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NEW DIRECTIONS IN BIOREACTOR DESIGN

PEER-REVIEWED
JAPAN COMMON TECHNICAL DOCUMENT: BRACKETING STRATEGIES FOR THE MANUFACTURING PROCESS

PROTEIN THERAPEUTICS DEVELOPMENT
FUSION PROTEINS POSE MANUFACTURABILITY CHALLENGES
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Keeping an Eye on Quality

With infrequent inspections by regulatory authorities—and time lapses between the identification of a violation and remedial or regulatory action—patients place trust in the self-enforcement of quality practices by API and drug manufacturers. How is the bio/pharma industry addressing quality practices?

The number of warnings for API and drug product manufacturing nearly doubled from 2015 to 2016 (1). Typical warning letter violations included failure to validate processes, determine a root cause, implement corrective action and preventive action (CAPA) plans, establish or follow written procedures for the prevention of microbiological contamination, conduct routing environmental monitoring tests, and investigate unexplained discrepancies in batches. The agency also cited firms for the lack of quality plans, supporting data, control over data and laboratory records, and scientific evidence to support procedures.

In fiscal year 2016, FDA issued almost 700 Form 483 observations, slightly above the average issued in the previous three years (2). The most frequently cited observations were:

- Quality control unit procedures were not written or fully followed
- Scientifically sound laboratory controls or procedures were not employed
- Failure to thoroughly investigate discrepancies or failures in batches
- A lack of written procedures for production and process controls designed to assure that the drug products have the identity, strength, quality, and purity they purport or are represented to possess
- Deficient environmental monitoring systems in aseptic processing areas.

What happens when FDA is not watching? In an industry-wide study, the editors of *BioPharm International* examined quality practices as described by experts in quality, regulatory affairs, analytics, and manufacturing and production roles at companies serving the US market. The results will be revealed in a session, Quality Practices: Meeting and Exceeding Regulatory Expectations, at the inaugural CPhI North America event (May 16–18, 2017) in Philadelphia.

The survey examined the role of the quality control department in a bio/pharma organization and its impact on GMPs, the prevalence of quality management systems and quality-by-design programs, factors contributing to quality problems, and the company’s approach to regulatory compliance.

Survey respondents also evaluated the performance and contributions of FDA in providing information to address quality-related problems; commented on the usefulness of FDA regulations, guidance documents, and agency staff expertise; and shared observations on key initiatives such as quality metrics, data integrity, and post-approval changes. Respondents also commented on actions FDA can take to improve the quality of drugs manufactured for the US market.

The study participants also commented on equipment failures, production errors, inadequate method development, and failures by the quality control team. Relationships with contract service organizations and materials and equipment suppliers—including quality agreements and supplier oversight—are also reviewed.

The presentation is scheduled for May 17 at 2:30–3:15 pm in the exhibit hall. Attendees with exhibit hall or higher passes are invited to attend this free session. See www.cphinorthamerica.com for details.

References

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Most Compact
FDA regulation of drug-biologic-device combination products has been a tricky business, with manufacturers struggling to contend with unfamiliar rules and development strategies for these complex therapies. The rise of personalized medicine and technological advances have led to new types of drug-eluting stents, prefilled syringes, autoinjectors, nasal sprays, inhalation devices, and transdermal patches, along with calls for more risk-based and harmonized review, manufacturing, and inspection procedures.

A main challenge for biopharmaceutical manufacturers is to incorporate engineering and design controls for medical devices into quality-based design strategies for a drug or biologic. Policy makers have responded with legislation that streamlines FDA oversight of combination products, while the pending FDA-industry agreement on drug user fees supports efforts to clarify requirements and expand staff involved with reviewing drug-based combination products (1).

These developments reflect increased focus on risk-based manufacturing approaches that adjust testing and oversight to the complexity and function of a therapy. Sponsors may request FDA’s Office of Combination Product (OCP) decide a product’s Primary Mode of Action (PMOA), which determines whether primary jurisdiction for product development, testing, and approval goes to the Center for Drug Evaluation and Research (CDER), the Center for Biologics Evaluation and Research (CBER), or the Center for Devices and Radiological Health (CDRH) (2).

Manufacturer concerns about too-slow FDA evaluation and approval of certain combination products prompted legislators to address these issues in the 21st Century Cures legislation. It clarifies the PMOA designation process, addressing device maker concerns that too many combination products are designated drugs largely based on evidence of chemical action in the patient. The legislation explains how sponsors may request meetings with FDA to discuss testing strategies and post-approval studies, and clarifies how exclusivity applies to combination products with a generic-drug component.

Medical device makers have proposed to further streamline FDA oversight of combination products through a “third track” process for more coordinated combination product review, but FDA officials first want to see how well the Cures legislation improves combination product evaluation, said CDRH director Jeffrey Shuren at a hearing in March 2017 before the Senate Health, Education, Labor and Pensions (HELP) committee on renewing FDA user fees. Shuren commented that FDA has improved combination product review and oversight and that the new initiatives should accelerate timeframes for bringing innovative therapies to market.

PDUFA SUPPORT

The Senate hearing, and similar sessions before House committees, aims to move forward legislation to reauthorize the Prescription Drug User Fee Act (PDUFA), which needs to be finalized by Sept. 30, 2017 to avoid massive FDA layoffs. One provision in the PDUFA VI performance goals negotiated by FDA and industry aims to advance development of combination products. The plan is to tap fee revenues to expand staff across FDA centers and in OCP to provide more expertise for reviewing manufacturing practices, engineering aspects, protocols for human factors testing, bridging studies, and instructions-for-use labeling.

FDA will start by mapping a “lean process” for combination product review and for tracking timelines for cross-center consultations. The agency also will establish a process by late 2018 for resolving scientific and regulatory issues, and to set standard operating procedures for quality assessments, coordinated inspections of different product com-
ponents, and labeling requirements for combination products (1).

A main goal is to clarify procedures for testing combo performance and safety. FDA will issue guidance on bridging studies for combination products with different device components for the same drug, or the same device component across different drugs and biologics. And it will confirm a process for review and evaluation of protocols for human factors studies, with the aim of clarifying how sponsors can assess the adequacy of study designs in terms of patient groups studied, critical tasks evaluated, and proposed endpoints. There will be additional staff training on combination product reviews to ensure consistent approaches in all centers, and an independent third party will identify areas that need better intercenter coordination.

STANDARDS AND SAFETY

These initiatives build on ongoing FDA efforts to improve combination product oversight and policies. A new Pre-Request for Designation option was announced in 2016 that offers manufacturers preliminary, non-binding feedback from OCP on likely classification of a new combination product. FDA issued draft guidance in January 2017 that seeks industry comments on preparing requests for such early advice (3).

Manufacturing standards have been a thorny issue for products with device and drug components. An FDA final guidance issued in January 2017 clarifies where to apply drug GMPs or device design controls and how a lead center should handle manufacturing compliance considerations (4). The guidance addresses industry concerns about cross-labeling requirements, design controls for investigational products, and how to present GMP information in marketing applications.

FDA also published an important final rule in December 2016 on post-market safety reporting for combination products (5). The regulation seeks more consistency in reporting adverse events based on whether a combination product is regulated primarily as a drug, generic drug, biologic, or medical device, and whether serious safety issues are involved. Adverse event reporting can vary notably, with five-day or 15-day reports required for different events involving different products. Beginning in July 2017, manufacturers will need to comply with reporting requirements for the constituent parts of their products, pending FDA clarification through additional guidance.

FDA officials and industry leaders are examining these issues at numerous industry conferences on drug quality and manufacturing innovation. At the PQRI/FDA conference on product quality in March 2017, Douglass Mead, senior director for regulatory affairs at Janssen Research & Development, explained how new GMP rules and design controls based on critical quality attributes of the drug should help devise a broader control strategy for the combination product. Ramesh Raghavachari, branch chief in CDER’s Office of Life Cycle Drug Products, discussed manufacturing processes and quality issues that impact transdermal patches, pulmonary delivery devices, and autoinjectors, all combination products where CDER is the lead regulator. He noted that the device housing for a drug over a long period can impact stability, and that human factors testing is important for ensuring that patients can use inhalers and autoinjectors correctly, particularly in emergency situations.

A January FDA guidance document describing human factors studies for combination products with generic-drug components (6) advises manufacturers to focus on whether differences in the user interface design for a generic or brand drug could impact clinical safety or effectiveness, explained Irene Chan, deputy director of CDER’s division of medication error prevention and analysis, at the PQRI conference. While the device component does not have to be identical in design to that used in the reference product, human factors studies may be needed to ensure acceptable error rates for generic products, and manufacturers should seek to minimize design differences as much as possible. At the same time, the drug component of a generic combination product can demonstrate therapeutic equivalency following usual testing standards.

Drug-device human factors studies were examined further at the April CMC workshop organized by the Drug Information Association. A Parenteral Drug Association interest group meeting on combination products in May 2017 is discussing a range of hot topics related to quality systems and regulatory expectations for these products.

REFERENCES

2. FDA, Combination Products, FDA.org, www.fda.gov/CombinationProducts/default.htm
A growing number of observers, including the Wall Street analysts that follow the publicly-traded contract research organizations (CROs) and contract manufacturing organizations (CMOs), have made statements recently suggesting that the CMO industry is following the trajectory that the CRO industry took in the past decade.

In particular, they are suggesting that CMOs will eventually fulfill a large percentage of global bio/pharma companies’ manufacturing requirements through strategic manufacturing relationships the way that large CROs established strategic relationships with large bio/pharma companies to run their clinical research operations. The corollary of that position is that a small number of large CMOs will come to dominate the industry thanks to their scale, scope of capabilities, and ability to win those strategic deals.

The CRO industry made a great leap forward in the years 2007–2009 when most global bio/pharmaceutical companies turned over their clinical research operations to strategic CRO partners. The strategic sourcing deals coincided with the patent cliff and the global financial crisis, events that created great uncertainty for the bio/pharmaceutical industry; these deals allowed the major bio/pharmaceutical companies to shed thousands of employees and convert fixed costs to variable costs. The five CROs that had attained industry leadership during the previous 10 years—Quintiles, PPD, Parexel, Icon, and Covance—were the major winners of those strategic deals, thanks to their billion-dollar scale, global operations, and financial wherewithal.

It may well be that CMOs will take on a bigger share of global bio/pharma’s manufacturing requirements, and that we will see greater consolidation within the CMO industry. But the nature of bio/pharmaceutical manufacturing suggests that a broad CMO takeover of manufacturing is a lot less likely than the CROs’ takeover of clinical research. Clinical research and manufacturing could not be more different, and extrapolating from one activity to the other can easily lead to the wrong conclusions.

The labor-intensive nature of clinical research has meant that CROs haven’t had to make the big upfront cash outlays that manufacturing requires.

MANUFACTURING IS KEY TO REVENUE
First and foremost, the global bio/pharma business model is to generate revenue by selling manufactured products, not by conducting clinical research. Product supply is crucial to meeting quarterly financial targets, and supply interruptions can damage both the stock price and the brand. Maintaining tight control over supply is a paramount objective, especially for the 20% of products that generate 80% of revenues. Clinical research is necessary for long-term performance, but delayed studies...
usually don’t have the dire near-term business implications that production problems do.

Secondly, manufacturing is capital-intensive while clinical research is labor-intensive. Capital intensity shapes the manufacturing industry because of the strain it places on cash flow: manufacturing capacity is extremely expensive and must be built years in advance of actual production. Thanks to their large size and hefty profit margins, global bio/pharma companies have the cash flow and capital resources to make massive investments in manufacturing capacity and technology. For CMOs, however, cash flow and borrowing capacity are limited and they can’t afford to build substantial capacity years in advance for products whose fate they cannot control.

Global bio/pharma companies spent nearly $125 billion (just 11% of operating cash flow) on new plant and equipment in the past six years, much of it to build the capacity they needed as they transitioned their pipelines to biologics (1). The major CMOs spent about 10% of that amount, but it consumed more than 70% of their operating cash flow. So it will be difficult for CMOs to maintain the scale of investment necessary to meet the manufacturing needs of global bio/pharma companies.

The labor-intensive nature of clinical research has meant that CROs haven’t had to make the big upfront cash outlays that manufacturing requires. CROs can generally hire staff as needed by the contracts in hand; they don’t have to staff up years in advance. So, CROs can fulfill their client’s capacity requirements without the strain on their cash flows.

**FIXED COST HEADWIND**
The fixed-cost nature of manufacturing is another headwind for the CMO industry. It means that as long as global biopharma companies have captive capacity, they will be tempted to keep products in-house or pull product back from their CMOs in order to absorb those fixed costs. It also means that CMO profitability will be negatively impacted whenever a contract manufacturing facility must be operated at a sub-optimal level of utilization.

In clinical research, CROs solve the fixed-cost problem for bio/pharma companies: bio/pharma companies just hire teams for projects as needed and don’t have to pay the staff once projects are completed. There is little need to bring projects back in-house, and generally they have no capacity to do so.

**The fixed-cost nature of manufacturing is another headwind for the CMO industry.**

Finally, bio/pharma companies have a tremendous reservoir of experience for running manufacturing operations. They are often not as efficient and cost effective as CMOs, but there is great depth of engineering, process, and quality knowhow and expertise that only a few CMOs can match. By contrast, most bio/pharma executives would admit that their companies are not great at running labor-intensive activities, and that the increasingly information technology-intensive nature of clinical research is well beyond their competence.

**UNBUNDLED RESPONSE**
CMOs have already won the battle for the manufacturing needs of small bio/pharma companies (at least 80% of their production is outsourced) and mid-size bio/pharma companies (at least 60% of their requirements are outsourced). The path to sustained organic growth runs through global bio/pharma but, as described previously, that path is strewn with some major obstacles.

Forward-thinking CMOs are responding to the challenge by breaking out the elements of the value propositions into its constituent parts, and allowing clients to customize an offering that fits their specific needs. Those elements include capabilities such as facility engineering, process development, overhead sharing, supply chain management, and regulatory knowhow. Already, major bio/pharma companies such as Vertex (with Hovione), Amgen (with Patheon), and Sanofi (with Lonza) have formed relationships with CMOs based on innovative combinations of those value components.

These new unbundled business models highlight one more big difference between the CRO and CMO trajectories. Before they became strategic partners, CROs generally sold individual services like site monitoring and data management to the big bio/pharma companies, but the strategic relationships they formed in 2007–2009 gave them the opportunity to bundle those offerings into full service packages.

CROs are bundling and CMOs are unbundling; clearly the two businesses are moving in completely different directions.

**REFERENCE**
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Enhancing Bioprocessing Efficiencies Through Run Reproducibility

Randi Hernandez

Can bioprocessing runs be consistently replicated in an inherently variable production environment?

Sean J. Morrison, the author of an editorial that analyzes scientific reproducibility, wrote, “Studies with revolutionary ideas commonly lead to many follow-on studies that build on the original message without ever rigorously testing the central ideas” (1). In addition to being a senior editor at the open-source journal *eLife* and an expert in stem cell biology, Morrison is the director of the Children’s Medical Center Research Institute at UT Southwestern, the Mary McDermott Cook Chair in Pediatric Genetics, and an investigator at the Howard Hughes Medical Institute.

If Morrison’s statement is true, the value of follow-on studies may be exponentially diminished, leading to an eventual erosion of scientific understanding over time. Revisiting and testing the protocols that were employed during groundbreaking studies, then, is a crucial element of damage control in the life-science industry. This idea is equally true for studies on bioprocessing techniques, especially as biologic drugs become more complex and new technology to manufacture them emerges.

The inability to reproduce results from a successful bioprocessing run is not only frustrating for manufacturers, but can also increase the costs of drug development and contribute to overall inefficiencies. In 2015, Leonard Freedman—who is the president at The Global Biological Standards Institute (GBSI) and has done work on reproducibility and cell lines—and coauthors estimated that the annual
economic cost of irreproducible research in the life sciences is approximately $28 billion (2). For each run that does not produce the anticipated result, several thousands of dollars are likely lost to reagent and reference material costs, says Freedman. While study design, laboratory protocols, data analysis, and reporting can also contribute to irreproducibility (2), researchers lose precious time when reagents don’t perform as expected—and this is one of the variables of irreproducibility that is likely easiest to control.

Although reproducibility exercises can be expensive, refraining from critically evaluating previous research and conclusions from past experiments may be costlier in the long term than investing in reproducibility studies. If a reagent’s purity and homogeneity is not scrutinized early on, it is possible that studies testing its uniformity will have to be conducted at a later date anyway, contributing to the overall cost of manufacturing operations. Thus, improving the validation and characterization of biologic materials reduces operating costs.

**REPRODUCIBILITY IN PRECLINICAL RESEARCH**

In 2011, researchers from Bayer found they could only validate approximately 25% of the studies on therapeutic drug targets from published data (3).

In 2012, researchers from Amgen were only able to validate 11% (6 of 53) of “landmark” papers that were used to justify the launch of various drug-discovery programs (4). And a 2016 survey published in *Nature* revealed that 70% of researchers have tried to replicate another author’s research findings, but nearly half of these researchers were unable to verify original conclusions (5).

**IN THE LITERATURE: RUN REPRODUCIBILITY IN BIOMANUFACTURING**

Efforts to monitor reagent origin and purity have not been the focus of widespread evaluation in academic papers on bioprocessing. The specific topic of reproducibility in bioprocessing has not been examined in much depth, either, although biomanufacturers do try to understand why high or low yields occur, says Veronique Chotteau of the KTH Royal Institute of Technology, given that yield has such a high impact on the economics of a process and on drug safety. Historically, Chotteau says that variation between runs in the same facility can be up to 50%.

Although process development and characterization improve reproducibility, biomanufacturers still regularly encounter differences between runs, notes Chotteau, who adds that cell growth is rarely exactly the same from batch to batch. Efforts to maintain reproducibility have prompted the biopharmaceutical industry to opt for serum-free media for the manufacture of cellular therapies (6); other studies stress that batch-to-batch reproducibility of glycosylation is essential to get a consistent end product (7).

Measurement of critical quality attributes (CQAs) for each run provides the most important information on run reproducibility, says Richard D. Braatz, the Edwin R. Gilliland professor of chemical engineering at Massachusetts Institute of Technology, who works on the mathematical modeling of bioprocessing plants. “In addition to direct measurement of CQAs for each run, reproducibility from run to run can be improved by operating a plant-wide mathematical model in parallel with manufacturing. Feeding process inputs from the real manufacturing process into the mathematical model would allow the model to make predictions that can be compared with the measurements.” Braatz adds, “Differences between the model predictions and measurements can be used to track changes in operations from run to run, and used to test hypotheses on potential causes,” a technique which he says has been applied in the oil refining, chemicals, and petrochemicals industry for decades, but has so far had limited applications to biologic pharmaceutical manufacturing.

Although some advances in design for manufacturing principles (8)—which factor cost of large-scale manufacture into design plans—have helped the industry plan for gaps in manufacturing reproducibility, and quality-by-design efforts have helped engineers gain better control over resulting outputs, there is little available literature testing the assertions of biomanufacturing models of best practice.

In addition, while there are many experiments in academic literature that use scale-down models to inform large-scale manufacturing, there are few examples of outside labs or researchers testing the so-called “optimal” set-ups touted by the original researchers in the academic papers. Nor are there studies that test what are thought to be the “optimal reference materials” for bioprocessing runs. Industry stakeholders say that a product is defined by a process, but who tests a process once it has been defined?

When a platform process does not deliver acceptable results, says Bruce Hamilton, head of characterization at antibody and life-sciences tool provider Abcam, suppliers refer to literature reports to examine relevant parameters of a product in development. Hamilton adds that process engineers “might also run a small-scale trial scouting lots of parameters
[of the monoclonal antibody (mAb)] as a first test, but this is not primarily guided by the literature."

The most efficient processes are those that can predictably be reproduced run after run. Given the concern circulating the industry about reproducibility in drug development experiments and clinical trials, there should also be industry concern that pivotal bioprocessing experiments cannot be replicated, both in scale-down models and at the commercial level. Production runs sometimes are not similar to one another after a process has been optimized for large-scale operation, or after a process has been transferred to a contract manufacturing organization. Are these run differences a result of subtle changes in protocol and methodology, or are materials the main culprit? When does the ability to reproduce a run correlate with good process understanding—and when is a good run simply a fluke?

"Reproducing a run does not mean a process is understood, only that the conditions perceived to be necessary could be performed again to obtain a similar result," notes Tim Errington, who is meta-science manager at the Center for Open Science and co-leader of the Reproducibility Project: Cancer Biology, a project which is attempting to replicate results from pivotal studies in cancer drug development (9). "Both runs could be the result of some unknown variable not thought to be a necessary part of the process—some aspect of the starting materials or a specific step." Adds Errington, "The reverse is also the case, that an inability to obtain the same result does not correlate with poor process understanding. It could be an undocumented mistake confounding the result. But, importantly, as a result is replicated, it increases the reliability of the process and as more variables not thought to impact the result are varied (different lots/sources of reagents, different personnel, different manufacturing location, etc.) and the same result is achieved, it further increases the reliability and utility of the process."

REPLICATION AND REAGENTS

In the five initial studies that are part of the Reproducibility Project: Cancer Biology, Errington and his colleagues were only able to validate the findings in two of the studies (10). The cancer project is similar to the Reproducibility Project, which was conducted to test the reproducibility of key psychology experiments (11).

Errington and colleagues concluded that while errors can be caused by the improper execution of an experimental technique, they can also be caused by problems with samples and materials. The researchers "undertook authentication of key biological materials (such as [short tandem repeat] STR profiling of cell lines)" to prevent the likelihood that a validation failure was due to error. To improve the probability of replication, the authors of the original studies were asked to "share any original reagents, protocols, and data in order to maximize the quality and fidelity of the replication designs."

C. Glenn Begley wrote in a Nature paper that validation of reagents is a key factor influencing whether a study’s findings can be replicated—and he found that many investigators relied on findings from earlier papers for information on reference material validation, even though many of those preliminary papers did not include specific validation data either (12). Begley added, “There are also examples of investigators using an antibody even when the manufacturer declares it unfit for that particular purpose.” Antibody supplier Cell Signaling Technology (CST) shares that even a validated reagent, if used incorrectly, “can induce changes in the specificity and sensitivity of an antibody.”

Anita Bandrowski, founder and CEO at SciCrunch, the company that runs the Antibody Registry, tells this publication that a reagent from a reputable company should include information about the protocols in which the reagents will likely work best. And, as CST notes, an antibody should be validated using the application in which it actually will be used in practice. The company says that validating with a Western blot, for example, is of little value to inform a researcher about an antibody’s ability to work by immunofluorescence or immunohistochemistry. Even if the correct validation method is chosen, Karin Lachmi, PhD, co-founder, chief scientific officer, and president of Bioz, a public website that chronicles reagent mentions in methodology sections of scientific studies, notes that achieving the exact binding or enzyme level in an assay is still difficult: “The cause of this inconsistency can be attributed to quality variability in antibody/enzyme batches, different target systems, and different techniques” used in labs.

Exact reagents are sometimes difficult to pin down because an original researcher has left an institution, taking his or her knowledge with him or her. A 2013 paper that reviewed several hundred journal articles found that 54% of resources were not uniquely identifiable in publications (13).

When reagents are not validated, researchers may draw conclusions from their experiments that are simply inaccurate. They run the risk of using an antibody that recognizes the wrong protein, or may base conclusions on a cell line that is not of interest.
to researchers. To make the situation even more problematic, sometimes antibodies cross-react and recognize the wrong targets (14), which is why some researchers use Western blots that rely on the recognition of at least two different epitopes. Gathering appropriate reagents, doing controls, and optimizing conditions is a major time sink, asserts Bandrowski, who is an expert on citing biological reagents. “If the original protocol is not adequately described, including a poor text description of the reagents, this part of the experiment can take weeks or months instead of days to weeks,” she says.

Although suppliers should make their best attempt to alert purchasers when changes in reagents occur, this can be challenging, says Bandrowski. “In academic labs there is a purchasing department and [supplier] companies often can’t track down who is actually making the purchase and using the product, so they are stuck with adding this information to their website and hoping that their customers come back before publishing,” Lachmi argues that revenue generation is a supplier’s top goal, and that currently, “changes that occur to reagents are insufficiently characterized and documented.” Because vendors have no incentive to alert customers about reagent changes, she reasons, a “vendor-neutral” clearing house such as Bioz is necessary to guide buying decisions.

GUIDELINES FOR REPRODUCIBILITY
NIH suggestions
Freedman says that to overcome the problem of reagent identification, standards and verification strategies for reagents must be developed for all reference materials (15). National Institutes of Health’s (NIH’s) Rigor and Transparency Guidelines, which went into effect in 2016, required that any researchers wishing to receive a grant for their research must incorporate a validation/authentication plan for every study (16). According to a section of the guidelines called “Authentication of Key Biological Resources in Grant Applications”, antibodies should be “validated by Western blot, ELISA, immunoprecipitation, immunofluorescence, or flow cytometry using knockdown cells and positive and negative controls, depending on the assay proposed” (17).

Industry stakeholders say that a product is defined by a process, but who tests a process once it has been defined?

A big part of the scientific rigor that NIH supports—through the thorough description of methodology and experimental design—relies on the ability of outside scientists to replicate the study’s findings in some incarnation. Whether it is statistical similarity, or similar effect size, some of the parameters must be met. As the American Statistical Association suggests, “Reproducibility shouldn’t be thought of as a binary state—either reproducible or not reproducible—but as a continuum from hard-to-reproduce to easy-to-reproduce” (18).

In 2014, the NIH developed a Data Discovery Index where researchers could analyze raw data from experiments, and a commenting system in which researchers could contribute their concerns about research protocols and methodologies (19, 20). NIH’s Resource Identification Initiative (https://scicrunch.org/resources) was launched to help study authors authenticate (as well as properly cite) organisms and cell lines (21). Errington argues that if all data and methodology items in a paper were automated and shared, this would further drive the discoverability and reuse of information. He says the burden to communicate research findings should not solely be on the researcher, but should be built into the “research ecosystem.”

CHARACTERIZING ANTIBODY/REAGENT PERFORMANCE
Because there are so many ways to get to a final product, and the types of products that are now in development are becoming increasingly complex (e.g., incorporating fusion proteins and bispecific antibodies with multiple active domains), researchers need to be as specific as possible when it comes to describing the starting raw materials used in studies and the characterization methods that were used to validate starting reagents. According to Freedman, there are some standards developed for antibody validation and cell-line authentication, but there is currently no standard for reporting serum characteristics (15).

Proper citation of an antibody is necessary for study reproduction, insists Tove Alm of the School of Biotechnology in the KTH Royal Institute of Technology. She says The Antibody Registry (http://antibodyregistry.org) assigns stable, unique identifiers (called Research Resource Identifiers, or RRIDs) to antibodies, which protects their identification should the companies that provide antibodies undergo mergers, acquisitions, or brand-name changes. Even antibodies under development (i.e., non-commercial anti-
bodies) are assigned RRIDs in the Antibody Registry, says Alm. Assigning an RRID assures a product is “machine readable,” says Errington, which is important to track a product’s use and utility in an experiment. According to those who run the portal Antibody Registry, in 2017, more than 1% of papers (approximately 1700 papers) in biomedicine publish RRIDs for reagents—which is an increase from 2014, in which approximately less than 0.02% of papers included this information (22).

The publication Nature requires that antibodies included in experiments be identified by genus and species and that trial sponsors list the citation, catalog number, and clone number for antibodies used in investigation (23). It also suggests adding information about the validation of an antibody, which is information that can be obtained through portals such as Antibodypedia, 1DegreeBio, and Biocompare. Other sites, such as Addgene Vector Database, allow users to search for expression vectors in published studies. Cell Press implemented STAR Methods in 2016, which are guidelines for reporting. Cell references Nature’s requirements, as well as the National Centre for the Replacement, Refinement, and Reduction of Animals in Research’s ARRIVE guidelines, which were first published in PLoS Biology (24).

Another reproducibility initiative is the Center for Open Science’s Transparency and Openness Promotion (TOP) guidelines, which seeks to encourage “shared standards for open practices across journals” (25).

Antibodypedia is a database that allows antibody manufacturers to submit validation data on antibodies, inviting them to divulge information on antibody performance in different applications using “pillars” of antibody validation—first described by Uhlen et al.—to help researchers and editors determine if an antibody is properly validated and appropriate for a specific experiment. The thought is that through use of at least one of these parameters measuring antibody specificity, researchers can more accurately predict antibody performance and reproducibility of antibody function (26). One of the pillars described by Uhlen et al. is the use of an orthogonal strategy where an antibody-based strategy is compared with an antibody-independent method; to verify antibody specificity, the level of detected expression of the antibody’s target protein using a specific application (e.g., Western blot) is compared with the level of the target protein that is identified using mass spectrometry.

Antibody supplier CST says it defines the models to be used to validate an antibody in tandem with the selection of new targets for antibody development. This information includes definition of the most critical applications for which the antibody should be used, which cell lines and tissues express high or low levels of the protein in question, the feasibility and availability of knockdown or knockout reagents, and what orthogonal methods would help validate a new antibody. CST adds that if an appropriate validation system is not known when an antibody is under development, when a method becomes available, the company retroactively validates the product. CST says besides being one of the few companies that validates its products, it is also one of the only companies that prefers to sell reagents by dilution instead of concentration, because “changes in assay performance are often specific to a single assay instead of antibody performance overall.”

CST admits that as its portfolio of polyclonal antibodies has grown, so has the burden to generate consistent product lot to lot—a main factor that drove the company’s decision to move toward rabbit mAbs, which they say are less variable. However, the company says it still sells polyclonal antibodies because they are inexpensive, easier to generate, and are still preferred by many scientists. The company says this preference is likely due to the fact that polyclonal products are “highly cited in the literature” already. Lachmi concurs that researchers rely on literature sources (typically through PubMed, she says) for reagent identification and selection. “Market studies have demonstrated that product use in journal publications is the number one attribute that drives the purchase of life-science reagents by researchers,” she remarks.

Some of the latest techniques to validate antibodies include using mass spectrometry, peptide arrays, and knockout cell lines, says Hamilton. By using knockout cell lines—which were produced using clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 and Horizon Discovery’s library of haploid human cell lines—Abcam was able to validate more than 1000 antibodies. Abcam’s push to authenticate antibodies, dubbed the Knockout Validation Initiative, began at the end of 2015 and is still an ongoing project.

SECOND-HAND ANTIBODIES

The resale of antibodies has become a particular concern as of late, especially when a researcher encounters an antibody that does not work as intended, or if he or she is looking for two antibodies binding to different parts of a target, notes Alm. It would be most helpful if antibody pro-
ducers shared target sequences or provided details about epitope mapping studies, says Alm, but she says this information is typically not provided. She says using the compare function in Antibodypedia can usually help researchers tease out when an antibody is the same as one they have already tried, but is just being resold by another company. She adds that when identical images are presented for antibodies with different names in the portal, it is likely a case of antibody reselling at play. CST comments that product clones are sometimes obvious because of the inclusion of a company’s clone name or identifier, but that some suppliers intentionally leave information about clonality obscure.

According to Bandrowski, antibody reselling is a dangerous game, primarily because problems with an original product being resold may not be detected if there is no clear chain of provenance from which the reagent came. “Think of it as the discovery of E. Coli on a batch of lettuce from a farm—that lettuce may have entered many products and a recall needs to be complete otherwise people will get sick,” she says. “In the scientific literature, there is no mechanism to create a recall and this cripples all efforts to improve reproducibility in science.”

Even with a properly referenced RRID, supplier changes still confound the source of some products, says Bandrowski, mostly because people hold onto their products in deep freezers. She recalls an instance with a Chemicon product that was listed with its RRID in a paper, but was cited in different ways by other researchers referencing the paper. “By this time, Chemicon had been part of Millipore for over eight years and Millipore was transitioning between EMD and Sigma. Another author using the same product cited the company name as Chemicon/Millipore and a third [cited it as] EMD Millipore. She adds, “This is actually one of the easiest cases to deal with because even though we had some confusion about which product came from which company, the smaller companies were bought in whole and their catalog numbers did not change.”

Verification studies could test processes that are considered “the gold standard” in the bioprocessing industry.

CHARACTERIZING CELL LINES

The misidentification of cell lines has likely affected the conclusions of hundreds of thousands of papers, according to researchers (27). An editorial in Nature Cell Biology found that during a five-month period in 2013, only 19% of papers performed cell-line verification studies (28). And, according to numerous studies, approximately one-third of cell lines contain a mixture of species types, are contaminated, or are misidentified (29, 30). Another paper found that only 43% of cell lines were identifiable (13). As Bandrowski points out, some argue that product codes (outside of an RRID) should be generated for the use of the same cell line by two or more companies, “because the quality control steps in various companies can vary drastically—or at least the products at the end of the process are different.”

Agencies such as NIH are asking that cell lines be authenticated by chromosomal analysis or STR profiling prior to the agency making any decisions on fund allocation for grants. Many journals are now asking that submissions identify the cell lines used in experiments. Nature guidelines require researchers to clarify if their cell line is among those that have been commonly misidentified according to the International Cell Line Authentication Committee (ICLAC) database, and provide justification for using the cell line they have selected. Of the ICLAC database’s 488 listed cell lines, 451 are misidentified and have no known authentic origin (26, 31).

Surprisingly, some stakeholders from the pharmaceutical industry think that regulatory agencies should focus more on the quality of a finished drug product than on the cell lines used to produce biologics. In a piece that appeared in the journal Biologicals in 2016, representatives from Eli Lilly, Amgen, Biogen, Genentech, Janssen, Bristol Myers Squibb, and Pfizer wrote that regulatory agencies should not focus on the “clonality assurance” of cell lines as heavily. They wrote the “genomic plasticity” of immortalized cell lines precludes “absolute genetic homogeneity,” so cell lines should not be referred to as clones; rather, cell lines are a population of cells that are “clonally derived” (32).

The representatives from pharma explained that when cells are grown in culture, genetic and phenotypic changes occur as a result of drift, regardless of where the precursor cells came from. The drift can occur even in the absence of any contamination. The ability of these cells to undergo genetic change is what allows them to accept transgenes via genetic engineering and adapt to changing process conditions,
the authors argued. They noted that cell lines cannot be adequately controlled, and say that genetic heterogeneity in cell lines has not been measured by most cell-line vendors. Furthermore, they pointed out that many vaccines originate from non-clonal cell lines—and despite this fact, vaccines have a good track record of safe and effective use. While the authors acknowledged that a clonally-derived cell line can reduce the resulting heterogeneity of a cell population, they emphasized that health authorities should put more of a focus on the purity and stability of the final proteins produced from the cell lines, rather than on the cell lines themselves.

However, because genetic changes to cell lines can occur simply as a result of being cultured repeatedly (15), Freedman counters that periodic cell-line authentication studies should occur numerous times during the course of an experiment. Errington notes that it is also valuable to measure the flexibility of raw materials, or their ability to undergo small changes while supporting the same experimental outcome.

**FURTHER EFFORTS TO STANDARDIZE BIOLOGICAL RESOURCES**

GBSI’s Freedman, an outspoken advocate of reagent validation, is currently working with stakeholders (including members of the industry, academic leaders, funders, and journal editors) to develop antibody validation guidelines. He says, “The producers engaged with our initiative are committed to assuring that quality antibodies are made available and developing new standards for quality assurance/quality control [QA/QC],” and shares that some biopharma players have already developed in-house QA/QC systems for the selection of cell lines.

The National Institute of Standards and Technology (NIST) launched a project in February 2017 to try to characterize the mouse cell lines that are using in the biomanufacturing of recombinant proteins (33). The number of short tandem repeat (STR) markers present in an assay is specific to an individual mouse cell line within a species, which NIST is able to measure with its new invention. NIST was granted a patent for this assay, which it says is the first of its kind for mouse cell lines (34).

The Medicines and Healthcare products Regulatory Agency (MHRA) in the United Kingdom is taking validation for stem cells into its own hands, announcing in February 2017 the launch of “regulator ready” stem cells for use in clinical development. The cell lines, released by the not-for-profit UK Stem Cell Bank (UKSCB) at the National Institute for Biological Standards and Control, will come with a certificate of analysis. UKSCB is also working on including a “starting materials dossier” for each of its cell lines to further inform researchers (35).

It’s clear that standardization, coupled with the public funding for the research of manufacturing best practices (such as the initiatives launched by NIST), can help shed light on the performance of a process by unearthing details about starting materials. Reputable suppliers should also support this approach, says CST: “Validating and optimizing a reagent prior to use should be a requirement for academic training, publication, and funding.”

**REAGENTS IN CONTEXT**

Although there are ongoing efforts to provide open access to certain experimental data published in scientific journals (The Gates Foundation; The Wellcome Trust; and various preprint servers, such as the OSF Preprints, arXiv, bioRxiv, PeerJ, etc.), there are also companies that are beginning to emerge that seek to bolster protocol transparency. Ultimately, access to protocols in high-profile journals may help drive reagent selection and/or purchasing decisions by researchers in the industry—but because of paywalls, much of this information remains under lock and key. As Alm tells this publication, the success of an antibody in particular is dependent on its application and the context where the antibody will be used.

To search for how reagents are used in a specific context—for a precise biomedical application—academics can use online tools such as Bioz. Lachmi argues that researcher authentication of every reagent is impractical, and instead, researchers need “structured scientific article data to bring to the surface unbiased reagent and assay ratings.” A sample search of antibody vendors in Bioz, for example, yields 37 suppliers, although two of the oft-mentioned suppliers located in the United States—Sigma-Aldrich and EMD Millipore—have since merged and are now one company (MilliporeSigma), but sell under two brands. The suppliers are organized by category and assigned an aggregated rating ranging from 1 (concern) to 5 (very good). The rating is calculated by way of an algorithm. The algorithm is governed by nine parameters, which include the consideration of how recent a paper is, its protocol relevance, and the impact factor of the journal in which the reagent was mentioned.

Invitrogen, Abcam, Santa Cruz Biotechnologies, Jackson Immunoresearch Laboratories, CST, Covance, Life Technologies, Aves Labs, and GeneFex are among the 10 most highly rated companies within the antibody space, according to a Bioz search. Although some
smaller antibody vendors appear in the list (albeit with mostly lower aggregated ratings), the largest vendors appear to dominate—a fact which is not surprising when one considers that most of the largest vendors actually buy antibodies from smaller vendors and relabel them to beef up their overall antibody portfolio offerings (36).

Lachmi states that the platform pulls information from 26 million life-science articles across 5000 academic journals, and the website already has 250,000 users from 191 countries. Plus, estimates Lachmi, “The number of Bioz users is growing rapidly, at a rate of 10% per week.”

**REPRODUCIBILITY BY DESIGN**

Rather than risk sourcing antibodies from a vendor with which a researcher has no experience, a better option may be for the industry to shift paradigms and use standardized antibodies made in recombinant technologies that could inform methods of antibody manufacture, and the techniques that are necessary to isolate and purify antibodies. Changing the way antibodies are made to better serve potential clients could also make an antibody supplier vulnerable to patent infringement claims (38). Regardless of clonality or the antibody production method chosen (i.e., recombinant vs. hybridoma), CST says a bulk of the company’s activities would still be ensuring that each new lot performs identically to the previous lot.

Instead of changing the composition of all of the reagents currently in commerce, GBSI supports a simpler approach—encouraging reagent vendors to refrain from selling any products that aren’t validated. This change of paradigm, however, must be supported by all industry stakeholders to be truly effective, insists Freedman.

**CONCLUSION**

Reproducibility is crucial to the advancement of the field of bioprocessing, especially when it comes to testing new methods or technologies that could inform more cost-effective manufacturing techniques. “What I find most amazing in all of this is that biopharma loses millions of dollars in dead-end R&D and has not, thus far, stepped up to the plate to help NIH and journals improve reproducibility in science,” comments Bandrowski. While unknowns about reagent origin and composition can dramatically skew reproducibility attempts, Freedman reiterates that flaws in study design, laboratory protocols, and data analysis/reporting can also render an experiment irreproducible.

In a “publish-or-perish” world, there is not as much pressure (or incentive) to reproduce previous experiments. The focus of many new studies is to explore a brand new concept, and there is also little interest in funding replication studies. Verification studies, however, could test processes that are considered “the gold standard” in the bioprocessing industry and could uncover significant economic inefficiencies. The ability to replicate may be especially important for the optimization of end-to-end continuous processing for biologics, as much of the new published material on continuous processing for biologics focuses on finding the technologies, the sequence of processing steps, and the combination of reagents that will result in the highest yield and purity possible. Errington says he thinks an initiative for informing bioprocessing techniques, one similar to the Reproducibility Project: Cancer Biology, would be “extremely beneficial,” adding, “I’d be happy to have discussions with others to organize a similar project.”

Errington says that another useful idea would be to “conduct a many-lab study to test the reliability of specific bioprocessing techniques” and to measure how certain techniques vary across facilities.

It is also possible, of course, that replication studies could be the source of new ideas, concludes Bandrowski. “The results that are not robust to replication may not be incorrect, but highly dependent on something that is not being directly controlled—some of the attempts to reproduce a study, when published, can lead to important discoveries.” She quips, “With a good, well-controlled experiment,
The bioreactor is a standard device in processing streams, and could be considered the workhorse of a bioprocessing train. Thus, ongoing research seeks to explore how to optimize their performance, with studies that focus on bioreactor modeling, feed schedule manipulation, designing tanks and impellers to support specific cell types, and efforts to switch fed-batch bioreactors to continuous modes.

Also important is a bioreactor’s ability to produce proteins at a consistent level, with reproducible levels of product from run to run. BioPharm International spoke to Ken Clapp, product manager in the bioreactor division at GE Healthcare Life Sciences; Peter Levison, PhD, senior marketing director of downstream processing at Pall Life Sciences; and Joe Capone, global product manager at Pall Life Sciences, to find out more about how engineers are optimizing the capabilities of bioreactors.

MULTIFEED PROCESSING

BioPharm: How could multifeed processing improve reactor productivity?

Clapp (GE Healthcare Life Sciences): Providing and controlling feeds necessary for optimal cellular metabolism would certainly improve bioreactor productivity. Keep in mind, a cell-culture bioreactor, regardless of physical design, instrumentation, and control system, acts as a substitute for the originating whole organism.
In the organism, productivity is a matter for staying healthy and alive; [cell health is characterized by the] adequate availability of nutrients and minimal exposure to waste with no physical damage. Commercially available bioreactors are used with ‘cells’ of the complex multicellular whole organism for the purposeful, preferential production of a therapeutic protein at an appropriate industrial scale. The more a bioreactor mimics the native environment, the better [it is] for the cells, and presumably, for their production of the protein of interest.

PERSONALIZED BIOREACTORS

BioPharm: Will bioreactors be optimized to support cells specific to certain diseases?

Clapp (GE Healthcare Life Sciences): Alternates to stirred-tank bioreactors have been under investigation for decades. Under the current paradigm, large-scale industrial bioreactors are used to produce proteins for the treatment of disease; the one-to-many approach.

In the future, will we see practical implementation of the one-to-one approach, in which each patient has or is the bioreactor? Yes, to a certain extent. Perhaps I see this question posed an alternate way: Will bioreactors emerge that are optimized to support specific cell types? There are many bioreactor designs in existence, from very simple to complex. Some are scalable, some are not. Some are commercially viable, others not. Research is ongoing for established designs as well as for new designs, with the intention to improve the growth environment for better, more specific productivity.

Fluid dynamics is a focus area, as chemical and physical cellular stresses can have catastrophic results. The productivity in terms of protein titer we see today was unheard of not so many years ago.

If the future of personalized therapies involves bioreactors tied to individual patients, then, yes, bioreactor design and optimization must occur to realize this possibility at the patient-disease level. The bulk of today’s therapeutic proteins are produced in stirred-tank bioreactors with adapted, industrialized cell lines.

RUN REPRODUCIBILITY

BioPharm: How can bioreactors be best designed to ensure reproducibility between runs?

Capone (Pall Life Sciences): Equipment robustness and automation are key drivers to performance consistency. Designing bioreactors using basic systems engineering principles, including bioprocess, mechanical, production, and control engineering, can ensure robust and reliable performance (1). In addition, a big effort has to be pushed towards eliminating human intervention—which is still a very large cause of process variation. As such, new single-use bioreactor designs—including ergonomics to ensure easy assembly and efficient operation of the equipment, automated biocontainer inflation sequences, and protective packaging solutions—are a large step in the right direction.

Clapp (GE Healthcare Life Sciences): There are certainly variables such as cell culture media formulation, operator error, and cell heterogeneity, that are beyond the bioreactor’s design space. Of these [variables], operator error can be minimized with an appropriate amount of automation and the application of good industrial design principles that make use of intelligent user interaction concepts within the operational context.

More generally, since the bioreactor is mimicking an organismal environment, concentration gradients and mechanical stresses on cells should be reduced or eliminated. Concentration gradients cover gas exchange, nutrients, and metabolic waste products. Mechanical stresses encompass shear and impact. A bioreactor designed to foster the necessary growth environment, while not causing excessive damage in its mimicry, along with enforcing operational consistency has the best chance for reproducible runs (note: ‘reproducible’ is taken as consistent protein production).

BIOREACTOR MODELING

BioPharm: If you had to estimate, how many manufacturing organizations actually perform bioreactor modeling experiments to drive their knowledge of their process?

Clapp (GE Healthcare Life Sciences): I would estimate that nearly all our customers are doing some level of modeling, inclusive of biological process, bioreactor, and operations (manufacturing) modeling. I see this as a prerequisite in quality-by-design (QbD) activities, process scale-up, and manufacturing optimization.

I would also suggest that the interest in continuous bioprocessing makes not only bioreactor modeling necessary, but also [requires] a broader modeling of the integrated process, such as the interdependence on upstream and downstream processes. This [approach] is imperative for a process destined for manufacturing, where reproducible, reliable protein production is required. Available computing power and modeling software tool improvements make modeling much more accessible and practical in all the areas mentioned.

MEASURING CELL HETEROGENEITY

BioPharm: What are typically the tools of choice to measure cell heterogeneity inside a bioreactor?
Clapp (GE Healthcare Life Sciences): In-situ measurement, or analysis of cellular heterogeneity measurement, is not something I typically associate directly with a bioreactor, especially not large-scale, stirred-tank pilot or production bioreactors. Tools useful for heterogeneity include techniques for gene expression, RNA analysis, and other single-cell sequencing techniques; none of which have been reduced to practice in real-time, in-situ bioreactor environments. The basic presumption is that the cell population within a bioreactor is homogeneous for the purpose of producing a desired protein.

Within this population, however, variation in cells’ genomic states, their external microenvironment, as well as their intra-cellular chemical constituents, are known to cause variations in protein expression and function. Extending this answer to encompass the consequences of protein variability on therapeutic effectiveness would be overreaching here. Considering a homogenous population, cell density and viable cell density devices are available and used routinely with bioreactors of all types—autoclavable, in-situ sterilizable, and single-use. Viable cell density has the advantage of determining living cell concentrations over dead or dying cells, which is more relevant. These cell density devices are practical as in-situ elements of real-time, closed-loop feedback control. Knowing that cell heterogeneity gives rise to a heterogeneous protein pool, measurement of a target protein to assess, indirectly, the cells’ condition has some possibility; but [is] difficult in practice.

END-TO-END CONTINUOUS MANUFACTURING

BioPharm: A truly, end-to-end continuous set-up is being investigated by numerous labs and research teams. What technology or advancement will be crucial to the seamless integration of an upstream bioreactor and downstream processes?

Levison (Pall Life Sciences): Currently fed-batch bioreactors dominate cell culture processes and these produce a bioreactor volume of harvested cell culture fluid (HCCF) from which the product is purified and formulated to give bulk drug substance (BDS). The HCCF can either be processed in batch or in a continuous manner to yield BDS, but in either case, the batch size is determined by the bioreactor volume used initially.

To move to a fully continuous state, there is a requirement to have a continuous feed of HCCF on a longer-term basis, which can then be processed on a continuous basis. To achieve this, there are two approaches that are being contemplated:

- A series of fed batch bioreactors being used in parallel, expressing the same product, but harvested on consecutive days in rotation. This method effectively provides a continuous supply of HCCF as you cycle around from bioreactor to bioreactor.
- The move to a perfusion cell culture process—which has been making a resurgence in market interest as of late—where a single bioreactor will be run for many weeks or months producing the same product. A volume of cell culture media, containing the product, is drawn off each day (typically 0.5–2 bioreactor volumes per day), which can be sent for processing to yield BDS; the bioreactor volume is continuously replenished with fresh culture media. By integrating a perfusion process, or using multiple fed-batch bioreactors in the downstream, we start to see the reality of continuous processing come to life (2).

Clapp (GE Healthcare Life Sciences): An end-to-end, continuous set-up is broader than just aligning the bioreactor and the receiving downstream process(es); it also must include feeds like media and nutrients; it must account for the removal of excess and unwanted constituents such as cells and cellular debris. There is a basic presumption that there is sufficient knowledge about the cellular protein productivity and the choice of filtration and chromatography mediums downstream.

With that understanding, advancements in control system creation that associate two or more unit operations seems obvious. Included in the control system design would be appropriate sensors for closed-loop, feedback control of the multi-unit operation process train. Absent sensors specific to, say, a protein of interest, an indirect measurement method may suffice. In-process measurements are preferred over those that require sample removal and processing prior to value’s availability, due to measurement lag and ineffective use in real-time control.

Technological advancements in sensors, especially those able to measure critical biochemical parameters, in-process and in real-time would be essential to progress in this area. Until which time, a mixture of existing devices could be pieced together for [an end-to-end continuous] result.

REFERENCES

As the number of biopharmaceuticals in development grows, there is a trend toward using a greater range of analytical techniques throughout product development. In particular, mass spectrometry is emerging as a routine technique in downstream laboratories for monitoring product quality and assessment of critical quality attributes (CQAs). In this webcast, we will discuss a streamlined mass spectrometry solution that enables users to generate high quality data for routine biologic analysis.

Key Learning Objectives

- How to make key biopharma assays routine with dedicated LC-MS workflows
- How to streamline biologics characterization with dedicated acquisition and processing software
- How to monitor CQAs across batches

Who Should Attend

- Research scientists and lab managers in biologics development, downstream bioprocessing, and process development

For questions contact Ethan Castillo at ethan.castillo@ubm.com
Continuous downstream bioprocessing is proving its worth, but connecting different operations and integrating upstream remains a challenge.

Today, downstream bioprocessing facilities are taxed as never before. Growth in demand for monoclonal antibodies (mAbs), expanded biosimilars research, and upstream productivity advances have meant larger numbers of smaller batches and much more product to purify.

Factor in the impact of patient-friendly formulas that require smaller, less frequent doses of more potent drugs, and the need to work with new materials with different impurities (e.g., antibody fragments and cell therapies), and many companies are questioning just how well, and how long, their batch-based bioseparation operations can keep up.

Adding motivation for continuous bioseparation is public outcry at drug pricing and the movement to reduce the cost of goods. Downstream batch bioprocessing, which relies heavily on multistep processes, large volumes of buffers, and tools such as Protein A resins, which can cost $15,000/L, contributes significantly to cost.

If a Toyota-trained engineer were to examine downstream batch-based bioseparation, it would be easy to find examples of waste. For instance, chromatography resins’ binding capabilities are never fully used, hold times are long, and equipment only runs a small percentage of the time, Michael Egholm, president of biopharmaceuticals at Pall Life Sciences, noted in a 2015 presentation at BPI Boston (1).

Adding motivation for continuous bioseparation is public outcry at drug pricing and the movement to reduce the cost of goods. Downstream batch bioprocessing, which relies heavily on multistep processes, large volumes of buffers, and tools such as Protein A resins, which can cost $15,000/L, contributes significantly to cost.

If a Toyota-trained engineer were to examine downstream batch-based bioseparation, it would be easy to find examples of waste. For instance, chromatography resins’ binding capabilities are never fully used, hold times are long, and equipment only runs a small percentage of the time, Michael Egholm, president of biopharmaceuticals at Pall Life Sciences, noted in a 2015 presentation at BPI Boston (1).

**PROCESS INTENSIFICATION**

Vendors and manufacturers are addressing these problems via process intensification, processing smaller volumes of material semicontinuously or continuously. “Process intensification with connected purification steps, and semi-continuous chromatography systems, is being tested and scaled up to pilot and production scales,” says Kevin Tolley, senior applications scientist with Thermo Fisher. “As additional product and process information is gathered and system complexities are better understood, the momentum in this space will increase to support much wider adoption,” he adds.

**SINGLE-USE SYSTEMS ARE PLAYING A MORE PROMINENT ROLE**

At the same time, single-use product development is progressing in continuous downstream separations. “When we look at how best to facilitate process intensification, continuous technologies designed as single-use systems show the most potential, while reducing set-up times by eliminating the need for cleaning and cleaning validation,” says Peter Levison, PhD, Pall’s senior marketing director of downstream processing.

New equipment and materials (e.g., resins, filters, and membranes) that are being tailored specifically for continuous downstream bioprocessing include:

- Continuous depth and tangential flow filtration via single-use membrane filtration systems such as Pall’s Stax and Millipore’s Clarisolve.
- Periodic countercurrent chromatography (e.g., GE AKTA’s PCC)
- Simulated moving bed (SMB) chromatography, such as Pall Corp.’s Cadence BioSMB; Semba Biosciences’ ProGMP; Sanofi’s Accelerated seamless antibody purification (ASAP); and ChromaCon’s CaptureSMB, the first GMP-scale version of its ContiChrom lab-scale systems, which is manufactured by Lewa Process Technologies and was commercialized in June 2016 (2)
- Sequential multicolumn chromatography (e.g., Novasep’s BioSC)
• Sequential continuous countercurrent tangential chromatography, such as Chromatan’s ICS
• Single-use chromatography membranes such as Natrix Separation’s HD
• Monoliths and novel adsorbents, including BIA Separations’ CIM, BioRad’s CHT, and Purifidify’s FibroSelect, which is being evaluated by GlaxoSmithKline (3)
• Expanded bed adsorption (EBA), as applied in DSM’s Rhobust platform (4).

“Innovators are still working hard to create large-scale purification or separation technologies that can be adopted in mainstream bioprocessing,” says Eric Langer, principal with BioPlan Associates. “It’s not problems with specific existing equipment or resins, but the lack of adaptable technologies that appears to be the challenge,” he says.

“Many unit operations need to work together if a continuous downstream biopharmaceutical processing strategy is to succeed. Incremental process intensification may be helpful, but won’t radically change manufacturing paradigms in the near-term,” says Langer.

Others see industry and regulator conservatism as the greatest challenge. “Continuous methodology is based on proven, existing batch methods, so there is not an introduction of anything ‘new’ per se. The real obstacles are more intangible. They come in the form of an industry that is not only very resistant to change, but also highly regulated, often with established platform processes,” says Levison. New facilities and new product introductions currently offer the greatest opportunities for continuous bioseparations, he says.

Biopharmaceutical manufacturers and technology providers are evaluating continuous processes and combining different solutions, a growing number of which are single-use, to reduce the overall number of downstream processing steps needed.

**REDDUCING THE NEED FOR CENTRIFUGATION STEPS**

Among the solutions are acoustic wave separation, which Pall developed to reduce the need for centrifugation and filtration steps in clarification; membrane separation; and DSM’s EBA, which is said to reduce filtration, centrifugation, and chromatography to a single step. Adoption of multicolumn chromatography and SMB is also moving forward, says Langer. “These technologies have been around for at least a decade, but now complexities and problems of multi-channel operations are being addressed, and scales are increasing,” he notes.

Process intensification efforts continue, but adoption of continuous downstream bioprocessing may reflect industry conservatism and familiarity with established platform processes.

Vendors have performed extensive studies on how continuous downstream platforms might reduce overall biopharmaceutical costs and timelines. In late 2015, Pall introduced its Cadence portfolio, with products designed to enable scalable continuous bioprocesses from process development through GMP production, says Levison. It included the Acoustic Separator to remove debris from harvested cell culture fluid and reduce the need for traditional centrifugation; BioSMB PD and Process platform of single-use multi-column chromatography solutions for bind/elute (using Protein A affinity, ion exchange, mixed mode, and hydrophobic interaction) and flow through methods such as size exclusion or ion exchange polishing; and the Inline Concentrator, which enables single-pass tangential flow filtration (SPTFF) technology for process intensification.

In 2016, Pall researchers evaluated the impact of this platform with anion exchange (AEX) membrane adsorber and mixed-mode cation exchange chromatography (5). They found that the productivity of Protein A separation improved by 74% with the continuous process. Productivity of the cation exchange step increased six fold, contaminant removal improved by 4.5 log; and soluble aggregates were reduced by 50%. In addition, the volume of Protein A resin required was reduced by 95%, and buffer, by 44%.

In 2015, studies by EMD Millipore (6) evaluated a bioseparation platform for mAbs based on capture chromatography using continuous multicolumn processes and incompressible Protein A, as well as precipitation and single-use depth filtration, anion, and cation exchange. Not only did the researchers find that the platform would reduce equipment costs by more than 55%, footprint by 32%, water use by 85%, buffer requirements by 58%, and overall time by 45%, but that it reduced overall unit operation costs by 21% at the 3-kg level, and by 29% at the 15-kg level (7), suggesting continuous downstream processes’ potential.

**INTEGRATING UP AND DOWNSTREAM**

Some research projects have the ambitious goal of integrating upstream with downstream continuous biopharmaceutical processing. In December 2016, for
example, Univercells, a vaccine and drug development company, received a $12-million grant from the Bill and Melinda Gates Foundation to develop a continuous biopharmaceutical platform that would incorporate continuous processing and process intensification, both up and downstream.

Using perfusion upstream and Natrix Separation’s single-use Protein A capture chromatography membrane downstream, the company plans to develop a portable micro-manufacturing cabinet that could be rapidly deployed wherever and whenever needed (8).

**MEMBRANE SEPARATION SYSTEMS UNDER DEVELOPMENT**

Downstream, the Natrix membrane is being designed to offer a 30-fold increase in productivity compared with Protein A resin, at a significantly lower cost. For antibody production, the company claims, it allows capture, virus deactivation, polishing, and sterile filtration to be accomplished in one cycle and within 24 hours.

Merck is also working with Natrix, EMD Millipore, and Novasep on a broad-based project, COMPAC2T, for Continuous Mode Purification and Cell Culture Technology, with the goal of establishing an integrated biopharmaceutical manufacturing process that would use continuous manufacturing both upstream and downstream. Xavier Le Saôut, associate manager of biotech process sciences technology and innovation at Merck Serono, described the project in a 2016 webcast (9).

It would use continuous downstream processing, operate on a single skid, and incorporate monitoring and process analytical technology. Studies so far have suggested that use of the integrated approach would allow working load capacity to be increased by 40%, so that 4.3 g of mAb could be purified in 48 h, and Protein A resin could be cycled 65 times, recovering more of its value.

The company has been evaluating both resins and membranes to compare costs and productivity, and planned to evaluate both fed batch and perfusion materials. So far, research suggests that global yield improvement could exceed 75%.

Another continuous downstream chromatography platform, ASAP, developed by researchers at Sanofi Pharmaceuticals, combines three connected operations: capture chromatography, ion exchange, and anion exchange polishing. In tests, all three were run simultaneously on a single skid, requiring only four (rather than the usual nine) buffers. The process was evaluated using single-use Sartobind membranes from Sartorius Stedim, and reportedly allowed purification to take place in 2.6 fours without any operator intervention, improving productivity by a factor of 50, allowing the processing of 125 g/L/h (10).

**IMPROVING CAPTURE**

Despite the high cost of Protein A resins, traditional capture chromatography will remain a downstream focus, particularly when used in continuous multicolumn systems that leverage more of the resin’s untapped value. Improving capture chromatography, whether with Protein A or other new affinity resins, is a top priority, says Tolley. “An effectively designed and efficiently operated capture step can reduce the burden on other purification steps and greatly improve the overall purification process,” he says.

Thermo Fisher has been addressing the need for consistent pressure/flow characteristics and high resolution required for semicontinuous chromatography applications, with new grades of POROS resins, Tolley says. An example is MabCapture A Select, which offers a lower cost Protein A alternative that can maintain binding capacity at higher processing flow rates, he says, allowing significant reduction in processing time, better resin utilization, and enhanced process efficiency.

**DIVERSIFYING RESINS**

Within the past year, the company has introduced resins specifically for the gene-therapy market, such as CaptureSelect AAV8 and AAV9 affinity chromatography resins, which offer high binding capacity and target specificity, permitting fewer unit operations and significant improvement in overall process recovery, Tolley says. Pall has diversified its resin portfolio with bulk, pre-packed, and custom solutions available for batch, continuous or semi-continuous operations, says Levison.

**MULTICOLUMN SYSTEMS REDUCE RESIN AND BUFFER CONSUMPTION**

Research has already shown the potential for multicolumn continuous chromatography to reduce the cost of resin and buffer. A few years ago, Genentech studied use of GE ÄKTa’s PCC system, and found that it could reduce resin and buffer consumption by approximately 40% (11).

In 2016, researchers at Merck and the University of Lorraine, France, compared the performance of five different commercial Protein A resins with Novasep’s BioSC continuous downstream technology (12). Although tests were primarily driven to compare resins, researchers found that use of the technology reduced the volume of resin required by 20–40%, and the need for buffer by 20%.

Pall is now exploring opportunities for a single-use perfusion application, Levison says. The company is also developing enabling technologies for continuous virus inactivation and filtration, as well as in-line diafiltration, he adds.
Incremental downstream efficiency improvements can add up. “Every step that we can streamline [downstream] improves the consistency and quality of the process, and, ultimately, the product,” he says.

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if we still don’t get the same answer as another lab, perhaps there is another force at play—and that is where the biology gets interesting.”

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Fusion proteins are engineered proteins that contain domains from different proteins that originate from different sources, such as a cell surface receptor or cytokine (i.e., interferon or interleukin) and an immunoglobulin. “These molecules are of interest because the domains may have functions that, when combined, are uniquely useful as therapeutics, such as binding/inhibition of cytokines and a longer half-life,” explains Steven Chamow, a principal with Chamow & Associates, a biopharmaceutical industry consultancy.

Specifically, two or more proteins are joined in one polypeptide chain, in some cases with a flexible peptide linker connecting the different parts of the fusion protein, according to Paul Carter, senior director and staff scientist of antibody engineering at Genentech.

Combining two different proteins in one molecule can lead to instability, which creates manufacturing challenges during both upstream and downstream processing. Solutions for overcoming these issues are being developed in academia and the pharmaceutical industry by equipment vendors and drug companies alike.

**Making Proteins ‘Druggable’**

Many proteins have short half-lives in human serum, making them unsuitable as recombinant protein drugs. When constructed as fusion proteins, however, they may become ‘druggable,’ according to Chamow.
Most pharmaceutical companies have active fusion protein programs. Early in 2017, for instance, Celgene acquired Delinia, which is developing DEL106, a novel IL-2 mutein Fc fusion protein designed to upregulate regulatory T cells (Tregs). Roche/Genentech is focused on cytokine fusion proteins, or immunocytokines, which provide targeted delivery of the cytokines into tumor cells to stimulate the local immune system and avoid responses in non-cancerous cells.

A variety of different fusion proteins have been evaluated in clinical trials and several products are on the market. Amgen’s Enbrel (etanercept) for the treatment of rheumatoid arthritis, and other moderate to severe plaque psoriasis is on the market. Amgen’s Enbrel clinical trials and several products proteins have been evaluated in cerous cells.

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provide targeted delivery of the
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avoid responses in non-cancerous
cells.

A variety of different fusion proteins have been evaluated in clinical trials and several products are on the market. Amgen’s Enbrel (etanercept) for the treatment of moderate to severe plaque psoriasis, rheumatoid arthritis, and other diseases, is one example. This TNFα blocker consists of a tumor necrosis factor receptor (TNFR) and an immunoglobulin (Ig) G1 Fc segment. The TNFR provides specificity and the Fc segment increases the stability.

In fact, the most successful fusion proteins to date have been so-called Fc fusion proteins, according to Carter. “These fusion proteins include an Fc region, are taken from an IgG, that extends the serum half-life of the fusion partner, facilitates expression, and allows purification by protein A affinity chromatography,” he says. The Fc region also provides the option for incorporating secondary immune functions in the fusion protein.

Fusion proteins are produced using genetic engineering by joining two or more genes that code for different protein components. In some cases, the full protein sequence is included, and in others only a portion. The fusion gene often encodes a peptide linker between the protein domains to allow the domains to fold correctly.

**THE NEED FOR STABILIZATION**

Achieving and maintaining proper folding for each of the components in fusion proteins is a major challenge in the molecular design of these products. “Issues can arise when domains are taken out of their natural context, or a domain is combined with a second domain with which it does not fold well,” Chamow observes. As a result, combining the different components of the fusion protein that do not naturally occur together can lead to instability with the composite molecule, creating manufacturing challenges such as aggregation during the cell culture or purification steps, according to Matt Kalo, associate director of the protein analytical chemistry department at Genentech.

For example, the low-pH elution step used in the initial protein A capture step may induce large amounts of aggregation, impacting product quality and yields. “Frequently, routine cell-culture and purification steps will need to be modified to address the tendency to form aggregates more easily,” notes Kalo.

Some fusion proteins are also prone to proteolytic degradation during production and/or after therapeutic administration, according to Carter. This issue can sometimes be overcome by modifying the junction sequence between the fusion protein domains. Kalo adds that modifying the junction sequence—either by moving it to a different location of the protein or by changing its length—may also alleviate incomplete disulfide bridging or disulfide scrambling. Development of a custom formulation may also be needed to minimize aggregation over time to enable long-term storage.

It is important to note, according to Kalo, that the design of fusion proteins can create novel epitopes, and thus the immunogenicity potential needs to be assessed to avoid any undesired strong hypersensitivity or an immune response.

“Overall,” he adds, “a fusion protein will often have liabilities that will need to be addressed by more process development than would be typically needed for monoclonal antibodies (mAbs), which are typically much better behaved from a processing standpoint.”

**LOW TITERS AND GREATER HETEROGENEITY**

In addition to stability issues, other manufacturing challenges have been encountered with fusion proteins, including low expression titers, and incomplete and heterogeneous glycosylation, according to Carter. The titers for fusion proteins are generally lower than those for mAbs due to less efficient translation, notes Chamow.

Non-human glycans—such as N-glycolyleucaminic acid and alpha-galactose—are also a concern with certain expression systems and may lead to immunogenic responses, according to Kalo. “In particular, the level of N-acetylgalactosaminic acid or sialylation, which can impact plasma half-life and the potency of the candidate, is a key quality attribute and can be challenging to control within a desired specification,” he says.

To address this issue, it is necessary to identify suitable levers in the cell-culture process that consistently generate the proper level of sialylation, according to Kalo. Different feed strategies are often used, such as the addition of galactose. Other approaches have also been employed to attain the desired level of sialylation, such as temperature and pH shifts.

*Contin. on page 37*
ABSTRACT

Japan’s pharmaceutical market is one of the top priorities for global pharmaceutical companies. Drug manufacturers are required to fulfill Japan-specific regulatory requirements. One of the requirements is to submit bracketing strategies for critical and non-critical aspects of the manufacturing process in a new drug application. These aspects include process parameters, quality attributes, operating and storage conditions, type and concentration of materials used, and other relevant aspects (also referred to as manufacturers’ proposals for handling post-approval changes). The critical and non-critical aspects of the manufacturing process are typically enclosed in major and minor brackets, respectively. Any post-approval changes to the bracketed aspects require appropriate regulatory action. Each bracketing strategy offers advantages and limitations, and determines whether a manufacturing deviation is allowed or not and whether a pre-approval for implementation of a post-approval change is required or not. Hence, it is important to submit a well-thought-out bracketing strategy. This article provides an overview on important aspects related to bracketing strategies.

Japan represents the second-largest pharmaceutical market in the world, accounting for approximately 10% of the total global pharmaceutical market (1, 2). Driven by new product launches and the healthcare burden of an aging population (1), Japan’s pharmaceutical market value is predicted to grow at a compound annual growth rate (CAGR) of 1.3% from $72.8 billion in 2013 to $79.8 billion by 2020 (3). Hence, entering the Japanese market is a top business priority for global pharmaceutical companies while aligning with ethical drivers to treat more patients in need. The Pharmaceuticals and Medical Devices Agency (PMDA) in Japan oversees the regulatory affairs for drugs and medical devices (4–7). It is essential to understand Japan-specific regulatory requirements to achieve successful approval for new drug products there. Accessing Japanese regulatory information, however, is challenging due to differences in language and culture.

The drug approval process in Japan involves a series of activities, including non-clinical and clinical (Phase I, II, and III) studies, bridging studies, submission of a new drug application (NDA) by the manufacturer, and review
of NDA by the PMDA (4–9). Application documents for a new drug should be prepared and submitted in the Japan Common Technical Document (Japan CTD) format (4–9). After review and evaluation of an NDA, the PMDA provides a recommendation and forwards the application for approval to the Ministry of Health, Labor, and Welfare (MHLW). The MHLW is the regulatory authority that issues NDA approval or rejection.

The Japan CTD dossier is organized into five modules (Figure 1). Module 1 is a legally binding document and consists of the application form (AF) (4–9). The content of the AF is considered registered detail and includes several categories of information (some categories are shown in the box on the right of Figure 1), including a description of the manufacturing process (4–9). In the Manufacturing Process section, the manufacturer must propose appropriate bracketing strategies for critical and non-critical aspects of the manufacturing process. These aspects include process parameters, quality attributes, operating and storage conditions, type and concentration of materials used, and other relevant aspects (also referred to as regulatory strategies for post-approval changes) (4–13). Critical and non-critical aspects should be enclosed in major and minor brackets, respectively (4–13). Any post-approval changes to the bracketed aspects in the AF require appropriate regulatory actions (9–13). This requirement is the key difference between Module 1 of the Japan CTD and the United States/Europe CTD. In the United States and Europe, Module 1 is considered an administrative section only (4–9). Module 2 of the Japan NDA should include a quality overview summary, non-clinical and clinical overviews, and non-clinical and clinical summaries. Modules 3, 4, and 5, respectively, contain more extensive information on quality, safety, and efficacy. In the Japan CTD, Modules 1 and 2 must be written in Japanese and are considered review documents. Modules 3, 4, and 5 are considered reference documents and may be written in English (4–13).

**WHAT IS BRACKETING?**

Bracketing is a Japan-specific regulatory strategy in which critical and non-critical aspects of the manufacturing process such as process parameters, quality attributes, operating and storage conditions, type and concentration of materials used, and other relevant aspects are enclosed in major (<< >>) or minor (“ ” or [ ] ) brackets in the written AF (10–13). The type of bracketing strategy proposed determines whether a post-approval change is subject to the partial change application (PCA) approval or minor change notification (MCN). The aspects enclosed in major and minor brackets are referred to as PCA and MCN matters, respectively (10–13). If an aspect is not enclosed in any brackets, it is considered a PCA matter (10–13).

**DIFFERENCE BETWEEN PCA AND MCN**

In general, a PCA is submitted for making a major change (change in a critical aspect enclosed in major brackets or submitted without any brackets) or a change that has significant impact on quality, stability, safety, and/or efficacy of the product (8–13). On the other hand, an MCN is submitted for implementing a minor change (change in a non-critical aspect enclosed in minor brackets) or a change that has a negligible impact on quality, stability, safety, and/or efficacy of the product (8–13). Other differences between PCA and MCN are listed in Table I.

**MAJOR BRACKETING STRATEGIES (PCA MATTERS)**

Critical aspects (e.g., process parameters, quality attributes, operating and storage conditions, or other aspects that are considered to
be critical) of the manufacturing process are expected to be enclosed in << >> or submitted without any brackets and are regarded as PCA matters. For example, the critical process parameters (CPPs) belong to the major bracketing category and are enclosed in << >> or submitted without any brackets (Table II and Table III) (9–13). The major bracketing approach proposed determines whether a manufacturing deviation is allowed or not. For example, a manufacturing deviation is allowed when a set/target value or midpoint of a normal operating range (NOR)/proven acceptable range (PAR) is enclosed in << >> (9–13). In contrast, the manufacturing deviation is not allowed if a process parameter or other aspect is submitted without a bracket (Table II) (9–13).

Different major bracketing strategies can also be adopted. For example, not less than <<lower limit of NOR>> or <<NOR>> can be proposed instead of <<target/set value or midpoint of NOR/PAR>>. For <<NOR>> approach, a PAR must have been established and documented in the manufacturing site documents (e.g., manufacturing guide, batch records, and control strategy).

Table I: The difference between a partial change application (PCA) and a minor change notification (MCN). PMDA is the Pharmaceuticals and Medical Devices Agency.

<table>
<thead>
<tr>
<th>Partial change application (PCA)</th>
<th>Minor change notification (MCN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submitted prior to implementation of change</td>
<td>Submitted within 30 days of implementation of the change</td>
</tr>
<tr>
<td>Data are essential. Data related to assessment of the impact of change on quality, stability, safety, and efficacy of product are submitted with the application.</td>
<td>Data are not essential. Data related to assessment of the impact of change on quality, stability, safety, and efficacy of product are documented internally.</td>
</tr>
<tr>
<td>PMDA reviews and approves the change prior to implementation.</td>
<td>PMDA reviews the change during next PCA or periodic GMP inspection.</td>
</tr>
</tbody>
</table>

Table II: Major and minor bracketing strategies, respectively, for critical and non-critical process parameters, and associated deviation acceptability and regulatory actions.

<table>
<thead>
<tr>
<th>Process parameter</th>
<th>Bracketing strategy in application form</th>
<th>Manufacturing deviation allowed?</th>
<th>Regulatory action for post-approval changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical process parameter</td>
<td>CPP without brackets</td>
<td>Major bracketing/PCA matter</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>&lt;&lt;Target/Set Value or Midpoint of NOR/PAR or NOR&gt;&gt;</td>
<td>Major bracketing/PCA matter</td>
<td>Yes*</td>
</tr>
<tr>
<td>Non-critical process parameter</td>
<td>&quot;Non-CPP without an acceptable range&quot;</td>
<td>Minor bracketing/MCN matter</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>[[Target/Set Value or Midpoint of NOR/PAR or NOR]]</td>
<td>Minor bracketing/MCN matter</td>
<td>Yes*</td>
</tr>
</tbody>
</table>

NOR=normal operating range; PAR=proven acceptable range; PCA=partial change application; CPP=critical process parameter; MCN=minor change notification.

* Acceptable range/NOR/PAR for bracketed value must have been established and documented in the site documents (e.g., batch records, manufacturing guide, and control strategy).
MINOR BRACKETING STRATEGIES (MCN MATTERS)

Non-critical aspects (e.g., process parameters, quality attributes, operating and storage conditions, or other aspects that are considered to be non-critical) of the manufacturing process are expected to be enclosed in minor brackets (“ “ or \[ \] \]) and are regarded as minor bracketing strategies or MCN matters. For example, non-critical process parameters (non-CPPs) belong to the minor bracketing category and are enclosed in minor brackets (Table II and Table III) (10–13). The type of minor bracket and minor bracketing strategy proposed determines whether a manufacturing deviation is allowed or not. For example, the deviation is allowed when the set/target value or the midpoint of NOR/PAR is enclosed in \[ \] (Table II) (10–13). Alternatively, not less than \[ \text{lower limit of NOR} \] or \[ \text{NOR} \] can be proposed instead of \[ \text{target/set value or midpoint of NOR/PAR} \]. In the case of \[ \text{NOR} \], a PAR must have been established for this parameter and documented in the manufacturing site documents.

The non-CPPs or other non-critical aspects of the manufacturing process without an established allowable/acceptable range are enclosed in “ ” (10–13). For example, the manufacturing process described in the AF includes a time limit for the bulk drug product hold at room temperature as follows: If sterile filtration of the bulk drug product will not be initiated within 24 hours of preparation, it is stored at 2–8 °C. The 24-hour time limit is enclosed in this bracket (“24 hours”). This approach is also used to bracket concentration of formulation excipients (e.g., “10% w/v”). A key limitation of this bracketing strategy is that an out-of-limit result cannot be treated as a deviation.

If the manufacturer would like to make post-approval changes in non-critical aspects enclosed in minor brackets, change(s) can be implemented immediately (10–13). However, an MCN must be submitted within 30 days of implementation of the change (implementation may refer to a process change or product release for Japanese market). The PMDA reviews the MCN during periodic site inspection or as part of manufacturer’s future PCA approval, whichever is earlier (Figure 2) (10–13).

### Table III. Examples of major and minor bracketing strategies.

<table>
<thead>
<tr>
<th>Type of bracket</th>
<th>Potential approaches</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major</td>
<td>Range without quotation marks</td>
<td>CPP: Mixing speed PAR: 200–250 rpm Proposed approach: PAR without brackets, i.e., 200–250 rpm</td>
</tr>
<tr>
<td></td>
<td>(&lt;\text{Target/Set Value or Midpoint of NOR/PAR or NOR}&gt;)</td>
<td>CPP: Mixing speed PAR: 200–250 rpm Proposed approach: Midpoint of PAR, i.e., (&lt;225\text{rpm}&gt;)</td>
</tr>
<tr>
<td>Minor</td>
<td>“Non-critical aspect without an acceptable range”</td>
<td>1) Non-CPP: Temperature, 20 °C; Approach: “20 °C” 2) The pH is adjusted by addition of “1.0 M” citric acid</td>
</tr>
<tr>
<td></td>
<td>[\text{Target/Set Value or Midpoint of NOR/PAR or NOR}]</td>
<td>Non-CPP: Mixing time NOR: 20–40 minutes Proposed approach: Midpoint of NOR, i.e., [30 minutes]</td>
</tr>
</tbody>
</table>

NOR=normal operating range; PAR=proven acceptable range; CPP=critical process parameter; RPM=rotations per minute.
WHY IS IT ESSENTIAL TO SUBMIT WELL-PLANNED BRACKETING STRATEGIES?

Part of PMDA’s regulatory responsibility is to monitor post-approval changes in the manufacturing process (8–13). Bracketing in the AF not only distinguishes critical and non-critical aspects of the manufacturing process, but it also represents manufacturers’ proposals and commitment to handling post-approval changes in those aspects. The manufacturer should carefully consider the advantages and limitations of brackets (major or minor brackets) and bracketing approaches (set point, lower limit of NOR/PAR, or range) when making a selection.

The content of the AF is an approved matter in Japan (8–13). Any post-approval changes in approved matter require appropriate regulatory actions (8–13), for example, a PCA approval to implement changes in critical aspects and an MCN to inform PMDA regarding changes made in non-critical aspects. The bracketing strategy also defines whether a manufacturing deviation is allowed or not. A bracketing strategy that allows for deviations to be handled internally by the manufacturer gives flexibility to manufacturers and minimizes the regulatory burden. For example, bracketing of the set point/target value/midpoint of NOR or PAR using \<\> or \[\] brackets allows manufacturing deviations, which can be handled via internal deviation control (9–13). The aspects enclosed in “ ” or without any brackets does not allow for deviations.

The manufacturer is required to maintain consistency between approved matters and routine manufacturing practices. For example, if it is discovered during the GMP inspection that an MCN had been submitted concerning changes in the manufacturing process that should have been addressed as a PCA, the MCN becomes invalid, and there may be a possibility that the manufacturer may be accused of violating the Pharmaceutical Affairs Law (4–13).

The level of information included in the AF on the manufacturing process should be a balanced approach. For example, too little information may lead to delay in approval and too much information may increase lifecycle management/regulatory burden. Typically, important aspects that ensure product quality and stability, safety, and efficacy are included in the AF. Note that no regulatory action is required for changes in aspects that are not included in the AF but described in Module 3.

CONCLUSION

Submission of a well-thought-out Japan-specific regulatory strategy for handling post-approval manufacturing changes is essential. The important aspects of the manufacturing process (e.g., process parameters, quality attributes, operating and storage conditions, type and concentration of materials used, and other relevant aspects) enclosed in major or minor brackets are considered approved matters in Japan, and any post-approval changes in these matters require appropriate regulatory actions. Whether a manufacturing deviation is allowed or not is governed by the bracketing strategy. The manufacturer should carefully consider the advantages and limitations of bracketing strategies when making a selection. Because the number of aspects bracketed in the AF may affect timely approval and lifecycle management/regulatory burden, a balanced approach (inclusion of those aspects that ensure product quality and stability, safety, and efficacy) should be adopted.

A number of factors play key roles in achieving this goal. Product and process understanding, design space around each process parameter (e.g., NOR and PAR), residual risk level, and control strategy are crucial to make appropriate selection(s). It is also noteworthy to consider prior feedback from PMDA on bracketing strategies. Finally, the bracketing strategy proposed in the AF is subject to negotiation with the PMDA and may be different from the final approved approach.

ACKNOWLEDGEMENT

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3. Pharmaceutical Executive editors, “Tepid Growth to Put Japan’s Pharma Market at $80 Billion by

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Finally, Chamow adds that Protein A capture chromatography can present problems for fusion proteins, not only with respect to their acid stability, but also in terms of lower binding capacities due to their larger size. He does note, however, that for other downstream operations, wider options for polishing chromatography steps to remove aggregates and viral inactivation methods that don’t require low pH treatment are now available. Improved linker sequences are also being developed that can modulate domain interactions and enhance domain stability, according to Chamow. He also expects that new technologies for cell-expression and processing methods, such as CRISPR/Cas9-based expression for cell lines, the implementation of ultraviolet C (UVc) radiation for inactivation of viruses at process scale, and continuous bioprocessing will be beneficial for fusion protein manufacturing.

NEW DESIGN STRATEGIES

While fusion proteins are a growing segment of the biologics market, manufacturing gaps do remain that must be addressed. Specific concerns for Chamow, as outlined previously, include increasing the productivity of processes, ensuring product stability, and minimizing immunogenicity.

Technologies are being developed to address these gaps, but Kalo would like to see an advance in up-front design innovation rather than technology advances across the various unit operations. “A key aspect of improving the manufacturability of fusion proteins lies with their design, which should be focused on minimizing the subsequent residual liabilities that remain for process development. Prospective molecular assessment and adjustments to the candidate earlier in the process by incorporating previous learnings and new discoveries obtained from rigorous assessment of candidates will permit faster and easier development of fusion proteins as drugs,” he asserts.

Chamow points out that more attention is being given to using in-silico design to ensure that fusion protein domains fold together well and are compatible. Kalo also believes that more time must be invested in identifying the underlying reasons for the manufacturability challenges, some of which are common across fusion proteins as a class, while others vary from candidate to candidate.

“Development of fusion proteins requires a more flexible mindset and less reliance on standard approaches, which are often exhausted first before the underlying reasons are thoroughly investigated. It is the latter efforts that often lead to a more sustainable and permanent solution for a candidate,” Kalo states. In practical terms, therefore, he notes that perhaps more investment in structure/function relationship studies should be pursued earlier in the development cycle than a standard mAb drug development strategy would encourage. ◆
The population of robots working on parenteral fill/finish lines is increasing rapidly. The expansion mirrors growth in biotech products, particularly self-injected therapies, which require autoinjectors that can deliver a safe, effective, and consistent experience. Robotic systems excel at maintaining aseptic conditions and protecting operators from toxic substances. In fact, Comecer Group has offered robot-equipped systems to handle radiopharmaceuticals for more than a decade.

Robots not only provide precise, consistent handling, but also offer a high level of flexibility so systems can accommodate a wider range of containers and components with minimal or no change parts. “Flexibility is crucial for high-value, relatively low-volume products,” says Eric Petz, senior marketing manager at Vanrx Pharmasystems. “Batches are smaller, so you can’t have long changeover and startup times,” he explains.

Robots also can reduce or eliminate glass-to-glass and glass-to-metal contact, which are major causes of container failure. But the most important benefit that robots provide is improved quality due to minimization or elimination of human intervention, the biggest source of product contamination. To prevent contamination, regulatory agencies worldwide are looking for less human intervention, and original equipment manufacturers (OEMs) are working on ways to remove operators from the process. “It’s all about risk mitigation,” says
Simon Cote, principal engineer, Technical Customer Support at West Pharmaceutical Services. In fact, “I would be highly surprised if the industry doesn’t incorporate more robots,” he says.

West Pharmaceutical Services is adding a robotic system to its equipment lineup with the purchase of a dual-robot KCP 5060 system from Bausch + Stroebel Machine Co. The model, which was shown at INTERPHEX 2017, features cleanroom-compatible robots that reach upward to perform their tasks. Mounting the robots below the containers maintains laminar air flow. Capable of handling vials, cartridges, or syringes in pairs or nests, the system can be equipped with up to four processing stations. Hydrogen peroxide decontamination cleans the work area including the robots.

The compact machine, which occupies less than 14 feet of linear space, operates at speeds up to 1200 units per hour, a rate particularly well-suited for self-therapy products. Container size ranges include vials from 16–52 mm in diameter with heights up to 94.5 mm, syringes from 0.5–20 mL, and cartridges from 6–14 mm in diameter with heights from 40–90 mm.

Biotech products have been a driving force behind robotics. In one example, the Model SF20 TaskMate robotic aseptic syringe filling and capping system from ESS Technologies integrates a FANUC robotic cell with an OEM-supplied restricted access barrier system. A FANUC LR Mate 200iD robot picks a cap and places it on the fill station. The second FANUC i Blender and uses vision and a mechanical gripper to pick the syringe and place it on the filling station. The second FANUC LR Mate 200iD robot picks a cap from the tray and places it into the torque station. A servo-driven pick-and-place unit transfers the syringe from the fill station to the torque station. Capped syringes descend a divided chute to a discharge/reject bin. Fills range from 50 μL–60 mL at speeds up to 15 syringes per minute. The machine also can handle vials and various cap styles. Changeover generally can be accomplished in less than 15 minutes.

Marchesini’s Extrafill syringe filling and stoppering machine features a robotic de-lidding station, monobloc design, and two tub opening and syringe filling/stoppering stations. Up to three more stoppering stations can be added to boost output to 12,000
syringes per hour. Dosing range is 0.1–50 mL.

The Steriline RVFCMS0 filling and capping machine, supplied in the US by MG America, handles vial sizes from 2 mL–100 mL without changing format parts. Rated at up to 3000 vials per hour, the system can be integrated with an RA-V4 rotary vial washer, ST2 CCS depyrogenation tunnel, and EDM-C external decontaminating machine.

### ROBOTIC INSPECTION AND PACKAGING SYSTEMS

Beyond the fill/finish operation, robotics play a role in inspection and secondary packaging of parenteral products. Robotic inspection systems can overcome problems with limited floor space. “A typical, commercially available manual inspection line for two operators that includes automatic de-nesting and re-nesting is a linear line using three machines for the primary operations and is in the order of 40 feet long,” explains Luciano. “The LPT robot cell [HPI-30M nested syringe inspection machine] is only 10 feet long because the robot is able to perform multiple operations—de-nesting, syringe presentation for flange inspection/rejection, and labeling path transfer or re-nesting. In this case, the robot is picking five syringes from the Hypak that supports a matrix of 10 staggered rows of 10 syringes per row. And since there is extra spacing between each tub, there are four different pick positions and 12 different re-nesting positions, so you can see how the programmable flexibility of the robot (in combination with a servo-indexing Hypak conveyor) is helpful in reducing mechanical complexity.” If higher speeds are needed, manual inspection can be replaced by automated vision systems, and a second robot can be added for discharging.

An example of a robot-equipped line for secondary packaging functions was shown by Marchesini Group at CIPM Qingdao (April 19–21, 2017, Qingdao, China). The customizable line integrates a deep-draw FBZ 320 thermoformer with a continuous-motion BA 400 Argento cartoner. Operating at up to 240 cartons per minute, the system thermoforms trays, loads syringes and vials, and cartons filled trays. A centerpiece of the thermoformer is a four-axis Robomaster system. Positioned between the forming and sealing stations, it picks syringes or vials and places them into trays. Infeed options include plug-in belts and vibrating tables.

### DESIGN CONSIDERATIONS

Many robot providers offer cleanroom models compatible with hydrogen peroxide sterilization for parenteral fill/finish applications. When designing robotic equipment, the first consideration is whether a robot is the best option. “You don’t want to use robots where simple mechanics could accomplish the task,” says Massimiliano Cesarini, global sales manager, Isolation Technology Division at Comecer.

A primary consideration when specifying a robotic fill/finish system is speed. “Robot lines are typically slower,” says Gregor Deutschle, business development manager at Schott North America. Batch size is another consideration. Shorter runs mean more frequent changeover. “Robots eliminate a lot of change parts and star wheels,” notes Deutschle.

Robots also replace traditional container handling mechanisms. “Eliminating turntables and infeed belts definitely helps reduce glass-to-glass contact,” he adds. As a result, robots can provide gentler handling and may be especially well-suited for polymer containers, which scratch easily.

End-of-arm tooling (graspers or end effectors) must be carefully designed to optimize handling. Factors to consider include weight, which directly impacts speed, and arrangement of pneumatic and vacuum lines and sensor wires so performance is not impacted by movement of the arm. The end effector also must handle the product without marring or other damage. Vacuum is a common choice, but may not work with all surface areas and product orientations. Nevertheless, “Vacuum is usually the gentlest,” says Luciano. However, vacuum may not provide enough support for the container(s). In that case, a rigid guide may need to be added.

Finally, programmability must be considered. Many of today’s robots do not rely on proprietary programming languages, but can be easily programmed from a library of functions or by simply moving the arm through the path it needs to take. ♦

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**Advantages of robots for parenteral packaging**

- Cleanroom compatible
- Sterilizable
- Limits operator interaction
- Flexible
- Minimizes change parts
- Precise
- Consistent
- Gentle handling
- Easier programming
- Competitive pricing
- High reliability
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laxoSmithKline (GSK)’s R&D Biopharmaceutical Pilot Plant in Upper Merion, PA is a R&D clinical trial material (CTM) manufacturing facility with an aggressive processing schedule that requires minimal shutdown interruptions. Utility reliability is paramount to achieve production demands and regulatory quality requirements. To meet the utility demands for increased CTM output, a capital investment project was required to replace the existing, obsolete high purity water (HPW) generation system and water-for-injection (WFI) generation system with new, reliable technology.

The existing, obsolete high purity water generation system and water-for-injection generation system were replaced with new, reliable technologies.

The project drivers were:
- Existing generation systems (HPW and WFI) had insufficient capacity for current operations and were unable to meet the demands of the growing pipeline.
- Spare parts for the existing systems were either not available or becoming more difficult to source.
- Existing systems were costly to maintain, not energy efficient, not reliable, and had insufficient redundancy, increasing the potential for unscheduled production downtime.

The primary objective for the project was to provide water system(s) generation reliability with the following additional requirements:
- Deliver more environmental sustainable systems (i.e., lower water and energy usage)
- Increase supply and storage capacity
- Replace obsolete equipment

Case Study: Retrofitting Two New High Purity Water Systems

Brian Lipko, Brian Termine, and Steve Walter

The existing, obsolete high purity water generation system and water-for-injection generation system were replaced with new, reliable technologies.

Facility Renovation
• Have no impact on ongoing GMP operations.

GSK engaged Hargrove Life Sciences to complete the design for this project. The conceptual design phase included evaluation of new equipment technologies, a sustainability evaluation including energy and operating cost comparisons, and visiting other recently installed GSK water systems at other locations. Also, because the pilot plant was land-locked, with no available space inside the facility for new equipment, investigation and analysis of where to install the new water systems was required.

Selecting the equipment and technology for the HPW and WFI systems was based on evaluations that took into consideration sustainability goals including reduction of energy, water, and carbon footprint. It was also determined that the best way to achieve system generation reliability was to have complete redundancy for all mechanical equipment (i.e., essentially two of everything).

REDESIGNING THE HIGH-PURITY WATER SYSTEM

The existing HPW distribution system provided a continuous flow of 30 gallons per minute (gpm) of purified water at 25 °C. Purified water was constantly circulated to the utility systems in the basement mechanical room and also to the three GMP operating floors using dual, sanitary variable-frequency drive (VFD) pumps rated at 150 gpm. The base-ment utilities supplied with HPW feedwater included a WFI still and two clean-steam generators.

This existing system produced water with a resistivity greater than 10 Mohm and total organic carbon (TOC) with less than 5 ppb, and GSK wanted to maintain this high quality for the new system while also incorporating a sustainable design.

Criteria for the new HPW system included the following:
• Redundant mechanical equipment (i.e., two of everything). Reliability would be achieved through redundancy, with dual multimedia filters, softeners, carbon filters, reverse osmosis (RO)/continuous deionization (CDI) skids, distribution pumps, and vent filters.
• The existing 34 gpm generation system did not always adequately maintain building operating demands. The new system(s) would require larger capacity (40 gpm) for existing building operating demands, increased WFI generation, and future building expansion or increase in users/processes.
• Start/stop technology was a requirement to reduce electrical energy and water consumption rates.
• The generation system must be designed for hot water sanitization at 80 °C (65 °C minimum).
• A mixed-bed polisher would be required on the new system design to meet resistivity quality requirements.
• A new stainless-steel, HPW storage tank must be provided that would include increased storage capacity to meet larger instantaneous demands of water from expected increases in production. The new tank would also retain a nitrogen blanket that was installed on the existing storage tank, which had proven to be successful in helping to maintain a low bioburden in the system.

Based on these criteria, it was decided that the RO system only needed to be single-pass technology to meet HPW quality requirements. The project team also decided to implement carbon filtration instead of bisulfite injection or ultraviolet light technology as the primary method to remove residual chlorine/chloramines. Although carbon filtration represents the most expensive initial cost, it is the most effective method of removing residual chlorine/chloramines. The downside of using carbon filtration is the carbon filters are an ideal breeding ground for bacteria. However, because the carbon filters would be hot water sanitizable, the concern of bacteria growth was reduced. Other design considerations included:
• Installing a new bulk brine storage system to eliminate the need for manual material handling of the salt required for softener regeneration
• Installing a RO reject water recovery system used for makeup water to the building’s cooling tower
• Supplying HPW to an adjacent biopharm process development facility, thus enabling the decommissioning of a second water purification system serving this adjacent building, which further reduced site operating expense
• Installing a new online microbial detection system.

The online microbial detection technology was released for commercial use in pharmaceutical clean utility systems just prior to the design phase of this project. After various on-site pilot testing scenarios, it was concluded that this online microbial detection technology would be installed on the HPW distribution system, greatly reducing the system’s water sampling and analysis work load. The project purchased and installed one of the first commercially available units in the United States. This online microbial detection system complements the online TOC and conductivity systems, which are typical to GSK water system designs.
The existing WFI generation system was a 25-year-old multi-effect (ME) still. The unit produced a maximum of 470 gph of WFI at 82 °C. As the facility's GMP processing manufacturing capacity increased, the WFI generation system had inadequate generation capacity, which resulted in rigid planning for various manufacturing users so that the WFI storage tank would not be completely drained during use. WFI manufacturing capacity studies indicated that the maximum WFI usage in the facility could approach 1325 gallons in a two-hour period. With the existing WFI still make-up rate, the WFI storage tank volume would fall dramatically and cause the WFI distribution system to shut down. It was determined that the existing WFI storage tank would not be replaced because of limited head room and access space into the basement utility area.

With maximum site plant steam pressure limited to 90 psig, replacing the existing still with another ME still would require an oversized (de-rated) system, as these are designed to normally operate with plant steam at 115 psig. In addition, the ME still would require an external cooling exchanger to remove excess heat, which would have an impact on the building’s process glycol system.

With reduced plant steam pressure and the desire to minimize process glycol loads, the engineering team determined that the replacement WFI stills would be vapor compression (VC) type stills. VC stills are designed to operate with 50 psig plant steam in lieu of 115 psig required for ME stills, which yields energy savings from reduced steam usage. Also, the cooling load could be virtually eliminated, which would reduce approximately 25 tons of process glycol that was required for the ME still. Although VC stills are more expensive from a capital installation cost perspective, they are more economical to operate from a utility demand.

The existing WFI distribution system consisted of three independent supply loops with a dedicated pump for each GMP operating floor of the facility. The new pumping distribution system design consisted of two new VFD-controlled redundant pumps each capable of serving all three floors simultaneously. If one pump were to fail, the remaining pump was designed with sufficient capacity to maintain WFI distribution for the entire facility.

**REDESIGNING THE WFI SYSTEM**

**Figure 1.** Courtyard between two buildings that was chosen as the site of the new, two-story mechanical room.

**RETOFITTING THE WATER SYSTEMS**

At the completion of engineering design, the project team determined that the two new water systems must be delivered using a phased construction and validation approach to minimize shutdown interruptions to the manufacturing areas.

Phase 1 included the construction, installation, and validation of a new HPW generation, storage, and distribution system in a location that would not impact the facility’s planned GMP manufacturing schedule. Phase 1A included replacement of the WFI distribution pumps and the main WFI control panel including validation testing. Phase 2 involved the demolition of the existing HPW generation and storage equipment in the building basement to establish the location for the installation of two new VC WFI stills. Phase 2 could only be completed after Phase 1 HPW generation and distribution systems were released for GMP use.

**Phase 1**

Phase 1 would entail the construction of a strategically placed new mechanical room in an unused courtyard that was isolated between existing facilities, as shown in **Figure 1**. The new mechanical room in the chosen location was required to be two stories, providing a location to install, commission, and validate the new HPW system prior to connecting to the operating pilot plant and decommissioning the existing HPW system.

The redundancy requirement proved to be a challenge because two HPW generation trains would need to reside in the proposed two-story mechanical room, which was constrained in...
a space that was only 14 ft. wide and 110 ft. long. A thorough and iterative study was done to confirm the equipment could be installed and be serviceable, which included the development of a three-dimensional design model (see Figure 2). Once the proposed space design was proved out, the detailed design of the water system and proposed addition began. The GSK/Hargrove team worked closely together to design every aspect of the project to ensure minimal impact to the existing adjacent operating facilities.

Phase 1 construction of the HPW mechanical room in the unused courtyard required the removal of a portion of Building 38’s exterior glass façade, potentially exposing the pilot plant to the elements of nature (e.g., weather, insects). Therefore, prior to removing any portions of the building’s existing façade, temporary weather-proof interior walls were constructed. As the façade was then being removed, the daily limit of removal was controlled to an area that could be sealed back up in the same day with a temporary facade.

Installation of equipment into the new HPW mechanical room

With the new mechanical room completely surrounded on all sides by existing buildings with no access for bringing in large pieces of equipment, it was necessary to install all the major pieces of equipment using a hydraulic crane. The challenge with doing this is that the room construction took place prior to equipment delivery. Therefore, the project team staged the construction of the new room to essentially have a section of the mezzanine floor and roof to be constructed after equipment installation. Once the missing building sections were constructed, equipment could then be shifted to its final location, as shown in Figure 3.

Phase 1A

Phase 1A included the installation of two new WFI distribution pumps and the new WFI control system. The existing WFI distribution system consisted of three independent loops for each GMP operating floor of the building, each with its own pump. The new distribution system consists of two new VFD-controlled redundant pumps each capable of serving all three floors. This phase was the most critical to ongoing operations because there was no backup plan (i.e., everything had to go right the first time to bring the WFI system back on-line in the shortest possible time frame). Therefore, extensive planning was required including: the development of process operational descriptions for the new control system, which included 86 instruments and 106 input/output points and the development of the WFI system hydraulics using Fathom Modeling for the entire building’s distribution system, which turned out to be extremely valuable for vetting the design. The plan to control the distribution system was to use flow control on the return from each parallel floor loop to maintain minimum velocities. Also, the third-
floor return pressure, which was the most remote location in the system, would be used to adjust the speed of the pumps to maintain minimum loop pressures.

Once Phase 1 and Phase 1A were completed, the pilot plant would be supplied with high purity water from the new HPW generation system and supplied with WFI using the existing ME still, new redundant WFI distribution pumps, and new WFI control panel. Phase 2 could then commence, which included the demolition of the existing HPW system in the basement mechanical room and the installation of two new WFI VC Stills.

**Phase 2**

Phase 2 construction activities were conducted similarly to Phase 1 given that the new WFI stills would be installed, commissioned, and validated while the Pilot Plant was fully operational. Due to the challenge with bringing the new equipment into the basement mechanical room through a constrained access door, the new stills were designed to be disassembled and shipped in multiple components that would then be reassembled onsite.

**Utility tie-ins for the different project phases**

All supporting utility system tie-ins (e.g., nitrogen, air, clean steam) and the connecting of generation systems to the building’s existing distribution systems were completed during preplanned windows when the pilot plant was not operating and had no water demand.

**Leveraged FAT qualification approach**

The project team decided early to use a leveraged factory acceptance test (FAT) qualification approach to reduce the overall project validation schedule. All GSK equipment FATs are executed and documented in a manner that may allow GSK to “leverage” the FAT execution documentation (FAT turnover package) during future commissioning and validation on-site at their facility by referring back to FAT-executed approved testing and results. The FAT is a GMP activity approved by all stakeholders including the equipment vendor, GSK Engineering and Validation group, Quality Assurance group, and Facilities Operations group to eliminate the need for redundant testing once the system has arrived on site.

Testing that has been successfully completed at the FAT and poses a low risk of being impacted by transport of the system from the factory to the site in a manner that would change the results of the testing will not be repeated. This testing is detailed and leveraged under a later lifecycle qualification document. Testing that has not been successfully completed within the FAT or that poses a higher risk of impact during transport is either repeated or conducted for the first time at the site under a later lifecycle qualification document. This approach reduced the qualification schedule by three weeks.

The total duration of construction was approximately 60 weeks. The majority of this time (44 weeks) was spent on Phase 1, which was the construction of the new HPW mechanical room and installation and validation of the HPW system. Phase 2, which was the removal of the existing HPW equipment in the building’s basement and the installation and qualification of the new WFI generation systems, took another 16 weeks to construct and validate.

**PROJECT CHALLENGES AND RESULTS**

The main challenges included the small area to construct the new HPW mechanical rooms and the limited utility shutdown opportunities available to tie-in two new water systems. Another major concern was installing the new WFI distribution pumps with a new WFI control panel because there was no turning back when the old pumps and control panel were removed. The WFI concern was reduced after full programmable-logic-controller simulations were completed along with hydraulic modeling of the WFI distribution system. In fact, the installation and validation of the WFI pumps and controls went exactly as simulated.

The phased approach for construction, validation, and testing provided for the installation of state-of-the-art water generation systems with minimal plant operation downtime and no impact to ongoing production. Since the release of the project for GMP use, benefits were realized quickly by the business, such as ample supply of water, reliable supply of water, lower energy usage, and lower overall operating costs. The GSK Engineering design team worked with selected equipment vendors to minimize water and electrical usage wherever possible, which has resulted in significant environmental sustainability benefits and operational cost savings. Annual operational expenses were reduced by approximately $170k per year, and annual carbon emissions were reduced by 348 tonnes CO₂.

The two new redundant HPW generation systems (see **Figures 4 and 5**) represent the latest technology for energy and water efficiency in the production of USP grade water. They are designed using commercially available technology that enables one of the systems to automatically be brought off-line to be sanitized and put back online when required. This control technology also consumes significantly less water and energy and produces significantly less wastewater compared to conventional systems. This system translates into operating savings, with more envi-
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**BIONE–SINGLE-USE BioreACTOR SYSTEM**

Convert your existing benchtop glass bioreactor to a single-use bioreactor in seconds. Introducing the BIONe by Distek, a benchtop scale single-use bioreactor system for mammalian cell growth and recombinant protein production. Engineered with a disposable headplate welded to a triple-layered liner, the BIONe significantly reduces turnaround time by allowing users to seamlessly transition to a disposable platform while utilizing their existing capital equipment. [Distek Inc](http://www.distekinc.com), tel. 732.422.7585, bione@distekinc.com, [www.distekinc.com](http://www.distekinc.com)
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Environmentally responsible, energy-efficient, purified water generation processes. In addition, customized software programming requirements were implemented to reduce potable water usage during the softener regeneration cycles, and the RO membrane cleaning is now determined on normalized differential pressure software monitoring in lieu of a traditional totalized flow rate approach.

Major benefits included in the implementation of the new HPW generation systems included the following.

- Water make-up and sewer savings were approximately 11,000 gallons per day (or 4M gallons per year).
- Electrical costs were reduced by 88% because the HPW generation system shuts down if there is not a demand for HPW storage tank make-up.
- The on-line microbial detection system has reduced manual quality grab samples by 20%.
- The bulk brine tank system reduced site labor costs because the manual salt replenishment process has been eliminated.
- Additional operational savings were realized by having the new HPW system supply the biopharmaceutical development pilot plant, thus eliminating a costly vendor service ion-exchange contract.

The two, new, redundant VC stills provide additional capacity and ensure reliability. The VC stills were also designed and validated to operate using variable compressor speeds to reduce electric demands when providing WFI to the storage and distribution system. For example, if the required WFI fill rate is minimized, the still compressor can operate on a slower speed to lower electrical operating costs.

**Figure 4.** First floor of the new water treatment room with the new high-purity water generation system (reverse osmosis and continuous deionizer skids).

**Figure 5.** Second floor of the new water treatment room showing the top of the high purity water storage tank with multimedia, softeners, and carbon filters in the background.

Major benefits included in the implementation of the new WFI generation systems include:

- Electrical costs reduced by 66% (VC does not have a feed water pump)
- Plant steam consumption reduced by 65%
- Chilled water consumption reduced by 97% (not required by VC for cooling)
- Blow down of high purity water to drain reduced by 91% (VC does not blow down in standby mode).

The water systems were installed with minimal planned downtime, and the two water-generation systems were constructed and validated in approximately one year. All project objectives were exceeded.

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Q: Our management is willing to pay for two industry association memberships of our choice. Can you offer any recommendations in terms of decision criteria we should focus on to evaluate which associations are the best fit for us? We are a contract manufacturing organization.

A: The decision to join industry associations is a positive and sensible one by your company’s management, as memberships with these associations provide a wide range of benefits. Membership can benefit the organization as well as individuals on a personal development level. To evaluate which industry associations are a best fit for your organization, it is best to first draw up a list of requirements or needs. Developing these criteria will help with decision making.

First, let us examine your business environment. Say, for example, that you are a well-established organization that covers the entire drug-development lifecycle, from the clinical phases to commercial production and to postmarketing. Industry associations can be broad in focus or specialized. Some are more focused on the engineering aspects, whereas others are more concerned with quality, compliance, and/or regulatory subjects. That said, the first step in deciding which association is best for you is to identify where your interests are focused. For example, if your company is involved in facility and equipment qualification, including automated systems, then an industry association whose members share this interest would be your first choice. Conversely, if you were in a quality unit-focused role, you would look for an association with a quality and compliance focus. It would not make sense to join an organization whose focus is entirely different than that of your company, thus it is key to hunt for those whose focus aligns with yours.

Before you make a final choice, there are additional considerations to make. One is regarding locality (i.e., does the organization cover the countries or regions where your company is located and is interested in expanding to?). These don’t necessarily have to be the same. You may prefer to be actively engaged with the association (e.g., through participation in interest groups, say for equipment automation, data integrity, or any other subject of interest), which means that you would be looking for a local branch or chapter of that association. On the other hand, your company may also be interested in selling your products globally, which would then make membership in an association with a global footprint preferable.

Next, consider the tangible benefits that you hope to gain from your association membership. Do you want access to strategic concept papers, technical or scientific reports, newsletters, interaction with peer groups, the possibility to interact with agency regulators, or perhaps benchmarking opportunities? Some organizations offer all of these benefits while others only offer some.

Finally, membership may offer you opportunities for personal development through active engagement, such as organizing events or conferences, authoring papers and reports, or even helping to shape the association as a board member. Such engagement can also provide major benefits to your company, as its name will become more prominently known. Its networking opportunities are increased, and its industry standing will be heightened due to its association membership. For this reason, it’s important to look for associations that encourage member participation.

With all this information in hand, you should be well prepared to make your association choices. Do not forget that to gain the most from your membership, you must remain engaged and committed. Not knowing your personal preferences, I cannot recommend individual organizations, but with these tips in mind, I am confident you can identify suitable industry associations.
Manually reading plates can be synonymous with time, frustration, and potential for error. EviSight™ Compact — our smart plate-reading incubator — can detect colonies at 40-250 microns; providing high-resolution color images and enumeration in real time. Get alarms for out-of-spec results earlier, reduce double reading of plates and ensure complete data integrity.

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