BIOMANUFACTURING TECHNOLOGY ROADMAP

PROCESS TECHNOLOGIES

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1.0 Summary

Process technology developments are at the heart of any efforts to increase the productivity and robustness of biopharmaceutical manufacturing. This Process Technologies Roadmap is intended to guide industry participants towards better technological solutions in their manufacturing plants mid to long term, thus enabling expanded patient access to safe and cost-effective biopharmaceutical medicine while maintaining the viability of an R&D-driven drug development.

This first edition of this roadmap places special emphasis on incremental advancements in current technologies that have the greatest probability of success for implementation in a manufacturing setting within a 10-year time horizon. Furthermore, the document focuses on the most common production scenarios in the industry, namely on 2,000L disposable and 10,000L (and larger) stainless steel (SS) bioreactor capacities for the commercial production of monoclonal antibodies (mAbs).

Following common practice in the industry, this roadmap groups its findings into upstream processing (centered on inoculation and cell culture production) and downstream processing (DSP) (centered on cell harvesting, purification and product isolation).

The document highlights unmet needs and improvement options in the process technology domain along the lines of increasing the productivity of the bioprocess itself, reducing the cycle times for the overall production campaign and optimizing the turnaround/downtime between two production runs. As examples, the authors consider the following improvement areas of particular importance for process development work in future years:

Media performance: in general terms, media design is still complex and the related process understanding is limited, which continues to be a problem in upstream process development. Furthermore, there are specific challenges with currently available media material, e.g. the often observed instability of concentrates. A desirable 2026 target scenario, therefore, involves a significant reduction of media cost – up to 50% compared to 2016 – and widespread implementation of newly developed stable media concentrates. Better media will result in higher titers, viable cell density and a lower cost of goods (COGS).

Robust harvesting: there is an identified need for new, robust harvesting systems that enable more cost-effective upstream processing options, e.g. handling high-density suspensions, or better/faster equipment cleanability or turnaround. Improvements in this field are expected to lead to higher harvest cell densities (>150^6 cells/mL) and substantially reduced downtimes within a 10-year horizon.

Cost-efficient viral clearance: currently available viral filter systems show limitations in key aspects, including lack of standardized absolute filter ratings, limited reusability options and low compatibility with continuous processing concepts. By 2026, the industry should be able to pick from a widely available and well adopted portfolio of filter systems (and/or alternative Viral clearance (VC) options) addressing these challenges.

Buffer management: the performance characteristics of DSP steps is highly dependent on an accurate and efficient buffer management. From today's perspective, buffer-related process fluctuations from cost-effective in-line dilutions and the incorrect tracking of buffer parameters (e.g. concentrations and pH) are not yet fully satisfactory and need to be further minimized. These efforts should lead to a target scenario where buffer characteristics are measured via highly accurate process automation and based on improved sensor concepts.

Higher capacity, longer lifetime resins: contributing to a lower overall COGS and helping to reduce the burden on buffer management.

Single-use technologies: to increase flexibility and improve closed systems, resulting in a decreased capital cost and a decreased total COGS over the lifetime of a product.

As stated above, entirely new, disruptive technology developments are not the focus of this roadmap. However, there are several concepts worth highlighting. In upstream processing, it is the implementation of virus-free/resistant cell lines that may include non-mammalian expression systems and the concept of biomarkers indicating the health state of the cell. In DSP, these include concepts of one-step purification/integrated unit operations and improving the understanding of protein structure-function relationships to allow the processing of highly concentrated formulations. The further elaboration of these (and other) emerging technology trends is intended to be included in a future edition of this roadmap.

2.0 Introduction

2.1 Vision

While great strides have been made over the past 20 years to increase the productivity and robustness of manufacturing processes for biopharmaceuticals, the cost and complexity of development and manufacturing remain high, especially in comparison to small-molecule pharmaceuticals. Further improvements are required to expand patient access while maintaining the viability of an R&D-driven biopharmaceutical industry. Process efficiency and economics, as judged by a facility's productivity (typically quantified as kg of protein produced per hour per liter of bioreactor volume), COGS and net present cost all have significant margins for improvement. Enhancing the process technologies available to the industry is a key element of achieving these goals, along with enhancements to the way in which these technologies are implemented, as facilitated by automation, analytics, modularity and knowledge management.

A challenge in realizing these goals is the intrinsic variations in product potency, market requirements and manufacturing productivity (i.e. cell culture titer) among the constellation of products under development. Due to these variations, the required scale of production varies widely among products, which is reflected in a broad range of facility designs. This complexity is amplified by the presence of substantial existing hard-piped SS facilities and the industry trend to minimize further investment through the pursuit of capital-sparing, single-use-systems-based facilities. As a result and to have the maximum impact, new process potential solutions must be adaptable across a broad range of scales and facility types. To address this, process technology solutions have been developed for different production scenarios defined in this roadmap, which vary in scale of operation, type of equipment (fixed-SS vs single-use systems) and mode of operation (batch-wise production vs continuous).

The technical advances that are proposed are generally intended to intensify the manufacturing processes. These include intensified ways of working, and intensified process designs using existing process concepts, to provide opportunities for short-term improvement; along with evolutionary changes to existing processes and the implementation of disruptive technologies to provide more substantial gains over a longer timescale. Ultimately, the cost and size of a facility required for a given product are envisioned to be minimized through a combination of these different types of advances, as follows:

- inoculum seed bags and operation at a high cell density in single-use systems will shorten the time in culture, while increasing titer
- continuous purification trains that utilize reusable filter elements and high-capacity adsorptive membranes with an 'infinite' lifetime will process the high-titer broth with fewer steps and in much smaller equipment
- enhanced process-monitoring capabilities will ensure each unit's operation is performed at its optimum condition
- closed-system operation will allow production in a less-costly unclassified space
- the combination of single-use systems and more robust reusable purification elements will reduce the time and cost of turnaround operations.

Taken together, these new technologies will not only reduce the cost of production for a biopharmaceutical product, they will also provide the flexibility needed to more easily manage the production of an entire portfolio of products.

2.2 Scope

This first version of this roadmap focuses on therapeutic protein (TP) production from Chinese hamster ovarian (CHO) cells, especially mAbs. There are many opportunities for technology enhancements in both the upstream and downstream production processes. In this first edition of this roadmap, the focus has primarily been placed on incremental advances in current technologies that have the greatest probability of success for implementation in a manufacturing setting within a 10-year time horizon. Some technology improvements that could have a profound impact on productivity and COGS (such as host-cell engineering, targeted integration and combined capture/clarification devices) are only mentioned as long-term disruptive technologies and not discussed in detail. The team also did not look at technology enhancements for specialized medicine at the 10L scale. In most instances, the technology improvements presented here could apply to other cell-culture processes expressing other TPs. For the sake of clarity, the technology enhancements proposed here, both incremental and disruptive, have been applied to specific facility-type scenarios.

Upstream – the proposed technology enhancements are primarily based on intermediate-scale, fully disposable production bioreactors with a fully disposable seed train. The mode of operation can be batch, fed-batch, process intensification at n-1 or production bioreactor,

or continuous. The use of long-term perfusion >2 weeks will be not be discussed here. The upstream scope also includes technology improvements for SS up to 30,000L production bioreactors operated in batch or fed-batch mode. The main goals for the technology improvements at this scale are for improving turnaround times and reducing cycle times. Some of the technology improvements at 2kL scale (production and seed-train) could be applied to the >10kL existing plant or considered during the design of new facilities at that scale. It should also be noted that the technology improvements mentioned here are targeted at typical cell-culture processes expressing mAbs. The team did not look at the technology requirements for specialized medicine at 10L scale but felt that, from an upstream production standpoint, the seed-train technologies for 2kL scale production could be directly applied for production at 10L scale.

Downstream – the downstream scenario recommended by the team for technology enhancements was required to be capable of processing material from any of the upstream scenarios, operated in any mode (fed-batch, intensified fed-batch or continuous). This is typically designed to some rate-limiting mass throughput which ultimately defines the productivity of a facility. As a specific unit operation may be operated in continuous mode in an overall batch-wise purification process (or continuous), the number of process permutations are too numerous to discuss each scenario individually. As a result, the strategy has been to breakdown individual unit operations and discuss the aspects relative to the different modes of operation (batch, semi-continuous or continuous). It is up to the process development engineer to implement the optimal configuration of the downstream process to suit the application. It is thought that the challenge in process technologies is scaling up, so that if a technology is robust at the 2kL scale, it could be scaled down to 500L, 100L or 10L scales with relative ease.

The report has mainly focused on the following unit operations:

1. Inoculum preparation
2. Production cell culture
3. Media preparation and storage
4. Bioreactor design
5. Harvest
6. Primary purification
7. Secondary purification
8. Viral clearance (VC)
9. Ultrafiltration (UF)/diafiltration (DF)
10. Buffer preparation and storage.

2.3 Approach

The roadmapping groups determined that the best way to map the impact of technology needs and solutions was to weigh these against manufacturing technology scenarios that are prevalent in the biopharmaceutical industry today. The approach was to identify these manufacturing scenarios as facility types and then to map the technology needs and potential improvements for each. The process/ facilities listed below were selected to represent the most probable scale for the operation as well as to represent each major upstream and DSP type:

1 **large-scale stainless steel fed batch** – low cost at high utilizations, high capital and long build times.
>10kL SS fed-batch bioreactors processed with batch or continuous downstream. This scenario is the current gold standard for production in the industry and a retrofit for more efficient operations must be considered.

2 **intermediate-scale single-use perfusion** – medium throughput production of a broad variety of proteins, more easily reconfigured or 'scaled across'.
2kL single-use continuous bioreactor (product transmission) with continuous or semi-continuous downstream. This scenario marries continuous bioreactor production with straight-through processing and minimal hold steps.

3 **intermediate-scale multiproduct single-use fed batch** – medium to low throughput production of a very broad variety of proteins, more easily reconfigured or 'scaled across'.
2kL single-use continuous bioreactor (product retention) with batch downstream or semi-continuous. This scenario can achieve very high titer and may include processes with single-use systems designed for the 2kL scale.

4 **small-scale <500L portable facility** – low throughput production units, can also be rapidly 'scaled across' and deployed into multiple regional markets.
<500L single-use continuous bioreactor (product transmission) with continuous downstream. This scenario is a scaled-down version of scenario 2.

5 **small-scale <50L for personalized medicine** – very low throughput, patient-specific preparation. Many production units, globally distributed
<500L single-use bioreactor (SUB) with batch or continuous downstream. This scenario is targeted for cell and gene therapy applications.

For each unit operation, a needs table has been constructed based on the identified needs in the text in Section 4. The table consists of a metrics heading at the top. The metrics are listed in order of importance to that particular need. Under the metrics, the individual needs are listed (along with associated metrics along the 10-year time horizon) followed by some key challenges and potential solutions. Please note that potential solutions were identified by the authors and other solution will also exist. The needs should dictate the best solution, whether identified in the tables or not.

For the sake of brevity and focus of the document, the technology improvements discussed here in detail are limited to what could be implemented in a commercial manufacturing plant in the next decade. Hence, some technology improvements that could have a profound impact on productivity and the COGS (such as host-cell engineering and targeted integration) are only mentioned as long-term disruptive technologies.

A concept that underpins more productive processes, and runs through all of the process scenarios listed earlier, but is not specifically addressed in the needs tables, is process intensification. This is a broad topic dedicated to increasing the efficient utilization of a facility. This may include ways of working to eliminate equipment and the steps associated with a white-space operation, combining or eliminating unit operations and modifying process parameters to make operations more efficient. A more in-depth discussion of process intensification is presented in Appendix A.

2.4 Benefits

Manufacturing scenarios

The manufacturing scenarios identified by the roadmapping teams are based on traditional and state of the art process and manufacturing designs, and on existing facilities (see Table 1). The inclusion of continuous upstream and downstream technologies provides a glimpse at the more modern facilities that exist today or will be implemented in the next 5-10 years. For the scenarios listed in Section 2.3, standard facility designs were taken into account to determine the number of each reactor configuration that would typically be in operation at one manufacturing site. For the SS item, a standard 6x15kL facility was assumed as a scenario that is commonly designed or in operation today at many sites. For the single-use item, a state of the art 6x2kL fed-batch design was chosen as a common platform with several of these facilities being implemented across the industry. For the continuous upstream scenarios, a traditional perfusion case at 2x2kL was included along with a 6x2kL concentrated or intensified fed-batch case. The former case is rather a large scale for perfusion (2x2kL), but this was selected to better compare to the other single-use scenarios using the same reactor size. The latter case (6x2kL concentrated fed batch) was included as a new technology that is in operation today for high throughput in a single-use platform. Each scenario incorporates the option for traditional or continuous downstream operations for comparison.

Modeling approach and results

The modeling approach identified by this roadmap teams was to use the identified manufacturing scenarios as a basis to keep the modeling work grounded to industry needs. BioSolve software was used for the modeling, along with the team parameter inputs to calculate the COGS for each scenario with variable annual throughput, as described in Appendix B in the Roadmap Overview document. The throughput ranges ~400–3,000kg are based on the general quantities required for the clinical trial and launch stages of a development and on typical outputs from the various facility types. It is important to point out that the COGS' analysis does not provide the full cost of manufacture due to differences in the upfront capital investment between scenario types. This limits the comparisons across facility types and future modeling efforts may incorporate net present value-based analyses to provide more cross-scenario analysis.

This high-level summary shows that at a high operational capacity (80% utilization) the 6x15kL fed-batch and 6x2kL concentrated fed-batch scenarios may provide a better COGS due to their high product output. However, these two scenarios also require a higher upfront capital investment, so the full cost of manufacture should ideally incorporate a net present value analysis to truly allow comparisons between scenario types when investing in new facilities. For example, if one can quickly add manufacturing capacity as it is needed via rapid single-use expansions vs a long-term SS facility build, then the return on capital spent is much faster. This leads to an overall lower cost of manufacturing of each gram of product needed. Overall, the COGS is not dramatically different between all scenarios. Only a minor impact was seen between traditional vs continuous downstream, which is shown in the range of cost per gram for each scenario with the lower value being the continuous downstream case. An initial response is that less than $100/gram of antibody is good, yet these values need to be weighed against the overall 5–10-year metrics identified in the Roadmap Overview document.

Table 1: Comparison of scenarios at full operating capacity (80% utilization)

Scenario	Scenario description (w/fed batch or continuous DSP)	Cost per gram ($/g)	Annual facility output (kg)
Scenario 1: large-scale SS fed batch	6x15kL fed batch	$43–46	2,974
Scenario 3: intermediate-scale multiproduct single-use fed batch	6x2kL continuous fed batch	$55–59	1,805
Scenario 2: intermediate-scale single-use perfusion	2x2kL perfusion	$73–88	452
Scenario 3: intermediate-scale multiproduct single-use fed batch	6x2kL fed batch	$71–79	405

DSP – downstream process, SS – stainless steel

To tie these results to the business drivers and the need for variable product output, it is important to compare the COGS at high and low product demand with the corresponding facility throughput. This analysis shows that the cost per gram at high throughput favors the 6x15kL SS and 6x2kL concentrated fed-batch cases, while the 6x2kL fed-batch and 2x2kL perfusion cases are favored with lower product needs. It is a key point that when looking at clinical- or low-throughput manufacturing, a facility with more flexibility and less capital investment is an advantage.

The overall scenario comparisons provide some insight into what operational strategies may be best for each organization depending on their product throughput needs, existing infrastructure, appetite for new technology and sensitivity to upfront investment.

Sensitivity analysis and metrics

Perhaps the greater value of the modeling for the roadmap is to test the impact of technology improvements within each scenario. Identifying technology areas that reduce the COGS within each scenario is very valuable in identifying which technologies will help us to meet our five- and 10-year goals. Targets for facility flexibility, speed, cost and quality are summarized in Table 3 below. The modeling results contained in Appendix B of the Roadmap Overview document can be reviewed against Table 3 to see which technologies may provide the biggest benefits and help us achieve our goals.

Overall, the factors of cell-line productivity, the cost of media, etc. showed a direct impact on the COGS for every case. Downstream costs of resins were surprising low due to the assumption of high resin cycling numbers (>100) that are typically used for commercial manufacture. When looking at clinical operations, the cost of chromatography resins can be very high so gains in resin capacity are assumed to be of benefit in those cases. Other less tangible costs – such as the impact on the cost of quality, real-time release (RTR), process analytical technology (PAT), etc. – have benefits that are harder to quantify. For individual organizations, the choice of manufacturing design and the benefits of each technology must be considered on a case-by-case basis depending on their product demands and the state of technology development or adaptation.

Table 2: Comparison of scenarios at high- and low-product throughput

Scenario	Scenario (batch or continuous DSP)	High throughput (1,805–2,974kg/year)	Low throughput (250–450kg/year)
		Cost per gram ($/g)	
Scenario 1: large-scale SS fed batch	6x15kL fed batch	$43–62	$199–362
Scenario 3: intermediate-scale multiproduct single-use fed batch	6x2kL continuous fed batch	$55–59	$105–168
Scenario 2: intermediate-scale single-use perfusion	2x2kL perfusion	$72–92	$73–120
Scenario 3: intermediate-scale multiproduct single-use fed batch	6x2kL fed batch	$75–83	$71–109

DSP – downstream process, SS – stainless steel

3.0 Scenario needs

A Scenario Needs Table below has been created to be a 'quick reference' guide for the reader. Here, one can quickly look up the top technology needs identified by the authors for each unit operation and cross check which process scenarios that need is relevant to. For a more detailed discussion on the identified technology, the reader is directed to the needs tables and descriptions in Section 4 according to the unit operation.

Table 3: Bioprocessing scenarios and key technologies and capabilities

	Unit operation		Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5
Description		BXR volume	SS 10kL–30kL BXRs	Disposable 2kL BXRs	Disposable 2kL BXRs	Disposable <500L BXRs	Disposable <50L BXRs
		BXR mode	Batch	Continuous[1]	Batch[3]/ continuous[2]	Continuous [1]	Batch/continuous
		DSP mode	Batch/continuous	Semi-continuous/ continuous	Batch/semi-continuous	Continuous	Batch/continuous
		Facility design	Segregated suites/ large footprint	Moderate footprint/ ballroom	Moderate footprint/ ballroom	Small footprint/ ballroom	Small footprint/ ballroom
		Processing	Low bioburden	Closed	Closed	Closed	Closed
		Product	mAb and other CHO TPs	mAb and other CHO TPs	mAb and other CHO TPs	mAb and other CHO TPs	Cell/gene therapy mAbs
		Comment	Adaptions on current facility designs/ retrofits	Continuous protein production through purification	High-titer batch upstream processes to match productivity of 10kL BXRs	Highly productive deployable facilities	Deployed at point-of-use

BXR – bioreactor, DSP – downstream process, CHO – Chinese hamster ovarian, DS – drug substance, DP – drug product, E&L – extractables/leachables, HCP – host cell protein, HTST – high temperature short time, mAb – monoclonal antibody, pCO_2 – partial pressure of carbon dioxide, PAT – process analytical technology, SS – stainless steel, TFF – tangential flow filtration, TP – therapeutic protein, USP – upstream processing, RV – reactor volume, VC – viral clearance, VF – viral filtration

Table notes: [1] N-stage perfusion with cellular retention and either product retention or transmission – assumes transmission
[2] N-stage perfusion with cellular retention and either product retention or transmission – assumes retention, termed 'concentrated, or intensified, fed batch'
[3] Shorter duration in production bioreactor enabled by n-1 cell retention

Table 3: Bioprocessing scenarios and key technologies and capabilities (continued)

	Unit operation		Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5
Upstream	Inoculum preparation		Cryobags to reduce time to production/n-1 perfusion	Cryobags to reduce time to production	Cryobags to reduce time to production/n-1 perfusion	Cryobags to reduce time to production/n-1 perfusion	
	Production cell culture	Cell density	30–60e^6 cells/mL	60–100E^6 cells/mL	60–100E^6+ cells/mL	60–100E^6 cells/mL	
		Perfusion exchange rates	NA	1–2 RV	NA; 1 RV average for concentrated fed batch	1–2 RV	
		Production culture duration	7–14 days [3]	30–60 days	7–10 days (batch)/10–15+ days (intensified fed batch)	30–60 days	
		qP	>40pg/cell/day	>40pg/cell/day	>40pg/cell/day	>40pg/cell/day	
		Titer/ productivity	5–15g/L	4–6g/(L/day)	30–50g/L	4–6 g/(L/day)	
		Product quality	Consistent throughout batch duration and between batches				
	Media	Media	Defined/stable/ low cost	Defined/stable/ balanced/low cost	Defined/stable/ balanced/low cost	Defined/stable/ balanced/low cost	
		Viral safety	HTST or low-cost VF	Disposable HTST/ low-cost VF	Disposable HTST/low-cost VF	Disposable HTST/ low-cost VF	
	Cell retention		N-1 microfiltration	Disposable filtration (non-fouling)/ acoustic wave	Disposable filtration (non-fouling)/ acoustic wave	Disposable filtration (non-fouling)/ acoustic wave	
	BXR design	Reactor design	Easily cleanable/ rapid changeover	Long duration, robust films and seals	Robust films	Long duration, robust films and seals	
		Polymers	New elastomers not requiring replacement	Defined and repeatable E&L profiles	Defined and repeatable E&L profiles	Defined and repeatable E&L profiles	
		Sensors	Multifunction, increased robustness (e.g. SU glucose, lactate, cell mass, pCO_2, etc.)				
Harvest	Harvest	Primary recovery	• Disc-stack centrifugation/ flocculation/ acoustic wave/ • Disposable centrifugation	See cell retention above	• Flocculation/ cell settling • Microfiltration/ acoustic wave/ disposable centrifugation	See cell retention above	
		Clarification	Reusable depth filtration	Gamma-compatible depth filtration	Reusable depth filtration	Gamma-compatible depth filtration	

BXR – bioreactor, DSP – downstream process, CHO – Chinese hamster ovarian, DS – drug substance, DP – drug product, E&L – extractables/leachables, HCP – host cell protein, HTST – high-temperature short-time, mAb – monoclonal antibody, pCO_2 – partial pressure of carbon dioxide, PAT – process analytical technology, SS – stainless steel, SU – single-use, TFF – tangential flow filtration, TP – therapeutic protein, USP – upstream processing, RV – reactor volume, VC – viral clearance, VF – viral filtration

Table notes: [1] N-stage perfusion with cellular retention and either product retention or transmission – assumes transmission
[2] N-stage perfusion with cellular retention and either product retention or transmission – assumes retention, termed 'concentrated, or intensified, fed batch'
[3] Shorter duration in production bioreactor enabled by n-1 cell retention

Table 3: Bioprocessing scenarios and key technologies and capabilities (continued)

	Unit operation		Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5
Purification	VC	Detergent/ solvent virus inactivation (optional)	Novel sewerable detergent with traceable removal from process stream				
Purification	Primary purification (e.g. affinity chromatography)		• Resin cross-use • Ultra-high capacity • SS continuous chromatography • Infinite lifetime adsorbents	• Disposable continuous chromatography • Resin cross-use • Ultra-high capacity • Infinite lifetime adsorbents	• Resin cross-use • Ultra-high capacity • Continuous chromatography • Infinite lifetime adsorbents	• Disposable continuous chromatography • Resin cross-use • Ultra-high capacity • Infinite lifetime adsorbents	
Purification	VC	pH viral inactivation	Modular claim	• Low pH, short time • Modular claim	Modular claim	• Low pH, short time • Modular claim	
Purification	Secondary purification 1 and 2		• Novel chromatography materials (flow through, or bind and elute) • Universal negative chromatography ligands (HCP/DNA scavengers) • High-capacity membrane adsorbers (flow through, and bind and elute) • Low hold-up scalable membrane devices for membrane adsorbers				
Purification	VC	VF	• High capacity – reusable viral filters • Absolute virus rating to streamline virus safety				
Purification	UF/DF	UF	• Membrane cross-use • Increased membrane lifetime	• Membrane cross-use • Robust single-pass TFF	• Membrane cross-use • Robust single-pass TFF	• Membrane cross-use • Robust single-pass TFF	
Purification		DF		Single-pass diafiltration	• High area disposable TFF • Single-pass diafiltration	Single-pass diafiltration	
Purification	Buffer management		• Buffer on demand • Buffer dilution • Novel in-process DSP formulations		• Buffer consolidation • Increased column binding capacity		
USP and DSP	Novel technologies		• Affinity partitioning • In-situ capture • Expanded-bed adsorption • High-resolution product-related impurity separations • Novel VC technologies	• Continuous crystallization • High-resolution product-related impurity separations • Novel cell retention devices • Novel VC technologies	• Aqueous 2 phase separation • Protein crystallization • Affinity partitioning • In-situ capture • Expanded-bed adsorption • High-resolution product-related impurity separations • Novel cell retention devices • Novel VC technologies	• Continuous crystallization • High-resolution product-related impurity separations • Novel cell retention devices • Novel VC technologies	
USP and DSP	General needs		• Cross-use of consumables among different molecules • Enhanced PAT tools • Advanced process control (e.g. multivariate statistical process monitoring with feedback-adaptive control) • Validation approach to continuous processing (e.g. batch definition)			• Rapid methods for column lifetime and cleaning studies • Standardization of connectors (both traditional and aseptic) • Normal flow filtration • Simplified VC validation • Developing new quality management systems to allow for straight-through processing from DS to DP	

BXR – bioreactor, DSP – downstream process, CHO – Chinese hamster ovarian, DS – drug substance, DP – drug product, E&L – extractables/leachables, HCP – host cell protein, HTST – high temperature short time, mAb – monoclonal antibody, pCO_2 – partial pressure of carbon dioxide, PAT – process analytical technology, SS – stainless steel, TFF – tangential flow filtration, TP – therapeutic protein, USP – upstream processing, RV – reactor volume, VC – viral clearance, VF – viral filtration

4.0 Future needs, challenges and potential solutions

Upstream operations for mAb production can be visualized in the diagrams in Figure 1. The needs for individual unit operations are discussed in separate sections. The linkages between unit operations can be seen below in the figure:

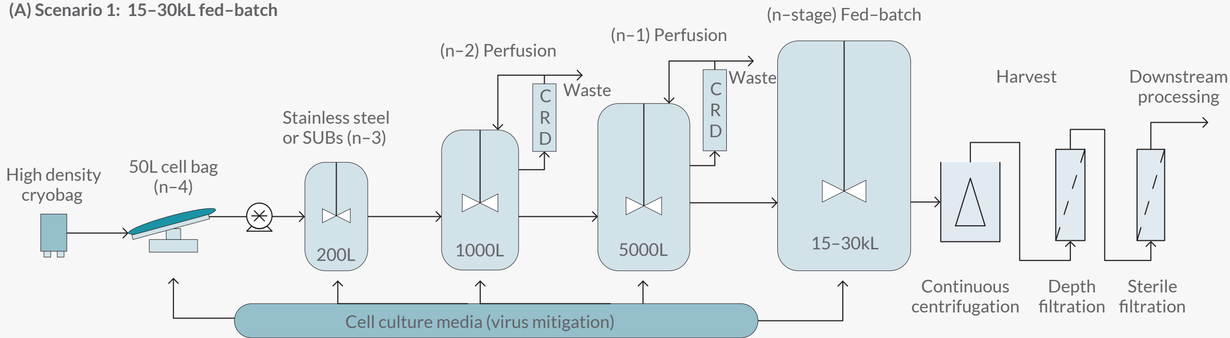
Figure 1: Flow diagram of upstream unit operations for manufacturing scenarios

(A) Scenario 1: large-scale SS production bioreactor (15–30kL) in an existing facility operated in fed-batch mode

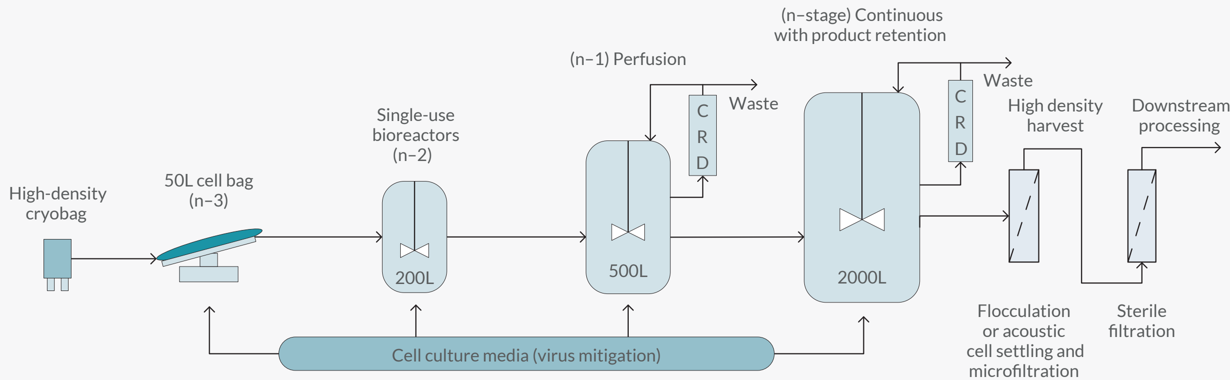
(B) Scenarios 2 and 3: single-use (2kL) bioreactor train operated in continuous mode with product transmission (not shown) or with product retention (as shown)

(C) Scenarios 4 and 5: smaller-scale (500L) SUB train for lower demand or personalized cell/gene therapy operated in continuous mode with product transmission (as shown) or batch mode (not shown)

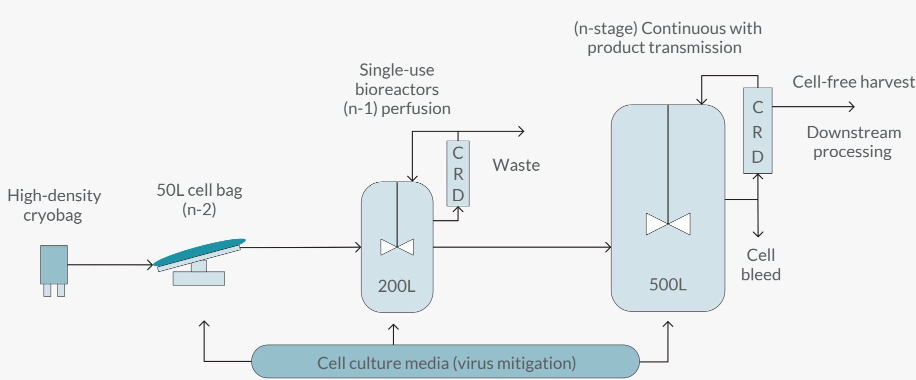
(A) Scenario 1: 15–30kL fed–batch



(B) Scenarios 2 and 3: 2kL continuous product transmission, product retention



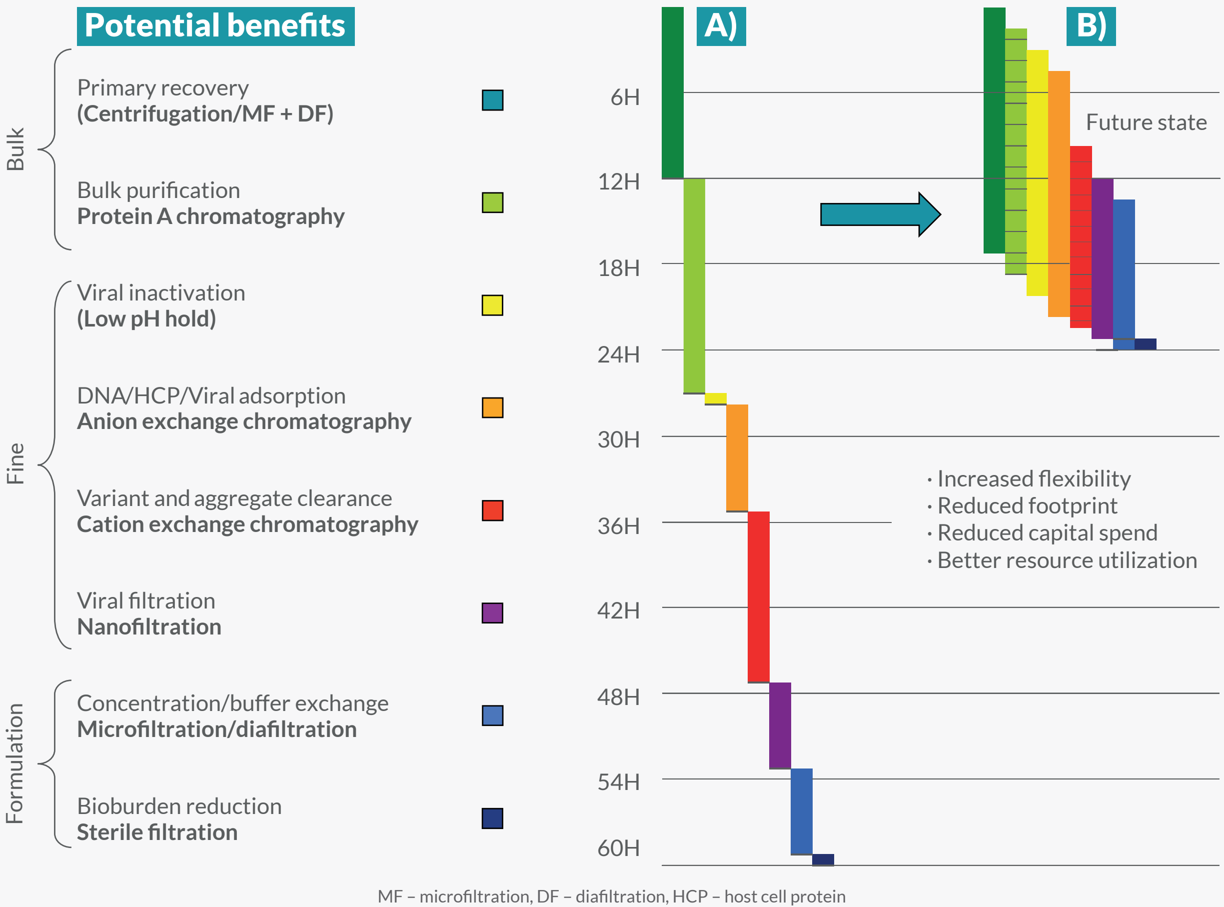
(C) Scenario 4a and 4b: 500L continuous product transmission, batch/cell therapy



SUB – Single-use bioreactor, CRD – cell retention device

Downstream operations can be visualized in the diagrams in Figure 2. The needs for individual unit operations are discussed in separate sections. The linkages between the operation can be seen below:

Figure 2: Example of A batch-based downstream processing time with linear processing for a generic three-column purification process as B) the productivity gain that can be realized if all of the unit operations are run concurrently

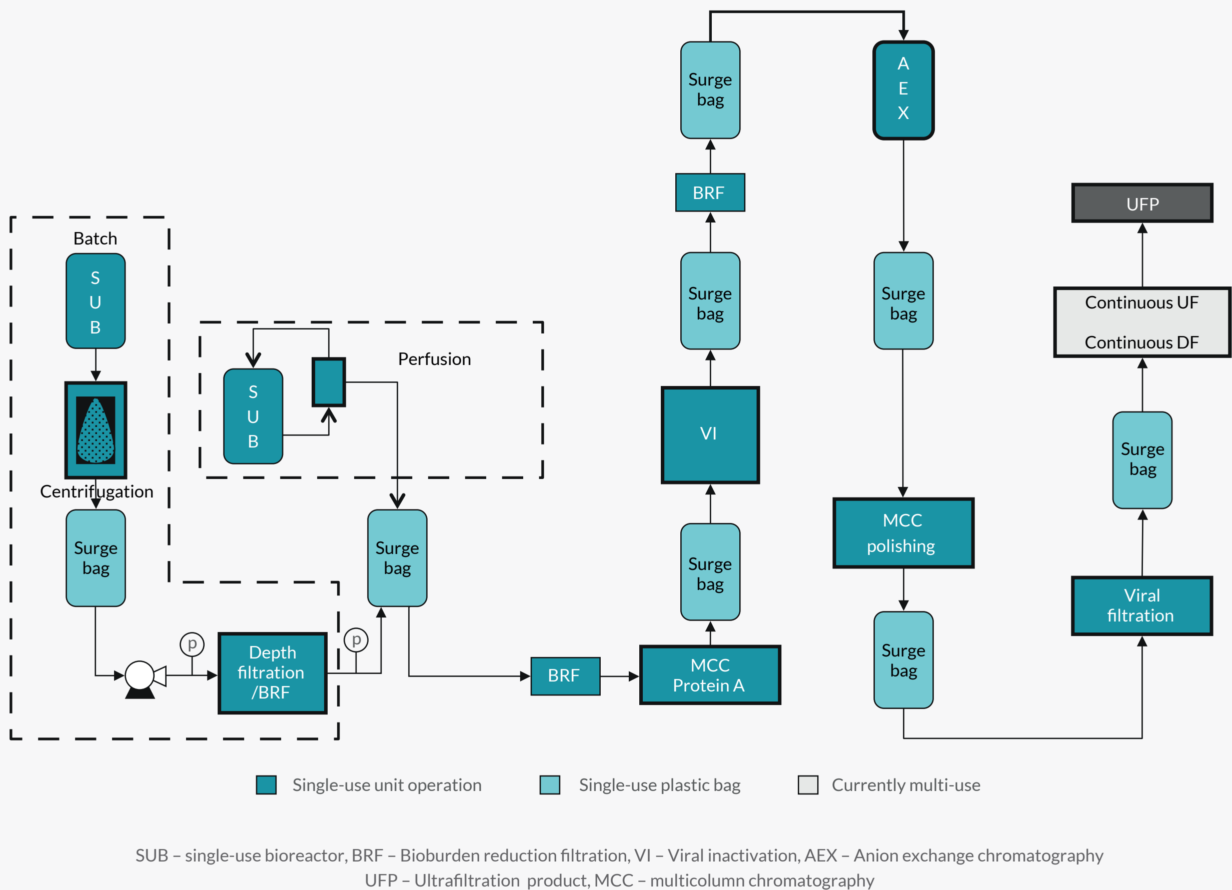


MF – microfiltration, DF – diafiltration, HCP – host cell protein

Batch processing can be accomplished with moderate automation outside of the bioreactor (automation is typically included for chromatography, UF/DF, 'sterilization in place' and 'cleaning in place' (CIP) procedures) and reliance on in-process sampling before progressing to the subsequent unit operation in the downstream space. Batch tracking and reporting are straightforward in this instance as material can be traced back through individual product pools to an individual bioreactor run.

The conversion of a batch-based process to a continuous one requires the introduction of different technologies to enable the material flow and transfer between unit operations. For example, multicolumn chromatography (MCC) skids replace standard single-column chromatography skids, and single-pass tangential flow filtration (TFF) and in-line DF replace UF skids. Also, strategies must be developed for time-dependent steps, such as viral inactivation. These strategies include pH-controlled tubular-flow reactors or sequential bag transfers of elution peaks from the continuous chromatography step. In a mature continuous process, reliance on in-process PAT may reduce the burden for off-line analytical testing and more rigorous feedback control to ensure consistent product quality. Material tracking and batch definition may also be complicated with continuous processing, especially in the case of perfusion bioreactors where multiple drug substance (DS) batches will be produced from a single bioreactor run over time.

Figure 3: Block flow diagram of a continuous downstream purification process (Scenarios 1, 2 and 4)



SUB – single-use bioreactor, BRF – Bioburden reduction filtration, VI – Viral inactivation, AEX – Anion exchange chromatography
UFP – Ultrafiltration product, MCC – multicolumn chromatography

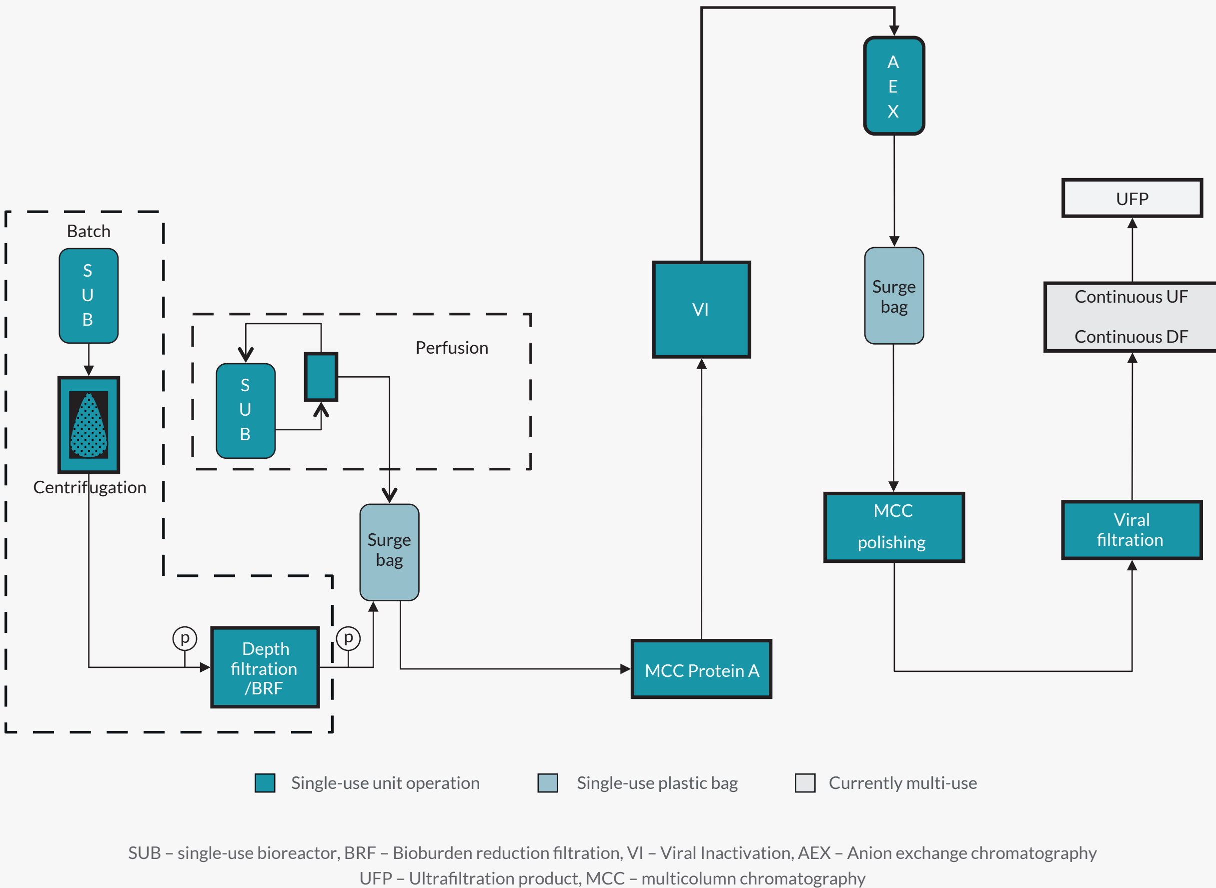
Options are shown in Figure 3 for batch/fed-batch upstream and continuous (perfusion) upstream with a cell retention device (CRD). All of the unit operations in the downstream are run at the same time using surge vessels as a place for stream conditioning for feed to the next step. The surge bags can be sized to accommodate small disruptions in the process (user-defined, up to 60 minutes) with optional containers between unit operations to collect flow for longer disruptions. Flow rates in the surge containers must balance per the following equation at normal operation:

$$0 = Q_{out} - Q_{in} - \sum_{i} Q_i \quad (1)$$

where Q_{out} is the take-off flow rate, Q_{in} is the incoming flow rate and Q_i is the flow rate for any component added for stream conditioning.

A higher degree of automation is required for this type of process that will primarily 1) control pH and conductivity between unit operations, 2) coordinate feed between the different unit operations, 3) divert or pause unit operations when there is a process upset, 4) interface with real-time analytics and multivariant process models, and 5) provide for feedback process control in the downstream space to account for disruptions or correct for 'critical process parameter' drift in real-time. Additional automation functionality will be required to trace material flow through the system and to give unit operation context information to a lot collected over a period that may not be traceable to an individual bioreactor run.

Figure 4: Block flow diagram of a continuous downstream purification process (Scenarios 2 and 3)



SUB – single-use bioreactor, BRF – Bioburden reduction filtration, VI – Viral Inactivation, AEX – Anion exchange chromatography
UFP – Ultrafiltration product, MCC – multicolumn chromatography

In Figure 4, options are shown for batch/fed-batch upstream and continuous (perfusion) upstream with a CRD. All of the unit operations in the downstream are run at the same time using minimal surge bags in the process. Here, surge bags are utilized to collect a defined volume of material that will be pushed through the subsequently linked unit operations as a collection of matched cycles before being pooled in a second surge bag before a second set of linked unit operations. In this instance, the surge bags are much larger to accommodate the user-defined amount of volume. For perfusion processes, the size of the first surge bag may equal the bioreactor volume (for those processes running at one vessel volume per day turnover ratio). A user may decide to operate with as many or as few intermediate surges as desired; the configuration above is used for illustration purposes only. Users may also incorporate bioburden reduction filtration and intermediate poolings on-skid to streamline the paired filtration analysis. In these types of processes, stream conditioning between unit operations must be accomplished in one of two ways. Either by matching buffer composition among unit operations so that pH and conductivity are compatible from the previous to the next, or by using in-line pH control.

As with the surge bag-reliant process, a higher degree of automation is required for this type of process covering the same aspects previously discussed above. Also, there is a higher degree of complexity associated with the in-line stream conditioning that will need to be accommodated, as well as strategies to align material flow between linked unit operations. Additional automation functionality will be required to trace material flow through the system and to give unit operation context information to a lot collected over a period that may not be traceable to an individual bioreactor run.

4.1 Inoculum preparation

4.1.1 Needs

The inoculum is the process to build up the required biomass to inoculate the production bioreactor. It starts with thawing a vial of cell bank in a shaking flask, followed by several passagings in shaking flasks and bioreactors. The steps involving shaking flasks are open operation and require segregated seed-train suites with higher room classifications (e.g. a laminar flow hood in class C area) to assure the sterility, which increases the capital expenditure and compliance effort. The current cell banking is usually 20–30x10^6 cells in a 1mL cryovial. The current cell line usually has a doubling time of 20–30 hours and a split ratio of 5–10, which needs about eight passages (24 days) to inoculate a 10kL SS, making the production scheduling inflexible.

Adapting the inoculum preparation to a fully closed scenario would lead to reduced room classification requirements, reduced quality control environmental monitoring sampling and less complex manufacturing operations. The use of cell bank in cryobags would enable this scenario.

Current processes need 20–30 days in the seed expansion stages before inoculation into the production bioreactor. This time spent in inoculum preparation can be an inefficient use of plant time and longer cycle time. Decoupling the early seed expansion stages by the use of high-volume, high-density cell bank in cryobags, instead of the vials used widely in the industry today, will help save time in plant by removing some steps associated with early cell expansion stages.

Current cell lines typically have doubling times in the 20–30-hour range. Host cell lines that can double faster with doubling times in the 18–24-hour range would enable faster cell mass build up. This build up will result in a shorter time to peak cell density for fed-batch cultures and to the steady-state cell density for perfusion scenarios. Since faster growing cell lines will need less time to produce an equivalent amount of product, the facility throughput will be improved.

An n-1 perfusion enabling a high seeding density in the production bioreactor allows a shorter production duration and more batches in certain facilities.

4.1.2 The needs, challenges and potential solutions

Table 4: Inoculum preparation – needs, challenges and potential solutions

		Current	2019	2022	2026	Scenario(s)
(Metric 1)	Cost					
(Metric 2)	Speed					
(Metric 3)	Quality					
(Metric 4)	Flexibility					
Need	Fully closed upstream to improve plant scheduling flexibility					All
Challenge	The current vial thaw and the first several steps for seed-train are with open handling under a laminar flow in a classification C area, leading to the complexity of the process and inflexibility of the scheduling					
Potential solution	Cryobags: large-volume (50–100mL), high cell density (100e^6 cells/mL), with long-term bag stability under low temperature, E&L addressed, easy handling and robust storage/shipment					
Disruptive technology	Vials that could be directly connected to a cell bag					

BXR – bioreactor, E&L – extractables and leachables, SS – stainless steel

Potential solutions manufacturing readiness level

Table notes: Business drivers have been listed in the table in order of priority for this unit operation. [1] Refer to Section 4.4: Cell Retention

Table 4: Inoculum preparation – needs, challenges and potential solutions (continued)

		Current	2019	2022	2026	Scenario(s)
Need	Fast-growing host cell with improved genetic and phenotypic stability (days from vial thaw to production BXR)	24	22	18	12	All
Challenge	The current cell line doubling time is ~24 hours; it takes 20–30 days to inoculate the production BXR-- making the production scheduling inflexible					
Potential solution	New generation of host cell line with shorter doubling time, ~12 hours	Research	Research	Research	Development	
Need	Robust scalable n-1 perfusion to enable high seeding density of production culture to reduce time in production tank, to improve productivity and throughput of facility (10^6cells/mL seed density)	10	10	15	20	All
Challenge	High-volume medium preparation/consumption, fouling of the filter membrane, scalability (2kL), to implement n-1 perfusion, the existing SS facility needs retrofit					
Potential solution	Robust, disposable scalable cell retention device [1]	Development	Development	Production	Production	
Need	A novel innovative process characterization/validation strategy					2 and 4
Challenge	For a perfusion process such as 60 days, the traditional validation will require significant time to perform the process validation, have a negative impact on time to market, hyaluronic acid interaction required					
Potential solution		Research	Research	Development	Development	
Need	Cell preservation technology other than liquid nitrogen					
Challenge	Cell banking stored in liquid nitrogen, difficult for storage and shipment					
Disruptive technology	Cryopreservation at -80°C	Research	Research	Research	Research	

BXR – bioreactor, E&L – extractables and leachables, SS – stainless steel

Potential solutions manufacturing readiness level

Research | Development | Production

Table notes: Business drivers have been listed in the table in order of priority for this unit operation. [1] Refer to Section 4.4: Cell Retention

4.2 Production cell culture

4.2.1 Needs

Typically, the production cell culture productivity has the largest overall impact on the COGS. A high-performing cell-culture process requires a high specific productivity (qP) cell line and maintenance of high cell densities. Accurate, real-time measurement of critical process parameters (e.g. temperature, pH, dissolved oxygen and carbon dioxide) is necessary for process control. Improvements in sensor and cell retention technologies, coupled with automation and advanced process control strategies, can allow for adaptive, continuous processes designed to deliver higher productivities and more consistent product quality. Some needs are described here, as the goals for next 5–10 years:

1. improve cell line specific productivity, growth and stability. Currently, it is common to see cell lines with qP ≥20pg/cell/day with decent cell growth achieving mAb titers ≥3g/L. To further improve titers for cost reduction, we must further increase qP or cell growth or both. Advancements in omics approaches, vector development and host-cell engineering (e.g. ZFN and CRISPR) can lead to more productive, stable and safer cell lines. Increased cell densities may be achieved through continuous and intensified fed-batch approaches where cell nutritional demands are met and harmful waste products are removed. In current perfusion processes, it is not uncommon to reach peak viable cell densities >60e^6 cells/mL, multiple fold higher than typical fed-batch operations. However, increased cell densities also introduce challenges to other areas, such as cell culture harvest and DSP (e.g. host cell protein (HCP)). Therefore, continued improvement to both stable cell line qP ≥50pg/cell/day and high cell density approaches will be needed for the next 5–10 years
2. consistent product quality over production time. Particularly in batch and fed-batch processes, product quality attributes can vary throughout the production bioreactor phase. This can limit the process duration and introduces the need for a robust manufacturing control strategy. Continuous upstream processing approaches can achieve a steady-state format and are expected to offer a more consistent product quality over time
3. the production bioreactor is the main unit operation and the driver of the COGS. Shortening the cultivation time in the production bioreactor can improve the facility output significantly, ultimately leading to a lower COGS. N-1 perfusion can build up the biomass to a high density enabling a high seeding process and shortening the production phase. However, in an existing multiproduct SS facility, the facility must be retrofitted to accommodate the n-1 perfusion process. The retrofit may have an impact on the processes in routine production, which must be evaluated
4. finally, improvements in online sensors and automation can enable the more complex control strategies needed to sustain intensified modes of operation. Currently, single-use pH and dissolved oxygen sensors are becoming more trusted and reliable in the industry and newer single-use glucose and cell mass sensors are available. It could be beneficial to have additional online sensors for titer, product quality, dissolved CO_2 and lactate, for example. However, proven quality and reliability is required before these technologies can be designed and implemented into manufacturing facilities.

4.2.2
The needs, challenges and potential solutions

Table 5: Production cell culture – needs, challenges and potential solutions

		Current	2019	2022	2026	Scenario(s)
(Metric 1)	Cost of goods					
(Metric 2)	Quality					
(Metric 3)	Speed					
(Metric 4)	Flexibility					
Need	Improved reliability of current in-line sensors. Further development of novel single-use and multifunction technologies					All
Challenge	Current sensor technologies are still not reliable and may need off-line recalibration during the process. The number of the ports in the BXR are limited. The disposable sensors' reliability are getting better, but still have room to improve					
Potential solution	Reliable and robust standard, single-use and multifunction sensors (e.g. pH, dissolved oxygen, CO_2, T, biomass and glucose) that do not require recalibration during the process and perform reliably over long culture durations					
Need	A new host cell line with higher qP (pg/cell/day) and enhanced phenotypic and genotypic stability (e.g. the specific productivity does not decrease). A new host cell line could produce defined product quality attributes	20 60	30	40	50 120	All
Challenge	Specific productivity is fundamental to cell culture productivity/COGS. The genetic and phenotypic stability is variable among different production cell lines. Cell stability is critical for long-term (20–60 days) perfusion processes					
Potential solution	Targeted hot-spot integration, innovative selection system. Genetic engineering of host cells (ZFN, CRISPR) and improved vector elements					
Disruptive technology	An engineered cell line with knockout of problematic HCPs and/or with defined quality attributes					
Need	Robust processes yielding consistent product quality					All
Challenge	Product quality varies during the scale up and technology transfer					
Potential solution	Improved process understanding and consistency, e.g. improved PAT, automation and real-time statistical modeling to enable robust, steady-state continuous processing. Automated feeding based on in-line sensors for cell mass, osmolarity, metabolite concentrations (in-line sensors or online measurement)					
Need	Disposable, scalable perfusion process (% used in process)					All
Challenge	High-volume medium preparation/consumption, fouling of the filter membrane, scalability (2kL), to implement n-1 perfusion, the existing SS facility needs retrofit					
Disruptive technology	Reliable, disposable, scalable CRD [1]					

BXR – bioreactor, COGS – cost of goods, CRD – cell retention device, HCP – host cell protein, PAT – process analytical technology, qP – specific productivity (per cell), SS – stainless steel

Potential solutions manufacturing readiness level

Research | Development | Production

Table notes: Business drivers have been listed in the table in order of priority for this unit operation. [1] Refer to Section 4.4: Cell Retention

4.3 Media

4.3.1 Needs

Compared to 10 years ago, generally speaking, current cell culture media have better-defined and more simplified formulations. The undefined and complex components, such as protein hydrolysates, have been gradually replaced by more chemically defined nutrients with an equivalent or better cell culture performance. This change has posted significant value to reduce bioprocessing variability due to raw material variables. With technology evolving, media formulation has been simplified by decreasing the number of media components from more than 100 to between 50–80, which has greatly improved the bioprocessing performance.

To meet overall bioprocessing performance goals in the next 5–10 years (including cost reduction and further improvements in process speed, flexibility and quality), some needs regarding cell culture media technology and capability are being discussed and considered, as briefly described below:

1. the need for cheaper media formulations. With increasing interest in intensified fed-batch and perfusion operations, it is critical to ensure that media costs are lower
2. developing and implementing high-quality media concentrates (liquid and powder). The high-concentrated media concentrates would greatly simplify the media make-up operations, reduce the chance of make-up error and the risks in manufacturing, therefore benefiting the overall bioprocessing performance. Simple in-line conditioning technologies to enable the use of media concentrates at scale
3. improving room-temperature stability. The longer the media stability at facility temperature adds good value to simplify operations, such as minimizing multiple make-ups, switching between 2–8°C and room temperature, etc. Improved room-temperature stability would also greatly help the long-duration cell-culture process (e.g. perfusion) and add significant value to single-use manufacturing with matching its simple and fast concept
4. improving media sterilization technology. More effective and robust high-temperature/short-time treatment (HTST) or other virus inactivation technologies would help reduce concerns about viral contamination and the impact on manufacturing. Also, there is a key gap in the availability of single-use technologies for viral inactivation at scale, i.e. disposable HTST or efficient/cheap VF
5. enabling the real-time reconstitution of media and creating the ability to make media on demand
6. creating better media formulations with reduced interactions with bag films, sensors, etc.

4.3.2
The needs, challenges and potential solutions

Table 6: Media – needs, challenges and potential solutions

		Current	2019	2022	2026	Scenario(s)
(Metric 1)	Cost					
(Metric 2)	Speed					
(Metric 3)	Quality					
(Metric 4)	Flexibility					
Need	Lower media cost (% relative to current media cost)	100%	80%	60%	50%	All
Challenge	Current chemically defined media formulations are still complex and a robust understanding of cellular metabolism is not available to optimize individual components					
Potential solution	Platform-based, chemically defined formulation with meaningful specifications that allow for multiple vendor supply					
	Supply forecasting and material handling methods that improve efficiencies and economies of scale [1]					
	Powdered media formats that are stable at room temperature					
Disruptive technology	Modification of the cell lines to reduce the dependency on high-cost media components					
Need	Optimized media formulation that supports higher cell growth, higher productivity and improves/maintains product quality					
Challenge	Cellular metabolism is complex and creates challenges in identifying the appropriate balance of components within the formulation that supports growth, productivity and quality					
Potential solution	Use of 'omics' analysis to develop a better understanding of cellular metabolism and physiology to optimize the media formulation					
Disruptive technology	Use of online probes to monitor cellular metabolism and provide specific media components on demand					
Need	Robust and cost effective methods of assuring adventitious agent safety in media (i.e. viral inactivation)	100%	80%	60%	40%	All
Challenge	Current solutions are cost prohibitive					
Potential solution	Cost-effective filters are provided for this application					
	Complete medium formulations that are more stable to HTST processing					
	Improved raw material safety and control					
	Disposable HTST technology is available					
Disruptive technology	New methods of viral inactivation in media are available					
Need	Ease of supporting high exchange rates of media for perfusion systems					2, 3 and 4
Challenge	Current media formulations cannot be formatted at high concentrations and require large storage capacity					
Potential solution	Simplification of media and introduction of alternative components to allow for higher concentrations					
	Point-of-use dispensing with a room-temperature, stable powdered formulation and in-line dilution					

HTST – high-temperature/short-time treatment

Potential solutions manufacturing readiness level

Table notes: [1] Refer to the Supply Partnership Management Roadmap report

4.4 Cell retention

4.4.1
Needs

In recent years, there has been an increased interest in perfusion and concentrated fed-batch bioreactor processing. These intensified modes of operation can offer increased cell densities and productivities that can drive down the COGS and provide more consistent culture conditions for product quality. High productivity perfusion processes (coupled with advances in cell line, media, in-line monitoring and automation technologies) can allow the supply from a flexible 2kL facility to match those achieved historically with large-scale stainless facilities. And, as advances in personalized medicine and cell therapies create therapeutics where cells are the product, these cells must be harvested safely and reliably.

One major aspect and potential limitation for executing a continuous bioreactor process or safely harvesting cells is the CRD. Historically, devices have been based on filtration, centrifugation, microcarriers, gravity or acoustic cell settling, yet challenges in complexity, scalability, fouling and implementation have hindered the widespread adoption of these technologies. Currently, most industrial applications rely on filtration techniques, such as alternating tangential flow (ATF), TFF or microfiltration. These systems have been proven in manufacturing environments, but operation at manufacturing scales can be challenging as systems lack robustness to achieve and sustain very high densities over long durations. To truly enable the biopharmaceutical industry to reach its goals of reducing the COGS and increasing manufacturing flexibility, further improvements to current technologies are needed, as well as new and novel approaches to cell retention.

Currently, most cell retention systems can only be modeled at the benchtop scale. As process development becomes increasingly automated, higher throughput, smaller (microscale) models and new approaches in microscale modeling of CRDs will be needed to maintain fast timelines and mitigate risk. Challenges with this include the following:

1. many low-shear pumps are scale limiting. The industry will need robust, large-scale, single-use pumps that can gently move cells through CRDs without fouling or imposing shear stress over long periods of time (up to three months)
2. ATF systems may be challenged at higher viscosities and cell mass conditions approaching $100e^6$ cells/mL. Diaphragm pump performance may be impacted by air leakage and fouling with challenging feed streams. And as filter area increases, so does the volume of culture residing outside of the controlled bioreactor at any point in time. This can cause difficulty in process control and differences in cell micro-environments. Finally, large-scale ATF systems are stainless, requiring parts to be autoclaved, which is undesirable for flexible, single-use facilities
3. TFF membrane filtration (MF) systems are subject to fouling with challenging feed streams approaching $100e^6$ cells/mL. New membranes with low fouling/sieving and high volumetric performance/efficiency will be needed to support densities up to $100e^6$ cells/mL over long durations (e.g. three months)
4. a disposable continuous flow centrifuge capable of handling large volumes/throughput
5. acoustic separators have shown promise at the laboratory scale, but have not been demonstrated as being robust in significant industrial applications. Current systems may be limited to under $50e^6$ cells/mL and lack the high separation efficiency needed to feed directly into downstream capture. A robust, reliable and scalable disposable acoustic separation/settling device is needed.

4.4.2
The needs, challenges and potential solutions

Table 7: Cell retention – needs, challenges and potential solutions

		Current	2019	2022	2026	Scenario(s)
(Metric 1)	Cost					2, 3 and 4
(Metric 2)	Flexibility					
(Metric 3)	Quality					
(Metric 4)	Speed to market					
Need	Robustness and performance: Cell retention systems and ancillary equipment that can handle challenging feed streams $>100e^6$ cell/mL over longer durations and provide feed streams direct to DSP to achieve a desired reduction in the COGS	$>60e^6$ cell/mL 2 months	$>80e^6$ cell/mL 2 months	$>100e^6$ cell/mL 2 months	$>100e^6$ cell/mL 3 months	2, 3 and 4
Challenge	Cell retention filtration devices are prone to fouling and loss of sieving with high cell densities and longer process durations High cross-flow provides improved sieving efficiency but can lead to cell damage Change-out of filters can pose risks to sterility and process performance Inefficient separation can lead to poor quality filtrate streams impacting DSP capture					
Potential solution	New membranes with low fouling/sieving and high volumetric performance/efficiency that can support densities over $100e^6$ cells/mL over longer durations (3 months)					
	CRD that can maintain sterility and allows for non-disruptive change-out that does not pose a risk to sterility					
	A robust, reliable and scalable disposable acoustic separation/settling device					
Need	Integration: Cell retention system that can be integrated with in-line monitoring and MES (feed rate, cell bleed and product harvest)	25% (degree of automation)	50%	100%	100%	2, 3 and 4
Challenge	Cell retention systems need to communicate with media feed/BXR/product removal/biomass removal Data needs to be acquired and parsed for knowledge management. In-line monitoring of process parameters is needed to support this					
Potential solution	CRD system coupled with ILM for cell density and key media components, which can integrate into MES for feedback control					

BXR – bioreactor, DSP – downstream processing, COGS – cost of goods, CRD – cell retention device, ILM – in-line monitoring, MES – manufacturing execution system

Potential solutions manufacturing readiness level

Research | Development | Production

Table notes: Business drivers have been listed in the table in order of priority for this unit operation.

Table 7: Cell retention – needs, challenges and potential solutions (continued)

		Current	2019	2022	2026	Scenario(s)
Need	Scalability and speed to market: Cell retention system that can be scaled reliably from development to commercial scales					2, 3 and 4
Challenge	Developing complex perfusion processes is not conducive to speed to market Lack of scalability creates challenges and risk in moving from clinical scales to commercial scales Benchtop scale-down models are difficult to achieve Currently, there is no small-scale device for perfusion modeling Cell culture impact to time outside of BXR for a large device may not be linearly scalable					
Potential solution	Device with capability to be run representatively from benchtop scale to manufacturing scale					
	Microscale – parallelized design of experiment system for fast process development and characterization					

BXR – bioreactor, DSP – downstream processing, COGS – cost of goods, CRD – cell retention device, ILM – in-line monitoring, MES – manufacturing execution system

Potential solutions manufacturing readiness level

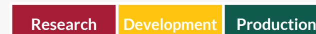


Table notes: Business drivers have been listed in the table in order of priority for this unit operation.

4.5 Bioreactor design

4.5.1 Needs

Production bioreactors are the workhorses for commercial production of biologics. Traditionally, large stainless steel bioreactors (>10kL) have been used for the commercial production of TPs, including antibodies. These SS vessels are designed to provide a well mixed, temperature- and pH-controlled environment, and provide sufficient oxygen and nutrients for the cells to grow and produce TPs. SS bioreactors are also designed for CIP so that the vessel and its components can be cleaned using an automated validated cleaning cycle. Also, the vessel and components are sterilized using steam to prevent contamination of the cell culture by microbes, such as yeast, fungi, bacteria, etc.

The technology behind the fabrication and installation of stainless steel bioreactors is established and will be used to produce biomolecules for the next 10–15 yrs. The challenges of building new stainless facilities are very well documented. High capital investment and long design, fabrication and qualification/validation times result in high business risks for the pharmaceutical industry. However, existing facilities will be retrofitted to reduce downtime, including sterilization, cleaning maintenance and changeover times. Also, process/technology improvements that can increase cell density/improve productivity will be part of the continuous improvements to realize reduced costs in these facilities.

For SS the following improvement opportunities have been identified:

- reduction in cleaning times: the reduction in cleaning cycle times can be achieved by improving cleaning agents that are more aggressive in removing standard cell culture soils. Sodium hydroxide- and potassium hydroxide-based cleaning agents are frequently used to clean large-scale bioreactors. The cleaning action is based on a combination of mechanical action via a liquid impinging on the surface of SS and the chemical solubilization of residue. The hardest to clean portion of the bioreactor is at the air-liquid interface. This deposition is caused by a combination of cell debris and antifoam carried by air bubbles. The deposition at the air-liquid interface requires a combination of sodium or potassium hydroxide in combination with surfactants to remove the deposit. In the future, a combination of improved cleaning agents, alternate foam control solutions (different to Antifoam C or improved mechanical foam breakers) and oxygen sparging systems (bubble-less aeration) could result in reduced cleaning times and less aggressive soil to be cleaned. Also, these should result in less water usage to rinse out residual cleaning agents. Phosphate-based detergents and phosphoric acid for neutralization need to be removed

- reduction in preventive maintenance times and changeover of the facility: in addition to cleaning equipment, the other major improvement opportunity in biopharmaceuticals processing is the reduction of downtime due to preventive maintenance activities and changeover activities between products. This involves changing out elastomers (such as gaskets, diaphragms, etc.) due to wear and tear or to prevent carry over from product to product. Improvements in construction materials for elastomers that result in less wear and tear and hence require less frequent replacements are required from the supplier of these materials. Also, regulatory agencies have to provide clearer guidance and expectations of elastomer change-out between products. Currently, a large proportion of the industry changes-out elastomers between products even though the surface area for the elastomer is a fraction of the total surface area (elastomer plus stainless steel) that the product sees. Improvements in the design of gaskets/diaphragms to prevent incorrect installation leading to inconsistent cleaning would help in moving towards eliminating or reducing the needs for elastomer change-out between products

  With stainless steel bioreactors and equipment, the other major preventive maintenance activity is derouging followed by passivation. Stainless steel vessels and pipes are prone to corrosion. High salt-containing solutions, water for injection (WFI), etc., are known to accelerate corrosion in SS surfaces. Regular activities such as passivation and electropolishing can prevent the onset of corrosion and increase the lifespan of SS equipment. However, these activities result in significant downtime in equipment. Austenitic steels that contain a high concentration of nickel, molybdenum and chromium, like AL^6XN and Hastelloy C-22, improve resistance to chloride-based pitting corrosion. However, the cost of these materials is prohibitive for fabricating entire manufacturing facilities. Improved cleaning agents that provide a passivation effect during the cleaning cycle can help prevent corrosion and thus eliminate/reduce downtime for passivation/derouging.

Over the last decade, the pharmaceutical industry has been moving towards single-use disposable bioreactors and associated tubing/connectors for producing biomolecules. The advantages of single-use systems have been very well documented in the industry. These include less downtime for changeover, reduced capital investment for both equipment and infrastructure, greater flexibility for manufacturing different profiles of products and eliminating the need for cleaning/validation of new products. However, the advantages of using disposables are offset to some extent by the following challenges:

- robust quality of film: the start of the supply chain is the resin that comes from the petrochemical industry for which the biopharmaceutical industry is a very small market. The initial manufacturers are also less aware and less willing to track the production process required by biopharmaceutical regulations. This leads to challenges in characterization of extractables and leachables and its effect on cell growth and productivity.

  The effect of extractable and leachables on cell growth and productivity has been documented in a variety of publications. The earliest reported effect was the study by Okonkowski et al., which showed reduced cell growth in disposable bioreactors compared to stainless bioreactors[1]. This was attributed to the extraction of cholesterol from the cell surface and adsorption of certain media components to the low-density polyethylene material. Wood et al. studied the effect of different biofilms from different suppliers on the growth rate of CHO cells[2]. They found that one of the films had demonstrated a negative effect on the growth of CHO cells.

  These findings suggest that cell culture-specific screening of single-use components needs to be performed in addition to extractable studies with model solvents based on the media composition. An industry-acceptable screening test for standard cell lines and media so that suppliers can provide that data before supplying new materials or changing certain components would mitigate this risk. Also, biofilm suppliers should provide data on extractables from standard off the shelf media for standard cell lines, such as CHO, non-secreting murine myeloma derived cells, VERO, etc. A supplier should also offer the capability for providing quick testing to facilitate testing with custom media that industry uses.
- single-use sensors: single-use, disposable pH and dissolved oxygen sensors that are critical for controlling the environment of the cells need to be as robust as their reusable counterparts, especially when gamma sterilized. In continuous perfusion cultures with campaign lengths that are longer than 15–30 days, the robustness issue becomes more important. Technology improvement to prevent deposition during long campaign lengths needs to be investigated and becomes critical for continuous production scenarios for upstream
- standardization and robustness of disposable bioreactors and holders: a SUB consists of the frame to hold the disposable bag assembly and the mixer motor.

[1] J. Okonkowski et al., "Cholesterol Delivery to NS0 Cells: Challenges and Solutions in Disposable Linear Low-Density Polyethylene-Based Bioreactors," Journal of Bioscience and Bioengineering, 103, No. 1, 50-59 (2007)

[2] J. Wood et al., "Strategy for selecting disposable bags for Cell Culture media applications based on a root-cause investigation," Biotechnology Progress, 29, No. 6, 1535-1549 (November/December 2013)

In addition, the entire unit contains a programmable logic controller. Several vendors manufacture disposable bioreactors up to the 2kL scale. The lack of standardization in bioreactor bag/mixer design, holder design and the programming language represents a challenge. End-users would prefer the flexibility and risk/benefit of interchangeability but suppliers are concerned about intellectual property and value-added differentiation. This balance has to be achieved via a conversation between end-users and suppliers to drive innovation and use of these technologies at a faster pace.

In addition to standardization, the other challenge with disposable systems is handling and leak rates. At scales larger than 500L, the handling and setup of the bags become critical both from an ergonomic perspective and also for prevention of leaks in bags. Leaking bags leads to loss of product and also questions the robustness of the manufacturing process. At lower temperature (2–8°C) the challenge with leaks/ruptures due to bag handling is increased. Improved materials of construction, packaging designs that lead to less creasing during setup and hoists/holders designs that result in less manipulation by operators can all lead to lower leak rates.

4.5.2
The needs, challenges and potential solutions

Table 8: Bioreactor design – needs, challenges and potential solutions

		Current	2019	2022	2026	Scenario(s)
(Metric 1)	Cycle time reduction					
(Metric 2)	Cost – reduced scrap rate					
(Metric 3)	Flexibility					
Need	Reduced downtime preventive maintenance	3–4 weeks per year	1–2 weeks per year	<1 week per year	1 week every 3 years	1,2 and 3
Challenge	Downtime is required for passivation/derouging of equipment, which is time- and labor-intensive. Also, lack of sufficient data on the lifecycle of elastomers results in an annual change-out of elastomers. The material of construction of elastomers has not changed in last two–three decades					
Potential solution	Development of cleaning solutions that can help in prevention/removal of rouge/pitting corrosion					
	Vendor data on elastomer lifecycle under process conditions					
	Improved material with increased lifecycle					
Need	Reduction in product changeover	2–3 weeks	1–2 weeks	No changeover between products	No changeover between products	1
Challenge	Regulatory guidance on requirements is lacking. No cohesive strategy on requirements for changeover from a cleaning validation perspective. Also, current cleaning times with existing cleaning solutions are long					
Potential solution	Improved materials for elastomers that are easily cleanable/less adhesion for products					
	Regulatory guidance on when elastomers can be changed out and what is required for cleaning validation					
	Optimized cleaning solutions that contain a combination of NaOH/KOH, surfactants, etc. Cleaning agents contain no phosphates					
	Cleaning agents are more soluble in water. Reduced complexity in equipment design					

BXR – bioreactor, COGS – cost of goods, E/L – extractables/leachables, USP<87> – United States Pharmacopeia 87 Biological Reactivity Tests

Potential solutions manufacturing readiness level

Research | Development | Production

Table 8: Bioreactor design – needs, challenges and potential solutions (continued)

		Current	2019	2022	2026	Scenario(s)
Need	Reduced leak rate/scrap rate for product	5–10%	2–5%	1–2%	0%	
Challenge	Current materials of construction are prone to leaks from bag handling, especially at low temperatures. Improved materials are also required for BXRs used for long-term cell cultures					
Potential solution	Improved materials of construction that are less prone to leaks due to bag handling					
	Improved design of hoists/packaging to reduce bag handling by operators					
Need	Well characterized E/L profile – based on cell culture components, used commercial media and effect on cell growth – robustness	USP<87> data from vendor	Well characterized films	Well characterized films	Well characterized films	
Challenge	Extractable studies are not currently done with standard components used in cell culture media. Standard test is based on United States Pharmacopeia guidelines, which can be improved					
Potential solution	United States Pharmacopeia testing changed – agreement in industry to test film using a base media that represents 90% of base media with respect to E/L					
	New films that eliminate E/L in standard media components					
Need	Multiple sourcing with minimal process validation for different bag vendors – COGs and supply chain risk reduction					
Challenge	Vendors design to differentiate from competitors and for intellectual property protection. Leads to non-interchangeability between different vendors					
Potential solution	Standardized design for hardware/automation allowing for interchangeable bags and connectors. Acceptance of reuse of buffer bags					
Need	Increased oxygen transfer for high cell densities					
Challenge	Venting/foam formation in BXRs at high oxygen sparging rates. Bubbling at high cell densities is achieved sometimes using microspargers (metal sintered), which results in large amounts of foam					
Potential solution	Alternate to Antifoam C – less challenge in cleaning or alternate design for foam breakers					
Potential solution (disruptive technology)	Disruptive – bubble-less aeration					
Need	CO_2 produced by cells at high cell densities needs to be removed from the BXR					
Challenge	No robust technology for removal of CO_2					
Potential solution	CO_2 stripping using silicon tubing to remove CO_2 by diffusion					1, 2, 3 and 4

BXR – bioreactor, COGS – cost of goods, E/L – extractables/leachables, USP<87> – United States Pharmacopeia 87 Biological Reactivity Tests

Potential solutions manufacturing readiness level

Research | Development | Production

Table notes: Business drivers have been listed in the table in order of priority for this unit operation.

4.6 Harvest

4.6.1 Needs

Current manufacturing technology standards for cell removal from production-scale bioreactors include one of two options – disc stack centrifugation followed by depth filtration, or direct depth filtration (typically the latter being used for bioreactor volumes up to 2kL scale).

Recovery for the large 10kL+ stainless steel scenario:
Cell removal from large scale 10kL+ SS bioreactors typically involves in-situ sterilizable disc stack centrifugation followed by (optional) depth and mandatory MF. These technologies are well established, robust and can be operated at reasonable operational process times and costs. Any further improvements to the 10kL SS manufacturing platform (e.g. intensified processes with higher cell densities or shorter cycle times) will easily be handled by the existing systems and no significant need for a revolutionary technology is required in the next 5–10 years at this scale. However, there is a need to improve the efficiency of product recovery from the recovery step for this scenario and improvements may have a significant impact as cell densities and product titers increase in the future.

Recovery in the single-use <2kL scenario:
In alternative production systems, such as 1kL or 2kL SUBs, operated in flexible facilities, single-use depth filters most commonly replace the centrifuges. At such scales, with usual final cell densities of up to 50 million cells/mL, the current depth-filter technologies reach their operational limits, with filter areas of about 20m² or more. Additionally, the current direct depth-filtration approaches have much lower solids removal capabilities. Such systems require a large footprint, have a cumbersome installation process and are expensive. With a further intensification of processes in the future, e.g. by perfusion (concentrated fed-batch systems with cell and product retention) followed by a batch-wise harvest at the end, there is a real need for new technologies, which could be:

1. newer, robust and improved depth-filter materials and construction of filter housings capable of removing larger amounts of cells (higher solids-removal capabilities) compared to current systems, while maintaining potential product loss at a minimum
2. depth filters that are reusable will become important with increased cell density scenarios as the cost of the disposable filter units used in the recovery process becomes large in comparison with other unit operations in this setting
3. new filtration technologies, such as body-feed filtration, where filter aids (e.g. diatomaceous earth material) in combination with membranes of wider mesh sizes allow for very fast flow rates and a much smaller filter area. Such systems have been recently commercialized[3]. It will take further time and effort to develop the applicability of such systems concurrent with the intensified upstream processes for the future
4. flocculation technologies could be used to complement the advances described in (1) and (2) above. Such a treatment could be used to reduce the depth filter area required to process broth that has not been centrifuged or could enhance the feasibility of other cell separation approaches. This is a difficult area for commercialization since so much knowledge is already in the public domain. A new category of polymers has emerged that has demonstrated the ability to remove process-related impurities, such as HCP and DNA[4]. The benefits of such technology may manifest themselves through extended lifetimes of the primary capture chromatography adsorbent (i.e. Protein A) and possibly the elimination of a unit operation targeted to remove these impurities. Also, a demonstration of clearance of the flocculants from the product requires additional assays and many flocculants are known to interfere with common impurity assays. However, development of a new flocculant that does not interfere with assays, and for which a sensitive assay could be provided, could be a very marketable product. This could even find a use in large-scale SS manufacturing settings
5. disposable centrifuge systems could be used analogously to the SS technology for a significant reduction of subsequent filter areas. However, currently available systems are limited in their design and operational procedures, in particular regarding the discharge of the solid matter. Further development of a single-use centrifuge that is easy to operate and robust in its design would be needed in the next 5–10 years
6. other available technology, such as acoustic (ultrasonic) filters, would require subsequent MF, which should not be a limiting technology in the future. For such systems, the scalability to a 2kL process currently requires further optimization
7. other cell removal systems are considered more suitable when the process is operated in a truly continuous harvest mode, e.g. a product containing the outflow of a perfusion bioreactor. In general, the CRD would already serve as a cell removal system and, dependent on its separation principle, would need more or less additional clarification

[3] van der Meer et al. 2014, BioProcess International, 12(8)s, 25-28-]

[4] Kang, Y., Hamzik, J., Felo, M., Qi, B., Lee, J., Ng, S., Liebisch, G., Shanehsaz, B., Singh, N., Persaud, K., Ludwig, D. L. and Balderes, P. (2013), Development of a novel and efficient cell culture flocculation process using a stimulus responsive polymer to streamline antibody purification processes. Biotechnol. Bioeng., 110: 2928–2937. doi:10.1002/bit.24969

8. ATF, or other cross-flow technology using membranes with defined pore sizes, would require a minimum of further clarification
9. technologies based on flocculation of cells in combination with separation techniques, such as hydrocyclones or inclined plate settlers, which are currently the least-developed options. Such systems would, in particular, be useful for truly continuous processes due to their operating principle, e.g. flocculation could be carried out in a defined reaction chamber.

For all of the above systems applied in a perfusion mode, there remains the challenge of cell separation of the bleed stream, i.e. the concentrated retentate from the perfusion device, which has to be removed from the bioreactor to keep cell densities constant. If the retentate is considerably low, e.g. 5–10% more likely it would be discarded from the process for ease of operations. If the amount is in a higher range, e.g. 10–20%, then an additional unit operation may be useful to harvest product from the bleed stream as well. A unit operation would be selected from the technology options mentioned above, e.g. filtration, centrifugation, etc. For ease of operations, it would be desirable if a perfusion device could have an integrated construction that allows harvesting the bleed stream, e.g. a series of integrated ATF devices, which further clarifies the bleed stream for optimized product yield.

Other novel ideas to improve the efficiency of the primary recovery process would be:

1. 'streamline' technology: fluidized bed-binding product while cell slurry passes through, essentially coupling capture and recovery in one step[5]
2. floating the cells to the top while harvesting through the bottom line?
3. aqueous two-phase separation
4. improved filters capable of reducing impurity levels, especially HCPs to help impact the lifetime of the capture column downstream.

4.6.2
Needs, challenges and potential solutions

Table 9: Harvest – needs, challenges and potential solutions

		Current	2019	2022	2026	Scenario(s)
(Metric 1)	Cost					
(Metric 2)	Flexibility					
(Metric 3)	Efficiency					
(Metric 4)	Quality					
Need	Cost: Reliable and cost-effective cell removal options for higher density processes in biomanufacturing (cost vs current scenario)	100%	90%	70%	50%	All
Challenge	Current depth filter capacities are low for direct depth filtration capabilities; depth filter capacity post-centrifugation can also be increased					
Potential solution	Better depth filters with increased solids-loading capacity					
	Cleanable, reusable depth filters					
	Body-feed filtration with filter aids					
	Flocculation with polyionic polymers					
	Acoustic separation methods – acoustic-wave settlers					
Disruptive technology	Combined harvest and capture steps – in-situ capture/fluidized bed binding; aqueous two-phase separation					

HCP – host cell protein, SS – stainless steel

Potential solutions manufacturing readiness level

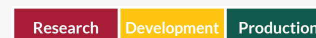


Table notes: Business drivers have been listed in the table in order of priority for this unit operation. [1] Cross Reference with cell retention section

[5] Beck, J.T., Williamson, B. & Tipton, B., Bioseparation (1999) 8: 201

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Table 9: Harvest – needs, challenges and potential solutions (continued)

		Current	2019	2022	2026	Scenario(s)
Need	FLEXIBILITY: Fully enclosed primary harvest operation to reduce room classification requirements for the harvest step	100%	90%	70%	<50%	All
Challenge	Current harvest operations, i.e. filter set up and operation, are not fully closed resulting in the need for operation in classified space					
Potential solution	Robust solutions for filtration (closed system set-up/connectors) and strategies to enclose a combined centrifugation/filtration operation					
Need	Flexibility: Robust, scalable, cheap single-use centrifuge option for a fully disposable facility supporting high-density processes					2, 3 and 4
Challenge	Current technologies have limitations on scalability, speed, ease of use and cost-limiting adoption					
Potential solution	Improvements in the existing technologies or newer technologies that provide cheap, robust, scalable single-use centrifugation options					
Need	Efficiency: Robust and rapid turnaround (and changeover) of disc stack centrifuge (reduced downtime)	2 days	<1 day	<1 day	<8 hours	1
Challenge	Disc-stack centrifuges are critical for use in the large SS facilities but are associated with cleanability concerns and changeover can be difficult and extensive					
Potential solution	Better cleaning agents and elastomers, designed to eliminate cleaning issues					
	Strategy and alignment with regulatory/quality to eliminate changeover					
Need	Efficiency: Increased step yields for primary harvest/cell bleed streams[1] without compromising impurity/cell removal	85–90%	95%	97%	99%	1, 2, and 3
Challenge	Product loss from the discharge stream of centrifuge/ filtration; product loss in cell bleed					
Potential solution	Disc stack centrifuge: product capture from discharge and/or strategies to reduce product loss; filtration: eliminate product loss during step; strategies for product capture from cell bleed stream in steady-state perfusion					
Need	Quality: Removal of process impurities in addition to solids removal (e.g. HCP, DNA, etc.), (reduction in impurity levels on capture column)	1x	10x	40x	100x	All
Challenge	Current filters can remove some HCP and DNA; however, improved feed streams with reduced impurity loads for capture steps may help improve capture column performance and lifetime					
Potential solution	Improved filters capable of removing impurities					
	Precipitation techniques to enhance impurity removal					
Disruptive technology	Novel solutions to identify impurities that impact capture step and remove them during the harvest step					

HCP – host cell protein, SS – stainless steel

Potential solutions manufacturing readiness level

Research | Development | Production

Table notes: Business drivers have been listed in the table in order of priority for this unit operation. [1] Cross reference with cell retention section

4.7 Primary purification

4.7.1
Needs

Primary purification, often described as the 'capture step', is the first true DSP unit operation with a key role in isolating and enriching a target molecule from residual clarified culture fluid. In the case of mAb processing, it almost exclusively employs an affinity chromatography step utilizing the inherent high affinity between the mAb Fc-constant domain and the proteinaceous Protein A ligand. This affinity to a broad range of monoclonal variants has created the cornerstone of mAb platform operations and is broadly utilized across the biopharmaceutical industry. The specificity of Protein A adsorbents comes at a high expense, however, in the order of $10,000–$15,000/L, making it typically the most costly raw material in the mAb production process (approximately 10x the cost of common ion exchange adsorbents). The high affinity between mAb and resin typically requires a low pH (~pH 3.0) to promote elution, which conceptually could be seen as a disadvantage to the unit operation; however, this conveniently combines with an orthogonal low-pH viral inactivation step (see Section 4.9). In fact, for the mAb industry, implementation of alternatives to Protein A are generally only explored when considering acid-labile mAb structures (where a low pH elution may irreversibly denature the target mAb). In this case, processing immunoglobulin sub-class 3 mAbs whereby Protein A displays a reduced affinity and thus is not deemed to be a suitable capture step, or in extreme commodity processes with atypical COGS drivers. For non-antibody products, the same level of affinity purification in the primary recovery step is the desired state, but natural ligands towards these products are not readily available and are costly to develop.

Throughout the growth of the biopharmaceutical sector, and the commercial success of numerous multibillion-dollar 'blockbuster monoclonals' based on such Protein A-based mAb platforms, the implementation of Protein A-related affinity resins highlights a list of needs if Protein A is to remain the key capture step in platform mAb processing. Historically, there has been an inability to sanitize and clean the resins with sufficient base molarity (≤0.5M sodium hydroxide), due to resin leaching and loss of dynamic binding capacity over a limited number of reuse cycles. This resulted in a vendor- and academic-driven evolution of the native (recombinant) Protein A to more highly engineered variants at the molecular level removing alkali labile amino acids together with improving chromatography immobilization-coupling strategies. The Protein A engineering, together with advanced chromatography resin (and membrane) adsorber base matrix technologies, continues to drive improvements in primary purification capture technologies.

In typical industrial immunoglobulin production processes, the primary recovery step is also considered to be a concentration/dewatering step wherein 10–35+ column volumes of product-rich clarified cell culture fluid are loaded to the Protein A column that in turn is recovered in two–three column volumes of elution buffer. As such it can, in many cases, be a bottleneck in mAb DSP with low specific productivities often in the range of 8–15g/L adsorbent/hr. It also represents a challenge as feed titers increase past 10g/L. As the fraction of bioreactor volume being loaded to a column decreases with increasing titer, more time is spent performing the washing, cleaning and equilibration of column per cycle, and with practical limits column diameter, the step productivity may ultimately be reduced even further. Biopharmaceutical supply partners have acknowledged this drawback and have designed novel hardware options for MCC as a means to increase step productivity. These systems also have synergy with continuous upstream processes to directly capture product from perfusion bioreactors. Here, these systems can isolate proteins, either stable mAbs or labile products, directly from the clarified permeate with significantly reduced residence time and tankage compared to batch-based chromatography.

The technology needs described below can, in many cases, be applied to the secondary purification unit operations (see Section 4.8) and refer to all four scenarios under consideration. Of specific interest to secondary purification include the following sections: ultra-high-capacity adsorbents, infinite lifetime adsorbents, MCC and pre-sterilized disposable columns.

Ultra-high-capacity adsorbents: higher-capacity absorbents in both primary and secondary purification (chromatography resins and membrane adsorbers) would contribute to process intensification, allowing processing of more material per cycle, i.e. higher productivity. While the benefits would be case-specific, it can generally be expected that increased binding capacities would also result in reduced buffer consumption per unit of product processed, and increased product concentration in the eluate, with a concomitant decrease in eluate volume (reducing processing tank or bag volume). This type of improvement should increase productivity, reduce the COGS and reduce the capital equipment cost for a given facility. Considering the case for primary recovery, the significant Protein A resin COGS often identifies this unit operation as the 'downstream process bottleneck'. In fact, in many cases, Protein A resin costs can contribute

to ~40–50% of the total downstream bill of materials and, to encourage resin utilization, three- to six-Protein A cycles are typical in most mAb processes. For secondary purification in mAb processing, anion exchange resins and membranes already have a high capacity, but cation exchange and hydrophobic interaction media have much lower capacities. Furthermore, traditional membrane adsorbers have a low capacity relative to resins, limiting their use for 'bind and elute' operations including Protein A. Advances in alternative bind and elute formats to traditional chromatography resin (i.e. membrane, monolith or other non-bead entities) may drive further increases in capture-step productivity, due to improved operational flow rates that can be achieved with such formats, together with reduced capital investments if traditional chromatography-column equipment can be replaced with disposable 'plug-and-play' approaches. It would be expected that new high-capacity adsorbents would be resistant to aggressive cleaning regimens so that they could be used across long lifetimes and are amenable to cross-use as described below.

'Infinite' lifetime adsorbents: improvements in the reusability of a resin, currently verified to approximately 150–200 cycles for a typical Protein A unit operation, are increasingly important when high cycle numbers are more readily reached, and thus are more rate limiting in a continuous chromatography mode. Resin reusability is inherently linked with the ability for the adsorbents to withstand rigorous CIP activities, while retaining dynamic binding capacities and avoiding in-process residual contaminants through ligand leaching. Likewise, such technology developments would also have a direct impact on more traditional batch processes (Scenarios 1 and 3). If residual impurities from previous molecule campaigns can be agreed and cleared from a regulatory perspective, increased resin lifetimes combined with adsorbent cross-use may become a key driver in significantly driving down mAb purifications costs for all scenarios considered. Cost modeling with traditional Protein A formats has shown minimal contribution for the adsorbent to the overall COGS after reaching cycle numbers >75. In reality, this cycle number may be difficult to achieve given current constraints, such as unpacking a fixed column for a new product campaign or exceeding the expiry date of the adsorbent. Prepacked formats and continuous MCC will now enable higher cycle numbers but will require extensions of shelf life and expiry times.

Agile methods to develop and manufacture custom affinity resins: affinity chromatography has clear advantages over lower-resolution modalities, but is rarely considered beyond the use of Protein A resins used to process mAbs. Current approaches to providing affinity resins for novel targets have long development timelines that are difficult to integrate into the desired product development timelines, and have uncertain success rates, often resulting in a reversion to non-affinity methods. Also, there is the complexity, cost and timeline of producing a 'good manufacturing practice' quality resin of using such a ligand. Improvements in ligand-discovery technologies combined with efficient integration into resin-manufacturing platforms would increase the frequency of utilization of custom-affinity resins, enhancing the efficiency of the manufacturing process. Specifically, the use of custom-affinity resins has the potential to reduce the number of steps in a purification process and so reduce process complexity, increase productivity and decrease COGS, while maintaining or improving product quality.

Multicolumn chromatography: an alternative to batch processing in mAb purification (as considered in Scenarios 2, 4a and 4b) is the application of continuous chromatography as a driver for reducing the cost of medicines by reducing overall COGS and operational expenses, improving facility utilization and turnaround, and opportunities for further advanced automation. There is no phase of the DSP where this is more relevant than when considering the primary capture step, again due to the high resin costs currently associated with this operation as described above in the Ultra-high-capacity adsorbents and Infinite lifetime adsorbents sections earlier. Although first-generation, continuous-chromatography hardware is current reaching the clinical setting, many believe that the fledgling technology is still in its infancy. SS systems that are amenable to larger-volume batch processing to debottleneck primary recovery (Scenario 1) and for high-titer processing (Scenario 3) have yet to demonstrate the robustness expected of established batch-chromatography alternatives. The perceived increased complexity of such systems must be compensated for by an increased ease of use of hardware and software components, as well as improved maintenance and lifetimes of components. Scenarios 2 and 4a on the other hand, where the continuous chromatography system may be directly coupled to smaller-volume perfusion production bioreactors (500L–2kL), lend themselves to a single-use format to facilitate low or no bioburden-closed processing. In addition to the requirements for the SS, the disposable alternative would need to demonstrate the long-term use of single-use components and sensors to match the timescales of the perfusion process (30–120 days in some cases). MCC methods may also be applied to secondary purification to similarly increase the productivity of the step.

True moving-bed chromatography: MCC has been demonstrated to increase the productivity of the capture step and allows users to invest in adsorbents in smaller aliquots over time. However, the technology is only periodic in nature and results in both concentration and pH gradients over time as individual columns are eluted. To ease the burden of step linkages in continuous processes, novel systems that result in a stream with a more consistent concentration and pH profile are desirable. Technologies in early phase development exist to provide this solution. One such technology, where the adsorbent is pumped through different fluid zones (loading, washing, elution, etc.), relies on TFF stages to provide sufficient buffer exchange in the platform of the desired operation, i.e. the washout of impurities or the elution of product[6]. Water efficient true moving bed processes pose to increase the productivity of the capture step while enabling continuous processing.

Pre-sterilized disposable columns: with disposable components entering the facility after gamma irradiation, there is also the potential for contamination from the columns themselves. As a result, there is a drive towards disposable columns in a pre-sanitized format that allows for a sterile connection to the continuous chromatography system with elevated operating pressure.

Adsorbent cross-use: a major advance for both primary and secondary purification would be the development of systems that would facilitate the 'cross-use' of the binding media – whether a chromatography resin or a membrane adsorber (both called 'adsorbent' from here on) – across multiple products. This would allow a unit of adsorbent to be used through its full lifetime (minimizing its contribution to the COGS) and would eliminate the need to stop and repack/reload a column/cartridge, thus minimizing facility downtime. This would also facilitate the use of larger columns in a facility, e.g. sized to process a batch in a single cycle, reducing the time required to process a batch, which would be advantageous as cell culture cycle times are reduced. The advantage of such a situation increases as the scale of operation trends toward the current maximum, which is typically regarded as a 2m diameter column. The realization of this vision requires that the adsorbent (base media and ligand) is able to withstand rigorous cleaning regimens without a loss of function, that there is an ability to test residuals to an extremely low level and that agreement on this practice can be reached with regulatory agencies. A key element of building a resin cross-use paradigm is agreement on the level of residual impurities (especially previous products) that can be considered safe as carry over into a product. This same approach could also be considered for cleanable filters used in the process, especially the tangential flow UF media.

Taylor-made affinity chromatography: affinity chromatography and the possibility to design ligands specific for single proteins has been under investigation for many years. Nevertheless, there is no theoretical reason why one could not develop a given single Protein A-specific ligand that can exhibit extreme selectivities, even superior of what Protein A can do for mAbs. To achieve this, the industry needs to advance its fundamental understanding of the structure of proteins and the principle guiding their interactions with other proteins, as well as with smaller chemical entities. This understanding could lead to the development of a general procedure, based both on silica and in-laboratory experiments, which could lead to the identification of a given protein to the appropriate ligand. Once the ligand has been discovered and produced, to support it on a chromatography or membrane backbone, proper technologies are currently available. Several companies and research institutions are active in this area but have not yet achieved this result.

To be disruptive, this technology should propose a ligand development strategy that is valid for large classes of proteins. The obtained ligand should be able to produce the ideal affinity chromatography that reaches specifications without further polishing steps and can be developed quickly to meet program and clinical timelines.

Affinity precipitation: another technology that has been shown to deliver a purification performance similar to Protein A chromatography is the use of affinity precipitation leveraging an ELP-Z stimulus responsive bi-polymer. Here, the ELP-Z is added to clarified cell culture fluid, complexed with the mAb that precipitates out of solution with a temperature or salt concentration adjustment[7]. To facilitate the adoption of this technology, industrialization is required to reduce process economics and improve scalability.

Combined clarification and capture technologies: A long-standing goal of biopharmaceutical process development engineers has been to combine the cell removal (clarification) and the initial bulk purification step for process intensification. One such example is expanded-bed adsorption, where the goal has been to capture the protein product while allowing the cellular debris to pass through the void space in the column by operating in up-flow[8]. Other approaches to process intensification have been proposed using adsorptive membranes for capture and cell retention, but no data is in the public domain on the topic. Alternative approaches to combined clarification and capture technologies are welcomed in a disruptive technologies mindset.

[6] Dutta, A. K., Tan, J., Napadensky, B., Zydney, A. L. and Shinkazh, O. (2016), Performance optimization of continuous countercurrent tangential chromatography for antibody capture. Biotechnol Progress, 32: 430–439. doi:10.1002/btpr.2250

[7] Sheth, R. D., Jin, M., Bhut, B. V., Li, Z., Chen, W., Cramer, S. M. "Affinity Precipitation of a Monoclonal Antibody From an Industrial Harvest Feedstock Using an ELP-Z Stimuli Responsive Biopolymer" Biotech. And Bioeng. , 111(8) 1595-1603, (2014)

[8] Chase, H.A. Purification of proteins by adsorption chromatography in expanded beds, Trends in Biotechnology, Volume 12, Issue 8, 1994, Pages 296-303, ISSN 0167-7799

4.7.2
The needs, challenges and potential solutions

Table 10: Primary purification – needs, challenges and potential solutions

		Current	2019	2022	2026	Scenario(s)
(Metric 1)	Cost					
(Metric 2)	Flexibility					
(Metric 3)	Quality					
(Metric 4)	Speed to market					
Need	Productivity: Current dynamic binding capacity is low, and diffusion characteristics limit operational flow rates and productivity batch and multicolumn productivity [g/(L/h)]	10 (30)	20 (60)	30 (90)	50 (150)	1, 2, 3 and 4
Challenge	The entire BXR volume must be processed through the column at relatively low flow rates and binding capacities					
Potential solution (incremental)	1) Improved ligand-coupling technologies and base-media chemistries to enhance 'usable' ligand density to reduce column volume and cycle number; 2) New high surface area materials with enhanced flow properties to enable higher operational flow rates; 3) Dewatering feed with single-pass TFF					
Potential solution (disruptive technology)	True moving-bed chromatography to increase productivity and enable continuous processing					
	Combined clarification and capture technologies					
Need	Adsorbent cross-use: regulatory approval to utilize chromatography media across multiple products and assets [number of cross-use campaigns]	0	0	2	5	1, 2, 3 and 4
Challenge	A step change in regulatory approaches to enable and facilitate mAb manufacturers to cross-use the capture media across multiple products					
Potential solution	Agency education, novel cleaning strategies, low-level of detection techniques for product carry over					
Need	'Infinite' lifetime adsorbents (average resin utilization (number of cycles)	100	250	500	1,000	1, 2, 3 and 4
Challenge	Unable to fully utilize maximum resin cycle number due to 1) resin shelf life expiration, 2) clinical success rate with no resin cross-use, 3) unpacking of columns to release column asset for other process use					
Potential solution	Improvements in ligand chemistries and resin/pore architecture to enable further efficiency in 'cleaning in place' strategies. Simplified verification of resin/column/membrane system cleaning/regeneration, to reduce time and material use, which do not directly contribute to product manufacture					

BXR – bioreactor, TFF – tangential flow filtration

Potential solutions manufacturing readiness level

Table notes: Business drivers have been listed in the table in order of priority for this unit operation.

Table 10: Primary purification – needs, challenges and potential solutions (continued)

		Current	2019	2022	2026	Scenario(s)
Need	Robust multicolumn chromatography operation downtime (days/month)]	7	5	2	0	
Challenge	Lack of standardization and complexity in currently available multicolumn chromatography systems lead to potential routes to failure					
Potential solution	Convergence toward standard formats. Simplification of components and skid hardware. Advances in single-use sensor robustness. Demonstrations of long-duration operation. Systems monitoring/diagnostics to prevent failures during operation. Advances in polymers to minimize component change-out and long duration of SU components					
Need	Sterility: Contamination of closed process trains in continuous manufacture risks batch rejection BXR failure. Bioburden contamination rate [%]	5	2	1	0	2 and 4
Challenge	Aseptic connectors and tubing welding as well as 'steam in place' procedures enable closed, multiproduct processing. However, bioburden can still enter the process through chromatography adsorbents, filters and tubing failures					
Potential solution	High-pressure aseptic connections, aseptic connections, pre-packed column and disposable membrane sterilization techniques that preserve binding capacity and molecular weight cut-offs					
Need	High-affinity ligand for non-platformable protein therapeutics (non-mAbs). Proportion of non-mAb processes that include high-affinity capture step [%]	10	20	30	50	1, 2,3 and 4
Challenge	Multiple platforms exist for the maturation of affinity ligands for non-platformable targets, yet they are all associated with high costs and long lead times, which are not conducive to clinical timelines. Stability of such ligand and coupling chemistry is less stable than mature Protein A					
Potential solution (incremental)	Rapid discovery, synthesis and conjugation technologies to yield protein-specific affinity ligands in a timeframe to enable clinical production					
Potential solution (disruptive)	Ultra-high-affinity ligand development leading to purification processes without further processing steps					
	Affinity precipitation					

BXR – bioreactor, TFF – tangential flow filtration

Potential solutions manufacturing readiness level

Research | Development | Production

Table notes: Business drivers have been listed in the table in order of priority for this unit operation.

4.8 Secondary purification

4.8.1 Needs

Secondary purification, the so-called 'polishing' steps that follow capture and provide most of the remainder of resolution required to produce a biopharmaceutical of acceptable purity, is most frequently achieved with some form of chromatography, using either a packed-bed or (compared to primary purification) membrane adsorbers. The main difference from the capture chromatography step is the binding ligand employed; with ion exchange, hydrophobic interaction and various mixed-mode ligands most commonly employed for secondary purification (vs some version of affinity ligands usually preferred for capture). Many of the ideas presented in the primary purification needs (see Section 4.7.1) can be equally applied in secondary purification considerations, including the following: 1) ultra-high-capacity adsorbents, 2) infinite lifetime adsorbents, 3) MCC, 4) pre-sterilized disposable columns, and 5) adsorbent cross-use; thus they will not be described again here in detail. As with the primary purification, these concepts should apply to all scenarios under consideration although, again, the impact on MCC used for continuous processing may be less dramatic than on batch processing.

Membrane adsorbents: distinct from primary purification, the secondary purification step in mAb processes is almost exclusively non-affinity in nature. To address productivity, it is preferred to operate polishing steps in the flow-through mode of operation, i.e. undesirable impurities (e.g. DNA, residual Protein A, HCP, virus, mAb-related impurities) bind to the chromatographic media at a relatively high loading, while the target mAb passes unbound remaining in the load flow through fraction. Thus, membrane adsorbers, typically composed of cross-linked cellulose or polyethersulphone, are often a cost-effective alternative to traditional chromatography beads. The more open and porous nature of such adsorbents enables much higher flow rates to be sustainably achieved, compared to traditional mass transport-limited beads, thus rapid polishing steps can be more cost effective in this membrane format. In contrast to chromatography beads, there are a limited number of commercial examples of such membrane absorbers available that offer both ion-exchange and hydrophobic interaction chromatography chemistries. When considering such membranes in a bind and elute mode, their open and porous nature can limit their application due to their limited dynamic binding capacity and poor hydrodynamic properties. In many cases, an ion-exchange bead can offer mAb-binding capacities five-fold greater than membrane-format alternatives. An alternative approach to operating such high-flow media is to accept the lower binding capacity of the material but compensate this with a higher cycle number, effectively processing feed material with a relatively lower adsorbent volume with the intention to reduce COGS. Through advanced membrane architecture, together with enhanced functional chemistry immobilization and grafting technologies, it is envisaged that improved binding capacities and impurity resolution can ensure membrane technologies continue to be implemented in future mAb process selection, with some in the industry anticipating all secondary purification activities could be membrane-adsorbent-based.

The concept of membrane adsorbers may be advanced even further through advanced materials design (both backbone chemistry and organized structure). Next-generation materials are beginning to enter the process development space where flow-through-type polishing has been demonstrated with functionalized hydrogels, nanofibers, foams and monoliths. Further research in material science may ultimately lead to capacities greater than that achieved in beads, while allowing the high-flow properties realized with the current generation of membranes.

Alternate approaches to secondary purification can be conceived, including precipitation or crystallization, and variations of liquid-liquid extraction, such as biphasic aqueous polymer systems and the use of 'smart polymers' carrying the same binding ligands used in chromatography.

4.8.2
The needs, challenges and potential solutions

Table 11: Secondary purification – needs, challenges and potential solutions

		Current	2019	2022	2026	Scenario(s)
(Metric 1)	Cost					
(Metric 2)	Quality					
(Metric 3)	Speed					
(Metric 4)	Flexibility					
Need	Resolution: insufficient product- and non-product-related impurity resolution in secondary purification leads to unit operation yield loss (secondary purification % step yield)	80–90%	92%	95%	98%	1, 2, 3 and 4
Challenge	Existing chromatography products and processes are insufficient to achieve necessary purification with high yield to separate product-related (e.g. charge heterogeneity, aggregates, glycoforms, fragments) and non-product related e.g. deoxyribonucleic acid (DNA), virus, HCP, endotoxin) impurities					
Potential solution	Alternative chromatography chemistries and ligands (e.g. mixed mode), format (e.g. membrane, monolith) and operational modalities (e.g. multicolumn countercurrent solvent gradient purification)	Development	Development	Production	Production	
Need	Productivity: current dynamic binding capacity, impurity capacity and diffusion characteristics limit unit operation productivity: Batch (multicolumn) (g/L/hr)	15 (40)	30 (80)	45 (120)	75 (200)	1, 2, 3 and 4
Challenge	Secondary purification unit operation productivity improvements through advanced chromatography media engineering, operational strategy and change in modality					
Potential solution	1) Novel high-throughput media and formats, 2) implement single-pass TFF to increase impurity binding capacity, 3) enhanced flow-through chromatography capabilities (for resin packed beds and membrane absorbers) away from bind and elute mode	Development	Development	Production	Production	

HCP – host cell protein, TFF – tangential flow filtration

Potential solutions manufacturing readiness level

Research | Development | Production

Table notes: Business drivers have been listed in the table in order of priority for this unit operation.

4.9 Viral clearance

4.9.1 Needs

Viral clearance considerations

Ensuring mammalian cell-derived products are free from contaminating viruses is an important part of the overall assurance of patient safety in biopharmaceutical product development. The risk is due to viruses' ability to infect and propagate in the production cells. Due to extensive controls and preventive safety measures, recombinant DNA-derived biopharmaceutical products produced from mammalian cells have a long history of viral safety.

One cannot rely solely on virus detection and quantitation methods to provide a high degree of safety assurance. Therefore, current practices require substantial validation activities to demonstrate clearance in each of the relevant downstream unit operation steps through spiking studies, in addition to extensive testing of cell line, cell bank, end of production cell and beyond. These activities not only require substantial costs, they also impose significant timeline restrictions, which can become a bottleneck of the future high-productivity process. Here are some examples that can potentially reduce the development burden without compromising the product and patient safety:

- **viral clearance modular claims:** within a typical mAb/recombinant protein purification process, low-pH inactivation, solvent/detergent and detergent treatment have been proven to be highly robust within a wide operational space to deliver effective inactivation on retrovirus. Similarly, 20nm nanofiltration has been shown to be highly effective and robust for removing large viruses, such as XMuLV. It will be valuable to have modular claims for these proven unit operations steps without having to repeatedly conduct the clearance studies if the industry could assemble a convincing data package. Some limited progress has been achieved in the past, including a modular claim of a Triton X-100 inactivation step[9], and a low-pH viral inactivation step[10]. It was helpful to have a regulatory submission database similar to what was published by Miesegaes et al. in 2010[11]. The recent emerging trend of collaboration across the industry to pool VC data from multiple companies, exemplified by the paper by Mattila et al[12]. is the first and positive step. Ultimately, regulatory agencies' adoption of a modular claim will be crucial to materialize the vision. For example, it is possible for the industry to assemble the data and justification on a standard claim of log reduction values for XMuLV within pH3.5+/-0.2 to reduce and potentially eliminate the need to validate the low-pH inactivation step
- in-line low-pH viral inactivation: the high-productivity DSP is moving towards linkage of the continuous DSP. The typical batch-mode, low-pH treatment-based viral inactivation would become a limitation in the continuous process. Work needs to be done to develop the relevant mixing device to enable an exposure time-based continuous flow for low-pH inactivation and neutralization to connect to the chromatographic steps in between. This would be similar to the ultraviolet inactivation technology that has shown success in the plasma-fractionation process but which is not yet widely adopted in the biopharmaceutical industry
- the enhanced capability of analytical technology and increasing scrutiny on critical quality attributes (CQA) might impose operational constraints due to the VC-validated ranges of the relevant unit operation steps
- **high-capacity viral filter:** currently, the viral filter throughput can be molecule- and matrix-specific and often requires significant efforts to optimize the throughput. A robust viral filter that can consistently deliver high capacity and high speed is much desired. It is equally needed for the filters to deliver consistently high performance that is independent of the molecule and matrix choices. Additionally, systematic prefilter selection (or using multiple prefilters) may improve VF throughput and lead to cost reductions
- **absolute viral filters:** it is highly desirable to produce an absolute viral filter that can be used with a standard claim on clearance (without having to do a spiking study) on a wide range of viruses based on absolute pore size assurance, similar to 0.2 micron filtration on bacterial reduction
- **viral filter reuse:** small-pore VF (20 nm) is a highly effective step for virus retention by size and is commonly used in the mAb production process. Currently, the viral filter is a single-use raw material and is often the highest cost component. It is possible to show reusability through demonstration of integrity (non-destructive) testing, cleaning effectiveness and filter lifetime for the benefit of reducing raw material costs and set-up time. Demonstrating thorough cleaning, performance consistency and regulatory acceptance can be some of the hurdles
- **alternatives to size-based virus retention:** with the emergence of complex protein drug constructs that add molecular mass and size, product recovery on virus filtration is becoming an issue. One way forward might be looking for means of achieving parvovirus clearance in these processes without relying on nanofilters. This challenge might be met with a focus on novel virus-inactivation agents, ultraviolet energy or alternative purification operations that can reliably and robustly deliver high levels of parvovirus clearance

[9] ASTM E3042-16

[10] ASTM E2888-12

[11] G Miesegaes et al. Biotechnol Bioeng 106 (2), 238-246, 2010 1994, Pages 296-303, ISSN 0167-7799

[12] J. Mattila et al, PDA J Pharm Sci and Tech, 70 293-299, 2016

- for chromatography steps, impurity profiles could potentially be used as surrogate makers for virus removal capacity, instead of conducting a VC-study on aged resin in resin lifetime studies
- develop chromatography media that can absorb viruses for reliable and robust VC
- a globally harmonized regulatory filing standard, with common technical documents, that could be expanded to include section templates. Since writing documents and addressing questions is often the bottleneck in development, a standard VC-regulatory template could further streamline the documentation activities and minimize the less-productive guesswork
- novel inactivation and clearance strategies, such as:
  - retrovirus particle-free cell lines
  - virus resistant cell lines
  - continuous VF
  - disposable HTST or HTST-treated media from suppliers
  - low-cost viral filter for media preparation in lieu of HTST
  - in-line adventitious agent monitoring, in-line real-time quantitative polymerase chain reaction. This is particularly valuable for live virus and cell entity, which are not feasible for low-pH and nanofiltration clearance.

4.9.2
The needs, challenges and potential solutions

Table 12: Viral clearance – needs, challenges and potential solutions

		Current	2019	2022	2026	Scenario(s)
(Metric 1)	Cost of goods on raw materials, such as VF	100%	95%	75%	50%	1, 2, 3 and 4
(Metric 2)	Cost of goods from VC studies	100%	90%	75%	50%	1, 2, 3 and 4
(Metric 3)	Critical quality attributes constraints					
Need	VC modular claims, e.g. low-pH inactivation and 20nm filter on retroviruses	Molecule- and company-specific. Resource intensive	Gather additional data across member companies for more publications	A few companies implement the strategies	General industry practices	
Challenge	Gain regulatory acceptance on modular claims					
Potential solution	Industry white paper to outline the matrix conditions and demonstrate effective and robust virus log reduction. Also valuable to have publication on regulatory submission VC database and cross-industry data in a publication, similar to what was published by Miesegaes et al. in 2010 and Mattila et al. in 2015[13]					

VC – viral clearance, VF – viral filtration

Potential solutions manufacturing readiness level

Table notes: Business drivers have been listed in the table in order of priority for this unit operation.

[13] Miesegaes et al in 2010 and Mattila et al in 2016

Table 12: Viral clearance – needs, challenges and potential solutions (continued)

		Current	2019	2022	2026	Scenario(s)
Need	Absolute and reusable viral filter	Research and development		Initial implementation	Well adopted by industry	
Challenge	Log-reduction values vary. Molecule-specific optimization of the viral-filtration process is labor intensive. Filter reuse is limited due to concerns associated with virus cross-contamination and product carry over					
Potential solution	Supplier to deliver absolute viral filter with standard claim, high performance independent of molecule and matrix against all viruses. Cost effective and reusable	Development	Testing	Widely available		
Need	In-line low-pH viral inactivation and continuous VF to support continuous processing					2 and 4
Challenge	Need a tool for viral-inactivation performance for the unit operation steps, challenging for scale-down model for VC validation					
Potential solution	Supplier to provide device with good performance in validation, e.g. integrate design concepts with mixing, time control acidification and neutralization to produce an easy to use device unit					
Disruptive technologies		Virus-resistant host cell lines Endogenous virus-free (low) host cell lines Novel viral-inactivation agent for non-envelop viruses Novel inactivation techniques Novel resin for robust virus removal In-line adventitious agent monitoring				

VC – viral clearance, VF – viral filtration

Potential solutions manufacturing readiness level

Research | Development | Production

Table notes: Business drivers have been listed in the table in order of priority for this unit operation.

4.10 Ultrafiltration/diafiltration

4.10.1 Needs

Tangential flow filtration UF for concentration and DF of purified protein to prepare a 'formulated' product stream, and 0.2um MF for bioburden control, to produce bulk DS is a standard manufacturing technology in the biopharmaceutical industry. The membranes and systems in place are generally robust, the membranes can be used to process several batches (following validation) so they are not usually a major component of the COGS, and the operation is not particularly difficult. For batch-purification systems, whether considering large-scale SS or 1–2kL SUB installations, current equipment and practices are adequate. However, current skid designs are typically large and have a high capital cost, so there are still opportunities for enhancements. For continuous purification processes, continuous systems are available for UF concentration but commercially available UF/DF systems are not, which represents a major opportunity for equipment manufacturers.

High-viscosity processing and process analytical technology: One challenge with conventional-batch UF systems is producing a product stream at high concentrations (>150g/L up to ~300g/L), which is desirable for many products. The ability to reach such high concentrations, accurately and reliably,

can be a challenge due to a combination of the large volume changes involved and, especially, the higher viscosities (can be >100cP) often encountered (which can also lead to high-yield losses). Novel approaches that address these issues would be valuable. Similarly, the robust integration of PAT into such systems to provide more automated control of operations, including the concentration endpoint, would also be welcome. The use of continuous single-pass TFF for the final concentration after a batch-concentration-DF operation begins to address some of these issues, but the technology is not yet widely implemented (and also this adds another process skid to the manufacturing facility).

Novel ultrafiltration membranes: The development of filters with longer service lifetimes would drive down the cost contribution of the UF step. At its simplest, this could be a matter of developing 'next generation' polymer membranes that would be used in the same engineered filter cartridges, in the same UF skids. The development of very robust membranes (such as ceramic-membrane filters) that could tolerate very severe cleaning regimens without a loss of filtration characteristics might be the next level of improvement. Such a system could even support the development of membrane cross-use, i.e. the ability to use the same membranes for processing multiple products, with just a rigorous cleaning between campaigns. Beyond the decrease in membrane costs, membrane cross-use would eliminate a significant amount of downtime required for membrane change-outs between campaigns, increasing overall facility utilization.

Continuous diafiltration: Continuous concentration by UF is a commercialized technology. It has various potential uses in a purification process, such as for the final concentration of DS after initial concentration and DF into a formulation buffer using a traditional UF system and before the final bioreduction filtration and DS packaging. To develop a fully continuous DSP, a commercially available continuous UF/DF system is needed, so that the complete concentrate-diafilter-concentrate sequence can be performed in a continuous mode. At present, such systems are being investigated by various vendors and other researchers; recent conference presentations suggest they are making good progress. But until a reliable, economical and commercialized continuous UF/DF system is available, only batch-continuous hybrid processing is possible, and it is not an attractive option. Introducing a batch process element in the midst of a continuous process requires larger-scale equipment and generally detracts from the benefits sought with continuous processing.

More desirable than a stand-alone continuous UF/DF skid would be a UF system capable of providing the full concentration-DF-concentration sequence in a continuous mode using a single integrated system. The systems currently available for precise continuous UF concentration are large, complex and expensive. The need to have three such units in a manufacturing line for a product (one for each of the three sub-steps of the UF step) would expand the footprint and budget of a continuous DSP facility, which is contrary to the intent of moving to such a facility. UF vendors are encouraged to develop an integrated continuous UF system capable of performing all three sub-steps as a single system. Such a system would likely require highly engineered components, especially the UF cartridges themselves, to minimize the requirement for active control elements and more intensive use of PAT. Vendors that can provide such a system would likely find a ready market.

Enhanced drug substance membrane filtration, packaging and integration with drug product manufacturing
Additional process improvements can be conceived beyond the UF step. Available platforms for freezing, shipping and thawing DS are expensive and involved. Novel approaches to providing the output of the UF step to drug product (DP) manufacture would be welcome. These might include ultra-high concentration bulk DS or the production of room-temperature stable solid DS. The direct coupling of the DS and DP manufacturing processes would result in significant efficiencies, but with major challenges for the integrated manufacturing site. This includes the development of a quality management system that could support such an approach, and regulatory acceptance of it.

4.10.2
The needs, challenges and potential solutions

Table 13: Ultrafiltration/diafiltration – needs, challenges and potential solutions

		Current	2019	2022	2026	Scenario(s)
Need	Method for reliably achieving high-concentration formulations for all molecules (mg/mL)	150	200	250	>250	
Challenge	• High viscosities, high-yield losses, longer cycling times • Missing PAT method for protein concentration					
Potential solution	• Improved PAT sensor for concentration • Improved UF cassette design for high viscosity • SU continuous system for single-pass UF concentration and DF	Development	Production	Production	Production	
Need	Continuous DF technology to facilitate continuous UF formulation step					
Challenge	• Robust control system capable of adapting to varying feed-stream concentrations while ensuring adequate DF and concentration to the desired final value • Extensive engineering investment required to develop integrated cartridges and systems					
Potential solution	Continuous DF as a stand-alone unit to be used with stand-alone continuous UF concentration systems	Development	Production	Production	Production	
	Continuous DF as part of an integrated continuous UF system for concentration-DF-concentration sequences	Development	Development	Production	Production	
Need	Increased lifetime for UF filters (to reduce COGS) • Increased lifetime for polymeric UF filters • Implementation of very robust (e.g. ceramic) UF filters with extremely long lifetime • Ability to use the same set of filters for multiple products, eliminating time for changeover and reducing cost					
Challenge	• Maintenance of membrane-retention characteristics after multiple exposures to potentially fouling feeds and potentially degrading aggressive cleaning solutions • Ability to demonstrate cleanliness of filters to extremely low levels, with respect to carry over of both impurities and prior product					
Potential solution	New polymeric materials for existing formats	Development	Production	Production	Production	
	New robust (ceramic) high-performance, low-cost membranes	Development	Development	Production	Production	
	New cleaning and validation techniques to allow filter reuse between products	Development	Development	Development	Production	
Need	More robust commercial platforms for DS freezing, shipping and thawing at the DP site					
Challenge	• Polymer materials get brittle at low temperatures • Cryoconcentration concerns – differences in freeze path length between frozen container sizes • Lack of cost-efficient end to end solution (container/freezing/shipping/thawing/closed systems) for various types of containers					
Potential solution	Cost-efficient, robust, scalable, controlled and closed end to end solution	Development	Production	Production	Production	

COGS – cost of goods, DF – diafiltration, DP – drug product, DS – drug substance, PAT – process analytical technology, SS – stainless steel, UF – ultrafiltration

Potential solutions manufacturing readiness level

Research | Development | Production

Table 13: Ultrafiltration/diafiltration – needs, challenges and potential solutions (continued)

		Current	2019	2022	2026	Scenario(s)
Need	Straight-through DS to DP processing at a single site (filling of DP at a DS manufacturing site to eliminate DS inventory and shipping, and associated issues)					
Challenge	• Developing new quality management systems to allow for straight-through processing from DS to DP					
	• Integration of sterile operations in a bulk DS manufacturing facility					
	• Reduced flexibility in production due to needing to select final DP image at time of DS manufacture					
	• Not all final DP containers are available in a ready-to-use format					
	• Conservative nature of DP manufacturing is slowing the adoption of flexible filling systems					
Potential solution	• Having required final DP formats available in ready-to-use format • Early solution adopter, open communication, supporting authorities and supporting community					
Need	Alternate DS presentations, including ultra-high concentration solutions (>300mg/mL) and solid DS forms (crystalline, dried powder), to increase DS stability and reduce cost and complications of DS storage and shipping					
Challenge	• Greater potential of highly concentrated systems to promote aggregate formation					
	• Increased difficulty of handling ultra-high concentrated liquids due to viscosity, yield loss, high value, etc					
	• Lacking availability of well characterized routine bulk DS protein-drying systems. Difficulty in handling high-value powders					
Potential solution	• Supplier to provide end to end solution for highly concentrated liquid product					
	• Supplier to provide end to end solution for dried-protein product					
Need	Improved 'closure' of buffer make-up and processing systems to allow final UF formulation, bioburden-reduction filtration and filling to occur in non-classified (or less classified) facilities					
Challenge	Lacking in industry alignment and standards of: • SS to SU interfaces • automation interfaces • tubing management systems • methods to achieve functional closure					
Potential solution	Aligned solutions for standardization of interfaces and systems					
Need	Process/quality – precise control of PS80 (polysorbate 80) and other excipients in continuous processing					
Challenge	PS80 formulation can be difficult to control					
Potential solution	New membrane materials that enable low sieving, and consistently and robustly pass PS80 and other excipients, e.g. Tweens and other polymeric, high-molecular-weight excipients					

COGS – cost of goods, DF – diafiltration, DP – drug product, DS – drug substance, PAT – process analytical technology, SS – stainless steel, UF – ultrafiltration

Potential solutions manufacturing readiness level

Table notes: Business drivers have been listed in the table in order of priority for this unit operation.

4.11 Normal flow filtration

4.11.1
Needs

Within biopharmaceutical processes, there is a multitude of processing steps, which utilize MF steps, either for bioburden reduction or sterilization. The individual steps have critical performance attributes, which need to be met to gain an optimal and safe process. The different steps are listed below with a description of the desired optimal performance criteria.

Cell culture media filtration:
This is essential to avoid any contamination input into the bioreactor system. These filters are required to produce a sterile filtrate into the bioreactor unit. In recent years, mycoplasma contaminations showed a rising trend within the cell culture media raw materials, therefore in this step typical membrane filters are not just 0.2 microns but also 0.1 micron rated. Since cell culture media volume can be elevated, the filtration systems can be complex due to a multitude of pre- or protective filtration steps, before the media is filtered through a 0.2 micron-rated filter followed by either one or two 0.1 micron filters. These large and multifiltration systems can have a high hold-up volume and therefore losses of valuable media. To reduce the filter system amount and configurations, as well as size, total throughput optimized filters need to be used. Filterability trial work can find such optimal filter solutions. In addition to the optimal configuration, one also has to validate the retention performance of the filter under process conditions and the unspecific adsorptive properties, as any higher adsorption may foul the filter faster, but can also retain valuable feed components.

In the future, the cell culture media streams may be concentrates, which run through in-line dilution and are filtered at that point. The other option is to filter the concentrate, depending on the viscosity. The in-line dilution would reduce the filter size and pre-sterilized filters may be used.

Buffer filtration:
Large amounts of buffer volumes are filtered to avoid any microbial contamination into the purification or formulation process. Buffers are not as difficult to filter as media or product streams since the solutions are commonly cleaner. In the case of buffers, the filters are typically optimized to flow. The faster the filtration step, the better the process utilization. Buffer filter systems are also not as large as media filtration systems and are typically filter capsules, which can be connected to single-use bags. An important factor in buffer filtration is the proof of retentivity – since buffers can have a high ionic strength, the Donnan equilibrium can set in and possible microorganisms within the fluid may shrink. Therefore, process validation of product bacteria challenge tests under process conditions are essential to verify the required retentivity.

Bioburden reduction filters:
Bioburden reduction or protective filters are used to protect critical processing steps, such as chromatography. The reduction of the bioburden is essential to avoid any microbial contamination of the larger column systems. Microorganisms can create a biofilm within the chromatography system, which needs to be avoided as such films are difficult to remove. Also, any potential fouling components are removed by the filtration step to gain the best efficiency from the chromatography column. In addition, the Committee for Proprietary Medicinal Products guidance of April 1996 distinctly asks for a maximum allowable bioburden of 10cfu/100mL before a sterilizing grade filter[14]. This means, if the bioburden is elevated, a reduction filter should be utilized to lower the bioburden to the described maximum allowable limit. A current Parenteral Drug Association comment paper is recommending a risk assessment regarding the bioburden and not specific levels. It remains to be seen whether the European regulators adopt the recommendation.

The bioburden reduction and protective filters are also utilized to protect UF/DF and virus-retention filter steps. It has to be analyzed whether the intermediate filter is really needed, as every filtration step creates a possibility for hold-up volume losses, unspecific adsorption and leachables. The process has to be reviewed and optimized to determine the absolute need of a filtration step or not.

Bulk drug substance filtration:
The product is not in its final stage, but often end-users design this step as the final filtration step, meaning it can be redundant with larger filtration devices. The filtration step needs to be optimized to determine the best size of the filter unit and to avoid, once again, excessive hold-up volume. Ideally, as in the case of final filtration, one wants to use single-use, gamma-irradiated, capsule filters, which would be connected to a multitude of hold bags.

Final filtration:
This is the most critical step and the fluid at that point is at its highest value. Robust retentivity performance is a must and commonly process validated. With low-volume, high-value products the last filtration step towards the filling line utilizes redundant filtration, meaning two 0.2 micron filters in a series. Redundancy is used with one filter as an insurance filter if the primary filter fails the integrity. These filtration devices need to be a small as

[14] Committee for Proprietary Medicinal Products (CPMP) Guidance of April 1996

possible to avoid elevated hold-up volumes, unspecific adsorption and leachable levels. Hold-up volumes can create a high level of valuable product losses, so optimized filter device design and connections towards the filters are essential. Also, the membrane polymer and the potential unspecific adsorption needs to be evaluated, as a higher adsorptivity would create losses of valuable target protein and stabilizers, such as polysorbate. Any adsorption of polysorbate requires detailed validation to avoid any out-of-specification final product.

4.11.2 The needs, challenges and potential solutions

Table 14: Normal flow filtration – needs, challenges and potential solutions

		Current	2019	2022	2026	Scenario(s)
Need	High recovery from filter devices regardless of stream tested (% recovery)	80-98%	90-98%	95-99%	>99%	
Challenge	High viscosities, high-yield losses, longer cycling times					
Potential solution	Low hold-up volume of the filter device, high total throughput, optimized filter process designs to reduce filtration devices, size and hold-up volumes, novel ways to recover entrained material					
Need	Low protein adsorption to membrane materials (% adsorbed)	<10%	<5%	<1%	None	
Challenge	Polymer materials tend to adsorb protein molecules					
Potential solution	Novel materials for membrane with low adsorption and high permeability					

Potential solutions manufacturing readiness level

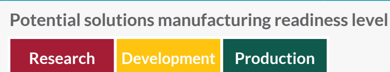

Table notes: Business drivers have been listed in the table in order of priority for this unit operation.

4.12 Buffer

Buffers and solutions are an inherent part of the DSP. They represent one of the most critical 'intermediates' in the manufacturing process as their control is critical to the performance and product quality obtained in each step. While this significance is definitely recognized, the emphasis has not been placed on buffers and solutions in terms of their control, because they are pre-made and released. This approach de-risks the buffers and solutions from impacting the processing.

Continuous processing and improvements in facility and process productivities have resulted in buffers' preparation being a bottleneck in existing facilities and a significant cost component in new facilities, therefore requiring alternate modes of manufacturing these solutions.

However, as we begin to automate the manufacture of these buffers and solutions, while significant benefits arise, some of the de-risking with conventional methods is lost, requiring novel approaches. This section summarizes the different drivers in buffer manufacture across the different scenarios. Table 15 provides a summary of the needs considering different dimensions of the problem, along with potential challenges and solutions for each of them.

4.12.1 Needs

Cost drivers in buffer manufacturing

Buffer production operations for antibody manufacturing are a significant portion of the facility footprint, labor needs and equipment costs. As the downstream operations are predominantly product-mass-based, with increasing productivity requirements or throughput requirements, the rate and/or amount of buffer needed increases in proportion.

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Stainless steel fed-batch scenario

A typical 10kL process at 2g/L titer requires about 40kL of buffers and solutions. As the titer increases, for example five-fold, this could translate to approximately four-fold higher buffer/solution needs in the facility. This level of increase in upstream productivity results in:

1. an increased amount of WFI that needs to be produced and stored
2. a proportional increase in the buffer preparation tanks and footprint
3. a proportional increase in buffer hold tanks and footprint
4. a proportional increase in waste treatment or disposal.

It should be obvious that the increase in harvest frequency, i.e. the number of bioreactor harvests to be processed, will also result in the above increased needs. The increased harvest frequency could be a result of an upstream operational strategy of

- decreasing production reactor cycle time
- increasing the number of bioreactors.

While building a larger WFI-production system and increasing the footprint of the buffer operations is not necessarily an infeasible solution, it has a direct impact on capital and operating costs, albeit in proportion to the product processed. In contrast, in an existing facility that now has a higher level of upstream productivity, clearly buffer production becomes a significant bottleneck, often limiting facility productivity.

Single-use perfusion scenario

The single-use approach to commercial manufacturing, while providing significant advantages (e.g. capital avoidance), poses additional challenges from a downstream perspective, with a direct impact on buffer manufacture, as follows:

- the scale size (and limitation) of single-use equipment, especially upstream, results in multiple batches to processed, for the same amount of material to be produced
- the increased frequency of harvest results in increased frequency of the downstream batches (given the limited hold-time typically allowable for the harvested material), with a direct impact on the rate of buffer production
- while the average buffer requirement is comparable to an equivalent fed-batch facility, the need for all of the buffers all of the time poses additional constraints in terms of being unable to stage the buffer production. For example, in a fed-batch facility, one may be able to produce the capture buffers first and then produce the downstream buffers in a sequential manner; whereas in the perfusion scenario, all the downstream operations are constantly occurring – requiring the need for the capture and downstream buffers within hours, if not at the same time.

In summary, there is clearly a need to identify alternate technologies in the area of buffer manufacturing and distribution operations, from a cost standpoint.

Quality drivers in buffer manufacturing

Current buffer manufacturing produces consistent solutions that address the needs of antibody manufacturing processes. However, there are various limitations both from the quality controls for the buffer, as well as impacting the quality of the resulting product. For example:

1. buffers are typically released based on the measurement of pH and conductivity, with inherent reliance on the controls in the measurement of raw materials and liquids
2. buffers are often made by titration resulting in significant variability in composition due to inherent variability in pH measurements
3. buffer-containing components (e.g. formulation excipients) that are not directly correlatable to pH or conductivity do not have appropriate controls to ensure the composition of the buffers
4. a buffer manufacturing strategy is not amenable to enable PAT, as the composition and pH of the buffers are not easily adjustable
5. pH measurement technologies are not reliable and often not translatable between probe to probe or meter to meter
6. the composition and identity analysis of buffer solutions is either unavailable or infeasible in routine buffer manufacture
7. the process hygiene aspects of the buffer manufacture heavily rely on operational controls with no ability to monitor them
8. buffers often contain salts, posing problems with corrosion, leaching and other significant issues
9. buffers often contain complex mixtures, resulting in difficulties in process understanding and control
10. buffers are invariably never reused or recycled – given that over 40% of the buffers are used for equilibration, it is very possible to reuse buffers, saving water and reducing waste.

While these quality needs apply to both fed-batch and single-use perfusion scenarios, given the frequency and magnitude of the number of solutions that need preparation in the single-use perfusion scenario, these needs are much more relevant.

Flexibility drivers

Flexibility in buffer manufacturing is a very critical need, both for fed batch and especially SU perfusion scenarios. For example:

1. current buffer manufacturing operations are invariably batch operations, resulting in poor adaptability to changing schedules in upstream and downstream operations
2. buffer production operations are inherently inflexible due to the fact that these buffers are manufactured and held for release
3. buffer manufacture is predominantly manual, requiring coordination and activities starting from raw material management, testing and release, weighing and dispensing, buffer preparation, buffer adjustment, quality control, buffer filtration and staging, release, hold, transportation and use during manufacture. Therefore buffer preparation is difficult to adapt to changing needs
4. due to the lack of flexibility in manufacture, significant overages are factored in and often 10–30% of the buffers manufactured are unused.

The flexibility drivers, as with other drivers, will likely be more relevant in SU perfusion scenarios, as large amounts at large rates will be required to support the manufacture. Considerations are:

1. buffer-on-demand system, solid tableting, in-line dilutions and stream conditioning
2. reducing asset footprint (buffer preparation, buffer storage capacity)
3. ease operational constraints in facility around buffer preparation
4. ease scheduling conflicts around buffer equipment
5. novel complex skids lose capability to do online buffer dilution (i.e. load conditioning)
6. sustainability: a green initiative to reduce water requirements.

Paradigm shifts

While the above drivers and needs were based on a framework of a manufacturing facility producing its own buffers, a paradigm shift that could disrupt the industry is the concept of 'buffer in a truck', which is conceptually modularizing buffer manufacture and delinking it from the facility itself.

In this concept, all the operations related to buffer manufacture, including the generation of purified water, can be built into this modular 'facility', with the concept of 'facility as equipment'. These operations can be managed independent of the manufacturing facility, much like gas supply, potentially by a third-party vendor that can provide an end to end supply chain, including water purification, chemicals, appropriate on-demand buffer preparation, testing and release equipment. Also, managing the supply of the solutions seamlessly with the manufacturing operations can be done with minimal infrastructure requirements from the manufacturing facility.

It is anticipated that this could cater very well for small- to medium-sized campaigns or facilities, where the scaling out is bottlenecked by buffer manufacturing capacity. It would also be beneficial to facilities where raw material sourcing and management is diverse from project to project (as in a contract manufacturing organization), where the end to end vendor can manage the supply chain.

Overall, the buffer manufacturing aspects of the typical biomanufacturing facility will likely see a significant shift in the next 10 years, as all the novel technologies are easier to implement in a non-product-containing part of the operations.

4.12.2
The needs, challenges and potential solutions

Table 15: Buffer – needs, challenges and potential solutions

		Current	2019	2022	2026	Scenario(s)
(Metric 1)	Cost ($/L)	$5–10	$3	$2	$1	
(Metric 2)	Flexibility	Batch/hold				
(Metric 3)	Quality	Test and release, pH, condition				
(Metric 4)	Speed	Batch/In-line dilute	In-line dilute	On-demand	Real-time adaptive	
Need	Reduce buffer storage volumes [metric: cost]	Batch primarily, some concentrates	Primarily concentrates	Concentrates only	Concentrates only	1,2,3 and 4
Challenge	Facility footprint increases with buffer volumes					
Potential solution	Buffer concentrates with in-skid dilution					1,2,3 and 4
	Buffer concentrates with buffer-dilution skid					1,2,3 and 4
	Primary component concentrates with buffer-preparation skids					1,2,3 and 4
Need	System to track buffer concentrates and buffer lineage [metric: quality]	Manual with some electronic systems	Electronic with some manual	All electronically tracked system	All electronically tracked and controlled	1,2,3 and 4
Challenge	Current systems are manual, prone to errors					
Potential solution	Barcoded or automated tracking system					1,2,3 and 4
Need	Better pH sensors [metric: quality]			Improved accuracy to 0.05	Improved accuracy to 0.005	1,2,3 and 4
Challenge	Accuracy at best 0.1 units and lack of consistency – not reliable for feedback control					
Potential solution	None today					1,2,3 and 4
Need	Alternate methods for composition and identity [metric: quality]	Off-line measurement	Online monitoring with off-line verification	Online monitoring and verification	Online verification and control	1,2,3 and 4
Challenge	Accuracy of pH at best 0.1 units and lack of consistency – not reliable for feedback control; not specific					
Potential solution	Raman spectroscopy					1,2,3 and 4
	Multivariate sensors, combining pH, conductivity and other systems in real-time					1,2,3 and 4

SS – stainless steel, WFI – water for injection

Potential solutions manufacturing readiness level

Research | Development | Production

Table 15: Buffer – needs, challenges and potential solutions (continued)

		Current	2019	2022	2026	Scenario(s)
Need	Reduce cost of buffer chemicals [metric: cost]	Batch/project-specific proactive procurement	Campaign-based procurement, on-demand procurement	Facility-based procurement	Facility-based on-demand procurement	1 and 2
Challenge	Chemical costs are driven partly by distribution and small batch sizes					
Potential solution	Procure materials from source in bulk					1 and 2
	Ship materials to users in bulk from distributor					1 and 2
Need	Reduce labor costs for weigh and dispense [metric: cost, speed]	Manual	Manual with some automation	Automation for large volumes	Automation for all volumes	1 and 2
Challenge	Buffer preparation, especially weigh and dispense, is manual					
Potential solution	Automated weigh and dispense systems					1 and 2
	Use pre-formulated granules, tablets, wafers, etc.					3 and 4
Need	Reduce corrosion [metric: quality, cost]	High concentration of salt reduced from process solutions of high volume	No high concentration of salt in process solution in high volume	No high concentration of salt in process solutions	No salt in process	1 and 2
Challenge	Process buffers often contain corrosive salts					
Potential solution	Eliminate corrosive salts from processes through redesign					1 and 2
Need	Reduce cost through WFI cost reduction [metric: cost]	Use WFI for most buffers	Use WFI only for final steps	Alternate WFI systems with distilled WFI only for final steps	No WFI for buffers	1 and 2
Challenge	WFI costs (manufacture, distribution and capital) can be a significant portion of the cost of buffers					
Potential solution	Use reverse osmosis systems for making WFI					1,2,3 and 4
	Use purified water for buffers					1 and 2
Need	Reduce buffer preparation needs by reducing number of buffers required [metric: cost, speed]	3 buffer systems, 7–13 raw materials	2 buffer systems, 3–5 raw materials	1 buffer system, 3–5 raw materials	1 buffer system, 2–3 raw materials	1, 2, 3 and 4
Challenge	Each step optimized with a unique buffer system					
Potential solution	Streamline process to reduce distinct buffers for each step					1, 2, 3 and 4
	Use same solutions for cleaning, regenerations and storage					1, 2, 3 and 4
	Reduce the number of solutions per step					1, 2, 3 and 4

SS – stainless steel, WFI – water for injection

Potential solutions manufacturing readiness level

Research Development Production

Table 15: Buffer – needs, challenges and potential solutions (continued)

		Current	2019	2022	2026	Scenario(s)
Need	Reduce buffer wastage [metric: cost]	Batch-based preparation and hold, with 25% overage and 10% hold-up	In-line dilution for majority of buffers	In-line dilution or on-demand preparation for most buffers	On-demand preparation for all buffers and solutions	1, 2, 3 and 4
Challenge	Need for contingency for process and inability to address contingency					
Potential solution	Prepare buffer on demand or use in-line dilution system					1, 2, 3 and 4
Potential solution	Enable top-off of buffer hold SU bags					2 and 4
Need	Reduce capital costs and cleaning needs associated with SS [metric: cost]	Bags size limit is 2kL	3kL bags	4kL bags	5kL bags	1 and 2
Challenge	Limit to the size of bags available resulting in large footprint for holding buffers					
Potential solution	Design and develop larger scale single-use bags					1 and 2
Need	Achieve operational efficiency in manufacture [metric: cost]	Buffer production completely managed by manufacturer				2, 3 and 4
Challenge	Requires raw material management and preparation management					
Potential solution (Disruptive)	Develop a 'modular buffer preparation system' that hooks to the water line of the manufacturer, generates water and prepares buffers					2,3 and 4
Need	Reduce footprint for buffer storage [metric: cost]	Storage efficiency = 1	2	4	5	3 and 4
Challenge	Buffer hold systems occupy significant footprint and do not leverage the height of the facility					
Potential solution	Build a stackable buffer storage system '2D-fillable bag'					3 and 4
Need	Enable adaptive buffers [metric: quality]	None today	None	Adaptive for critical steps	Adaptive for all steps	1, 2, 3 and 4
Challenge	Today's buffers cannot be adjusted on demand					
Potential solution	On-demand, fully automated buffer preparation system					1, 2, 3 and 4
Need	Enable real-time monitoring of process hygiene [metric: quality]					1, 2, 3 and 4
Challenge	No method to directly assure process hygiene					
Potential solution	Novel sensor design for bioburden					1, 2, 3 and 4

SS – stainless steel, WFI – water for injection

Potential solutions manufacturing readiness level

Research | Development | Production

Table 15: Buffer – needs, challenges and potential solutions (continued)

		Current	2019	2022	2026	Scenario(s)
Need	Reduce buffer volumes for process					1 and 2
Challenge	Significant amount of buffers are still useful after a single pass through column (especially equilibration buffers)					
Potential solution [disruptive]	Enable reuse of buffers that are used for equilibration	Research	Research	Research	Research	1 and 2
Need	Reduce human error in buffer manufacture	All manual, prone to errors with significant consequences	All manual, prone to errors with significant consequences	All errors prevented from affecting process	All automated and errors completely prevented from process impact	1, 2, 3 and 4
Challenge	Difficult to detect errors until later					
Potential solution [disruptive]	Automated buffer preparation	Development	Development	Production	Production	1, 2, 3 and 4
	Composition monitoring with Raman, etc.	Development	Development	Production	Production	1, 2, 3 and 4

Table notes: Business drivers have been listed in the table in order of priority for this unit operation.

Potential solutions manufacturing readiness level

Research | Development | Production

4.13 Novel technologies

4.13.1
Needs

Through the dialogue between end-users, technology vendors and academics, some disruptive concepts were discussed that were considered key for supporting the USP and DSP process technologies towards their future long-term goals. It is not the intent to describe or expand on these concepts but to highlight them to the reader to encourage further investigation and exploration so they may lead into disruptive technologies in the future.

Upstream process concepts:

- virus-free or resistant cell lines incapable of virus replication
- implementation of non-mammalian expression systems to avoid all viral safety needs
- biomarkers to indicate the health state of the cell.

DSP concepts:

- one-step purification
- no VC requirements
- integration of subsequent and orthogonal unit operations (e.g. a combination of capture and virus removal in one chromatography resin).

Some existing ideas and technologies were also recognized that may still have an impact in achieving future process technology goals and objectives. Although described in research literature and with some implementation in industry, they have yet to gain widespread application in industrial therapeutic antibody production. The technologies are listed below:

1. affinity partitioning
2. Protein A biomimetic ligands through low-molecular-weight ligand synthesis
3. aqueous two-phase separation
4. expanded-bed adsorption
5. continuous precipitation (including PEGylation)
6. crystallization (batch and continuous).

4.13.1
The needs, challenges and potential solutions

Disruptive technologies have been added to each unit operation section where relevant. Please see those tables for detail.

4.14 General needs

4.14.1
Needs

General needs that span across multiple unit operations and supporting processes are listed in Table 3 in Section 3. These needs are considered in other roadmap reports (for example, standardized single-use technology and its implementation across a facility are included in Modular and Mobile).

5.0 Linkages to other roadmap teams

5.1 In-line Monitoring and Real-time Release

1. In-line, fast methods to measure CQAs, e.g. high molecular weight, DNA, HCP
   - single-use disposable robust sensors with a multifunctional capability
   - shortcomings of current sensors still include stability issues related to irradiation, drift and lifetime
   - single-use sensors to include various controls, such as pH and dissolved oxygen, dissolved CO_2, viscosity (≥high protein concentration), osmolality, biomass and metabolites
   - consider hybrid concept with (validated) soft sensors
   - sensors should be an increasingly integral part of purchased SUBs at different scales with a seamless transfer between scales
2. Facility analytics
   - development of analytical tools that have the capability to analyze the multiple sources of monitoring data in an entire facility and embrace a high-level of collaboration between applied systems
   - analytics may focus on optimizing facility/ unit operation load factors, increasing process robustness and/or supporting RTR concepts
3. Product quality and process parameters real-time monitoring
   - both process intensification as well as continuous processing will require accurate, real-time monitoring instrumentation to fully leverage the concepts' potential
4. Minimize quality assurance/quality control full-time equivalents in facility
   - a tendency towards using smaller-scale disposable (≤2kL) set-ups for a given capacity may increase the number of batches produced and therefore put pressure on quality assurance/quality control costs. Automated monitoring concepts are required to counter this effect. Also, robotics concepts could increasingly support/complement human inspection.

5.2 Automated Facility

1. Minimize human intervention
   - upstream manufacturing operations (such as media and buffer preparation, fermentation and harvesting) should be increasingly controlled by automated process control systems, but also CIP/'sterilization in place' and utility-usage management. Also conceivable is the application of robotics in the assembly/handling of equipment.
2. Fully automated process; minimize human interaction with process
   - integration of SU automation hardware (automated valves, sensors, etc.) and SU software needs to be improved to approach the current state of SS systems.
3. Multivariate data analysis with standardized data management (controls)
   - based on advanced monitoring and sensor technologies, complex datasets will become available and need to be managed appropriately. Validated and robust multivariate data analysis tools have to be developed to ensure effective and efficient controls for automated facilities.

5.3 Knowledge Management

1. Batch and raw material genealogy for RTR
   - for RTR concepts, the capability to instantly track and trace the location, status and genealogy of raw materials and batches is mission critical. This is especially a challenge in a cross-system manufacturing environment.
2. Electronic batch records/manufacturing execution systems
   - there are still challenges (validation, inflexibility) to establish fully electronic process documentation.

5.4 Modular and Mobile

1. 'Ballroom' concept
   - having mobile equipment in a ballroom-type facility will provide benefits regarding low capital expenditure, rapid reconfiguration and cost savings in operations (heating, ventilation and air conditioning/room classification). However, certain shortcomings or risks need to be addressed
   - full flexibility may increase the risk of operational errors, which needs to be managed through a hierarchy of risk assessment and error-proofing approaches
   - modular and mobile concepts need to be well aligned with the corresponding automation concepts to allow for a simple and seamless transition between mobile skids, allowing both flexibility and automated/low full-time equivalent operations

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- lean manufacturing concepts need to be developed that are tailored to the new set-up (e.g. ergonomic clustering, length of tubing and tubing management, storage)
- linking the ballroom concept to the continuous manufacturing concept mentioned in this report

2. Closed 'sterile' systems
   - single-use systems, challenges on sterile tubing connections and pumps
   - standardization of componentry and single-use units across suppliers.

5.5 Supply Partnership Management

3. SU supply chain
   - since single-use equipment has a major impact on the production capability for a specific product, cooperation with suppliers has to be more intense and SU solutions are typically customized. Alternatively, companies are traditionally building their supply chain strategy on dual-sourcing of standardized material to mitigate an 'out of stock' risk, reduce inventories and control costs. New cooperation models, potentially including cross-supplier standardization efforts, need to be developed taking into account the needs of manufacturers and suppliers.
   - SU component cost reduction (columns, flow paths, etc.)
   - Standardization (data management, connectors, films, etc.)

5.6 Other industry initiatives

Several relevant initiatives are active in the industry. A selection is:

- **Engineering Conferences International – Integrated Continuous Biomanufacturing Conference Series:** in its third installment (November 2017), the conference will bring together leading scientists and engineers from academia, industry and regulatory authorities who are actively engaged in integrated continuous bioprocessing development to debate how industrialized our sector can become and the scenarios where continuous platforms will better serve our needs.
- **International Symposium on Continuous Manufacturing of Pharmaceuticals:** this symposium will bring together pharmaceutical company end-users, suppliers, regulators and academics to look at accelerating the adoption of continuous manufacturing for both small molecules and biologic products and how research groups, globally, might collaborate more to help drive this. This is an enormous opportunity to guide the way in which new technologies and new approaches in the pharmaceutical industry can transform quality, cost and service for the benefit of the patient. The output from the first conference is summarized in the 2014 white papers link below. A regulatory white paper will be posted to the 2016 white paper link below once finalized later in 2017.
  - 2014 white papers
  - 2016 white paper
- **Industrial Biotechnology Catalyst:** UK Continuous, Integrated Biologics Manufacturing Project.

6.0 Emerging and/or disruptive technologies

As the biopharmaceutical industry moves to the business mainstream, it will increasingly need to find new ways to maintain competitiveness by ensuring affordability, quality and delivery performance. Continuous processes have been proposed as a solution as they are scalable, offer higher productivity with reduced running times and materials usage, and require smaller footprints and less capital-intense facilities.

Emerging technologies are discussed throughout Section 4 as they are related to each unit operation. Novel technologies that could impact multiple unit operations are discussed in 4.13. Each needs table refers to disruptive technologies and the challenges associated with them; potential disruptors are flagged as such.

7.0 Regulatory considerations

This document outlines a technology roadmap for biopharmaceutical manufacturing based on the collective vision of the industry and other stakeholders. Implementation of this vision will require the adoption of new technologies and approaches to improve operational efficiency, reliability and product quality. Regulatory acceptance and support are key to enabling the attainment of this vision. Therefore, a collaboration between industry, suppliers and regulatory authorities is key to successful progress towards the described vision of a continuously improving future state for biopharmaceutical manufacturing. A willingness of regulators to collaborate with industry on the implementation of changes that will improve efficiency, reliability and quality, and to establish uniform global guidance where possible, is vitally important. Equally critical is recognition on our industry's part that substantial changes to manufacturing processes and approaches require data to address the inherent risks associated with new methodologies and to justify their adoption based on their scientific and engineering merits.

Two levels of regulatory challenge are envisaged: 1) current or already defined/envisaged scenarios with new approaches (e.g. continuous process verification, RTR), and 2) new approaches and challenges. There is a gray area between the two in some instances, e.g. RTR is not a new concept, but implementation raises many new challenges. Current continuous manufacturing in active pharmaceutical ingredient production has been successful with small molecules. Large, three-dimensional mAbs provide a significant challenge to PAT, feedback control and continuous process verification/RTR.

Current process characterization and validation practices (including process validation, VC, column lifetime, etc.) require significant amounts of resources (including technical staff workforce, facility time and substantial operational costs) and are often on the critical path towards marketing application submission. These activities aim at gaining a process understanding, defining process boundaries and ensuring a high degree of consistency from a given process for a high-quality product. However, these exercises are done with the limitation of only a small number of batch histories and have to be done at risk ahead of demonstrating clinical safety and efficacy. Moving to continuous processing, there will be even fewer batches to be made and so a further limitation on historical data, therefore it is important for the industry to redefine validation assumptions and conditions, and to find alternative means to ensure product quality and consistency. These changes will require regulatory input and alignment.

Regulatory review plan

Many of the technologies discussed will improve processing concerning scale and cost. With the introduction of single-use technologies, the development of a truly closed process can provide cost benefits in qualification and required heating, ventilation and air conditioning environmental control, while adding flexibility to plant configuration. Of equal importance will be proof of product quality and efficiency within the defined design space. A quality by design approach will tie product performance back to the manufacturing design space. Small-scale development studies with improved single-use systems, such as supplier development scale systems with scaled down manufacturing systems, can help to develop the design space for production scale runs.

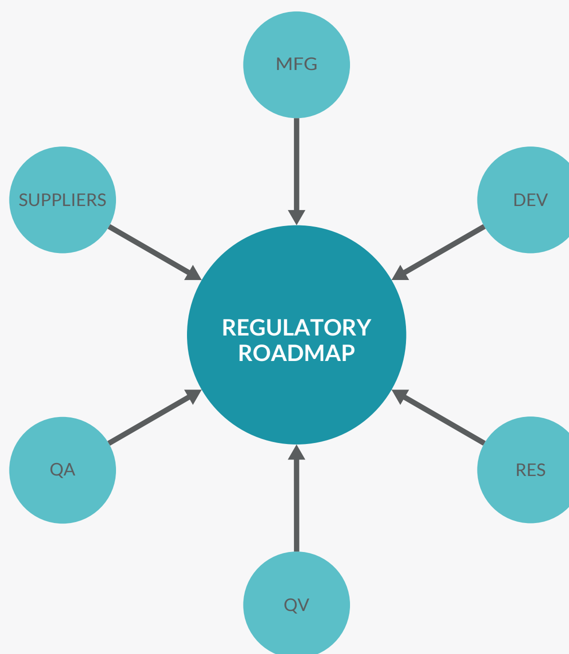
Development of a regulatory roadmap

Through the development of a regulatory roadmap, one can develop a work plan for addressing the regulatory concerns for many of the envisioned improvements. With the coordination of the information, simplification of qualification proof and design space definition, a robust work plan can be developed for review by regulatory agencies. Future revisions to this roadmap report will include regulatory feedback and qualified person (QP) reviews.

A basic plan to include:

1. identify disruptive technologies with a plan for qualification
2. identify supplier disposable technology systems and assemblies and qualify for design space
3. verify the plan to establish closed system processing throughout upstream and downstream production
4. verify the plan for environmental qualification to address temperature and humidity requirements with all controlled non-classified (CNC) space, except media and buffer preparation. (Closed system media and buffer formulations are not considered within the 10-year window)
5. VC plan for proof for raw materials, in-process, suppliers and log reduction basis addressing enveloped and non-enveloped virus
6. area segregations are limited to open processing in media and buffer preparations in DS manufacturing

Figure 5: Development of a regulatory roadmap



MFG — manufacturing, DEV — development, RES — research, QV — qualification validation, QA — quality assurance

With the development of a regulatory roadmap and coordination with regulatory agencies, it is possible that many opportunities exist for reduced production and capital cost of development, approval, total installed cost and manufacturing. This proactive approach can also provide a platform for discussion that evolves with the needs of the biopharmaceutical industry.

Biologic and TP production utilizing a continuous process has attracted significant attention in the industry recently as a potential strategy to provide processes with smaller footprints, ability to scale out instead of scale up and steady-state operations, while maintaining a tightly controlled product quality. However, continuous processing also poses several challenges in term of the process development and validation timelines, which will have a significant impact on time to market and lifecycle management.

Process validation
Stage 1 (process development) timeline

For upstream processes (culture initiation through clarification/harvest of intermediate product), continuous processing is defined as a perfusion process or a perfusion of fresh nutrient media into the fermentation vessel (production bioreactor) and collection of intermediate product via a strategy that retains the producing cells. This strategy allows the production bioreactor to achieve a quite lengthy production period with operation times exceeding 60 days or even longer. Completion of the development package for this production strategy would require additional time, compared to a standard fed-batch fermentation process, due to the requirement to demonstrate consistent product quality over the life of the production reactor. If a fed-batch process requires six–nine months for development, one would expect 12 months or longer for the perfusion process, based on target production lengths and the number of required independent experiments. It is important to note that the developer needs to clearly understand the associated CQAs of their final product and how the upstream process may impact on those.

A different mixed fed-batch/continuous strategy may offer an alternative to the development challenges listed above. This strategy would involve operating the production bioreactor in fed-batch mode and then employ a perfusion strategy to lengthen the production period to 14–20 days. This development process length would not differ significantly from the common fed-batch process, may limit potential issues of achieving CQA targets and have no significant impact on the development timeline while delivering the benefits of a lengthened production phase.

DSP is less impacted by the issues regarding the upstream process development time. Generally speaking, continuous DSP optimizes the strategies for connection of the various DSP unit operations.

Process validation
Stage 2 (process performance qualifications)

The qualification of a continuous perfusion process will potentially require more time and effort compared to the standard fed-batch process and developers should be aware of this. Regulatory agencies will anticipate that an applicant clearly understands how the production term and cell age impacts on product quality, including a demonstration of product consistency across the full production period of all batches utilized in clinical manufacture and qualification. If a common fed batch needs one year of characterization work and three–six months of validation (wet lab work), perfusion process qualification could easily exceed two years.

Consistent with a DSP continuous process development time, the continuous DSP qualification will not negatively impact the overall product process performance qualification (PPQ) timeline.

Control strategy

One opportunity to develop and validate a continuous process is to gain a greater process understanding through in-line monitoring and predictive multivariate modeling. If a predictive model could be built up based on development and scale up data, it is possible to change the current practice for process characterization and validation. The regulator’s acceptance would be critical.

Table 16: Regulatory considerations for process technologies – key themes

Regulatory issue/ challenge	Regulatory opportunity/benefit	Regulatory engagement plans	Stakeholders	Proposals
Real-time release	• Provides additional rationale to support wider adoption • Demonstrates how process technologies enables secure supply and reduced lead times • Illustrates how real-time release would drive newer PAT and release testing methodology development and adoption	• Industry events • Papers • White papers	• Food and Drug Administration • European Medicines Agency • Parenteral Drug Association, International Society for Pharmaceutical Engineering	• Build case studies to show concept to practical experiences
Single-use standards	• Supports broader use of consumables and systems • Enables harmonization of expectations and requirements • Aligns expectations and enables addressing of critical regulatory concerns	• Industry events • Papers	• Food and Drug Administration • European Medicines Agency • Parenteral Drug Association, International Society for Pharmaceutical Engineering, vendors	
Reduce and/ or eliminate changeover	• Enables efficient multiproduct facilities of the future • Demonstrates cleanability/no product carry over • Reduces costs and ensures timely supply of medicines to patients	• Industry events • White papers • BPOG (BPI BioProcess International article)	• Food and Drug Administration • European Medicines Agency • Parenteral Drug Association, International Society for Pharmaceutical Engineering	• Write a white paper to detail risk-based approach for addressing cross-contamination
Ballroom design and declassified facility	• Drives more focus on production and product testing rather than environmental monitoring • Enables multiproduct manufacturing in the same facility with overlapping campaigns • Demonstrates reduction and possibly elimination of open operations to address microbial risk/cross-contamination • Includes interchangeable parts • Reduces costs and ensures timely supply of medicines to patients	• Risk-based approach to show how cross-contamination is managed • Quantify how this impacts on the security/robustness of supply and lead times to patients	• Food and Drug Administration • Food and Drug Administration's Office of Biotechnology Products • Food and Drug Administration's Emerging Technology Team • European Medicines Agency	• Provide education on ballroom concept and closed systems • Build case studies to show concept to practical experiences • Write a white paper to detail microbial control strategy and risk mitigation for addressing cross-contamination
Global regulatory harmonization	• Consistency of regulatory expectations will ensure consistency of process and product • Reduces costs and ensures timely supply of medicines to patients	• Quantify how this impacts on the security/robustness of supply and lead times to patients	• Food and Drug Administration • European Medicines Agency and other major regulatory agencies • International Council for Harmonisation	• A consortium of industry and regulatory agencies to drive harmonization

Table 16: Regulatory considerations for process technologies – key themes (continued)

Regulatory issue/ challenge	Regulatory opportunity/benefit	Regulatory engagement plans	Stakeholders	Proposals
Resin cross-use for downstream processing	• Sufficient data and risk assessment/management could be generated to justify an appropriately low risk of cross-contamination, enabling reuse of chromatography resins for certain operations (i.e. Protein A capture) and could provide significant benefits to industry			
Viral validation strategy	• While this may be possible within companies at present, sufficient data may exist to support the adoption of this strategy on an industry-wide basis			
Parallel processing of multiple products	• Use of dedicated single-use systems with fewer open operations reduces the risk of cross-contamination from multi-process operations in a common processing area			
Regulatory challenges associated with continuous processing	• Implementation of continuous processing in downstream operations is likely to create regulatory challenges in viral validation, demonstration of bioburden control and batch definition			

8.0 Conclusions and recommendations

This document presents a vision for improvements in process technology over the next 10 years that will result in a substantial improvement in productivity and COGS in the manufacture of biopharmaceutical products. The authors hope that this vision will provide a starting point for the engagement of manufacturers, suppliers, regulators and academics, and translate it into a reality. For some challenges, the path may already be clear, but for others, where disruptive shifts are needed, developments and collaborations will need to be longer term and include multiple stakeholders to bring new technologies into the innovation space, including academic and other research communities. In almost all cases, it is clear that collaboration across multiple organizations will be required to bring such innovations into routine use.

The choice of which process technologies to pursue will depend heavily on the desired outcome and specific situation of a given company. One trying to significant reduce the COGS for a single product will likely require different technology improvements than a company looking for increased flexibility in the manufacture of a multiproduct portfolio. Before perusing any given technology, companies should consider what intensification strategy (outlined in Appendix A) makes the most sense for its circumstances. Several additional factors should be considered, including existing manufacturing capacity, prior knowledge with existing technologies, size of company and portfolio, potential market for the product and stage of a product's lifecycle. The impact of technology can then be weighed against the risk to its successful implementation.

No single technology will be right for every company. However, a focused development effort on a select few could have a significant and broad impact in our industry. These include:

1. process intensification to increase titers, reduce volumes, reduce the number of unit operations, more streamlined ways of working and bringing lower capital facility cost
2. richer, chemically defined medias, feeds and supplements that enable higher cell densities, higher titers, simplified media make-up and longer media stability
3. robust, scalable harvest technologies and CRDs that minimize large capital investments and can handle ever increasing cell densities
4. standardized modular claims for robust VC approaches that provide streamlined regulatory processes and ease process development
5. buffer management approaches that reduce operational constraints and space requirements for buffer preparation and hold, and result in cost-efficient in-line dilution solutions
6. longer-lifetime, higher-capacity resins for chromatography to reduce costs
7. single-use technologies to increase flexibility and improve closed systems, resulting in decreased capital costs and total COGS over the lifetime of a product.

Most of the technologies identified in this document will have an incremental impact on the COGS and operational flexibility but are still important in an increasingly competitive landscape.

Looking farther into the future, disruptive technologies capable of revolutionizing biomanufacturing should also be considered. The role of an individual biopharmaceutical company is likely to be limited since it does not want to put clinical and commercial programs at risk. Industry consortiums can address the biggest challenges such as CHO understanding and VC but would benefit from support by both academia and vendors. Development of these future disruptive technologies carries an increased risk but has the potential for higher reward.

Continuous processing holds a unique position among the process technology improvements considered in this work. Implementation of continuous processing significantly reduces the size and cost of a facility required to produce a given amount of product, and this translates into reduced fixed costs and hence reduced COGS, so it would seem a natural approach for implementation. And most of the other technology improvements discussed herein can be implemented to the benefit of continuous processing. But currently, there is a high threshold for the development and implementation of continuous processes. There are a few specific technology barriers, e.g. the lack of commercially available continuous DF systems, and centrifuges and control systems, that can be employed at the smaller scale required for the development of a continuous process. However, these purely technical issues should yield fairly easily to focused development efforts. More important are the systematic barriers to its development and implementation. First, many companies have installed capacity for batch processing that they must continue to leverage. Second, the ways of working in R&D and manufacturing, employed at most biopharmaceutical/biotech companies, have taken decades to develop and do not translate easily to continuous processing. These ways of working focus mainly on the approach to process development in R&D; in manufacturing, they would include issues such as batch definition, deviation management and inventory management for raw materials and product. Circumscribing all this, is the approach to licensure and other regulatory issues. Given all these issues, the promise of continuous processing may take many years to realize, despite its many benefits, if companies have the long-term vision to allocate effort to its development.

The advances described in this document will provide a meaningful level of benefit over current practices, which will reduce manufacturing costs and enhance patient access over the 10-year horizon that was considered by the authors. This would be a first step in reducing the manufacturing cost of biopharmaceuticals towards those of small-molecule drugs. Even more pronounced benefits become possible with the addition of disruptive technologies, which will require a longer time horizon and broader collaborations among industry, suppliers, regulators and research communities for development and commercialization.

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10.0 Acronyms/abbreviations

Acronym/abbreviation	Definition
ATF	Alternating tangential flow
BOM	Bill of materials
BXR	Bioreactor
CAPEX	Capital expenditure
CHO	Chinese hamster ovarian
CIP	Cleaning in place
CMO	Contract manufacturing organization
CNC	Closed non-classified
CONT DF	Continuous diafiltration
COGS	Cost of goods
CPMP	Committee for Proprietary Medicinal Products
CPP	Critical process parameter
CPV	Continuous process verification
CQA	Critical quality attribute
CRD	Cell retention device
DF	Diafiltration
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DOE	Design of experiment
DP	Drug product
DS	Drug substance
DSP	Downstream process or downstream processing
E&L	Extractables and leachables
E/L	Extractables/leachables
EM	Environmental monitoring
EMA	European Medicines Agency
FDA	Food and Drug Administration
FDA ETT	Food and Drug Administration's Emerging Technology Team
FDA OBP	Food and Drug Administration's Office of Biotechnology Products
FTE	Full-time equivalent
GMP	Good manufacturing practice
HCP	Host cell protein
HTST	High-temperature/short-time treatment
HVAC	Heating, ventilation and air conditioning
ICH	International Council for Harmonisation
ILM	In-line monitoring
IP	Intellectual property

Acronym/abbreviation	Definition
ISPE	International Society for Pharmaceutical Engineering
LDPE	Low-density polyethylene
LRV	Log reduction values
mAb	Monoclonal antibody
MCC	Multicolumn chromatography
MCSGP	Multicolumn countercurrent solvent gradient
MES	Manufacturing execution systems
MF	Membrane filtration
MVDA	Multivariate data analysis
NSO	Non-secreting murine myeloma derived cells
PAI	Pre-approval inspection
PAT	Process analytical technology
pCO_2	Partial pressure of carbon dioxide
PDA	Parenteral Drug Association
PFA	Paired filtration analysis
pg	Picogram
PLC	Programmable logic controller
PM	Preventive maintenance
PPQ	Process performance qualification
QA	Quality assurance
QC	Quality control
QMS	Quality management system
qP	Specific productivity (per cell)
QP	Qualified person
QPCR	Quantitative polymerase chain reaction
R&D	Research and development
RO	Reverse osmosis
RTR	Real-time release
RV	Reactor volume
SIP	Sterilization in place
SPTFF	Continuous single-pass tangential flow filtration
SS	Stainless steel
SUB	Single-use bioreactor
TFF	Tangential flow filtration
TFUF	Tangential flow ultrafiltration
TP	Therapeutic proteins
UF	Ultrafiltration

Acronym/abbreviation	Definition
USP	Upstream processing
USP<87>	United States Pharmacopeia 87 Biological Reactivity Tests
UV	Ultraviolet
VC	Viral clearance
VERO	Cell line derived from African green monkey kidney epithelial cells
VF	Viral filtration
VVD	Vessel volumes per day
WFI	Water for injection
WoW	Ways of working

11.0 Appendices

Appendix A – Process intensification

Process intensification efforts can be divided into different categories, each of which needs to be considered separately:

1. **Intensify existing processes through intensified operations: without changing operating parameters within unit operations**
   Intensification through intensified operations, i.e. through ways of working in the production facility that eliminate the 'white space' in equipment utilization charts, minimize inventory of materials (both in the warehouse and on the production floor) and minimize the labor required to operate the facility (including floor coverage, document handling, analytical support, etc.). These require little or no change in the process or our understanding of it. Implementation of such ways of working has a very high probability of providing improved productivity and COGS, and the magnitude of the benefit should be predictable. There is no regulatory burden on the process itself, although facility validation could be more difficult. All organizations that are sensitive to productivity and COGS should implement the technologies that facilitate these ways of working.
2. **Intensify existing processes through intensified process designs: change parameters within existing unit operations**
   This approach examines the ability to optimize (re-optimize?) existing unit operations to achieve global optimization, i.e. consideration of the 'total cost of ownership'. Steps are often optimized in isolation without consideration of their impact on the overall yield, productivity or COGS for the process as a whole; this can lead to costly 'excessive robustness'. A global optimization of the process could meet product quality requirements with better productivity, or even with a reduced number of steps. Some process parameters are based on past experience with other products and, to minimize development effort, conservative parameter values are selected. Furthermore, a concern for process robustness reinforces the selection of sub-optimal parameters (e.g. chromatograph wash volumes or DF volumes). Operation at the optimum values for such parameters can have a real impact on efficiency (productivity and COGS) without impacting product quality or yield. Intensified use of PAT, using existing or yet-to-be-developed technologies, is critical to relieving the robustness concern with such an approach. The impact of the change should be predictable (assuming characteristics are understood for new materials). There should be a minimal regulatory burden to implement such approaches, assuming appropriate development and characterization activities are performed, and the robustness of any new PAT technology itself is demonstrated. (Who should pursue such an approach?)
3. **Intensify by changing the process design: evolutionary changes to existing processes**
   This approach considers the use of improved materials that fit into existing process flowsheets (or only with minimally impact on the process flowsheet) to achieve intensification and small changes to the process flowsheet using existing technologies. For the former, the intensification achieved can be intrinsic to the process itself (e.g. high-capacity chromatography resins, high-flux filters/membranes, etc.) or can intensify the facility operation (e.g. long-life resins or filters that reduce the frequency of repacking). The latter could include the use of enhanced approaches to existing steps to improve the performance, or eliminate the need, for other steps (e.g. the use of flocculants to eliminate the need for a centrifuge or to reduce the area of depth filter required). There will be a moderate regulatory burden for early adopters of these technologies although, in general, the development and characterization effort required to support licensure should be roughly equivalent to that required for current processes. (Who should pursue such an approach?)
4. **Intensify by implementing disruptive technologies: that substantially change the process flow from current designs**
   Intensification by application of disruptive technologies has the greatest potential to achieve more than marginal improvements in the development timeline, productivity, yield and COGS. But our ability to predict the success of any such new technology, and the extent of an improvement after it is integrated into the full production process (or process development workflow), is very poor. Such technologies also face a significant regulatory hurdle; the first company to bring forward a process with one of them will carry a major burden of proof that their implementation does not impact patient safety. A portion of effort should be allocated to exploring the feasibility of such ideas, with further developments pursued opportunistically.

Appendix B – Antitrust statement

It is the clear policy of BioPhorum that BioPhorum and its members will comply with all relevant antitrust laws in all relevant jurisdictions:

- All BioPhorum meetings and activities shall be conducted to strictly abide by all applicable antitrust laws. Meetings attended by BioPhorum members are not to be used to discuss prices, promotions, refusals to deal, boycotts, terms and conditions of sale, market assignments, confidential business plans or other subjects that could restrain competition.
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- Criminal prosecution by federal or state authorities is a very real possibility for violations of the antitrust laws. Imprisonment, fines or treble damages may ensue. BioPhorum, its members and guests must conduct themselves in a manner that avoids even the perception or slightest suspicion that antitrust laws are being violated. Whenever uncertainty exists as to the legality of conduct, obtain legal advice. If, during any meeting, you are uncomfortable with or questions arise regarding the direction of a discussion, stop the discussion, excuse yourself and then promptly consult with counsel.
- The antitrust laws do not prohibit all meetings and discussions between competitors, especially when the purpose is to strengthen competition and improve the working and efficiency of the marketplace. It is in this spirit that the BioPhorum conducts its meetings and conferences.

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