Process Validation
How Much to Do and When to Do It

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The trick to process validation, these industry experts argue, is to understand that it is a process that stretches through the whole product life cycle. Some secrets of success: Take a team approach; focus on the timing of the various stages of validation; avoid some common mistakes (see page 20); and build your documentation as you go.

Process validation is defined in the supplementary information section of the *Federal Register* as "a QA function that helps ensure drug product quality by providing documented evidence that the manufacturing process consistently does what it purports to do" (1). It has also been defined as the act of "establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes" (2).

Process validation is required in license submissions for all products regulated by CBER or CDER and usually is a subject of intense scrutiny and lots of activities that culminate in an acceptable validation package. FDA expects that each step of a CGMP manufacturing process must be controlled to maximize the probability that the finished product meets all quality and design specifications.

Validation often requires a joint effort — planning and expertise from several groups. Units or departments that help create the validation package often include process development, engineering, manufacturing, quality assurance, and quality control. Some of the critical process validation activities are described in the "Critical Activities box" (2–4).

A big dilemma during process validation development is how much to do and when to do it. Although it is essential to be thinking of validation as early as possible, the validation process often changes as the product goes from phase 1 to phase 3 and beyond. Creating the package too early — doing too much too soon — can mean redoing a lot of the work. Insights into those "how much" and "when" questions are shared here by some process experts from our industry.

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Validation has become one of the pharmaceutical industry's most recognized and discussed subjects. It is a critical success factor in product approval and ongoing commercialization. Defined, it is the "act of establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes." Despite the simplistic definition, validation is subject to variable interpretations both by industry and by regulators.

Approach and philosophy. Our industry's approach to validation is often flawed. We tend to think deductively — indeed, companies train us to think deductively. We order our thoughts and approach problem resolution by listing options and selecting the optimum course, prioritizing issues and selecting actions. Deductive thinking identifies a solution and looks for all the problems that might be solved by the solution. Inductive thinking results in an analytical rather

than a systemic approach to problem resolution. *Analysis* focuses on structure; it reveals how components work. *Synthesis* focuses on function; it reveals why components operate as they do. Systems thinking is synthesis, putting things together. Analysis is taking them apart. The two approaches are complementary: Analysis yields knowledge; synthesis yields understanding (5).

Process validation requirements. Validation is a dynamic process. It is expected to follow a timeline stretching from initial design through ongoing commercial operation — the product life cycle. Typical expectations are that design qualification (DQ), installation qualification (IQ), and operational qualification (OQ) should be nearly complete early in the development process, at phase 1 and 2. Process qualification (PQ) should be complete at end of phase 3. But everything evolves with the process, and that process is usually a moving target until registration.

Controls must be applied to all manufacturing steps, critical starting materials, components, and the master cell bank. Those controls need to increase as the process develops toward final isolation and purification. Those controls must cover all process steps identified as critical — those steps that can affect the quality and purity of the final product. The subcomponents that affect the process include equipment, facilities and utilities, systems, computers, cleaning, analytical method transfers, and sterilization among others. A frequent problem is the failure to address the life cycle of the system as a whole — most validation efforts focus on individual subcomponents of the process and stop after three commercial runs.

Process validation requirements for active pharmaceutical ingredients (API) differ from those for finished dosage forms (drug products). The standards vary with the type of API, the range of specifications, and "other factors." For drug products, the regulators expect validation of all manufacturing steps: cleaning, weighing, measuring, mixing, blending, filling, packaging, and labeling. For an API, the expectation is that all critical processing steps determined to affect the quality and purity of the API be validated.

Frequent problems. Regulatory expectations at each functional stage often exceed the best efforts of those charged with translating management directives into direct action. The "Validation Steps Often Missed" sidebar illustrates each functional stage and the problems inspectors frequently find there. It is not intended to be all-inclusive and should not be construed as definitive.

Validation is about control — that is, adequate controls supporting and surrounding the process — without which, the validation will fail. For biopharmaceuticals, the more frequently *misvalidated* activities include maximum cell age, impurity profiles, column resin life, and viral clearance.

Critical success factors. Success requires leadership and management with a focus on quality across all functional areas. Preparing and planning can never be overemphasized. The small costs incurred up front pale when compared with reperforming the entire exercise. Validation should be written as if FDA were the customer. The agency *is* the reviewer. And of course, corrective actions to solve any validation problems need to be implemented before a preapproval inspection.

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Regulations mandate compliance with Good Manufacturing Practices (GMP) for the manufacture of clinical trial materials (6,7). Validation is one component of the GMPs, but it is not feasible to complete validation before a process is fully developed. Instead, it is important to understand what is required for phase 1, 2, and 3 clinical trials. Understanding the requirements means that the needs of the patient and the expectations of the regulatory agencies are considered. From both patient and regulatory perspectives, safety is critical. An understanding of the risks associated with product source, manufacturing methods, and the product itself is essential for making decisions about what has to be validated for the various clinical trial phases.

Phase 1. Validation "must-haves" for the earliest clinical trials include those related to safety. Annex 13 to the *EU GMP Guide: Manufacture of Investigational Medicinal Products* states that "Validation of the sterilization process is no different than for licensed product. Virus clearance, where relevant, and removal of other impurities of biological origin should be no less rigorous than for licensed product and should, therefore, be validated" (7). The product must also be shown to be stable during the time it is at the clinic, and that can require validation or, at least, qualification of stability-indicating assays. The catch is that validation of a process requires validated assays. Before phase 1, sponsors' assays are often still in the research unit, performed by only one or, at best, a few people.

Sterility and mycoplasma testing. Sterility and mycoplasma testing are crucial to the safety of a biotechnological product produced in mammalian or insect cells. Sterility assays are essential not only for aseptic processing validation but also for establishing product stability. Sterility assays are

Critical Activities

Some of the critical process validation activities include the following (2–4).

- Create a validation master plan (VMP) that shows "when" and "what" activities will be performed.
- Develop a strategy that allows revisiting the VMP as the process "changes" from a phase 1 process to a phase 3.
- Identify components that will take place at small and at large scale.
- Know the implications of CGMP on raw material usage, facility maintenance, documentation requirements, utilities, equipment cleaning, and personnel training.
- Define "critical" process parameters.
- Identify process parameters as "critical" and "noncritical."
- Determine the proven acceptable range (PAR) and the normal operating range (NOR) for each critical process parameter.
- Demonstrate that critical parameters can be monitored and controlled during manufacturing runs.

—Courtesy of Anurag Rathore

also used to determine the acceptability of unprocessed bulk for further processing. These assays must be validated according to the latest regulatory requirements, such as 21 CFR 610.12 (8), United States and European pharmacopoeia standards, or FDA *Points to Consider*.

Sterility and mycoplasma assays can't be considered validated without stasis testing to ensure that the test article doesn't interfere with the assays, which can cause false negatives that can lead to adverse patient reactions that can potentially terminate clinical trials. If samples are to be shipped, then shipping conditions must also be validated to demonstrate no loss of bacteria, fungi, or mycoplasma.

Viral clearance validation is usually contracted out: The assays are validated by a testing company and each sponsor's test article is evaluated for interference and cytotoxicity. But validation of process scale-down is often overlooked in the rush to get into the clinic.

Scale-down. Time and money are wasted if virus clearance evaluation studies are performed without a validated scale-down model of the process. Validation requires that the sponsor understands critical process and control parameters, uses qualified equipment and validated assays, and follows a protocol defining the study and the expected outcome. A sponsor must also understand what each unit of operation does and how output is measured. Unfortunately, at this early stage of development, many of the assays that enable that understanding are not validated. At worst, a sponsor might lack understanding of what a unit of operation accomplishes.

Take, for example, an immobilized protein A column used to purify a monoclonal antibody (MAb). Initially, product yield might be determined using high-performance liquid chromatography (HPLC) and a total protein assay. But what about activity and impurities associated with the eluted antibody? Without an impurity profile or without knowing how much of a MAb's biological activity has been retained, the control parameters (such as flow rate and pH) cannot be established with certainty. A sponsor in this situation should go back and understand both purity and impurity profiles. The assays used to determine those profiles are unlikely to be validated at this stage, but they must be "qualified." Typically, a reference standard is run along with each assay to ensure the assay is working according to protocol.

Which viruses? Another issue in viral clearance studies is how many and which viruses to use in the first studies so that clinical trials can begin. The opinion of regulatory agencies varies. If a sponsor intends to begin clinical trials worldwide, it is essential to understand the latest regional concerns related to viral safety so that validation of virus clearance will stand up to regulatory scrutiny.

Other impurities of biological origin. Developing a validated assay that demonstrates the removal of impurities (such as host cell proteins) for phase 1 is seldom possible. There is one exception. By using a parental cell line and similar culture conditions to produce multiple products, a generic assay can be validated and qualified for each new product. However in other situations, the host cell

Validation Steps Often Missed

Regulatory expectations at each functional stage often exceed the best efforts of those charged with translating management directives into direct action. The following list of items that frequently cause problems if missed is not intended to be all-inclusive and should not be construed as definitive.

At the design qualification (DQ) stage, elements often missed include:

- Adequate description of the equipment's intended use
- Clear specifications for all critical design parameters
- Setting design parameters that allow future flexibility (for example, the process will likely change, but the equipment may not)
- Specifications that take CGMPs into account.

At the installation qualification (IQ) stage, elements often missed include:

- A list of all equipment that, when operating, has the potential to affect product quality or process performance
- As-built drawings and specifications for all purchased equipment, new or used
- Verification that all purchased equipment and its installation meets the original intent (functional specifications and design parameters), including applicable building, electrical, plumbing, and other such codes
- Preventive maintenance plans and schedules for all such equipment.

At the operational qualification (OQ) stage, elements often missed include:

- Process operating parameters for each module, including those designated as critical
- An OQ protocol designed to demonstrate that the equipment used in each module operates as intended throughout each process operating parameter range
- Task reports describing the successful execution of each OQ protocol
- A list identifying each module (step, unit of operation, or stage) of the process.

At the performance qualification (PQ) stage, elements often missed include:

- A fully defined process, including identifying critical processes and their acceptable operating parameter range (traceable to development reports or small-scale supporting studies) and defining potential adverse consequences
- Completed product specifications
- Scientific rationale or basis for criteria — usually exists but is poorly documented
- IQ and OQ steps completed and reports written, reviewed, and approved
- Operating personnel trained and qualified
- Change control procedures in place.

—Courtesy of Joseph F. Noferi

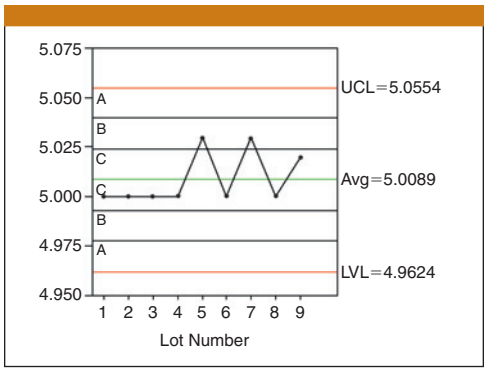

Figure 1. Control chart showing that operator skill, manufacturing equipment, and written instructions are sufficient to adjust oxidation pH to the same set-point from lot to lot. —Courtesy of Peter Watler

protein population is unlikely to be consistent until the scale and conditions of the final culture have been established.

Enzyme-linked immunosorbent assay (ELISA) kits on the market may be sufficient for clinical trial material, but companies are still required by today's regulations to develop their own assays for licensed product. The development of those assays is time-consuming — they can take more than a year. Planning ahead is essential. In addition to host cell proteins, other impurities for which validated assays may be required include any toxic or potentially immunogenic substances.

Phase 2. During phase 1 and 2 clinical trials, the process is generally improved. Both upstream and downstream processes change, analytical methods are usually transferred to QC during this stage, and potency assays validated. No specific process validation activity is required at this stage, unless the changes made to improve the process have the potential to affect the results of previously performed validation studies for sterility, virus clearance, and specific impurity removal. However, while the process is being optimized, assay validation efforts should continue so that process validation at pilot or full scale for licensure can take place during phase 3.

Phase 3. Sometime before phase 3, the process is, hopefully, finalized to avoid bridging studies in the clinic. Assays not yet validated must now be validated so that process validation required for biologics licensure can be completed during phase 3. This is the phase of heavy-duty validation and requires equipment qualification, then process validation during three or more consecutive batches. Cleaning validation and lifetime studies for chromatography columns are important validation elements. Clearance studies to remove host cell proteins, DNA, viruses, and other impurities may eliminate lot release testing. Some clearance studies are performed at a smaller scale than manufacturing, so the small-scale model must be validated. Once the process is finalized, viral clearance studies are carried out again. At this stage, however, larger virus panels are used, mass balance analyses are attempted, and duplicate runs are tested. For chromatography steps that claim to remove viruses, end-of-resin lifetime studies are performed to demonstrate consistent virus clearance.

Preventing surprise. One of the most common FDA form 483 observations is the lack of process validation. Planning ahead during early development can prevent unpleasant surprises, such as specifications that are so tight they cannot be met, analytical assays that can be performed only by one operator and so are not validatable, or a process that cannot be scaled up or down without redesign. Surprises like that result in processes that cannot be validated and often multiple failed batches.

I find that for new processes and products, the greatest validation problems arise when insufficient resources and insufficient time are budgeted for understanding and optimizing production. For biological and biotechnological products, phase 1 validation activities are carried out to ensure safety and the manufacturing process should be controllable. Phase 2 manufacturing processes should be well-controlled and better understood with validated assays that demonstrate that control. Phase 3 should be very well controlled and full process validation should take place during this phase.

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Process validation demonstrates the consistency of multiple batches at full-scale. The validation shows that the process is operated in a consistent manner and that contaminants are reproducibly reduced to acceptable levels. That is accomplished by monitoring those parameters that demonstrate consistent operation of the process, consistent formation of the product, and consistent removal of the contaminants. Processes can be validated through two distinct metrics: operational parameters (inputs) and performance parameters (outputs). *Operational parameters* are process control set-points for variables such as flow rate, temperature, and concentration. These parameters define the process recipe and are used to demonstrate that the facility, equipment, and staff can execute the process consistently. *Performance parameters* reflect the outcome of a given step and indicate that the process gave the desired result.

Process validation is an investment in future production because it sets the bar by which the process will be judged at future inspections. Process validation is the point at which the science of the process can be explained to regulatory agencies. Following validation, regulatory agencies are looking for consistent process operation within the ranges and

meeting the criteria specified in the validation. Scientific arguments explaining operation and performance outside of specified ranges are likely to fall upon deaf ears. Therefore it is essential to generate bench and pilot scale process data that establish key parameters and their acceptance criteria before process validation in the GMP facility.

When to validate. Process validation is expensive, involving copious sampling, extensive analysis, and detailed documentation. Because of the complexity and cost, process validation is best performed during phase 3 trials following the decision to file a Biologics License Application (BLA). This "delayed" strategy offers several advantages. The process is better understood, which means that key parameters and acceptance criteria can be better specified. In addition, the commercial process is in place at this point, and validation does not have to contend with process changes, adjustments, and inexperience that can lead to deviations from the validation protocol. By the time products enter the regulatory review phase, the likelihood of product success increases to 90%, meaning that there is less risk that process validation will have been for naught. Then too, although process validation is a required component of a BLA submission, it is not required for clinical trials.

What to validate. Demonstrating process consistency requires multiple, full-scale batches. Three to five consecutive purification runs from three consecutive fermentation lots should generate sufficient consistency data. Process validation can be made more efficient and more consistent by using templates that identify key input and output parameters for a unit operation. For example, consistent operation of a tangential-flow filtration step can be shown by monitoring cross-flow rate, transmembrane pressure (TMP), temperature, retentate tank volume, and diafiltration volume. Consistent performance of this step can be shown by monitoring excipient removal, protein contaminant removal, product concentration, product recovery, pH, and conductivity. Such parameters demonstrate that the step achieved its purpose in the process. These validation templates for unit operation can be customized to address any unique specifics of the process or product.

Operational control parameters are input variables with set-points or ranges specified in the manufacturing procedure to define how a process is executed. Control charts can be used to demonstrate that the set-points are consistently achieved within the specified ranges. Typical operational parameters include pH, raw material quantities, reaction times, flow rates, temperature, and pressure. For example, the control chart in Figure 1 shows that operator skill, manufacturing equipment, and written instructions are sufficient to adjust oxidation pH to the same set point from lot-to-lot.

Performance parameters are output variables that reflect the outcome of a given step, indicating that the process performed as expected. Validation should demonstrate that the process is capable of consistently removing three classes of contaminants: process-related, host cell-related, and product-related. *Process-related* contaminants are reagents required by the process (for example, guanidine, glycerol, and antifoam). *Host cell-related* impurities are derived from the organism used to generate the product (for example, nucleic acids, CHO proteins, and endotoxins). *Product-related* contaminants are variants and isoforms of the target protein (for example, oxidized, deamidated, aggregated, and clipped forms). The validation should also demonstrate at which step the contaminant is removed and at what point in the process it meets acceptance criteria. Table 1 shows that *E. coli* proteins were reduced by the filtration step, significantly reduced at cation exchange 1, and further reduced to below the acceptance criteria by cation exchange 2.

Process consistency is also shown by monitoring yield and product concentration at each step. With sufficient advance planning, earlier process data, and careful execution, the data from the validation should demonstrate that the manufacturing procedure consistently and effectively yields product that meets specifications. It will also demonstrate that the process can be operated consistently and that the process, product, and host cell contaminants are reduced to acceptable levels.

Table 1. Validation results demonstrating that *E. coli* proteins were reduced by the filtration step, significantly at cation exchange 1, and further reduced to below acceptance criteria by cation exchange 2.
—Courtesy of Peter Watler

	Purification Lot Number (% w/w)					
Process Stream	**23004**	**23006**	**23007**	**23008**	**23010**	**Average**
Oxidation	6.39	4.60	13.4	6.78	11.8	8.60
Filtration	4.13	3.26	2.42	4.83	7.31	3.58
Cation exchange 1	0.915	0.494	0.64	0.532	1.18	0.65
Cation exchange 2	<0.009	<0.003	<0.004	<0.003	<0.011	<0.006
Purified bulk	<0.007	<0.014	<0.003	<0.009	<0.023	<0.011

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Manufacturing processes for biologicals are continually optimized as the product moves through various stages of development, from the preclinical stage through the investigational new drug (IND) filing to the common technical document (CTD) filing and on to approval. The early-stage product may have a basic and somewhat unpolished manufacturing process. By midstage the process is optimized and becomes more robust. By the end of development, the final manufacturing process is fully characterized, validated, and qualified.

Development validation activities. At Genentech, our goal is to have the manufacturing process completely finalized before the manufacturing campaign that supplies the phase 3 clinical trials. That means that the critical process parameters have been identified, the operating ranges have been set, and the process is deemed robust enough to tolerate the manufacturing environment and produce marketable product.

Phase 1 process validation activities. Validation evolves along with development of the process, and different types and levels of validation are appropriate at the various developmental stages. At the time of IND filing, the only validation requirement is a demonstration that the process removes retroviral particles. If modifications are made as the process moves through development, the process for removing retroviruses must be reassessed. Complete process validation is not required until the CTD is filed.

Phase 2 and 3 process validation activities. Validation is performed after the process is fully developed and finalized. Process validation can be a combination of manufacturing-scale and scaled-down studies. The qualification runs are performed on validated equipment, at full manufacturing scale, in the facility in which the product is to be routinely manufactured. The validation studies performed at manufacturing scale typically include the validation of the process to clear impurities (host cell proteins and DNA) and small molecules and the cleaning of resins and membranes.

Scaled-down studies typically include the validation of resin lifetimes, in-process hold times, buffer stability, virus validation, harvest criteria, filter extractables, resin leachables, and cell age. Genentech has found that a combination of manufacturing-scale and scaled-down studies provides the best overall process validation plan.

Before phase 3, evaluate robustness. Before the process is finalized several scaled-down runs are performed to evaluate process robustness. These runs provide an adequate history of the process' variability. Such runs may include running the process at the extremes of its operating ranges, testing different feedstocks, using different lots of resins, testing cell culture components or peptone lots, among others. Sometimes we perform these runs at the 400 liter scale, more often at lab scale. The timing of these scaled-down runs also provides the scientist with the ability to incorporate last minute changes that result from data gathered during these runs to further optimize and improve the robustness of the process.

It is sometimes easy to overlook the importance of a complete evaluation of the robustness of the process for both cell culture (or fermentation) and recovery before finalizing the process. Although it is hoped that the manufacturing process will run at its optimum operating conditions on a routine basis, knowing how the process works under conditions beyond its optimum set points (such as flowrate, pH, and temperature excursions) is important. If the process is running close to the edge of failure, the time to discover and correct that is before finalizing the process. The studies performed at this stage require fully developed analytical assays, and those assays are examined to ensure the process meets the acceptance criteria set in the Certificate of Analysis. Although this phase of development is not considered part of the official validation process, if well documented, it can become the foundation on which the process validation program is based.

Final process with product in phase 3 trials. Genentech finalizes the manufacturing process for the start of the phase 3 clinical trials — that is, the material that is made in the manufacturing campaign to supply the phase 3 trials is from the fully developed, fully characterized, and finalized manufacturing process. No process changes are expected beyond this stage. At this point, the analytical assays are also finalized and are used to release the material to the clinic. Once that product is manufactured, some of that material can be used to support process validation. Validation of the analytical methods and the definition of the control system begin at this stage.

Generic process validation activities. Some of the validation studies we perform are common across many of our processes: viral validation, resin and membrane sanitization and storage, buffer stability, filter extractables, and resin leachables. Rather than continuing to repeat these studies time and time again, we developed matrices to support a wide variety of conditions — conditions that typically reflect our most common operating parameters.

We try to assess the worst-case situation, after which all other conditions are well covered by

those conditions. For example, by testing the ability of a particular resin matrix (such as Sepharose) to be sanitized in the presence of a protein mixture, we can use that study to support the same sanitizing agent for all other similar protein mixtures, on similar resin matrices. If we did not adopt such an approach, it is likely that the huge workload currently required for process validation would be doubled. The ability to develop such large matrices for our various processes is reflective of the large number of products that have been validated in Genentech in recent years. A smaller company might not have the luxury of developing the necessary database of information to support a matrix design, but it could use the worse-case scenario to cover not just a single product, but also many future products.

Process validation is a combination of efforts. The importance of documentation is also frequently overlooked. Throughout all phases of development, we try to capture all development work and all decisions in development reports. These reports, in turn, support any questions that the regulatory agencies may have about how a process was developed or why certain decisions were made. These reports are stored in a database for easy access. They also serve as a resource into the development history if an employee leaves and as guidance for new employees.

The biostatisticians are also on hand to assist in the design of characterization studies and to evaluate process robustness, frequently using fractional factorial designs. Sometimes these results are incorporated into resin lifetime studies. Quality assurance, analytical chemistry, and process development groups work closely together to ensure that all appropriate studies are performed, that the data are audited for accuracy, and that reports are generated and approved.

Developing a manufacturing process that will ultimately receive regulatory approval and yield a marketable product requires a development plan that follows regulatory requirements. The development plan must have goals that are met during development before it moves onto the next stage. In addition, we have found that having standardized study designs, protocols, and reports have led to successful validation programs. The program described has undergone four FDA preapproval inspections since 1998.

MEETING THE CHALLENGE

In this article, experts from different companies in our industry have outlined the strategy that they use to arrive at a validated process that can be submitted to regulatory agencies. Although it is interesting to see the different approaches that can be taken, distinct commonalities underlie all the approaches described. The validation approaches discussed here seek to overcome challenges often encountered during development of a validation program for biotechnology products. An acceptable validation program addresses the following difficulties encountered by others:

- Lack of overall strategy
- Failure to consult early with the regulatory authorities
- Inadequate product definition
- Failure to follow CGMP regulations
- Poorly defined cell bank genealogy
- Inadequate analytical procedures
- Too many process changes after validation activities have been completed
- Not enough process characterization or qualification
- Commencing process validation too early
- Inappropriate acceptance criteria.

VALIDATION PERSPECTIVES

We hope that this article is useful to those in the biopharmaceutical industry who are currently involved in validation activities and especially useful to those who are planning to start validation activities in the near future.

This article series presents opinions and viewpoints of some of the industry experts on issues that are routinely faced in process development and manufacturing of biopharmaceuticals.

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