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DOWNSTREAM PROCESSING

AN OVERVIEW OF MAB PURIFICATION METHODS

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Downstream Processing

An Overview of MAb Purification Methods

by Shada Warreth

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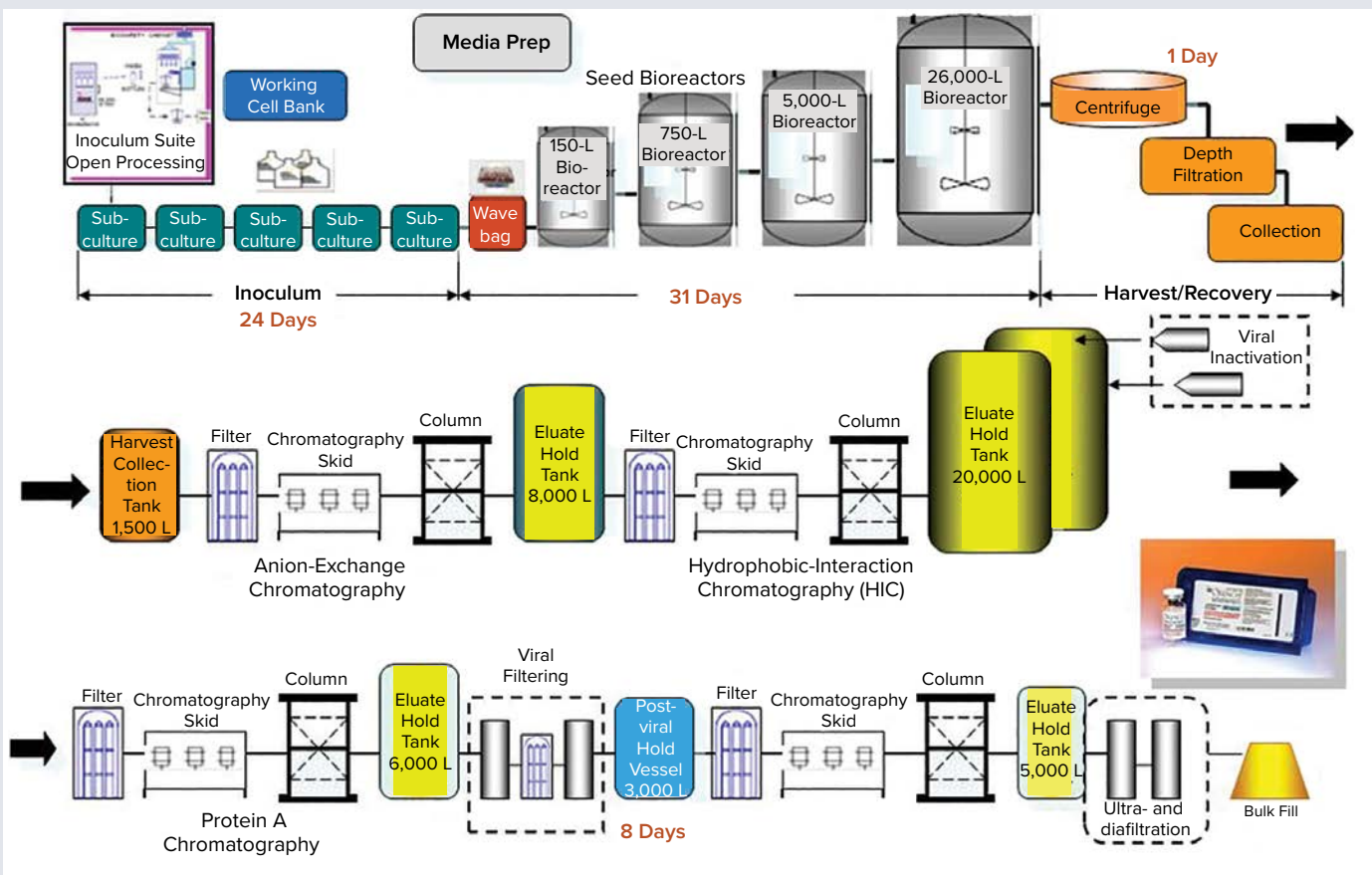
Biopharmaceutical products are developed using a number of biotechnology methods. These products include recombinant proteins such as monoclonal antibodies (MAbs), bispecific antibodies, tissue therapies, vaccines, cell and gene therapies, and antibody–drug conjugates. They are manufactured by methods other than by direct extraction from nonengineered biological sources and used to treat different diseases such as rheumatoid arthritis, psoriasis, cancer, and immune disorders. The first biopharmaceutical approved for patient use was recombinant human insulin developed by Genentech (marketed by Eli Lily) in 1982. Today, these products have many different applications in diagnosis and therapy. Specifically, MAbs are a prominent part of the development and commercialization portfolios of many biomanufacturers.

MAb biomanufacturing is a complex process and differs extensively from manufacturing traditional (small-molecule) pharmaceuticals. A MAb process can be divided broadly into three platforms: upstream production, harvesting and purification (downstream processing), and fill and finish operations.

Upstream production involves culturing and scaling up cells that have been genetically modified to produce a protein of interest. Downstream processing refers to operations that are applied once cell scale-up has been accomplished. They begin with harvesting from a production system and clarification using centrifugation or

Figure 1: A flow diagram representing a typical MAb process (**1**)



microfiltration technologies. Depth filtration typically is used after the harvest stage as well. Some manufacturers have chosen to perform their harvesting stages by using only a series of depth filtration steps. Further along a process line, downstream operations include ultrafiltration and diafiltration (UF/DF), viral clearance (inactivation and removal), and a number of chromatography methods.

Below, I provide an overview of a typical MAb purification process. It focuses solely on downstream operations. Other process areas are outside the scope of this review.

Literature Review

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The manufacturing process for MAbs has progressed considerably since the first approved MAb (Orthoclone OKT3) was approved in 1986 for use in averting kidney transplant rejection (**2**). Since then, the MAb industry has grown dramatically and is now worth billions of dollars (**3**).

Generally, MAb production processes are based on the use of Chinese hamster ovary (CHO) cell culture and protein A affinity capture column chromatography (**4**). Figure 1 illustrates a typical MAb manufacturing process.

Today, the "gold standard" production for a MAb begins with cell-line engineering and development through to seed-train culture. Production generally takes place in fed-batch cell culture over several weeks with the antibody harvested through centrifugation and depth filtration (**5**). The feed stream then enters purification. The order of downstream process steps can vary from company to company, but the methods used are mostly the same.

In many cases, a downstream process begins with a capture chromatography step, which is designed to capture the protein of interest. Protein A is the most commonly used affinity capture chromatography method used for antibody purification. Two common polishing types are ion-exchange (IEX) and hydrophobic-interaction chromatography (HIC). Transitional filtration steps for concentration and buffer exchange use ultrafiltration and diafiltration (UF/DF), which take place between those chromatography steps. To clear viruses from a MAb stream, some methods use detergents, nanofiltration, and/or controlled high-temperature short-term (HT-ST) intermediate holding.

The final step of antibody purification typically is a UF/DF process, which is designed to formulate the MAb with suitable excipients and buffers. Thereafter, the formulated MAb is ready to be sterile filtered and filled into final containers such as vials, syringes, cartridges, or ampules.

Downstream processing begins with the separation of large insoluble impurities from a harvest "feedstock" solution, typically whole cells and cell debris (**6**). But for the purpose of this review, *harvesting* refers to the clarification steps such as centrifugation, microfiltration, depth/sterile filtration, and flocculation; *purification* refers to all other processes described above. All of these methods are discussed below.

Harvest and Clarification

Harvesting is performed by separating cells from cell culture solution, which contains the antibody of interest (extracellular protein) (**5**). If an expressed protein is intracellular, which is the case with bacterial cells (prokaryotes) such as *Escherichia coli*, then harvesting begins with cellular disruption or lysis. In that process, a cell membrane or outer boundary is disrupted or completely destroyed to release intercellular components such as proteins, DNA, RNA, and organelles from cells (**7**). (Figure 2)

Cellular Lysis: Different methods of cell lysis have been established, including use of sonicators, homogenizers, and chemicals such as detergents, enzymes, and reagents (**8**). The selected method depends on the molecule of interest, ease of the purification, and desired quality of a final product.

Cellular lysis methods are either mechanical or nonmechanical. Nonmechanical methods can be physical, chemical, or biological (Figure 3). If a solution contains a relatively low density of cell debris, then the harvest method typically will be depth filtration. If a solution contains a high density of cell debris, then a primary clarification step with a centrifuge is implemented before or in place of depth filtration.

Centrifugation: By contrast with traditional laboratory centrifuges that process large volumes in several batches, a continuous-flow disk-stack centrifuge separates solids (e.g., cell debris) from liquid phase (cell culture media) constantly using high centrifugal forces. This process benefits from the density differences between solids and liquids (**5, 9, 10**). A centrifuge bowl contains a stack of disks that are placed on a vertical spindle that rotates inside a stationary centrifuge housing. The stack of disks increases the surface area and speeds up the separation process. A harvest solution to be separated enters the bowl and flows through the stack of rotating disks, which spin at high revolutions per minute (e.g., 12,000 RPM). Because a solid

Figure 2: Structure of mammalian (eukaryote) and bacterial (prokaryote) cells (**11**)

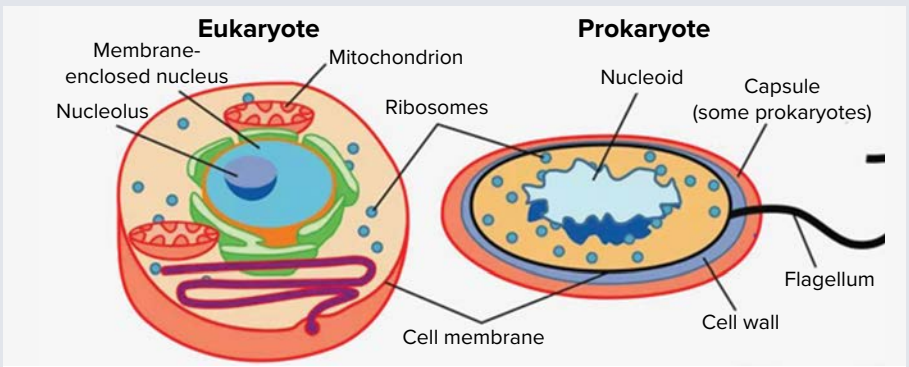


Figure 3: Cell lysis methods (**7**)

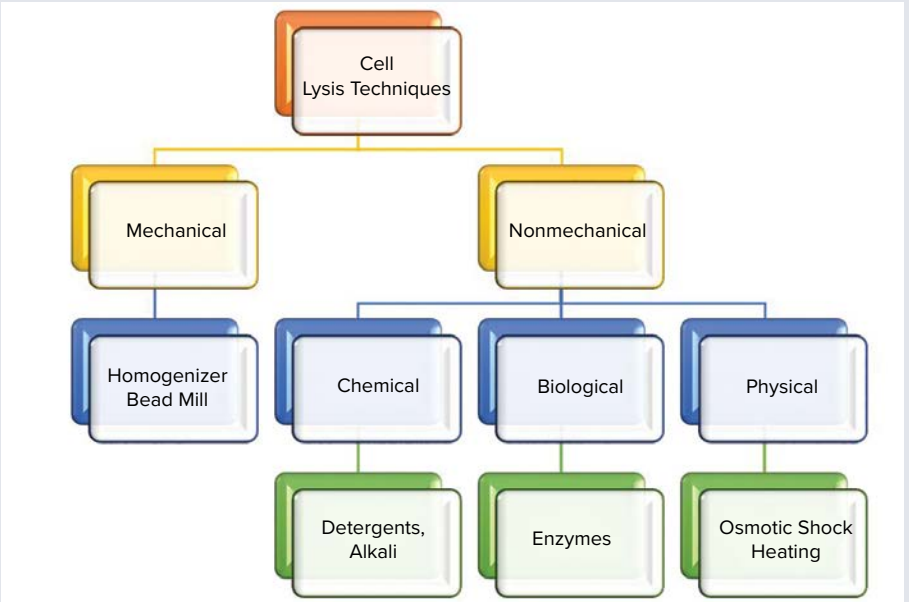


Figure 4: Fluid pathway of a disk-stack centrifuge

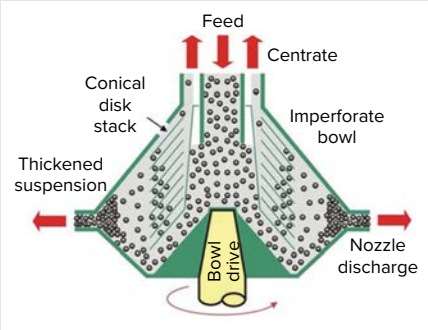
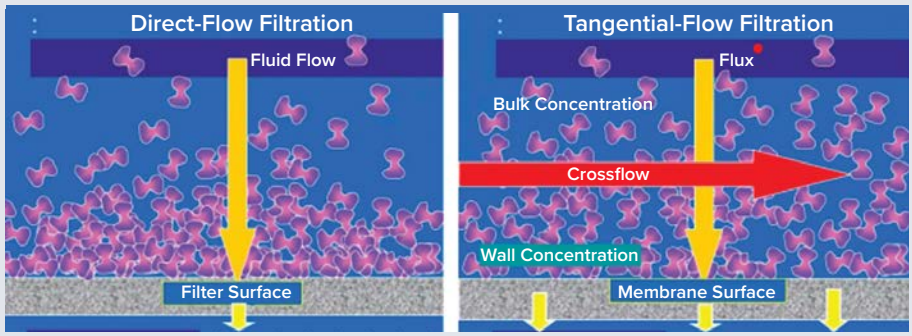


Figure 5: Flow mechanism through dead-end filters (LEFT) and crossflow filters (RIGHT) (**12**)



phase (waste) has greater density than a liquid phase (supernatant), solids are forced outward against the spinning bowl wall and discarded. Liquids with lower densities are collected (centrate) for further downstream processing (**10**) (Figure 4). Parameters associated with a cell culture harvest solution (e.g., cell density and viability at harvest) and those related to the centrifuge system (e.g., centrifugal force, residence time, and solids-ejection frequency) influence the efficiency of a clarification process (**13**).

Crossflow microfiltration, also referred to as *tangential-flow filtration* (TFF), can be used as an alternative process to centrifugation. In fact, microfiltration was used as the primary recovery step for many biopharmaceuticals in the past, when it was implemented before centrifugation (**14**). In microfiltration, a starting solution passes at a tangent along the surface of a filter. The pressure differential across the filter drives components smaller than the pores of the filter through it (filtrate) (Figure 5). Particles that are smaller than the pore size will flow freely through the filter's pores. Components larger than the filter pores (cell debris) are retained (retentate), swept along the membrane surface, and discarded (**12**).

The pore size of a microfiltration cassette (membrane) ranges from 0.1 µm to 10 µm (**12, 15**). However, in most cases, particles adsorb onto a filter pore, causing a drastic drop in pore size. Particles also can accumulate on the top of a filter and form a cake-like filter layer. That also reduces pore size, and separated particles are much smaller than the initial pore size of the filter. In such cases, filters can be reused after cleaning (**16**, **17**). But after several runs, the filters eventually will become blocked beyond use (fouling) and must be replaced. Another disadvantage of using microfiltration is that set-up and processing times are long compared with centrifugation systems. However, microfiltration provides some benefits over centrifugation. For example, microfiltration can achieve a particle-free harvest stream, which then requires few filtration steps.

Depth Filtration: After large insoluble impurities (cell debris) have been removed with centrifugation or microfiltration (occasionally), a clarified harvest solution goes through a polishing filtration step, which uses a depth filter to ensure that all cells are removed. That filtration step safeguards further downstream

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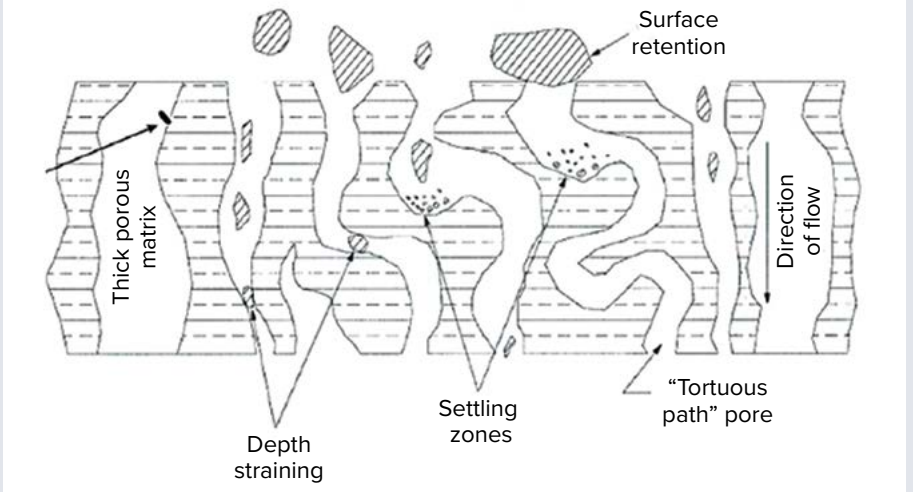
processing steps and helps remove cell debris.

In some irregular cases, a series of depth-filter membranes are used to clarify a harvest without the need for other clarification methods (**13**). Such depth filters have porous membranes, which retain particles through their "depth" and not just on the surface (**9, 18**) (Figure 6). Depth filters for bioprocessing generally consist of cellulose fibers, perlite (volcanic microporous material with a high surface area), diatomaceous earth, and a positively charged resin binder (**19**).

Figure 6: How depth filters operate at capturing particles (**9**).



Depth filters retain more particles in their convoluted flow paths than can size-based filters because depth filters retain particles that are both smaller and larger than the pore size. Particles are retained by adsorption through ionic and hydrophobic interactions (chemical separation) and by size exclusion (mechanical separation) (**13, 19**). Because of their hydrophobic features and charge, depth filters have been used to remove endotoxins, DNA, host-cell proteins (HCPs), and viruses (**20**, **21**). Sometimes, depth filtration also is implemented to clarify a product stream after capture chromatography to remove trace levels of HCPs and DNA (**22**).

Because low-shear centrifuges are commercially available, use of microfiltration for harvesting mammalian cell culture has declined. Centrifugation with depth filtration has become the method of choice for primary recovery from cell-culture broth (**23**). If scaling up a centrifugation process is complex, microfiltration with depth filtration is the method of choice (**9, 15, 19**). Flocculation (adding multivalent cations, metal salts, or polymers to aid in cell aggregation) also has been shown to increase clarification performance by improving centrifugation efficiency, as demonstrated by increased filter capacity (**13**). In some cases, clarification is taken one step further with the application of a normal-flow filter (also known as a dead-end filter) for bioburden reduction. Figure 7 illustrates the different options for harvesting.

Purification

Downstream purification focuses on the capture and separation of MAbs and elimination of all impurities for final formulation. That is achieved through diverse processes such as column chromatography, viral inactivation and removal, and dead-end and cross-flow filtration (**24**). Those applications can entail chemical, mechanical, or dual chemical–mechanical separation: A MAb can be separated from impurities chemically by electrical charge or by interaction with other molecules, mechanically by shape or size (or a combination of

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both). Generally in downstream processing, product purity increases as volume decreases through each process step. A MAb's environment might be altered to obtain a desired stable and suitably formulated bulk drug substance. Such alterations are unique with each product and can consist of minor or extreme alterations, including the following (**24**):

- protein refolding
- increased concentration of product in solution
- addition of excipients to boost product stability
- attachment of molecules to the product to improve product stability
- attachment of molecules to the product to improve immune response.

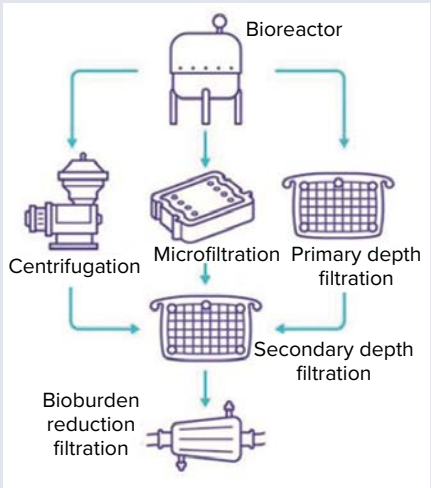
Column chromatography is the primary method used in downstream processing, in which a liquid phase encompassing a MAb of interest passes through a solid phase. Typically, the initial step after harvest/clarification is an affinity or capture chromatography step. In almost all cases, protein A capture chromatography is the first chromatography used and the most expensive step in a purification process (**4, 25**). In this type of chromatography, a MAb binds to a protein A resin while impurities pass freely through the column. The bound MAb then is eluted from the resin and is collected off the column for further purification. In most cases, two polishing steps follow: IEX chromatography and (less frequently) size-exclusion chromatography (SEC) and HIC (**4**). Those additional steps are implemented to remove impurities such as HCP and host DNA. On occasion, HIC clearance is enhanced by adding selective washes to a protein A step (**26**). All of those chromatography steps are designed specifically for a protein's characteristics and for properties identified during product development phases.

The purification pathway also includes two separate orthogonal viral clearance steps, as directed by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (**27**). Generally, low-pH viral inactivation and viral filtration through a parvovirus-grade nanofilter are used. The low-pH incubation step occurs directly after protein A chromatography as the column elutes in a low-pH buffer (pH 2.5–4) (**28, 29**). The low-pH condition inactivates enveloped viruses (**28**).

Figure 8 shows Genentech's and Biogen's purification processes. Both begin with protein A chromatography followed by viral inactivation and anion-exchange (AEX) chromatography, usually in flow-through mode. AEX chromatography is used when HCP clearance is required and high–molecular-weight (HMW) aggregates are not a concern. Cation-exchange (CEX) chromatography is used in bind–elute mode, or HIC in flow-through mode will be applied if HMW aggregates are present and must be removed. Both processes also contain viral filtration and UF/DF steps (**26**).

If specific product-related impurities are present, then hydroxyapatite (HT/HTP) chromatography can be used. However,

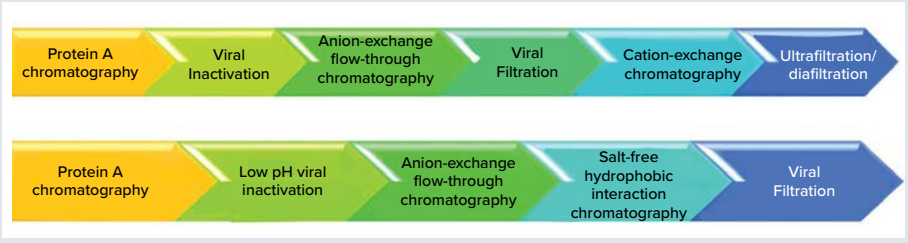
Figure 7: Different clarification options available (**19**)



that method is not a popular choice because of its relatively low capacity and difficult handling. HT/HTP chromatographic behavior also can be complicated (**30**).

Recent advancements in downstream processing include multimodal chromatography and inclusion of a hydrophobic moiety into a ligand structure for IEX chromatography. Increased resin hydrophobicity enhances removal of HMW on IEX columns. And IEX chromatography (e.g., AEX and CEX) are proficient at clearing DNA and HCPs (**31**).

Figure 8: Genentech's (TOP) and Biogen's (BOTTOM) monoclonal antibody downstream processing approach, including their chosen viral clearance methods (**26**)



Ultrafiltration/Diafiltration

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UF/DF is a pressure-dependent procedure for MAb concentration (ultrafiltration) and buffer exchange (diafiltration) (**32**). Like microfiltration, ultrafiltration is a size-based separation technique and typically is carried out in TFF mode. Impurities larger than the pores of a filter membrane are retained, and smaller impurities flow through freely. The diafiltration step (buffer exchange) is performed in diafiltration mode, for which a buffer of a final preferred conformation is added to retentate (holding vessel) at the same rate that permeate (filtrate) is removed. Ultrafiltration membranes with pores between 1 nm and 20 nm separate impurities ranging in molecular weight from 500 Da to 1,000 kDa (**33**). That process differs from microfiltration, which has micrometer-sized pores that allow proteins to pass through. A UF/DF cassette pore size is selected based on the size of a desired MAb to be retained. Typically, a cassette has a molecular weight cut-off point that is two to three times the size of the desired MAb. That will ensure that the MAb is retained on the upstream side of the membrane (retentate) while all other impurities flow through the cassette (permeate).

Like MF cassettes, UF/DF cassettes can be cleaned and are manufactured from a range of materials, including polyvinylidene fluoride, polysulfone, polyethersulfone, and regenerated cellulose. Those synthetic polymers have a strong resistance to acids, bases, and alcohols, so they are compatible with cleaning agents and high temperatures. Cellulose is the material of choice because cellulose membranes are less prone to protein fouling, are mechanically strong, and are easier to clean than other polymers (**28, 34**). Some manufacturers choose to use these cassettes as single use and dispose of them after each batch.

Viral Clearance

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Viral clearance methods are a major part of downstream purification platforms. However, because viral contamination also can happen in bioreactors, some manufacturers choose to add viral clearance screening and mitigation techniques to their upstream processes

Table 1A: A range of different platforms that can be used to clarify and purify MAbs (**28**)

Process	Method	Role	Mode of Operation	Drawbacks	Place in Purification Process
Centrifugation	Mechanical equipment	Centrifugal separation of host cells, with monoclonal antibody (MAb) present in filtrate (supernatant)	Liquid and solid separation	Good system design required to limit cell shearing	Protein clarification (harvest)
Microfiltration (MF)	Membrane filtration	Separation of host cells (retentate) with MAb present in filtrate	Size exclusion	Liable to fouling when cell culture stream contains high solid contents; liable to higher shear and cell lysis	Protein clarification (harvest)
Depth filtration	Filter	Removal of cell debris	Size exclusion and adsorption	Handling and disposal	Primary and secondary clarification
Flocculation	Chemical	Aggregation of solids to aid in separation (can be used with centrifuge, tangential-flow filtration, and normal-flow filtration)	NA	Sensitive to shear, and aggregates affect further purification processes	Protein clarification (harvest)
Protein A affinity chromatography	Resin	Primary purification step to capture MAb; highly efficient at removing DNA, host-cell protein (HCP), and other impurities	Bind and elute	Expensive; low MAb binding capacity	Initial chromatography step
Cation-exchange (CEX) chromatography	Resin	Polishing step used to remove impurities (e.g., aggregates, HCP, DNA)	Bind and elute	Low MAb binding capacity	Second or third chromatography step
	Resin	Capture step to capture MAb from high-capacity cartridge filter (HCCF); reduce process and product impurities; can be used as alternative to protein A	Bind and elute	Low MAb binding capacity	Primary chromatography step
	Membrane	Polishing step to bind impurities and aggregates	Flow through	Low impurity binding capacity	Prefilter for nanofiltration

(specifically for cell-culture media and components) as well as during protein purification (**35**). *Viral clearance* includes viral inactivation and viral removal methods. High-temperature–short-time (HTST) treatment and UV-C (UV light at wavelengths between 200–280 nm) both are used in upstream processing areas (**36**). But those methods are outside the scope of this review.

Examples of viral inactivation methods in purification processes include low-pH viral inactivation (effective at targeting enveloped viruses) and use of solvents and detergents. Viral filtration (described below) removes viruses. Both viral inactivation and filtration are described below. When devising a viral-clearance strategy, biomanufacturers test a clearance method independently and calculate the total amount of clearance obtained for the entire process (**37**). Regulatory agencies require that biomanufacturers determine and report viral clearance as log reduction (**36**).

Low-pH viral inactivation involves adding an acid such as phosphoric acid to a protein solution and holding it at a low pH for a validated time. A pH ≤3.9 is considered to be robust (**37**). This method is appropriate for inactivating enveloped viruses. The pH of that solution then is adjusted to a higher pH, generally pH ≥7. It is important to ensure that the low pH targets viruses and does not

Table 1B: A range of different platforms that can be used in the clarification and purification of a MAb (**28**)

Process	Method	Role	Mode of Operation	Drawbacks	Place in Purification Process
Viral inactivation	Chemical	Inactivation of enveloped viruses	NA	Risk of protein damage if not used correctly	After second or third chromatography step
Ultrafiltration/ diafiltration (UF/DF)	Membrane	Concentrate and buffer exchange	Size exclusion	Precise adjustments needed to ensure complete buffer exchange, high concentration formulation, and high product recovery	Different intermediary pools and final formulation
Sterile 0.2-µm filtration	Membrane	Bioburden reduction and particulate removal; provides sterile barrier for intermediate hold steps or final storage	Size exclusion	Not suitabile for large protein loads; prone to blocking	All points in the purification process
Anion-exchange chromatography (AEX)	Resin	Polishing step to remove residual HCP, DNA, viruses, and other impurities; remove leached protein A if it is used as a capture step	Flow through and bind and elute	Loading conductivity must be low; predilution of load pool might be required; MAb binding capacity constraint in bind-and-elute mode	Second or third chromatography step
	Membrane	Polishing step to remove residual HCP, DNA, viruses, and other impurities; remove leached protein A if it is used	Flow through	Loading conductivity must be low; MAb binding capacity constraint in bind-and-elute mode	Second or third chromatography step
Hydrophobic interation (HIC) chromatography	Resin	Polishing step to remove unwanted aggregates, viruses, residual HCP, DNA, and other impurities	Flow through and bind and elute	MAb binding capacity	Second or third chromatography step
Hydroxyapatite (HA) chromatography	Resin	Polishing step to remove unwanted aggregates, viruses, residual HCP, DNA, and other impurities; remove leached protein A if it is used as the capture step	Bind and elute	MAb binding capacity; incompatible with citrate salt and chelating ethylene-diaminetetraacetic acid (EDTA)	Second or third chromatography step
Viral filtration	Membrane	Removal of large-size viruses, depending on pore-size of filter	Flow through	Expensive; prone to fouling	After second or third chromatography step

compromise the stability of a protein product. Low-pH viral inactivation generally is performed directly after protein A chromatography because those columns elute in low-pH buffers (**26**).

Solvent and detergent viral inactivation involves incubating a protein solution in a detergent with an organic solvent such as tri-n-butyl phosphate for a set time. After inactivation is complete, the solvent and detergents are removed by using a sorbent such as a polymer (**36**). Other less common methods for inactivating enveloped viruses include microwave heating, irradiation (UV and gamma), pasteurization, and HTST treatment.

Viral Removal Methods: Size-exclusion techniques such as chromatography and viral filtration (nanofiltration) can remove nonenveloped viruses. Those that are chemically resistant can be difficult to remove (**38**). Although the technique is not designed to eliminate viruses, column chromatography can remove both enveloped and nonenveloped viruses (**39**). Such methods are controlled by several operating parameters (e.g., temperature, flow rates, buffers and wash volumes) that can limit the extent of viral reduction achieved. Consequently, column chromatography is less favored than filtration (**40**). Nanofiltration is a popular method for viral removal because it can remove small and large enveloped

viruses and nonenveloped viruses through size exclusion. Membrane chromatography is another popular method because it uses virus-binding ligands with ion-exchange adsorbers. It also operates at flow rates that are higher than those for traditional column chromatography (**36**).

Regulatory agencies advise performing multiple orthogonal methods for viral clearance (methods that are independent and have unrelated clearance methodologies) (**41**). Thus, a successful viral clearance strategy is one that can ensure that the risk of product contamination with a virus is less than one in a million (**40**).

On reviewing the above clarification and purification methods, you are likely to conclude that several methods are needed to achieve a pure formulated drug substance that will be ready to be filled in a final container. Table 1 (presented in two parts) lists those clarification and purification methods discussed above.

Process Understanding

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Bioprocessing can be complex and time consuming. The development and purification journey comprises many process steps, including clarification and combinations of different unit operations such as centrifugation, microfiltration, and depth and sterile filtrations. Purification steps combine several types of chromatography, UF/DF, and viral clearance steps. If genetically modified cells produce an intracellular protein, then an additional step is required to lyse those cells and collect the protein before proceeding to harvest and clarification steps.

It is essential to understand how different process steps influence MAbs and how one step can affect the next process steps. The main challenge is to select equipment and devices that work together, offer the best-fit option, and keep up with rising product titers and increasing complexities of cell-culture process fluids to be clarified, purified, and filled into final containers. Many biopharmaceuticals continue to progress through all process stages toward commercialization as therapeutic products. A more in-depth review of all cell lysis methods and of current disposable downstream processing applications available for protein clarification and purification would be useful for future studies.

References

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1 Lannon K. Quantitative Risk Assessment of Bioaccumulation Attributable to Extractables and Leachables in Cellular Immunotherapy Biomanufacturing. *BioProcess Int.* 13(11) 2015; https://bioprocessintl.com/manufacturing/cell-therapies/quantitative-risk-assessment-of-bioaccumulation-attributable-to-extractables-and-leachables-in-cellular-immunotherapy-biomanufacturing.

2 Leavy O. Therapeutic Antibodies: Past, Present, and Future. *Nat. Rev. Immunol.* 10(5) 2002: 297; https://doi.org/10.1038/nri2763.

3 Liu, JKH. The History of Monoclonal Antibody Development: Progress, Remaining Challenges, and Future Innovations. *Ann. Med,. and Surg.* 3(4) 2014: 113–116; https://dx.doi.org/10.1016/j.amsu.2014.09.001.

4 Center A, Scott C. Monoclonal Antibodies: Beyond the Platform in Manufacturing. *BioProcess Int.* eBook. 17(3)e1 2019; https://bioprocessintl.com/

manufacturing/monoclonal-antibodies/monoclonal-antibodies-beyond-the-platform-in-manufacturing.

5 Rose P. Cell Harvesting: Getting Cultural. *Filtr. Separat.* 18 March 2009; https://www.filtsep.com/food-and-beverage/features/cell-harvesting-getting-cultural.

6 Conner J, et al. *The Biomanufacturing of Biotechnology Products. Biotechnology Entrepreneurship: Starting, Managing, and Leading Biotech Companies*. Shimasaki C, ed. Academic Press: Waltham, MA, 2014: 351–385.

7 Islam MS, Aryasomayajula A, Selvaganapathy PR. A Review on Macroscale and Microscale Cell Lysis Methods. *Micromachines* 8(3) 2017: 83; https://doi.org/10.3390/mi8030083.

8 *Cell Lysis/Cell Fractionation Market: Global Forecasts to 2021*. Markets and Markets: April 2016; https://www.marketsandmarkets.com/Market-Reports/cell-lysis-market-260138321.html.

9 Shukla A, Kandula JR. Harvest and Recovery of Monoclonal Antibodies from Large-Scale Mammalian Cell Culture. *BioPharm Int.* 21(5) 2008.

10 Richardson A, Walker J. Continuous Solids-Discharging Centrifugation: A Solution to the Challenges of Clarifying High–Cell-Density Mammalian Cell Cultures. *BioProcess Int*. 16(4) 2018: https://bioprocessintl.com/downstream-processing/separation-purification/continuous-solids-discharging-centrifugation-a-solution-to-the-challenges-of-clarifying-high-cell-density-mammalian-cell-cultures.

11 *Science Primer*. National Center for Biotechnology Information: Bethesda, MD; https://www.ncbi.nlm.nih.gov.

12 Maher J, et al. Filtration for the Bioprocessing Industry. *BPE J. Sys. Des.* 2(1) 2017.

13 Roush DJ. Lu Y. Advances in Primary Recovery: Centrifugation and Membrane Technology. *Biotechnol. Prog.* 24, 2008: 488–495; https://aiche.onlinelibrary.wiley.com/doi/pdf/10.1021/bp070414x.

14 Russotti G, Goklen K. Crossflow Membrane Filtration of Fermentation Broth. Membrane Separations in Biotechnology. 2nd ed. Marcel Dekker: New York, NY, 2000: 85–159.

15 Besnard L, et al. Clarification of Vaccines: An Overview of Filter Based Technology Trends and Best Practices. *Biotechnol. Adv.* 34(1) 2016: 1–13; https://doi.org/10.1016/j.biotechadv.2015.11.005.

16 Pattnaik P, Louis I, Mahadevan MS. Use of Membrane Technology in the Bioprocessing of Therapeutic Proteins from Inclusion Bodies of *E. coli*. *BioProcess Int*. 7(3) 2009: 54–62; https://bioprocessintl.com/downstream-processing/filtration/use-of-membrane-technology-in-bioprocessing-therapeutic-proteins-from-inclusion-bodies-of-e-coli-183674.

17 Raghunath B, et al. Best Practices for Optimization and Scale-Up of TFF Microfiltration Processes. *BioPro. J.* 11(1) 2012: 30–40; https://doi.org/10.12665/J111.Raghunath.

18 Fiore JV, Olson WP, Holst SL. Depth Filtration. *Methods: Plasma Protein Fractionation*. Curling JM, Ed. Academic Press: New York, NY, 1980: 239–268.

19 Cherradi Y, et al. Filter-Based Clarification of Viral Vaccines and Vectors. *BioProcess Int*. 16(4) 2018: 48–53; https://bioprocessintl.com/downstream-processing/filtration/filter-based-clarification-of-viral-vaccines-and-vectors.

20 Charlton HR, Relton JM, Slater NK. Characterisation of a Generic Monoclonal Antibody Harvesting System for Adsorption of DNA By Depth

Filters and Various Membranes. *Bioseparation* 8(6) 1999: 281–291; https://doi.org/10.1023/A:1008142709419.

21 Yigzaw Y, et al. Exploitation of the Adsorptive Properties of Depth Filters for Host Cell Protein Removal During Monoclonal Antibody Purification. *Biotechnol. Prog.* 22(1) 2006: 288–296; https://doi.org/10.1021/bp050274w.

22 Soloamo F, et al. Utilization of Depth Filters for Removal of Non-Product Related Impurities in a Large-Scale Monoclonal Antibody. *Purification of Biological Products, 1st Annual Meeting*, 5–7 December 2005, Santa Monica, CA.

23 Shpritzer R. Evaluation of a Continuous Disc Stack Centrifuge for the Clarification of Mammalian Cell Cultures. *225th ACS National Meeting,* 24 March 2003, New Orleans, LA American Chemical Society: Washington DC.

24 Conner J, et al. *The Biomanufacturing of Biotechnology Products. Biotechnology Entrepreneurship: Starting, Managing, and Leading Biotech Companies*. Shimasaki C, Ed. Academic Press: Waltham, MA, 2014: 351–385.

25 Liu Z, Mostafa S, Shukla A. A Comparison of Protein A Chromatographic Stationary Phases: Performance Characteristics for Monoclonal Antibody Purification. Biotechnol. Appl. Biochem. 62(1) 2015: 37–47; https://doi.org/10.1002/bab.1243.

26 Shukla AA, et al. Evolving Trends in MAb Production Processes. *Bioeng. Translat. Med.* 2(1) 2017: 58–69; https://dx.doi.org/10.1002%2Fbtm2.10061.

27 ICH Q5A: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin. *US Fed. Reg.* 63(185) 1998: 51074; https://database.ich.org/sites/default/files/Q5A_R1_Guideline.pdf.

28 Liu HF, et al. Recovery and Purification Process Development for Monoclonal Antibody Production. *mAbs* 2(5) 2010: 480–499; https://dx.doi.org/10.4161/mabs.2.5.12645.

29 Shukla A, Aranha H. Viral Clearance for Biopharmaceutical Downstream Processes. *Pharm. Bioprocess.* 3(2) 2015: 127–138; https://doi.org/10.4155/PBP.14.62.

30 Doonan S. Chromatography on Hydroxyapatite. *Protein Purification Protocols*. 2nd ed. Humana Press: Totowa, NJ, 2004: 191–194; https://doi.org/10.1385/159259655x.

31 Wolfe L, et al. Multimodal Chromatography: Characterization of Protein Binding and Selectivity Enhancement Through Mobile Phase Modulators. *J. Chromatogr. A*. 1340, 2 May 2014: 151–156; https://doi.org/10.1016/j.chroma.2014.02.086.

32 Bhangale A, et al. A UF–DF Screening System for Bioprocess Development: Efficient and Cost-Effective Process Fit and Scale-Up to Manufacturing. *BioProcess Int.* 16(9) 2018: 50–59; https://bioprocessintl.com/analytical/downstream-development/a-uf-df-screening-system-for-bioprocess-development-efficient-and-cost-effective-process-fit-and-scale-up-to-manufacturing.

33 Zhou JX, Tressel T. Basic Concepts in Q Membrane Chromatography for Large-Scale Antibody Production. *Biotechnol. Prog.* 22(2) 2006: 341–349; https://doi.org/10.1021/bp050425v.

34 van Reis R, Zydney AL. Protein Ultrafiltration. Flickinger MC, Drew SW, eds. *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation*. Wiley: Hoboken, NJ, 1999: 2197–2214.

35 Remington KM. Fundamental Strategies for Viral Clearance – Part 1: Exploring the Regulatory Implications. *BioProcess Int.* 13(1) 2015: https://bioprocessintl.com/downstream-processing/viral-clearance/fundamental-strategies-viral-clearance-part-1-exploring-regulatory-implications.

36 Sandle T. Current Methods and Approaches for Viral Clearance. *Am. Pharm. Rev.* Sept.–Oct. 2015; https://www.researchgate.net/profile/Tim_Sandle/publication/282216764_Current_Methods_and_Approaches_for_Viral_Clearance/links/5607f17208aeb5718ff9c07b/Current-Methods-and-Approaches-for-Viral-Clearance.pdf.

37 Aranha H. Virus Safety of Biopharmaceuticals: Absence of Evidence Is Not Evidence of Absence. *Contract Pharma* 14 November 2011; www.contractpharma.com/issues/2011-11/view_features/virus-safety-of-biopharmaceuticals.

38 Challener CA. Viral Clearance Challenges in Bioprocessing. *BioPharm Intl.* 27(11) 2014; http://www.biopharminternational.com/viral-clearance-challenges-bioprocessing.

39 Zhang J. Mammalian Cell Culture for Biopharmaceutical Production. *Manual of Industrial Microbiology and Biotechnology.* 3rd ed. Baltz RH, Davies JE, Demain AL, Eds. American Society of Microbiology: Washington, DC, 2010: 157–178; http://www.asmscience.org/content/book/10.1128/9781555816827.

40 Remington KM. Fundamental Strategies for Viral Clearance – Part 1: Exploring the Regulatory Implications. *BioProcess Int.* 13(1) 2015: https://bioprocessintl.com/downstream-processing/viral-clearance/fundamental-strategies-viral-clearance-part-1-exploring-regulatory-implications.

41 *Virus Validation Studies: The Design, Contribution, and Interpretation of Studies Validating the Inactivation and Removal of Viruses*. European Medicines Agency, CPMP/BWP/268/95; 14 February 1996.

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