated to those used for release testing of either DS or DP. It will be equally important to develop more advanced technologies for monitoring critical process parameters (CPPs) and critical material attributes (CMAs) coupled with enhanced knowledge of their impacts on the product critical quality attributes (CQAs) (primary effects and interactions). Thus, an in-process objective could be detection of deviations as a signal for diverting a fraction of the process stream (Section 8.5.3).

Chapter 8

Managing the Process in Real Time: Deviations in Product Quality

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8 Managing the Process in Real Time: Deviations in Product Quality

8.1 Introduction and scope

The purpose of this chapter is to discuss considerations for managing deviations to critical controls in the context of continuous manufacturing. For this chapter, critical controls are defined to include critical process parameters (CPPs), critical material attributes (CMAs), critical in-process controls (IPCs), and drug substance specifications. Although, under cGMP, critical controls also apply to facilities, instruments, personnel, utilities, etc., the general cGMP considerations for deviations that impact these controls are not unique to continuous manufacturing and will not be discussed in this chapter.

In general, deviations should be opened whenever there is a departure from a state of control with the potential to impact product quality. This chapter will focus on a subset of potential triggers for deviations:

- Excursions of CPPs from registered operating ranges with the potential to impact critical quality attributes (CQAs)
- Excursions of critical IPCs from registered action or reject limits
- Out-of-specification results for drug substance

If whole-lot in-process pools are available for testing, or if more advanced on-line or at-line process analytical technologies (PAT) are deployed, drug substance specifications may be verified using real-time release from the in-process test, rather than through off-line Quality Control lab tests. For this chapter, the distinctions between in-process or real-time release tests and tests performed on the drug substance are relevant only with respect to the time of detection of the unexpected test result. The chapter discusses the implications of a "real time" test result on the ability to make timely decisions about forward processing and other immediate actions to intervene in the continuous process.

This chapter covers key technical and Quality Management System (QMS) considerations and requirements to ensure that investigations and associated actions are effective for continuous manufacturing. QMS requirements include the following:

- Identification and segregation and/or disposal of potentially impacted drug substance lots or constituent portions of these lots
- Rapid root cause analyses (RCA) and product impact assessments for which the time available to investigate and the access to in-process samples for non-routine investigational testing may be constrained by continuous manufacturing
- Immediate corrective actions required to continue processing additional portions of the lot, as documented within the batch record

The chapter will not cover lot disposition decisions or corrective and preventive actions (CAPAs) because their decision framework and impact to operations are not unique to continuous manufacturing. A continuous manufacturing run ultimately results in one or more discrete drug

substance batches, which will be dispositioned according to typical requirements. A deviation tied to a given batch could result in lot rejection, quarantine, or release according to normal QMS procedures. If an investigation results in CAPAs, the corresponding actions will likely be implemented in future batches, after the completion of any ongoing portions of the continuous run wherein the deviation originally occurred.

Critical material attributes (CMAs) are an important consideration for continuous manufacturing because of the intensified use of critical single-use systems. Materials are generally managed using a batch-like procedure wherein each lot of materials is made and released for production off the critical path for continuous manufacturing. As a result, deviations to CMAs would normally be detected off the critical path for continuous manufacturing decisions, either well in advance of use, or after the fact in the case of undetected defects. For this reason, deviations to CMAs will not be discussed in depth in this chapter. An important exception to this is the case wherein a previously unknown relationship between a particular material attribute is identified during the course of manufacturing using multiple lots of a given raw material. In a case such as this, acceptable ranges for a given CMA need to be reestablished to maintain an orderly process of preventing unacceptable materials from entering the process. Two examples of this were given in **Section 4.3** and will be listed in the relevant tables in this chapter.

To further focus the discussion, the CPPs and CQAs related to adventitious agent control and to general drug substance properties determined by the final ultrafiltration/diafiltration (UF/DF) step (e.g., pH, osmolality, and protein concentration) are excluded. The adventitious agent control strategy is discussed in **Chapter 5**.

8.2 Framework considerations for the N-mAb process

This discussion of deviation management for the N-mAb process is dependent on the N-mAb process flow framework (**Chapter 1**) and associated control strategies (**Chapter 7**). The minimum requirement for a deviation management system is to inform the disposition of the resulting drug substance batch. The management of product quality deviations requires at least three elements:

1. Detection of an excursion of process control beyond a defined limit that potentially impacts product quality for either a critical process parameter (input), a critical process control (output), or an acceptance criterion for drug substance quality.
2. An investigation to determine the root cause(s) for the loss of control
3. A determination of the potential impact of the excursion/deviation on the resulting drug substance batch and any other impacted batches associated with the root cause(s)

In addition, for continuous manufacturing processes, a fourth element is expected to be an integral part of the deviation management approach, which is dependent on the specific process framework design.

4. An intervention to divert to waste a portion of the process stream (or batch) that is predetermined to have a high potential to be negatively impacted by the detected deviation to prevent subsequent impact to the remainder of the batch. With considerations of the N-

mAb framework process design, diverting to waste can be active (prospective) or static (retrospective). For prospective diversion, a portion of the process stream under active movement/processing is diverted at an intervention point prior to being added to a batch pool until the process is considered back in control or the root cause for the deviation has been identified and remediation actions have been put in place. The detection of the deviation would therefore either occur as part of active process monitoring or it would be part of rapid testing of a sample representing a cycle surge tank or a point-in-time sample. In contrast, static or retrospective diversion is dependent on testing an intermediate batch pool, which can be held until testing for the impact of a deviation is complete or forward processed at risk if the potential for the deviation to impact product quality is low. Diverting a batch pool prior to complete processing removes all material processed up to the detection of the deviation.

The intervention to divert to waste a portion of an active process stream is considered the basic approach to be taken with continuous processing; however, if the process includes intermediate batch pools, alternate approaches that include segregation of material, confirmation testing, and re-introduction for forward processing can be considered if the systems hardware, automation, and quality systems allow this and are validated for such use. The segregation of non-batch pools (cycle surge tanks) or portions of an active process stream with the potential for re-introduction into the process will need to be considered on a case-by-case basis and will be dependent on having the appropriate systems in place to accommodate segregation, testing, and re-introduction into the process stream. This approach will not be expanded upon in this chapter. The formal QMS implications of these elements are discussed in more detail in **Section 8.3**.

The active management of a CPP deviation will be determined based on the integration of monitoring instruments and equipment, an understanding of the response time for detection with respect to potential action, and the capability to remove the impacted process stream from the system. A risk-based assessment of the potential impact to the final batch will also be part of the CPP deviation management approach to inform the decision as to whether the action taken is diverting to waste or forward processing at risk. Considerations for removing potentially impacted process streams or for segregating material for potential re-processing or re-entry into the process are not part of the primary deviation management approach outlined in this chapter. **Table 8.1** presents an example deviation management strategy for addressing CPP deviations. This table lists the potential CPPs as well as considerations for defining and detecting a deviation and the immediate/potential actions to be taken.

Table 8.1. Examples of CPP controls and deviation actions.

Step	Process parameter	Established Range	NOR	Monitoring strategy (Discrete or Continuous)	OOR Duration triggering a deviation	Action to be taken immediately (Pause, Continue, Divert)	Data and/or samples available for investigation (Step output, subsequent step output, DS testing)
AEX	Load pH	6.8 – 7.2	6.9 – 7.1	Continuous (post VI neutralization)	Outside of NOR (inside established range) for ≥ 0.2 CV or outside of established range for 0.05 CV	Continue processing. Impacts DNA levels and there is redundant clearance from subsequent steps	VI inactivation pH (root cause investigation), VI protein concentration (root cause) AEX (DNA non-routine), CEX (DNA non-routine) DS is not tested for DNA
CEX	Load pH	6.8 – 7.2	6.9 – 7.1	Continuous (post load adjustment)	Outside of NOR (inside established range) for ≥ 0.2CV or outside of established range for 0.05CV	Divert (prior to loading) Impacts DS aggregates levels and this is last step to clear	CEX product (aggregates non-routine test), DS (aggregates routine test)

AEX	Anion exchange
CEX	Cation exchange
CQA	Critical quality attribute
CV	Column volumes
DS	Drug substance
NOR	Normal operating range
OOR	Out of range
VI	Virus inactivation

8.3 Framework and control strategy implications related to excursions to CQA in-process controls (IPC)

Sampling of in-process material for testing of CQAs is generally performed to support process monitoring adjacent to the critical unit operations controlling the CQA. In some cases, in-process testing can substitute for drug substance release testing. However, if the CQA will be formally assessed in the drug substance, there should be a valid, specific motivation for performing potentially redundant in-process testing. For example, if two orthogonal steps are required to control a CQA, then an IPC for monitoring the first step may be desirable to provide more granular monitoring, control, and deviation management.

For the purpose of this discussion, each IPC is expected to have defined limits along with established actions to be taken if those limits are exceeded. In addition, IPC trending should be used to identify potential risks to process performance drift that also requires an action based on the rules defined in the QMS. Those rules are informed from process characterization studies that establish the process operating targets and limits.

For continuous processing, the use of IPCs to detect and react to a loss of control with the potential to impact a CQA is dependent on the processing framework and associated control strategy. In addition, the use of PAT in continuous processing as an element of the overall control strategy is dependent on the availability of robust and readily available technologies that can provide outputs of CQAs in a time frame that allows active management of the process. Two approaches can be taken into consideration when establishing an appropriate control strategy:

1. **A lean approach**, where the framework design is truly continuous with no intermediate pools, e.g., Option 1, and thus IPC testing of CQAs is of limited value unless performed using in-line, rapid PAT technologies. In this instance, CQA testing is performed on the final drug substance only. In lieu of in-line PAT, in-process sampling and off-line testing for a truly continuous system can only be performed retrospectively and can serve to provide supportive data for deviation investigations if the framework design includes routine sampling across the process. If PAT approaches that enable continuous monitoring of IPCs are integrated into the framework design, then real-time IPC monitoring can be applied, although the capability to react to real-time monitoring must be evaluated during process characterization studies to establish the criteria for a required action.
2. **A hybrid approach**, where the framework design involves intermediate pools or intervention options, e.g., Option 2, and thus IPC testing can be used to inform as well as enable actions to remove impacted process flow or pools from the final drug substance. This approach is dependent upon the timely availability of the IPC result to enable a response to a deviation in real time or in a time frame that does not impact other integrated manufacturing operations. Thus, the use of IPC testing will be dependent on enabling sampling and testing technologies.

Other considerations for the use of IPCs for managing deviations in product quality may depend on whether IPC testing is considered part of a dynamic operational feedback approach or a requirement prior to further forward processing, especially if intermediate batch pools are part of

the framework design. If product quality can be ensured by incorporating the monitoring of a CQA via in-process testing, then the potential to enable real-time release testing during manufacturing and reduce the amount of CQA testing for drug substance may be realized. The use of IPCs to obtain data used as part of an adaptive process control approach is considered a future-looking state and will not be discussed in greater detail as part of deviation management.

If the framework option omits any intermediate pools that represent the entire drug substance batch, then the control strategy would likely rely predominantly on monitoring and control of CPPs in lieu of measuring IPCs. In cases where process characterization studies have established the linkage of IPC sampling frequency and location to final drug substance attributes, then IPC testing using point-in-time sampling can be incorporated. Management of in-process testing for CQAs might be possible via automated at-line sampling and analytical instrumentation but would add complexity to the control strategy. For example, as there is no longer a 1:1 relationship between a sample and the resulting drug substance batch, multiple in-process CQA measurements would be required or a comprehensive understanding of the relationship between point-in-time sampling and potential impact to the DS batch would be necessary to inform a decision to forward process at risk or to divert to waste.

If the framework option selected includes a homogenous, intermediate pool representative of the entire drug substance batch, it would be feasible to sample and test this pool for conformance of a CQA to an IPC limit. In such a situation, the implications of a deviation from the IPC limit would default to conventional batch processing scenarios. As the entire batch would be contained in the intermediate pool, it is unlikely that there would be any incentive to divert the pool to waste to prevent product impact to the batch. Instead, unless it is grossly contaminated or otherwise defective, the material can be forward processed through the remaining unit operations, and the investigation and lot disposition activities could proceed decoupled from ongoing operations. Other considerations for this approach are dependent on whether intermediate batch pools are part of a longer duration process where multiple bioreactors are used and >1 drug substance (DS) batch is produced per production run or if a sustained perfusion process is used where multiple DS batches are expected per production run.

It is expected that deviations associated with a potential safety risk (e.g., pH out of operating range during viral inactivation for greater than a pre-defined time frame) will be predetermined to result in immediate diversion to waste or otherwise removal from the process flow to minimize any impact to the DS batch.

Within the context of the N-mAb framework, a sampling point can be defined as a place in the process flow where either process data or product quality data is collected. Data can be obtained from in-line sensors/probes that are considered integrated into the framework design and provide the essential process information to ensure that the process operates within defined limits or to pre-established targets.

Assessments of product quality during processing can be determined using a multitude of options, including in-line, on-line, at-line, and off-line testing. Each of these options is associated with a sampling point, and the details regarding the specific attributes of the sampling point/sampling system are dependent upon the current enabling technology intended for the targeted application

(e.g., on-line HPLC versus a Raman probe). In addition, if intermediate batch pools are included in the framework design, then traditional sampling and testing of this process pool should be considered because the batch pool is expected to contain all the material that could be processed to a single DS batch. For the testing of intermediate batch pools, the approach taken is not considered different from that used for conventional batch processes, and therefore deviation management at this step is to be aligned with current standard batch processes.

An intervention point is a location in the process stream where a deviation detected by the outcome from process monitoring or sample testing can result in an action to the process flow. The minimal actions available at an intervention point are to proceed with further processing at risk or divert to waste the appropriate amount of the process stream that has the potential to be impacted by the deviation event. An intervention point is most likely downstream of a sampling point for continuous process operations but could be the same as a sampling point for intermediate batch pools. It is expected that process characterization efforts will establish the stability limits for a batch pool and thus enable the definition of the timeframe required to react to a deviation.

8.4 Deviation investigation requirements

A validated manufacturing process is expected to operate in a state of control, subject to continuous verification through mechanisms including statistical process monitoring of various types and formal assessments of process performance against established controls for CPPs, IPCs, and product specifications. A deviation from formally established criteria for CPPs, IPCs, or product specifications differs from statistical process monitoring practices in that the apparent loss of control must be investigated prior to disposition of the lot. Depending on the nature or severity of the loss of control, an investigation of root cause, a product impact assessment, and implementation of corrective or preventative actions (CAPAs) may also be required. Considerations for categorizing the severity of the deviation according to the potential for product impact, and the criteria for determining the required scope of the investigation, are typically captured within the QMS.

Following a confirmed deviation from established controls, the lot disposition decision will depend on the product impact assessment, which in turn, may depend on the findings of the root cause investigation. While the root cause and/or impact may be immediately apparent based on routinely collected information, in some cases, additional data collection, potentially including non-routine sample testing, may be required either to confirm a root cause or to reach a conclusion on product impact. If the root cause potentially impacts other portions of the same lot or associated lots, then the product impact and disposition assessments may also extend to these other portions or lots.

For the purpose of this chapter, deviations are categorized as excursions from IPC limits, out of specification (OOS) results, or excursions from CPP acceptable ranges. IPCs may include reject limits, which function similarly to specification acceptance limits, and control or action limits. Confirmed excursions outside of IPC reject limits or confirmed OOS events typically result in rejection of the entire lot or of the impacted portion of the lot. In contrast, excursions outside of IPC control or action limits or outside of CPP acceptable ranges may require an investigation of root cause and product impact, but they do not necessarily result in lot rejection.

Please note that manufacturers may define IPC control or action limits in different ways within their QMS, and they may or may not file these limits with health authorities. This chapter is concerned with those IPC limits for which excursions would require a lot-specific investigation prior to disposition. If the QMS does not require an investigation for an excursion, those limits and events are not in the scope of this chapter. For example, control limits may be referenced in internal procedures as alerts that would drive procedurally described interventions to restore control, but such limits would not necessarily require an investigation prior to dispositioning the lot.

Several relevant aspects of the integrated, continuous bioprocess (ICB) framework are unique or intensified relative to batch processing, and these aspects may motivate enhancements to the QMS to meet the challenges. The ICB framework poses constraints of *limited time* and *limited access*, and it enhances the potential for incidental *comingling* of impacted portions of a lot into the remaining, unimpacted contents of a lot.

8.4.1 Limited time

Continuous processing implies that, in many cases where a loss of control is detected at a given step, ongoing processing will occur through the same step, often using the same equipment, while the investigation proceeds. This processing could be for additional portions of the same lot or additional lots deriving from the same continuously harvested bioreactor run. Thus, unlike the case of a batch process, the manufacturer will have limited time to react to a loss of control and investigate the root cause, intervene to correct any issues, and thereby prevent impact to additional lots or portions of the same lot. In the absence of intermediate pools with options for extended holds, rapid, risk-based decisions may be required to either forward process any potentially impacted portion of the lot or to divert the impacted portion of the lot to waste.

Furthermore, it may not be possible to perform effective forensic analysis of equipment, materials, or controls associated with a suspected out-of-control unit operation if the associated equipment is in near-continuous use with ongoing production. In some cases, a conflict between ongoing operations and the ability to conduct an effective investigation may drive a decision to discontinue processing.

8.4.2 Limited access

The ICB framework limits access to in-process samples that would be representative of the output of the portion of the operation experiencing a loss of control. Depending on the framework options, there may be no representative intermediate batch pool downstream from the impacted step, or if there is such a batch pool, it will typically contain the combined contents of multiple processing cycles, in some cases deriving from parallel chromatography columns. In these scenarios, it will be difficult or impossible to perform forensic testing on in-process material to facilitate the investigation into root cause or product impact if an excursion impacts a sub-portion of the lot.

In the absence of an intermediate batch pool, the potential exists to include slip-stream sample collection from processing surge vessels for purposes of either routine or non-routine testing. However, this practice is not common in current ICB implementation, and it introduces complications in sample management, synchronization of samples with specific processing cycles, and potentially managing the test result data in real time. It is unlikely that a slip-stream sampling option would be considered necessary for a robust and well-controlled ICB process.

8.4.3 Comingling of impacted material

The continuous processing framework typically implies that a loss of control in a downstream processing step may impact only a small portion of a given lot. The N-mAb framework mitigates the costs of downstream processing capital equipment and materials with a very high utilization rate accomplished by using multiple chromatography processing cycles for a given lot, and typically uses dozens of cycles to process the output from a bioreactor run. In such a paradigm, a loss of control impacting a single downstream processing cycle would impact only a small percentage (<10%) of a given lot. If the impacted portion of the lot is incidentally comingled with the remaining, unimpacted portion of the lot, then any impact to product quality may be diluted in the final batch.

It is clearly unacceptable to deliberately combine out-of-specification product with conforming product to meet specifications. However, if a loss of control occurs at a given step, and routine IPC verification is not performed prior to further processing, then any comingling with unimpacted portions may be considered incidental to normal processing. In this circumstance, an investigation should be performed to determine the product impact. Even in a scenario when CQA measurements occur in real time and prior to further processing, if the excursion does not exceed an IPC reject limit, comingling may be justifiable. The QMS should address the criteria and circumstances under which the incidental dilution of impacted material through comingling may be acceptable, provided there is no unacceptable impact to the quality of the final, combined batch.

8.4.4 Adjustments to the Quality system required with integrated continuous bioprocessing

To manage the three factors of limited time to avoid impact to ongoing production, limited access to representative in-process materials, and comingling of impacted material into a combined lot, the QMS may be enhanced by reference to documented and approved heuristic algorithms. These algorithms may address three orthogonal characteristics following an initial observation of loss of control:

1. How does the severity and duration of the CPP excursion relate to the characterized range(s) of the parameter(s)?
2. Is the root cause clearly assignable?
3. What is the proportion of the lot impacted by the loss of control?

8.4.5 Severity of excursion

When a CPP excursion is detected, a rapid decision may be required either to divert impacted material to waste or to continue forward processing. Ideally, process characterization studies should define at least two thresholds for each CPP, separating the operating condition into three regimes to inform processing decisions.

First, when the process is operating within the normal operating range (NOR), no deviation should be triggered. Although trend investigations deriving from statistical process monitoring programs may identify opportunities to tighten the NOR to improve process consistency, those QMS decisions should be prospective, and would generally have no retrospective impact to disposition of previously manufactured batches.

Second, operations outside of the NOR, but within a characterized acceptable range (AR) contain the regime in which a deviation should be opened, but the product quality may remain acceptable. In

this regime, a decision to forward process at risk may be prudent if the alternative is to divert material to waste. The ultimate disposition of the lot will depend upon the outcome of the investigation, but the documented AR could provide a basis for a rapid decision to forward process. If multiple CPPs are impacted outside of their NORs, and if their interaction effects could exceed the characterized design space, then forward processing at risk may not be prudent. These decisions may also be informed by whether the excursions are transitory or persistent (see next section).

Third, forward processing at risk would generally not be advisable for confirmed CPP excursions outside of the AR. CPP excursions in this regime are either unknown territory (because process characterization studies did not explore this space), or they are known to produce product of unacceptable quality. In either case, the prudent rapid decision would be to divert impacted product to waste. Again, this decision may be subject to information about whether the CPP excursion was transitory or persistent.

8.4.6 Duration and persistence of excursion

For some unit operations that operate in continuous or semi-continuous mode, CPPs may experience transitory departures from the NOR or AR without necessarily impacting product quality. For example, the pH control loop of a bioreactor may commonly experience fluctuations, but the physiological impact of pH excursions is generally limited if the duration of the excursion is short relative to the time constant of metabolic responses. Similarly, transitory temperature excursions for product pools would have limited impact on quality.

To limit the impact of such transitory deviations to operations and to the investigation burden, the process characterization studies should ideally assess excursion impact by depth and duration. Data from experimental studies may be supplemented by sound scientific rationale justifying, from first principles, how a transient excursion would not impact a given unit operation. If the resulting two-dimensional (extent x time) design space is well documented, the need for a deviation investigation could be mitigated in some cases, and in other cases, the decision to forward process at risk would be informed.

8.4.7 Assignability of root cause

The root cause of a loss of control may be immediately apparent if it is related to a rapidly diagnosed equipment malfunction or immediately reported operator error, or the root cause identification may require a more deliberate investigation. If a root cause is immediately assignable, this information may assist in rapid decision-making. For example, if the issue has been or can be quickly corrected, or if it is unlikely to recur, then the decision to continue processing the remaining portion of the lot through the impacted unit operation can be supported. Similarly, the rapid identification of the root cause should enable timely decisions in the QMS about the potential for product impact to the impacted and associated portions of the lot.

In contrast, if the root cause is not immediately apparent, there may not be confidence as to whether the issue will recur, whether the root cause may have implications for a product impact assessment, or whether there are additional, associated portions of the lot impacted.

8.4.8 Proportion of impacted lot (transitory, recurrent, or persistent loss of control)

Deviations in ICB frameworks may impact only a portion of a given lot because of the relatively large number of process cycles involved in most or all of the downstream processing steps. Transient loss of control in the bioreactor, or episodic loss of control in one, or a few, of the downstream processing cycles could result in an isolated impact to product quality in a minor portion of the lot.

Loss of control could also be recurrent or persistent. For example, if the framework uses a dual-column or multi-column processing step, and if only one column experiences a sustained loss of control (e.g., due to a loss of bed integrity), the impact to process performance would recur every time that column is cycled. In contrast, if there is a single processing unit, or if a defect in raw materials or other inputs impacts all of the processing units simultaneously, the loss of control would persist for all impacted process cycles.

For transitory loss of control impacting a small portion of the batch, when this portion of the lot is comingled with the remainder of the lot in a downstream "whole lot" pool, the quality of the drug substance may be considered acceptable, subject to QMS considerations as discussed in Section 8.4.3.

In contrast, recurrent or persistent loss of control for several or all subsequently processed portions of the lot could result in significant impact to the overall quality of the lot. The QMS decision framework should include diagnostics to quickly determine whether loss of control is likely isolated to a small portion of the lot or if the issue should otherwise be considered recurrent or persistent.

8.4.9 Supportive data and documentation

The aforementioned considerations may be integrated for decision support by access to a database of relevant, up-to-date experimental data, which is the foundation for establishing proven acceptable ranges (PARs). The outputs from process definition, process characterization, and process validation can be captured in such a database and combined with some pre-defined heuristic considerations, for example, respecting the severity of the deviation impact and the proportion of the lot potentially impacted. This reference database should remain a living entity by being updated with new learnings from inspections, investigations, additional studies, etc. Note that some companies use certain electronic tools for consolidating data that supports criticality determinations or data management for providing structured content for regulatory submissions (Ahluwalia et al., 2021); these tools could be adapted to support extended applications of a process information database and could also serve as pre-requisites for automated decision support, discussed in Section 9.4.

8.5 Scenarios for managing deviations

We consider three scenarios for the conduct of a product impact assessment following a potential deviation in product quality from established conditions:

1. The deviation is detected via monitoring of CPPs (Section 8.5.1)
2. The deviation is detected via off-line CQA testing of an intermediate batch pool, or via a drug substance specification test (Section 8.5.2)

3. The deviation is detected via "real time" in-process monitoring of CQAs in either a batch pool or other sampling point representing a portion of a lot (**Section 8.5.3**)

Due to the nature of continuous manufacturing, the timing of detection relative to the location and time of occurrence have significant implications on the management of deviations.

8.5.1 Scenario 1: Deviation detected via monitoring of CPPs

8.5.1.1 Excursion of CPPs from established controls

In-process control strategies for continuous manufacturing will rely principally on monitoring CPPs in lieu of obtaining real-time analytical results for CQAs. Excursions of CPPs from established controls are therefore likely to be the most common deviation scenario in the ICB framework.

Decisions made regarding excursions from established CPP controls are impacted by whether or not the excursion happened at the principal point of control for a CQA impacted by that CPP. For example, for glycan attributes, the bioreactor is typically the principal point of control, whereas for a process- or product-related impurity, the last chromatography step with significant clearance capability will typically be the principal point of control.

If a CPP excursion impacts the principal point of control, and if a batch pool intermediate is included in the framework, the pool may be held in place pending a decision to reprocess, forward process, or divert to waste. More commonly, a decision to either divert to waste or immediately forward process at risk should be reached if the point of control is part of connected unit operations without an intermediate pool, or if reprocessing is not allowed. As described in **Section 8.5.1.3**, this decision will depend, in part, on the severity of the excursion, the assignability of the root cause, and the proportion of the lot impacted.

If the excursion to the CPP is upstream of the principal point of control, then there remains a possibility that the process can recover from any resulting impact to the CQA, and a decision can be delayed until the material reaches the principal point of control. However, if the CQA impurity value will likely exceed what has been characterized as the maximum limit for clearance, then a decision to divert to waste can be performed immediately.

Concurrent with the rapid triage decisions regarding forward processing, diversion to waste, or holding an intermediate, the CPP deviation event will typically trigger root cause and product impact assessments. While it may be necessary to terminate a continuous production run to investigate a significant failure of control, in many cases, a CPP deviation may be a transient and recoverable event, and continued processing of the production run will be justifiable. In these more common scenarios, it will be desirable to employ rapid, heuristically driven assessments of root cause and product impact to minimize unnecessary disruption to ongoing continuous manufacturing.

8.5.1.2 Root cause considerations for CPP excursions

In many cases, the root cause of a CPP excursion may be immediately apparent (e.g., transient control system perturbation with known proximate cause), and a formal root cause evaluation may not be required. If the root cause is known to be transient and reversible prior to the next processing cycle, a decision to continue processing can be justified. The root cause and any immediate corrective actions can be documented in the batch record.

In other cases, the root cause may not be obvious (e.g., undetected maintenance issue impacting equipment performance). The triage process may then require a more deliberative root cause investigation that, in some cases, may require interventions impacting ongoing production.

If the root cause investigation identifies potential impacts to the validated state of operation beyond the immediately apparent CPP excursion, non-routine testing may also be required for CQAs that have been "validated out" of routine testing. It should be cautioned that frequently resorting to non-routine testing to disposition lots following repeated losses of control could be considered a cGMP violation. Instead, the root cause(s) of the deviations should be clearly identified, and appropriate corrective and preventive actions should be implemented prior to additional manufacturing.

Special considerations apply to excursions in the production bioreactor CPPs. If the CPP excursion has an unknown root cause and no obvious reversibility, it may be necessary to terminate the run to permit an investigation.

For continuous manufacturing, it is essential that an agile Quality mechanism exists to drive timely decisions about the scope of the root cause investigation and its impact to ongoing production.

Examples:

1. Bioreactor pH excursion due to controlling probe failure. Root cause of pH excursion is obvious, immediate corrective action is to replace the probe and/or switch control to the monitoring probe.
2. Polishing step buffer conductivity out of range due to buffer mixing and control system failure without obvious root cause. Immediate corrective action to reverse the issue may not be possible. Polishing operations may be paused, with any upstream material diverted to waste, while a forensic analysis of the control system components proceeds.

8.5.1.3 Product impact considerations for CPP excursions

The QMS should also address the product impact implications of a CPP excursion. Aside from CPPs tied to adventitious agent controls (e.g., pH of viral inactivation), most CPPs are not classified to cause an automatic lot rejection if exceeded. If the impacted lot portion has been diverted to waste, a formal product impact assessment may not be required for this portion, but an assessment may still be required for associated portions of the lot depending on the findings of the root cause investigation.

If the lot portion is forward processed, or if other portions of the lot could have been impacted by the root cause, the product impact decision will depend on the investigation findings. The quantitative impact of the CPP excursion on a CQA may depend on multiple factors, including the severity of the CPP excursion (extent and duration), the interaction with other CPPs, and the proportion of the lot that is impacted. Until confirmatory CQA testing results are obtained, the product impact may be unknown.

A transitory or isolated CPP excursion associated with an impact to CQAs may not cause the entire lot to depart from established criteria for the CQAs, even if the impacted portion of the lot is comingled prior to detection of the deviation, or if it is deliberately forward processed as the result of a decision taken in the QMS. For example, impurity specifications are generally tolerant of

process variability and could accommodate partial failure of a small portion (<10%) of the lot because this portion would be comingled with the remainder of the processed material. If the impurity impacted by the CPP excursion is routinely assessed in the drug substance specification, then it may be acceptable for the QMS to conclude that there is no product impact if the drug substance passes the corresponding specification test.

8.5.1.4 Examples of managing deviations to CPPs

An overview of the considerations and decisions involved in managing deviations to a CPP in an integrated continuous process is shown in Figure 8.1.

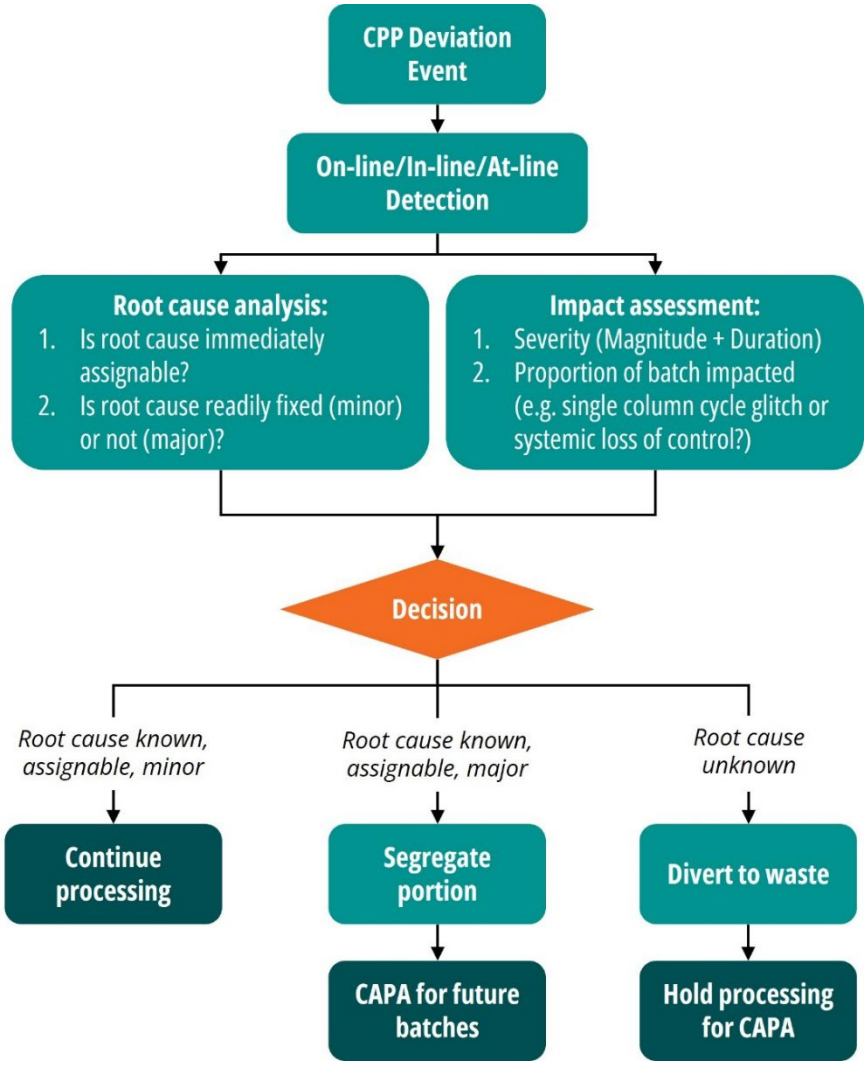

Figure 8.1. Considerations for CPP deviations in an ICB framework.

ICB Integrated Continuous Bioprocess
CPP Critical Process Parameter
CAPA Corrective and preventative actions

It may be helpful to consider some examples of CPP deviations such as the following:

Examples:

1. Bioreactor pH excursion for several hours outside of the acceptable range during which the pH dropped to a value of 6.9 (control range = 7.0 – 7.2), combined with atypically low starting viable cell density within the acceptable range. This combination has the possibility of impacting glycosylation due to cellular metabolic stress based on either product-specific or platform experience and this potential impact is supported by the PC study that evaluated the impact of pH on glycosylation (**Figure 3.3** and **Figure 3.6**) for an extended duration. The investigation triage team decides to perform confirmatory non-routine CQA testing at the capture step surge tank for the cycles immediately following the CPP excursion. If the glycosylation attributes are within the validated ranges or specifications, there is no product impact, the impacted portion of the lot may be retained, and the overall control strategy may be updated to indicate an acceptable duration for low pH. If the glycosylation attributes are not within the validated ranges, there may be product impact, and the impacted drug substance lot should be dispositioned according to the QMS.

2. A single Protein A column cycle is loaded above the acceptable range due to an operator error. The overload has potential impact to high molecular weight species (HMWS) or host cell protein (HCP) levels, but downstream steps are the primary control points for these CQAs. The cycle is combined with material from at least five other cycles to produce a single drug substance batch. It is estimated from process characterization studies that the quantitative impact to HMW and HCP is modest and that the levels of those impurities are well within the capability of downstream polishing steps when considering the 5:1 dilution of the atypical cycle combined with results from some of the worst-case linkage studies performed during PC. The investigation determines that non-routine HCP purity should be confirmed in the final control point process pool (if available) or in the drug substance, along with routine HMWS release testing of drug substance. Provided that these attributes remain within validation acceptance criteria (for HCP) and specifications (for HMWS), there is no product impact.

These and other examples illustrating the integrated QMS decision framework considering severity, root cause, and proportionality of CPP deviations are described in **Table 8.2**.

Table 8.2. Scenarios for managing deviations to critical processing parameters or material attributes.

Critical control point	CPP excursion	CQA Potentially Impacted	Detectability of potential CQA impact	Severity of Impact (extent, duration, proportionality)	Root cause assignable?	Deviation management considerations (including confirmatory QC tests)
Bioreactor	Bioreactor pH	Glycosylation, HCP, Aggregates	Glycosylation: No routine test HCP: DS release test Aggregates: DS release test	Transient excursion outside NOR, inside PAR	Yes	*Immediate:* Forward process at risk *Long term:* Assess need to investigate ultimate root cause
				Prolonged excursion outside PAR	No	*Immediate:* Divert to waste *Medium term:* decide if PC data suggest process can recover to SS. If so, resume processing at risk after restoring SS. *Long term:* Assess need to investigate ultimate root cause
Protein A Capture Chromatography	Capture column load ratio	HMWS	DS release testing	One cycle exceeds PAR	Yes	*Immediate:* divert to waste or forward process to polishing depending on severity and proportionality
Protein A Capture Chromatography	Process hold time examined in PC exceeded	HMWS	DS release testing	One cycle exceeds PAR (Table 3.20)	Yes	*Immediate:* divert to waste or forward process to polishing depending on severity and proportionality *Long Term:* Evaluate impact of extended pause on product quality using an expanded set of product characterization tests and adjust PAR for process pause accordingly after validating new total process pause in PPQ
Polishing Chromatography	Polishing elution ionic strength	HMWS	DS release testing	One cycle outside NOR, inside PAR	Yes	*Immediate:* forward process at risk *Long term:* assess requirement for ultimate root cause analysis (RCA)

Critical control point	CPP excursion	CQA Potentially Impacted	Detectability of potential CQA impact	Severity of Impact (extent, duration, proportionality)	Root cause assignable?	Deviation management considerations (including confirmatory QC tests)
Non-specific	Low pH	Deamidation	DS release testing	Transient excursion outside NOR, inside PAR	Yes	*Immediate:* Forward process at risk *Long term:* Assess need to investigate ultimate root cause

CEX Cation exchange
CQA Critical quality attribute
CPP Critical process parameter
DS Drug substance
HCP Host cell proteins
HMWS High molecular weight species
NOR Normal operating range
PAR Proven acceptable range
PC Process characterization
QC Quality control
RCA Root cause analysis
SS Steady state

8.5.2 Scenario 2: Off-line detection of deviation to CQAs

When a deviation in a CQA is detected in a "whole lot" pool IPC or in the drug substance, the product impact investigation may proceed according to typical QMS practices, whether it is for a batch or continuous processing framework. It is noteworthy that there may be no representative "whole lot" pools in some versions of the N-mAb framework, and hence CQAs would only be assessed through final testing of drug substance.

In conventional batch bioprocessing, analytical tests for CQAs typically occur off-line in a QC laboratory, and results can be reported with a delay of days to weeks after the time of sample collection. Exceptions commonly exist for simple tests of the general properties of the drug substance (e.g., pH, osmolality, or protein concentration), which may be tested on the production floor in near real time. However, discussion of the management of these general property CQAs is outside of the scope of this chapter.

For the more complex CQAs related to purity and impurities, the delay in testing results applies equally to a critical in-process control or to a drug substance release test. In either case, if the reported results deviate from defined action or acceptance criteria, an investigation will be required. For continuous manufacturing, the delay in reported results will preclude timely decisions for handling in-process materials. Instead, the impacted portion of the lot will likely have been forward processed to a drug substance batch, and the entire batch will therefore either be accepted or rejected based on the findings of the investigation, with no opportunity to divert a portion of the batch to waste.

Similarly, the root cause investigation will proceed long after the initial deviation, and it will have no immediate implications for managing the ongoing production of that batch. However, if production of additional drug substance batches is ongoing from the same production bioreactor run at the time that the deviation is detected, the Quality Unit and Manufacturing will need to take a decision either to maintain ongoing operations at risk or to terminate the production run. In principle, this decision framework is no different from a similar scenario in a conventional batch production campaign where a deviation detected after the completion of one batch may implicate ongoing production of other batches using the same materials, equipment, procedures, or controls.

Because the off-line detection of CQA deviations will be managed conventionally by the QMS in a continuous manufacturing framework, no further discussion of this scenario is required.

8.5.3 Scenario 3: Real-time detection of deviations to CQAs

In contrast to the off-line CQA testing scenario, emerging technologies permitting rapid on-line, in-line, or at-line testing may enable continuous or intermittent testing of CQAs. For the purpose of this discussion, we will refer to the collective scenarios of on-line, in-line, and at-line detection as "real-time" detection.

Real-time CQA monitoring in the ICB framework would require repeated sampling and testing, with accompanying QMS requirements for managing the impacted material following a detection of loss of control. Such a real-time IPC paradigm has been reduced to practice in pharmaceutical manufacturing. For example, at-line fill-weight checks are commonly used in drug product manufacturing, with associated interventions to pause production, segregate impacted portions of

the lot, correct the control issue, etc. Similarly, continuous processing frameworks for synthetically manufactured drugs include continuous or semi-continuous in-line testing for CQAs. It would be possible to introduce a similar paradigm for biologic drug substance manufacturing.

A real-time CQA monitoring capability would permit implementation of IPCs in surge vessels in addition to in-process batch pools, and it would enable more agile decision-making in a continuous manufacturing environment. These real-time results can inform the decisions for managing deviations. In addition, these results could be used to feed-forward or feed-back for adaptive control strategies, but this topic is outside of the scope of this chapter.

As with the CPP excursion scenarios discussed in **Section 8.5.1**, real time detection of CQA excursions will require the QMS to specify decisions in real time about forward processing impacted material or diverting it to waste, with considerations for whether deliberate co-mixture in downstream processing is acceptable.

QMS decisions should be informed by the extent of the CQA deviation relative to action or rejection limits, as well as by the other considerations in **Section 8.5.1**, respecting root cause identification and proportionality of impact to the quality of the whole batch. If an IPC for an impurity does not meet an established action limit, and if the deviation is transient, it is likely that the combined drug substance lot will have acceptable purity. This outcome can occur because impurity specifications are generally tolerant of process variability and could accommodate partial failure of a small portion (<10%) of the lot. If the same impurity CQA is also routinely measured in the drug substance specification, then it may be acceptable for the QMS to conclude that there is no product impact if the drug substance passes the corresponding specification test.

We consider several scenarios for real-time detection of CQA excursions with different degrees of certainty about the root cause of the CQA excursion and corresponding implications for the deviation investigation and decision process.

8.5.3.1 Excursion to CQA with obvious linkage to known relevant CPP excursion

In many cases, the real-time detection of a CQA deviation can be immediately correlated with an observed CPP control excursion. Process characterization studies would have demonstrated the cause-and-effect relationship between the CPP and the CQA, and the near-simultaneous detection permits rapid decision-making.

If the framework includes an in-process batch pool tank downstream from the CPP excursion, the capability of real-time IPC monitoring supplements the QMS decision considerations described in **Section 8.5.1** with an added benefit: the CQA levels in the batch tank can be immediately determined, providing a more solid basis for assessing the impact of co-mingling the affected upstream portions of the lot with the remainder of the batch. This assessment can be performed prior to forward processing the batch pool, and the real-time information can help expedite the assessment of product impact for the deviation.

In contrast, without an in-process batch pool tank downstream from the CPP excursion, the decision framework largely reverts to the considerations in **Section 8.5.1** with the added confidence that would be instilled by correlated deviation signals.

8.5.3.2 Excursion to CQA with a tentative or unknown linkage to CPP or non-CPP excursion

In some scenarios, a real-time CQA excursion may be detected in the absence of a definitive linkage to a CPP excursion. These scenarios could include no correlated CPP excursion, or a correlated observed CPP excursion with a plausible linkage to the CQA.

When there is no correlated CPP excursion, the QMS must proceed with very limited insights into the potential root cause of the CQA excursion. If the CQA exceeds an action limit in this scenario, a decision may be made to continue processing the batch, but such actions may impede an effective root cause investigation. The decision may be informed by whether the action limit excursion is transient or sustained. However, if the CQA exceeds a rejection limit with no known root cause, it is advisable to divert to waste and pause operations at the point of control for the CQA to permit an effective investigation.

If there is a correlated CPP and CQA excursion, the deviation triage team may be able to formulate a rapid hypothesis. For example, a specific failure mode may not have been studied in deliberate process characterization studies, but a plausible root cause may be generated based on first principles of the unit operation design and other prior knowledge. In this scenario, the QMS must manage the investigation decisions based on a tentative linkage to a CPP excursion. Again, if the CQA exceeds an action limit in this scenario, a decision may be made to continue processing the batch as informed by the ability to return the CPP to a state of control and the ability to investigate the tentative root cause. If the CQA exceeds a reject limit, the QMS will likely require diversion of impacted material to waste until a state of control can be reestablished. These considerations may also apply for an excursion of a non-CPP well outside of the NOR.

8.5.3.3 Examples of managing deviations to CQAs with real-time monitoring

Examples illustrating the integrated QMS decision framework considering severity, root cause, and proportionality of real-time CQA deviations are described in **Table 8.3**.

Table 8.3. Scenarios for deviation management with real-time CQA monitoring.

CQA Impacted	Point of detection	CQA Impact (extent, duration, proportionality)	CPP or Material Attribute causal factor assignable?	CPP Impact (if identified) (extent, duration, proportionality)	Deviation management considerations (including confirmatory QC tests)
Aggregation	Post-VI tank	Action limit, transient impacting <10% of lot	Yes	VI low pH excursion	*Immediate:* Forward process at risk *Medium term:* Confirm root cause of VI pH excursion and monitor closely *Long term:* Confirm DS specification conformance and evaluate upgrade to pH control scheme at VI step
Glycosylation (Galactosylation, High Mannose Species, etc.)	Post-VI tank	Action limit, sustained impacting >50% of lot	Not immediately Cell culture media trace metal levels identified as root cause after investigation	N/A	*Immediate:* Forward process at risk *Medium term:* Investigate plausible root causes, monitor for worsening trend *Long term:* Confirm DS specification conformance; Assessment of raw material quality identifies cell culture media lot-to-lot variation in trace metal (Cu, Mn) levels as root cause
Aggregation	Post CEX B/E Polishing Surge Vessel	Reject limit, recurrent for 1 column cycle	No	N/A	*Immediate:* Divert to waste *Medium term:* Investigate root cause; if one column identified, take off-line and operate with reduced yield
			Yes (Resin Material Attributes)	Yes – reduce elution pH PAR to achieve robust performance	*Immediate:* Divert to waste *Medium term:* Investigate root cause; if one column identified, take off-line and operate with reduced yield. Root cause investigation identifies variation in lot-to-lot resin material attributes that could result in tighter

CQA Impacted	Point of detection	CQA Impact (extent, duration, proportionality)	CPP or Material Attribute causal factor assignable?	CPP Impact (if identified) (extent, duration, proportionality)	Deviation management considerations (including confirmatory QC tests)
					acceptance criteria for resin lots or, if that is not possible, modification of the CEX elution conditions could result in robust performance over the variation expected from multiple resin lots
HCP	Post CEX B/E Polishing Surge	Action limit, sustained	Tentative assignment	AEX F/T Polishing load outside of PAR	*Immediate:* Forward process at risk *Medium term:* Correct AEX load conditioning controls, monitor for worsening trend *Long term:* Confirm DS specification conformance

B/E Bind and elute
CPP Critical process parameter
CQA Critical quality attribute
DS Drug substance
F/T Flowthrough
HCP Host cell proteins
PAR Proven acceptable ranges
QC Quality control
VI Virus inactivation

Chapter 9

Future Directions

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

9 Future Directions

9.1 Overview

This N-mAb case study has attempted to present an overall story of the development of an ICB from process design through commercial manufacturing to support teaching and learning for both industry and regulators around adoption of advanced manufacturing process technologies for mAbs. The focus was on an ICB for a monoclonal antibody with the thought that other, more complex protein therapeutics could be considered as part of a future update. The process options and approaches presented herein were based on current consensus thought so as to make the move to an ICB appear as approachable as possible and not dependent on a raft of seemingly futuristic technologies. But another important goal of this work was to enable effective continual improvement across the process development and, more importantly, commercial arenas by provoking and challenging current thinking to stimulate discussion and advance new concepts. The focus was admittedly on how today's process technologies can be used to create more efficient processes. Discussion of how advanced process technologies would further impact such processes is left to future publications. Similarly, the focus in this document was around application of current analytical technologies. However, advances in the development and integration of analytical technologies would be of great benefit even for processes built with today's process technologies.

The continuous evolution of advanced analytical measurement systems has already contributed to the rich knowledge surrounding process parameters and their influence on product quality attributes. This trend is readily apparent in the development of the N-mAb framework and process characterization strategy, where various surge tanks may be used for daily product quality testing to enable model building. Advanced analytics help identify and monitor CPPs that can be used to infer product attribute control when operated within the normal operating range (NOR). Such correlations allow sampling frequency to be minimized in the commercial setting as appropriate process parameter ranges have been established (Section 7.1). Building this deep understanding of the influence of the process on product quality is a critical component of state-of-the-art CQA control wherein a combination of CPP and CMA monitoring is used to assure product quality. While direct monitoring of CQAs in-line or on-line would provide more direct evidence of clinically relevant attribute control during a process run, current state-of-the-art analytical technology requires additional improvements in manufacturing readiness levels to be implemented in a cGMP facility. Commercial manufacturing requires a more rapid data acquisition-to-action timeframe, for example, to assure process control and inform deviation management. Great strides have recently been made in process integration, advanced analytics throughput, operator usability, and cost, and these advances may eventually allow implementation of process analytical technologies to enable more real-time measurements and potentially be used for in-process controls (IPCs). The N-mAb framework has been built in a manner to allow incorporation of these advanced analytical tools as a future effort, and an introduction to a subset of promising analytical techniques is provided below.

Advanced manufacturing processes (i.e., continuous manufacturing) may benefit from advanced process measurement tools. Among other reasons, drifts in product quality may arise from known sources of variation over long production runs, albeit within limits observed during process validation. Direct knowledge of this drift during a run may enable the downstream unit operations

to be adapted to ensure that final product quality is maintained. Several recent literature reports have demonstrated proof-of-principle for online advanced analytics as process development tools as noted in the previous Section. Another interesting example details how testing for early clinical manufacturing was performed using a detailed peptide map method, but a switch was made to a method that is more quality control (QC) lab-friendly for the registrational and PPQ runs, (Evans et al., 2021). With continuous manufacturing, the potential for segmenting batches and the need for continuous information throughout the duration of runs may increase business drivers and product quality relevance of truly online characterization with advanced analytics. Initially, at-line attribute-relevant measurements will aid in correlating CQAs to CPPs. These novel measurements may evolve to be applied in-line or online, thus becoming IPCs capable of replacing downstream drug substance characterization upon proper process validation. In addition, detailed real-time product knowledge would enable agile investigations of product quality deviations as detailed in **Section 8.4**. A future state of using adaptive control to anticipate and prevent excursions by learning from the accumulation of process history could be very effective in reducing deviations. Process monitoring with advanced on-line or in-line analytics will play a critical role in managing deviations.

9.2 Considerations for future state of real-time CQA monitoring

Chromatographic approaches are frequently used as drug substance/drug product characterization and/or lot release tools, and they are often considered for use in direct bioprocess monitoring. For example, size exclusion chromatography (SEC) is commonly used to monitor size heterogeneity, and ion exchange chromatography (IEX) is commonly used to evaluate charge heterogeneity. In fact, both of these techniques are currently applied as part of sequential offline multidimensional separations for bulk drug substance/drug product (DS/DP) monitoring after Protein A (Pro A) or polishing chromatography, or both. The use of Pro A chromatography in a two-dimensional format enables the integration of at-line testing into the production environment. For example, Pro A x SEC-HPLC can be used for at-line monitoring of monomeric purity.

As an example, high levels of HMWS that are above historical limits can be detected in the production bioreactor by an online HPLC system or an in-line Raman sensor. This event can trigger a notification to the operators to be on alert and closely monitor downstream steps. The intermediate drug substance can then be monitored following the capture step, and the automation can target the high end of the pH range and the lowest allowed incubation time to avoid the further growth of aggregates at the low pH inactivation step. An aggressive loading scheme on the polishing step can also be triggered through automation to load at a low ratio of protein to liters of resin (L_{resin}), thus sacrificing some of the yield but maximizing the removal of HMWS. If the process has the capability to adjust the concentrations of the charge variants, a similar scheme can be followed.

Mass spectrometry, a critical tool for characterization of mAb primary structure, has more recently been added to the critical process development tools, and it is a leading technology in the quest toward real-time release. LC-MS can be used to evaluate the intact mAb with little to no sample preparation after Pro A purification. As a result, two-dimensional liquid chromatography (i.e., Pro A and reversed phase chromatography (RP-HPLC)) has been directly coupled to high resolution mass spectrometry for intact mAb characterization. The evolution of top-down liquid chromatography/mass spectrometry (LC-MS) approaches for the analysis of intact mAb or mAb

subunits may be an avenue for more rapid at-line or even on-line primary structure characterization. Recent advances in novel gas-phase fragmentation technologies can provide deeper sequence coverage, thus avoiding sample preparation bottlenecks. Top-down mass spectrometry does, however, require continued evolution of fragmentation techniques, library searches, and quantitative capabilities to be readily deployable as a process analytical technology (PAT).

A detailed primary structure readout can be achieved through peptide mapping, which involves reduction and proteolytic digestion followed by LC-MS. An evolution of peptide mapping, coined "multi-attribute method" (MAM), has sought to advance LC-MS of proteolytic digests as a simultaneous test for attribute and purity control (Xu et al., 2017). In MAM, a library of peptides reliably observed by peptide mapping is generated using multiple production batches and/or forced degradation samples. The peptide library then allows a simpler and more quantitative LC-MS strategy to monitor various CQAs identified at the peptide library level in subsequent samples (e.g., deamidation, oxidation, and C- or N-terminal variants). A direct signal comparison to a primary reference sample, known to be representative of the process and product, also allows a sequence-agnostic evaluation for the presence of new or changed masses, thus evaluating the sample for low level impurities. The MAM has garnered significant attention for process development and as a potential lot release strategy. As a process development tool, MAM offers significant advantages because it enables direct detection of numerous attributes from a typical CQA list, including glycosylation, deamidation, and host cell protein identity and quantity (Song et al., 2021). However, it is limited in the total throughput due to rather long at-line sample preparation (1-4 hours or more), LC-MS gradients of an hour or more, and the requirement for data analysis by subject matter experts. These limitations are being actively targeted with the development of innovative technologies such as online multidimensional chromatographic sample preparation, gas phase ion mobility separations instead of chromatography, and various automated MAM software workflows. The ideal process analytical LC-MS-based technology has yet to be realized, but the elements for success are increasing in throughput and specificity, and they may soon be approaching the required performance metrics.

On-line LC-MS analysis could be used to measure glycosylation in the bioreactor, and because most downstream processes do not impact glycosylation, a decision could be made to divert the material to waste immediately as it comes out of the bioreactor until the appropriate profile and levels are reached. On-line LC-MS analysis of the production bioreactor output could also be used to detect higher than normal levels of HCPs, and the downstream process could be adjusted based on process characterization to adjust the loading conditions to attempt to reduce HCPs. This approach would be particularly useful in the case of known "hitchhiker" HCP species that might be problematic for a given product. On-line liquid chromatography/mass spectrometry (LC-MS) systems can also sample the eluate of the capture step. If intermediate pools are used at the end for testing as a batch, no decisions can be made prior to pooling. However, if material is collected and released in a continuous fashion, then decisions based on process characterization could be made prior to release to divert the material with the high levels of HMWS to waste.

9.3 Considerations for managing a multi-variate design space

The QMS may need to accommodate advanced control strategies enabled by multi-variate design space or artificial intelligence applications to enable automated decision support for detection of and management of deviations. Conventional design space definitions apply constraints "one variable at a time" on the acceptable ranges for CPPs. The CPP ARs are set with the assumption that the process will perform acceptably if each CPP is controlled within its respective AR. Ranges may be defined conservatively to minimize the probability of unfavorable interactions between CPPs. These design space definitions are readily managed in the QMS by reference to one-dimensional alarms and documented limits in the batch records that can be referenced for triggering a deviation investigation.

Organizations have also developed more complex, multidimensional design space definitions that may allow more flexible operations within a non-orthogonal combination of CPP acceptable ranges. They may have also developed sophisticated ways of linking data supporting criticality assessments such that it can be readily and quickly retrieved. As a result, ongoing learnings from investigations, inspections, etc. may be included as part of the design space. These design spaces could also include adjustments for the duration of an excursion. Operationalizing such design spaces may require the alarm systems, batch record, and QMS to access computational tools to determine if a deviation from the multi-variate design space has occurred.

In parallel, novel technologies, such as Raman spectroscopy (Liu et al., 2021; Rafferty et al., 2020; Ryder, 2018; Tulsyan et al., 2020; Yilmaz et al., 2020), viscometers, online multi-angle laser light scattering (MALLS), and biocapacitance also known as dielectric spectroscopy (Braasch et al., 2013; Downey et al., 2014; Shek & Betenbaugh, 2021; Ma et al., 2019; Mercier et al., 2016) will be required to monitor process performance attributes in real time. While proof of concept has been established for these technologies, collaboration will be needed between instrument vendors and industry to fully develop them to become reliable and robust for broad use in manufacturing. Also, as noted earlier, the value of these measurements is enhanced significantly when the links with impact to product quality attributes are well established and preferably built into a multivariate predictive model. In the future, systems could be developed to constantly adapt downstream unit operations to ensure consistent end-product quality (adaptative control strategies). However, this approach would require tackling important technical hurdles as well as developing modeling tools built on this prior knowledge (e.g., process "digital twins") that may be used to support rapid decision-making after a CPP perturbation has been detected.

Indeed, a truly effective PAT strategy requires the seamless interplay of different technological domains:

- Availability of reliable in-line sensors and instruments to enable real-time measurements
- Establishment of a robust digital environment and computing power to:
    - Manage the flux of data generated by the sensors and instruments
    - Ensure data integrity storage
    - Rapidly evaluate the data collected using models, possibly with an artificial intelligence (AI) component

    - Control the process skids accordingly (feedback or feedforward) given the limitations of the overall process dynamics
- The development of reliable process models (statistical, mechanistic, hybrid) to predict responses to process variations, adopt the proper control strategy, and properly synchronize adjustment of process parameters (taking into account unit operation residence time, skid reaction delay, etc.)
- On-demand CPP adjustment and buffer preparation

Based on these considerations, the following information would therefore be required in order to establish an effective control strategy:

- The identification of the process steps where product CQAs are created or modified, and the process steps constituting critical control points for each CQA
- An in-depth understanding of how the CPP/CMA variations affect the product CQAs for each unit operation and the ability to collect and organize that information over the product lifetime
- The definition of clear "algorithms" supporting process control decisions, based on accumulated prior knowledge

9.4 Considerations for automated decision support tools

As discussed in Section 8.4.4, the QMS may be required to support rapid decisions to either forward process or divert to waste any potentially deviating material from a batch. Automated decision support tools could integrate deviation signals from non-conforming CPPs, supportive information about conforming CPPs, design space knowledge, and decision heuristics to provide recommended immediate steps. Such tools should be validated to provide reliable outputs for pre-defined scenarios, and these outputs could be referenced immediately on the manufacturing floor to drive real-time decisions. When the automated support tool is unable to map a scenario to the predefined knowledge space, the issue would likely be escalated for a more deliberate, expert-driven assessment.

Multi-variate analysis tools rely on statistical models developed after establishing a "golden batch" by collecting the data of an acceptable number of batches. A golden batch is defined as the statistical norm for well-behaved batches, against which other batches may be compared. The behavior of new batches is then monitored on how close or far they are from the golden batch. Data used in multivariate models can include different elements in addition to process monitoring, allowing operators to immediately identify the sources of variation contributing to the diversion from a golden batch, thus avoiding a deviation. Process characterization data can assist in building a design space that can be used to assist in-process monitoring and in the response to a variation in the process.

We anticipate an increased role for automation, including artificial intelligence (AI), in the QMS systems as the technology advances. We believe that AI will initially be involved in deviation detection and advanced warning, beyond existing process monitoring, and through comprehensive monitoring of multiple factors, including bioburden, weather, utilities, training logs, increase in numbers of new operators, and personnel movement within the site, in addition to all activities that

may elevate the risk of deviations. With time, this involvement may evolve to enable automatic process control through artificial intelligence (AI) without the reliance on operators to make decisions. Eventually, automation may evolve over the next decade to perform batch release without relying on human operators as computing power increases and leads to better scenario analysis, risk mitigation, and decision making.

An additional point to consider is the addition of automated detection of deviations. Machine learning/AI can assist in model building to challenge and potentially exploit our traditional "rules-based" thinking. Product deviations are unforeseen, i.e., they have not yet been realized in process history runs. Machine learning can learn from process/product deviations or intentional robustness runs in scale-down models and prevent recurrence by identifying the signatures that led to the initial failure. Once built, models could explore long-term unforeseen potential deviations and possibly identify novel signatures before they happen.

The advanced control strategies described in the preceding sections may still be considered as too forward-looking for systematic implementation at this time and could require advancing the analytical technologies beyond what is currently available. However, it is important to keep these end goals in sight as instrument and control capabilities continue to mature.

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NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

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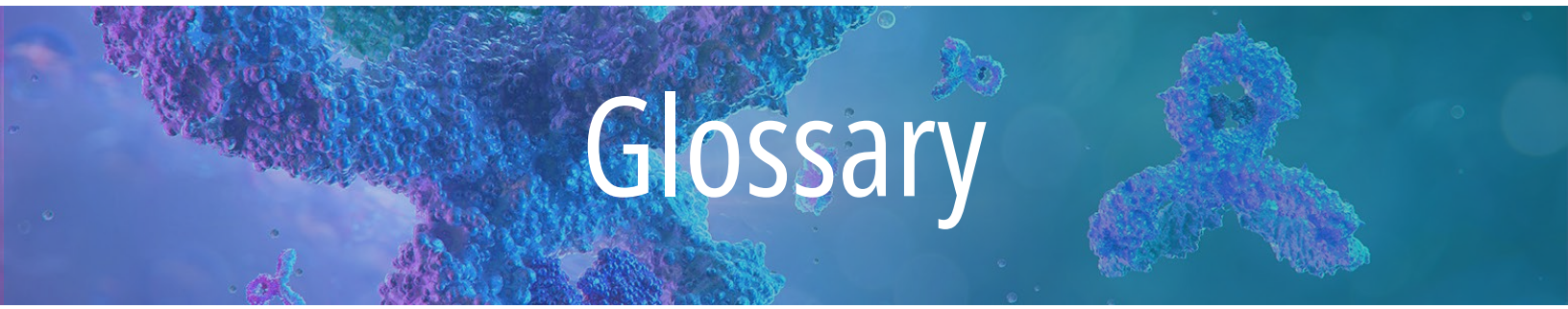

Glossary

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Glossary

Term	Definition	Sources/ References
Acceptance Criteria	Numerical limits, ranges, or other suitable measures for acceptance which the drug substance or drug product or materials at other stages of their manufacture should meet to conform with the specification of the results of analytical procedures.	ICH Q6B, 1999
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. Exceeding an action limit typically triggers a quality event and an investigation	ICH Q6B, 1999
	Established criteria, e.g., microbial levels, requiring immediate follow-up and corrective action if exceeded.	PIC/S (PI 007-6), 2011
Alert Limits	Established microbial or particulate levels giving early warning of potential drift from normal operating conditions which are not necessarily grounds for definitive corrective action, but which require follow-up investigation. Note that some sponsors may extend the use of this term to IPCs	PIC/S (PI 007-6), 2011
Batch	A specific quantity of material produced in a process or series of processes so that it is expected to be homogeneous within specified limits. In the case of continuous production, a batch may correspond to a defined fraction of the production. The batch size can be defined either by a fixed quantity or by the amount produced in a fixed time interval.	ICH Q7, 2000
	A specific quantity of a drug or other material that is intended to have uniform character and quality, within specified limits, and is produced according to a single manufacturing order during the same cycle of manufacture.	21 CFR 210.3

Term	Definition	Sources/ References
	A specific quantity of purified product of interest having a unique identifier that enables traceability of raw materials, production bioreactor days, subsequent downstream unit operations, and a pre-defined, unique data set confirming adherence to in-process control limits and final product quality release specifications that enable disposition by quality systems and forward processing to drug product.	This document
Bioburden	The level and type (e.g., objectionable or not) of micro-organisms that can be present in raw materials, API starting materials, intermediates, or APIs. Bioburden should not be considered contamination unless the levels have been exceeded or defined objectionable organisms have been detected.	ICH Q7, 2000
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)	ICH Q10, 2008
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.	ICH Q1A(R2), 2003
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.	ICH Q5E, 2004
Contaminants	Any adventitiously introduced materials (e.g., chemical, biochemical, or microbial species) in the drug substance/drug product not intended to be part of the manufacturing process.	ICH Q6b, 1999
Continued Process Verification (CPV)	An alternative approach to process validation in which manufacturing process performance is continuously monitored and evaluated.	ICH Q8(R2), 2009
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 A-Mab, 2009

Term	Definition	Sources/ References
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.	ICH Q10, 2008
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.	ICH Q7, 2000
Critical Quality Attribute (CQA)	A physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.	ICH Q8(R2), 2009
Critical Process Parameter (CPP)	A process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality.	ICH Q8(R2), 2009
Design Space	The multidimensional combination and interaction of input variables (e.g., 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.	ICH Q8(R2), 2009
Detectability	The ability to discover or determine the existence, presence, or fact of a hazard.	ICH Q9, 2005
Drug Substance (DS) Batch – see Batch	A specific quantity of purified product of interest having a unique identifier that enables traceability of raw materials, production bioreactor days, subsequent downstream unit operations, and a pre-defined, unique data set confirming adherence to in-process control limits and final product quality release specifications that enable disposition by quality systems and forward processing to drug product.	This document

Term	Definition	Sources/ References
Drug Product (DP) (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.	ICH Q6B, 1999
Edge of Failure	The boundary to a variable or parameter, beyond which the relevant quality attributes or specification cannot be met.	ICH Q8 (R2), 2009
Established Conditions	Legally binding information considered necessary to assure product quality.	ICH Q12, 2019
Formal Experimental Design	A structured, organized method for determining the relationship between factors affecting a process and the output of that process. Also known as "Design of Experiments."	ICH Q8(R2), 2009
Harm	Damage to health, including the damage that can occur from loss of product quality or availability.	ICH Q9, 2005
Hold Tank	Surge Tank: Small stirred tank with ~5-10 min RT used to match flow between unit operations Cycle Surge Tank: Larger stirred tank with a capacity of approximately one column cycle (~4-6 hr RT) Batch Pool Tank: Stirred tank that allows for collection of whole batch as a homogeneous pool. An accumulation point to account for stability and for traceability within and across bioreactor(s) for example. Its use may be dependent on time-constant of PQ variation so will need to be included in an intermediate hold time study	This document
Hazard	The potential source of harm (ISO/IEC Guide 51).	ICH Q9, 2005
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.	ICH Q6B, 1999

Term	Definition	Sources/ References
In-Process Control (IPC), 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.	ICH Q7, 2000
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.	ICH Q6A, 1999
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.	ICH Q5C, 1995
Intervention Point	A location in the process stream where a deviation detected by the outcome from process monitoring or sample testing can result in an action to the process flow such as diversion to waste or to a segregated hold vessel. Can also be called a diversion point.	This document
Knowledge Management	Systematic approach to acquiring, analyzing, storing, and disseminating information related to products, manufacturing processes, and components.	ICH Q10, 2008
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 A-Mab, 2009
Lifecycle, Product Lifecycle	All phases in the life of a product from the initial development through marketing until the product's discontinuation.	ICH Q8(R2), 2009 ICH Q9, 2005

Term	Definition	Sources/ References
Lot	A batch, or a specific identified portion of a batch, having uniform character and quality within specified limits; or, in the case of a drug product produced by continuous process, it is a specific identified amount produced in a unit of time or quantity in a manner that assures its having uniform character and quality within specified limits.	21 CFR 210.3
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 Process Robustness
Normal Operation	Behavior of the process which can be expected or predicted, or both, based on an understanding of the process. Unforced variability in the process or product which can be expected, predicted and characterized statistically or predictable variability, or both, which is forced by an external stimulation may be considered as normal operation.	ASTM E2968 – 14, 2016
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.	ICH Q10, 2008
Pharmaceutical Quality System (PQS)	Management system to direct and control a pharmaceutical company with regard to quality.	ICH Q10, 2008
Process Analytical Technology (PAT)	A system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes with the goal of ensuring final product quality.	ICH Q8(R2), 2009
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 A-Mab, 2009
Process Control	See In-Process Control.	ICH Q7, 2000

Term	Definition	Sources/ References
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.	ICH Q5B, 1995
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.	ICH Q6B, 1999
Process Performance Attribute (PA)	An output attribute within a unit operation related to process performance rather than a quality attribute, used for monitoring and/or adjustment of the process	ICH Q8 (R2), 2009
Process-Related Impurities	Impurities that are derived from the manufacturing process. They may be derived from cell substrates, culture (e.g., inducers, antibiotics, or media components), or from downstream processing (e.g., processing reagents or column leachables).	ICH Q6B, 1999
Process Robustness	Ability of a process to tolerate variability of materials and changes of the process and equipment without negative impact on quality.	ICH Q8(R2), 2009
Product-Related Impurities	Product-related impurities are molecular variants of the desired product arising from processing or during storage (e.g., certain degradation products) which do not have properties comparable to those of the desired product with respect to activity, efficacy, and safety.	ICH Q6B, 1999
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.	ICH Q6B, 1999
Proven Acceptable Range (PAR)	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. Operating limits proven through development or engineering studies. The proven acceptable range may or may not be near the known edge of failure.	ICH Q8(R2), 2009

Term	Definition	Sources/ References
Quality	The degree to which a set of inherent properties of a product, system or process fulfils requirements.	ICH Q9, 2005
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.	ICH Q5E, 2004
Quality by Design (QbD)	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.	ICH Q8(R2), 2009
Quality Control (QC)	Checking or testing, that specifications are met.	ICH Q7, 2000
Quality Management System (QMS)	See Pharmaceutical Quality System (PQS)	ICH Q10, 2008
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.	ICH Q9, 2005
Quasi-Steady State	Conditions where some individual process parameters are consistently varying in time but with a set pattern of variation (for example, PCC). In this guide, quasi-steady state conditions are considered equivalent to steady-state conditions.	ASTM E2968 – 14, 2016
Raw Material (RM)	Raw material is a collective name for substances or components used in the manufacture of the drug substance or drug product.	ICH Q6B, 1995
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.	ICH Q8(R2), 2009

Term	Definition	Sources/ References
Reference Standards or 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.	ICH Q6B, 1999
Representative Sample	A sample that consists of a number of units that are drawn based on rational criteria such as random sampling and intended to assure that the sample accurately portrays the material being sampled.	21CFR201.3
Risk	The combination of the probability of occurrence of harm and the severity of that harm (ISO/IEC Guide 51).	ICH Q9, 2005
Risk Analysis	The estimation of the risk associated with the identified hazards.	ICH Q9, 2005
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.	ICH Q9, 2005
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.	ICH Q9, 2005
Severity	A measure of the possible consequences of a hazard.	ICH Q9, 2005
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.	ICH Q1A(R2), 2003

Term	Definition	Sources/ References
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.	ICH Q6B, 1999
Steady State	Consistent operation over a period of time where all relevant process parameters and product qualities are not subject to variation outside of a defined range of values.	ASTM E2968 – 14, 2016
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 A-Mab, 2009
Testing or Measurement Terminology	**At-Line**: measurement where the sample is removed, isolated from, and analyzed in close proximity to the process stream. **In-Line**: measurement where the sample is not removed from the process stream, and can be invasive or non-invasive. **Off-Line**: measurement where the sample is removed, isolated from, and analyzed in an area remote from the manufacturing process. **On-Line**: measurement where the sample is diverted from the manufacturing process, and may be returned to the process stream.	ASTM E2363 – 14, 2016
Transient Conditions	Conditions where the process is disturbed from steady state or is in transition between one steady state condition to another (that is, the process conditions or product quality are not in steady state or quasi-steady state). Transients may be due to either external disturbances or intentional changes in the selected operating conditions.	ASTM E2968 − 14, 2016

Abbreviations

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Abbreviations

AA	Amino acid
ADCC	Antibody-dependent cell-mediated cytotoxicity
AI	Artificial intelligence
AR	Acceptable range
ASTM	American Society for Testing and Materials (former name), now known as ASTM International
ATF	Alternating flow filtration
AVA	Adventitious agent
B/E	Bind and elute
Biocap	Biocapacitance
BLA	Biologics license application
BT	Breakthrough
CAPA	Corrective and preventive action
CDC	Complement-dependent cytotoxicity
CEX	Cation exchange
CFR	Code of Federal Regulations
cGMP	Current good manufacturing practices
CHO	Chinese hamster ovary
Chrom	Chromatography
cIEF	Capillary isoelectric focusing
CIP	Clean in place
CM	Culture medium
CMA	Critical material attribute
CMC	Chemistry, manufacturing, and controls
CNC	Controlled non-classified environment
COGS	Cost of goods sold
CPP	Critical process parameter
CPV	Continued process verification

CQA	Critical quality attribute
CSPR	Cell-specific perfusion rate
Ctrl	Control
CV	Column volumes
DF	Diafiltration
DO	Dissolved oxygen
DOE	Design of experiments
DP	Drug product
DS	Drug substance
DSP	Downstream process
EOP	End of production
EOPCB	End-of-production cell bank
EQ	Equipment qualification
FDA	US Food and Drug Administration
FMEA	Failure mode and effects analysis
F/T	Flowthrough
FTIR	Fourier transform infrared (spectroscopy)
G0F, G1F, G2F	Galactosylation variants in glycosylation profiles
GPP	General process parameter
GUR	Glucose uptake rate
HCCF	Harvested cell culture fluid
HCP	Host cell proteins
HPLC	High performance liquid chromatography
HMWS	High molecular weight species
HTST	High temperature short time (heating)
ICB	Integrated continuous bioprocess
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEX	Ion exchange chromatography

IND	Investigational new drug (application)
IPC	In-process control
IQ	Installation qualification
ISO	International Organization for Standardization
ISPE	International Society for Pharmaceutical Engineering
KPP	Key process parameter
LC-MS	Liquid chromatography/mass spectrometry
LIVCA	Limit of in vitro cell age for production
$\mathbf{L_{resin}}$	Liters of resin
LRV	Log-reduction value
MA	Material attribute
MAA	Marketing authorization application
mAb	Monoclonal antibody
MALLS	Multi-angle laser light scattering
MAM	Multi-attribute method (used for peptide mapping)
Man	Mannose
MCC	Multi-column chromatography
MCMC	Markov Chain Monte Carlo
MOA	Mechanism of action
MVA	Multivariate analysis
MVM	Minute virus of mice (sometimes abbreviated as MMV)
Myco	Mycoplasma
NCPP	Non-critical process parameter
NIIMBL	National Institute for Innovation in Manufacturing Biopharmaceuticals
NGS	Next-generation sequencing
NME	New molecular entity
NOR	Normal operating range
OFAT	One factor at a time

OOR	Out of range
OOS	Out of specification
OOT	Out of trend
OQ	Operational qualification
OR	Operating range
PA	Process performance attribute
PAR	Proven acceptable range
PAT	Process analytical technology
PC	Process characterization
PCC	Periodic countercurrent chromatography
pcd	Picograms per cell per day
pCO_2	Pressure of CO_2
pCPP	Preliminary critical process parameter
PCR	Polymerase chain reaction
PD	Process development
pCQA	Preliminary critical quality attribute
pFMEA	Preliminary failure mode and effects analysis (beginning of Chapter 4)
PFR	Plug-flow reactor
PIC/S	Pharmaceutical Inspection Convention/Pharmaceutical Inspection Co-Operation Scheme
PK	Pharmacokinetics
PK/PD	Pharmacokinetics and pharmacodynamics
PP	Process parameter
PPQ	Process performance qualification
PQ	Performance qualification
PQRI	Product Quality Research Institute
PQS	Product quality system
ProA	Protein A
PTM	Post-translational modification

PV	Process validation
QbD	Quality by design
QC	Quality control
QMS	Quality management system
qP	Cellular specific productivity (pg/cell/day)
Qp	Volumetric productivity (g/L/day)
QTPP	Quality target product profile
RBIA	Risk-based impact assessment
RCA	Root cause analysis
RCC	Regulatory Considerations Committee (of NIIMBL)
RM	Raw materials
RPLC	Reversed phase liquid chromatography
RSM	Response surface model
RT	Real time
RVLP	Retroviral-like particles
SAR	Structure-activity relationship
SEC	Size exclusion chromatography
S/F	Structure-function
SDM	Scale-down model
SIP	Steam in place
SMB	Simulated moving bed
SME	Subject matter expert
SPTFF	Single pass tangential flow filtration
SP-UF	Single pass ultrafiltration
SS	Steady state
SUB	Single-use bioreactor
SVRF	Small virus retentive filter
TEM	Transmission electron microscopy

TFF	Tangential flow filtration
TMP	Transmembrane pressure
TPP	Target product profile
UF/DF	Ultrafiltration/diafiltration
USP	Upstream Process
vc	Viable cells
VCD	Viable cell density
VF	Viral filtration
VI	Viral inactivation
VVD	Vessel volumes/day
WCB	Working cell bank
WFI	Water for injection
XMuLV	Xenotropic murine leukemia virus