UNRAVELING PROTEIN CHARACTERISTICS

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VACCINE DEVELOPMENT INNOVATIONS

UPSTREAM PROCESSING
CELL-CULTURE SUPPLEMENTS

DOWNSTREAM PROCESSING
CONTINUOUS OPERATIONS INTEGRATION

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As face masks start to come off, bio/pharma’s COVID-19 battle goes on.

Moving Into the Next Phase

With some indicators showing vaccines are helping to ease the number of the COVID-19 infections in some regions, many people are asking when they will be able to resume previously routine activities.

The US Centers for Disease Control and Prevention attempted to answer some questions about when it is safe to stop wearing a face mask for typical social situations in an April 27, 2021 guidance for fully vaccinated people (1). The underlying message: if you are fully vaccinated, you can participate in more normal activities without wearing a mask compared with people who have not been vaccinated.

In the United States, the initial frenzy to secure a COVID-19 vaccine calmed as vaccine production ramped up to meet demand; as of late April 2021, almost 30% of the adult US population was fully vaccinated (2). The next concern is convincing the vaccine-hesitant that the risks of not getting vaccinated far outweigh potential side effects of the injection. One positive sign is that as more people are vaccinated, more people holding a “wait-and-see” approach are lining up to get vaccinated. However, a persistent group of approximately 20% said they would get the vaccine only if required or not at all (3).

Various statistical models suggest that the combined number of vaccinated adults and people with some immunity due to exposure to the virus may mean herd immunity is within reach for the US. There are warnings, also, that the nation can slip back and forth over this crucial threshold if a sufficient number of people are not vaccinated.

With the virus raging in hotspots such as India and Brazil, however, a quick end to the pandemic is not in sight. Evolving variants threaten to set back advances made thus far. Some patients have lingering, and sometimes debilitating, effects from COVID-19. The task of healthcare professionals and pharmaceutical researchers to control the pandemic—and deliver new treatments—is not yet done.

NEW AND REPURPOSED TREATMENTS

In the 15 months following the initial outbreak of the SARS-CoV-2 virus, bio/pharmaceutical companies launched more than 850 research programs for vaccines and treatments for COVID-19, including 218 vaccines, nearly 250 antivirals, and nearly 400 treatments; approximately 46% were in clinical-phase development. Approximately one-third of the antivirals and 90% of the treatments were redirected or repurposed therapies (4).

Meanwhile, the National Institutes of Health has announced a large Phase III clinical trial to evaluate existing prescription and over-the-counter medicines for people to self-administer at home to treat COVID-19 symptoms. The goal is to determine treatment options for patients with mild-to-moderate symptoms, who are not sick enough to be hospitalized, to take at home. Currently, there are no approved medicines that patients can take at home to address symptoms of the disease. The trial will study up to seven drugs already approved by FDA for other conditions (5).

While the public may be eager to throw away the masks and get back to “normal” life, the reality is that COVID-19 will not magically go away any time soon. Treatments to manage the symptoms will be a big part of the bio/pharma picture for the foreseeable future. For bio/pharma companies, the work goes on.

REFERENCES

Following several months of negotiations involving regulators, industry, and patient and consumer advocates, updated programs supporting the development and review of prescription medicines are being readied for approval by federal agencies and ultimately by Congress. The fee programs so critical to FDA operations for regulating drugs and other medical products must be renewed every five years, this time in 2022, and a range of entities need to sign off on various changes and updates to the programs. The negotiators are finalizing “commitment letters” that spell out what industry will pay and what policies and programs FDA will implement with those funds, to be referenced in implementing legislation.

Although it may seem that there’s plenty of time to finalize new user fee packages and to gain legislative approval before the current authority expires in September 2022, there is pressure to move the proposals forward in the next few months to meet this deadline. The discussions involve separate user fee packages for drugs and biologics (Prescription Drug User Fee Act [PDUFA] VII), generic drugs (Generic Drug User Fee Amendments [GDUFA] III), and biosimilars (Biosimilar User Fee Act [BsUFA] III). The legislators usually look to authorize the new fee packages as part of a broader bill enacting a host of projects and requirements for drug regulation, development, and marketing, possibly including an updated version of the 21st Century Cures Act with provisions governing clinical research and data disclosure.

A new program may test broader use of “split real-time” review of certain innovative drugs. The program also will continue support for innovative manufacturing technologies and efforts to address more clearly chemistry, manufacturing, and controls issues during development. There is agreement on enhancing oversight of postmarket drug safety and risk evaluation and mitigation strategies, and to utilize more broadly analytics from FDA’s Sentinel

An important goal is to further implement digital health technologies and bioinformation to streamline drug development and review.

for manufacturing and inspections, digital health and informatics, finance, regulatory decision tools, and pre- and post-market review (1). A main topic has been the need to increase fee revenues allotted to the Center for Biologics Evaluation and Research to provide additional support and resources to oversee a fast-expanding volume of applications for cell and gene therapies, including expedited development of regenerative medicine therapies. And industry continues to press FDA for progress in hiring and retention of a “world-class technical and scientific staff” (1).

PDUFA agreements direct the Center for Drug Evaluation and Research to utilize fee revenues to streamline and manage multiple meetings with sponsors and to advance rare disease therapies and use of real-world evidence, model-informed drug development, and complex clinical trial designs. A new program may test broader use of “split real-time” review of certain innovative drugs.

The program also will continue support for innovative manufacturing technologies and efforts to address more clearly chemistry, manufacturing, and controls issues during development. There is agreement on enhancing oversight of postmarket drug safety and risk evaluation and mitigation strategies, and to utilize more broadly analytics from FDA’s Sentinel

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Updates to user fee programs that fund FDA operations are being finalized by industry stakeholders for approval by Congress.
Initiative. An important goal is to further implement digital health technologies and bioinformation to streamline drug development and review.

All these initiatives will continue to boost industry fees. Drug application fees are slated to reach $4 million over the five years of the program ending in 2027, up from nearly $3 million today. This increase reflects the steady rise in the portion of FDA operations funded by industry user fees, which now account for about half of the agency’s budget for regulating drugs and biologics. While some observers complain that this arrangement gives industry too much influence over FDA decisions, Congress seems fairly content with the financial arrangement. And FDA officials maintain that increased reliance on fee revenues does not sway approval decisions.

FEES FOR GENERICs, BIOSIMILARS, OTC:

Negotiations also are winding up for reauthorization of GDUFA III, which was launched at a July 2020 public meeting. After making notable changes in fee categories and rates in 2017 revisions, current discussions have focused on plant inspection processes, drug master file use, application review goal dates, and enhancements to the inactive ingredient database (2). Main innovations aim to facilitate the review of complex generics, of the continued rise in supplements seeking post-approval changes, and for controlled correspondence. These and other changes aim to achieve more application approvals in the first review cycle, possibly by assessing facility status earlier in the review process.

Similarly, FDA is considering revisions to BsUFA III to further streamline the approval process and facilitate patient access to less costly biotech therapies. That discussion began with a public meeting in November 2020, where FDA officials presented their goals for updating the program, acknowledging the need for additional guidance on interchangeable biosimilars (3). FDA seeks to better manage meetings with sponsors, who want advisory sessions to begin earlier in product development. Manufacturers emphasized the importance of building a capable staff to manage this program, along with upgrading information technology supporting BsUFA. Consumer advocates would like FDA to address how current reimbursement policies and misinformation stymies biosimilar marketing, but that may lie outside the user fee agreement.

And, separately, FDA is launching its new user fee program for over-the-counter (OTC) drugs, after agreeing to drop levies on manufacturers that jumped in to produce hand sanitizer to combat the spread of COVID-19. FDA included fees for such firms in its December 2020 publication of policies governing the new Over-the-Counter Monograph Drug user fee program (OMUFA), which was authorized by Congress in March 2020 as part of broader pandemic legislation. But an outcry from firms such as liquor distillers forced FDA to drop the fees for companies that only produce hand sanitizer in response to the public health emergency. A new fee schedule was published March 26, 2021 with revised fees for facilities and for OTC products for the current year (4).

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FDA Weighs in on Remote Inspections

On April 14, 2021, FDA issued guidance on conducting remote inspections, or, as the agency is calling them, “voluntary remote interactive evaluations (VRIEs)” at pharmaceutical and biologics facilities, using videoconferencing software, 360-degree video inspection, and other tools (1). The agency had been criticized for failing to take a stand on the use of remote inspection technologies in light of a growing backlog of plant inspections and delays in new drug approvals due to concerns about putting inspection staffs at risk of contracting COVID-19.

The guidance’s wording makes it clear that the agency views use of these technologies as a temporary measure, during the COVID-19 pandemic. However, it also suggests that FDA may use remote technologies in the post-COVID-19 world, as a way to supplement inspections, gain additional insights into issues found during a traditional inspection, or clarify questions or support a new drug application.

Reference

—The Editors of BioPharm International
Evolving Analytical Technology Unravels Protein Characteristics

Mass spectrometry and automation are growing in importance, but further improvements are still needed.

CYNTHIA A. CHALLENER

While monoclonal antibodies (mAbs) still dominate the biologics drug market, many novel therapeutic modalities are in the biopharma pipeline, with some already commercialized. Many of these next-generation therapeutics present new requirements with respect to analytics. Accelerated approval designations create further challenges with respect to shortened development timelines.

Protein characterization is an evolving and critical application for the biopharma industry that enables the evaluation of critical quality attributes (CQAs) such as identity, potency, purity, and stability, according to Anis H. Khimani, senior strategy and product portfolio leader for life sciences at PerkinElmer. “Analytical technology platforms that can help biopharma companies deliver on CQAs and create reproducible and robust methods of development and validation are important,” he states.

Proteins are large and complex molecules that require methods with high sensitivity, accuracy, and specificity, adds Patrick Tishmack, general manager of AMRI’s West Lafayette, Ind., facility. The characteristics of such large molecules challenge the capabilities of most analytical methods, which then push the limits of available technology. “Primary, secondary, and tertiary structure, as well as purity and potency, are critical performance characteristics to be determined for protein biologics. Aggregation and immunogenicity are also critical issues to address. Protein impurities that are similar to the molecule of interest require techniques with sufficient selectivity to differentiate and separate them. Small-molecule impurities require techniques that maintain reliability over a wide molecular weight range,” he says.

As a result, the biopharma industry is pushed to innovate and be at the cutting edge of analytical technology development. Drug manufacturers, contract manufacturing and research organizations, instrument suppliers, and regulators must therefore work together to advance capabilities for protein analytics to ensure the rapid development of safe and effective biotherapeutics.

CYNTHIA A. CHALLENER, PhD, is a contributing editor to BioPharm International.
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Visit usp.org/biologics/reference-standards/bivalirudin or to contact someone directly about USP Reference Standards please email RSTech@usp.org.
RISE COMPLEXITY AND THE NEED FOR SPEED

Biologics continue to rise in use as therapeutics, especially in cases where small molecules have not fully addressed diseases, such as oncology. This rise has, according to Trevor Jones, head of marketing for analytical chemistry at MilliporeSigma, highlighted the need for easily integrated characterization and quality control methods that can be seamlessly integrated and transferred among sites.

“Speed and accuracy are the drivers,” Jones asserts. “Increasing the speed of assays to approach real-time release and increasing precision or repeatability of experiments provide greater data quality assurance. Structure-function relationships is always key to understanding performance and setting specifications.”

In addition to increasing the speed of workflows, Ulrik Lytt Rahbek, vice-president of assay, analysis, and characterization with Novo Nordisk, notes that automating sample workup and workflows and increasing the resolution and sensitivity of analytical techniques are additional drivers.

For mAbs, platform characterization methods have been established. More complex biologics such as multi-specific antibodies introduce significant complexity, with the number of different charge variants—many of which can be undesirable from a safety and efficacy standpoint—increasing exponentially, according to Susan Darling, senior director for capillary electrophoresis (CE) and biopharma product marketing with SCIEX. “Many of the assumptions made about mAbs don’t apply to multi-specifics, and defined analytical tests are needed that provide as much detail for as many parameters as possible,” she says.

Other increasingly complex modalities include fusion proteins, bioconjugates, recombinants, nanobodies, and oligomeric structures, as well as newer approaches such as lipid nanoparticles, mRNA, and exosomes. Outside of the traditional biotherapeutics, there are the gene therapies that use viral vectors comprising 70 proteins and their encapsitated transgene that all require analytical characterization and development of their product quality attributes, according to Scott Berger, senior manager of biopharmaceutical markets with Waters Corporation.

“As these modalities enter the market, the challenge to assess CQAs and characterize them becomes more difficult. The challenge is to develop methods that are suited to the assessment of very high concentration drug substances, highly-glycosylated species, complex formulations/excipients, and even alternative approaches to traditional host-cell protein (HCP) coverage,” observes Todd Stone, senior manager of analytical development at Catalent.

Not only are new analytical methods required for the more diverse format of protein molecules, the chemistry, manufacturing, and controls (CMC) development cycle has decreased from approximately two years to less than six months, according to Gang Huang, senior vice-president of analytical sciences and clinical quality control for WuXi Biologics. To support CMC development within this compressed timeline, faster and higher throughput analytical methods are needed to meet such challenges. He adds that development of process analytical technologies (PAT) is essential to support continuous processing with real-time analysis solutions.

In addition, comments Berangere Tissot, director of biochem method establishment and biologics characterization with Eurofins BioPharma Product Testing, material availability is becoming an issue. “Protein characterization takes place at all steps within the drug product cycle and for many different reasons. Whether it is the first [investigational new drug] (IND)-enabling characterization or a reference-standard qualification post-commercialization, characterization data are embedded in any regulatory submission but are generally the last items to get checked off the list. The amount of material left to perform these characterization assays is therefore often limited, and the time allocated to get the results is inversely proportional to the depth of the results required,” she explains.

Novel, rapid methods are also needed in the research/discovery phase where thousands of highly complex molecules must be quickly analyzed for various properties and assessed for developability, according to Maria Wendt, head of US large-molecule research and global head of digital biologics platform at Sanofi. “More functionality is demanded from single molecules to afford multi-targeting products. Even so, protein characterization assays for these complex molecules need a certain throughput while still providing important individual molecular information. Melanie Fischer, group leader for assays and analytics at Sanofi, adds that “a comprehensive analytical toolbox composed of high-throughput compatible assets complemented with lower throughput high quality assays is thus key to success to react to the growing zoo of multi-specific drugs.”

COMPLEXITY AND SPEED CREATE CHALLENGES TOO

The complexity of new modalities also makes their production, purification, and characterization more difficult than traditional mAbs, according to Wendt. These products, according to John Rogers, director of chemistry and biochemistry, protein and cellular analysis, Thermo Fisher Scientific, require extensive characterization for structural and chemical modifications, assessment of host cell and other contaminants, and identification of reference standards and methods. “These efforts are often limited by an inability or incompatibility of existing systems to meet the characterization needs,” he observes.

Conventional purity methods such as CE and high-performance liquid chromatography (HPLC), for instance, which are used to quantify product-related variants and impurities, have been powerful tools but suffer from relatively low resolution and insufficient sensitivity for the detection of low-abundant protein prod-
uct variants, particularly in the analysis of multi-specific antibodies containing new variants such as chain-mispairing and other closely related post-translational modifications (PTMs), notes Huang.

Existing methods may also not allow characterization of proteins within complex mixtures, such as in the case of gene therapy products comprising both proteins and oligonucleotides, according to Tissot. “For this new class of products, the data sets are more complicated and there are no platform methods, controls, or reference materials in place yet. Therefore, characterization of these new products requires a different perspective on the existing toolkit, with more controls built into methods and more optimization required,” she remarks.

Drug formulations, meanwhile, are a complex mix of APIs, excipients, buffers, and depending on the mode of delivery, adjuvants, lipid carriers, emulsifiers, and surfactants. “With each novel formulation component, new analytical methods are needed to detect and evaluate their effects on the stability, immunogenicity, efficacy, and safety of the drug,” comments Bettina Kehoe analytical development scientist with Catalent.

In addition, for many new analytical and characterization techniques, in fact, the maturity is often at prototype level, and additional development is required to implement new methods that have demonstrated robustness for higher throughput of sample analysis, according to Rahbek. “Technology is either moving too fast or too slow,” adds Tissot. “Some assays are still using old technology that needs to be improved, such as Edman sequencing, while complex instrumenta- tion is being introduced that only provides meaningful information in an academic research environment,” she explains.

Darling comments that the most common challenge customers are having in the development of new protein characterization methods is ensuring sufficient reproducibility and robustness. This can often mean that exponentially more effort must be invested in achieving the re- ability necessary for the analysis of drugs that are injected into humans. “With the wide range of newer, highly complex and diverse modalities, that goal is becoming more critical to reach,” she says.

Furthermore, new methods may not be adequately scalable and may require unique skills, instrumentation, and software, Rogers adds. Adopting new methods requires dedication of significant time to understanding any new application theoretically as well as specific issues around successful day-to-day operation, agrees Andrew Hanneman, a scientific advisor at Charles River. The appropriate setting (quality control, process development, characterization, etc.) for new methods may also not be initially obvious. As diverse biologics and formulations move from academic laboratories toward commercialization, there is also continuous need for updated analytical methods and instrumentation, according to Kehoe.

Specific issues outlined by Jones include reagents for sample preparation and sample isolation/purification and derivatization, which can cause poor reproducibility and development of reference standards can be difficult due to limited availability. Biologics with newer mechanisms of action will require novel approaches to cell-based assays. Antibody-drug conjugates and protein capsids in viral vectors present their own unique sets of requirements with respect to understanding how specific properties affect functionality.

Regardless of whether protein characterization is needed for a conventional mAb or new therapeutical modality, choosing the appropriate set of orthogonal analytical techniques is crucial and challenging, because one or two methods are rarely sufficient to adequately characterize a biologic, according to Tishmack.

Consolidating assays carries risk too, though, says Tissot, because each individual technique for characterizing a protein—or a more complex biologic—has inherent bias that could lead to a mis-estimation or misleading assignment of quality attributes when combined with other methods. “Some consolidation can happen, but the only way to overcome the need for speed is to develop better interpretation software, because interpretation is often the most complex and lengthy part of a characterization study,” she asserts.

The need to conduct numerous orthogonal techniques to ensure full protein characterization is a challenge not only due to time and resource demand, but also from compliance and data management perspectives. “Addressing software challenges from a compliance perspective is critical to meet regulatory guidelines,” Khimani observes. He points to electronic records and signatures, as well as strong security and audit trails, as hallmarks for 21 Code of Federal Regulations (CFR) Part 11 compliance. For characterization methods that may end up as quality control assays in the absence of equally specific or equally performing quality control-friendly assays, Tissot notes that more may need to be done from a method-readiness standpoint than is usually performed on purified proteins, particularly with respect to 21 CFR Part 11 considerations.

The large amounts of data generated by the array of analytical technologies used for protein characterization, while welcome, is also presenting another layer of challenges. “Many organizations are struggling with both the volume of data and the capacity to leverage it fully in service of their drug-development goals, Bill McDowell, group leader and head of analytics at Abzena, observes.

“We have found that while our clients ask for their data in raw form, some of them just do not have the infrastructure in place to use, manage, and store it properly. It may be that the industry needs to lend even more scrutiny to data handling and sharing practices with labs and service providers while adopting a more common base of standards and technologies to suit the current and emerging needs of biopharma’s developers,” McDowell concludes.
The possibility to automate and increase robustness of current manual workflows has led to expanded use of existing technologies, which has in turn led to measurable advances in protein characterization capabilities, according to Ulrik Lytt Rahbek, vice-president of assay, analysis, and characterisation with Novo Nordisk, pointing to the increased use of capillary electrophoresis techniques and mass spectrometry (MS)-based analysis. Multi-attribute monitoring (MAM), Rahbek says, is of particular note because has the potential to replace traditional methods with one liquid chromatography (LC)-MS based method.

The main overall trend, says Trevor Jones, head of marketing for analytical chemistry at MilliporeSigma, is the movement of LC-MS out of the laboratory setting and into the quality control lab because of its value in biotherapeutic characterization. “We can start to contemplate having fewer assays moved into quality control that are faster and more accurate,” he states. The ability to monitor product and process variation at the intact, subunit, or peptide levels with greater specificity and sensitivity while conducting fewer assays is an objective many labs are now investigating for both development and manufacturing/quality control applications, Scott Berger, senior manager of biopharmaceutical markets with Waters Corporation, agrees. “It comes back to the value statement of what can you discover if you can see more,” observes Colette Quinn, marketing manager for biopharmaceuticals at Waters Corporation. “Not only is it the high resolution of new mass spectrometers but also the incorporation of ion mobility into quadrupole, time-of-flight mass spectrometers that is enabling scientists to deconvolute complex spectra and better identify peaks that were overlapping,” she explains.

Important recent advances in protein characterization include intact protein analysis with ultra-high mass range-mass spectrometry, and simpler and less costly instruments for functional assessment of binding activity and protein secondary structure as, says John Rogers, director of chemistry and biochemistry, protein and cellular analysis, Thermo Fisher Scientific.

Sensitive detection and quantitation of sub-visible viral particles and protein aggregates are improving in several areas as well, according to Andrew Hanneman, a scientific advisor at Charles River. In addition to instruments capable of measuring several aspects of protein structure within a single run and MAM solutions, specific examples include improved in-vitro bioassays, analytical methods runnable under good manufacturing practices using fully 21 Code of Federal Regulations Part 11-compliant instruments and software, native LC-MS of large intact proteins and glycoproteins by various high-performance (HPLC) methods including ion-exchange and size-exclusion chromatography, and alternate fragmentation approaches for MS/MS of modified peptides and branched structures, including electron capture dissociation/electron transfer dissociation-MS and multi-stage MS.

Ion mobility-mass spectrometry (IM-MS) and hydrogen-deuterium exchange-mass spectrometry (HDX-MS) are two other MS-based techniques that should find many applications with protein biologics, according to Patrick Tishmack, general manager of AMRI’s West Lafayette, Ind., facility. He notes that IM-MS adds a unique dimension to MS because the shape of the molecule can differentiate multiple conformations, aggregation states, or small mass differences that cause heterogeneity of proteins. HDX-MS, meanwhile, is useful for studying protein-protein and protein-ligand interactions, conformational changes related to protein activity, and protein stability among other phenomena. “The detailed understanding of protein behavior that HDX-MS affords therefore has direct application in biologics drug discovery and development,” Tishmack observes.

In Sanofi’s research lab, there is real need to access high-throughput methods that perform at a high level even for highly complex biologics. One recent advance noted by Maria Wendt, head of US large-molecule research and global head of digital biologics platform at Sanofi, is a method coupling size-exclusion chromatography with MS for the rapid characterization of tri-specific antibodies. “With this beautiful characterization capability for such complex molecules, Sanofi is placed in a much better position to deliver more complex drugs with a higher likelihood of success for addressing more challenging diseases,” she states.

“It has been difficult to date to couple really high-resolution techniques with mass spectrometry, but real progress has been made, which is giving scientists the ability to achieve deep characterization quickly,” agrees Susan Darling, senior director for capillary electrophoresis and biopharma product marketing with SCIEX. She highlights capillary isoelectric focusing-MS as a method providing rapid separation and identification of charge variants with resolution high enough to identify isoforms on intact proteins. The possibility of identifying both charge and size variants at high resolution down to the PTM level for peptides is a game changer, according to Todd Stone, senior manager of analytical development at Catalent. “Efficiency gains from the elimination of bench-scale fractionation and sample preparation are enormous, while the increased depth of data provides a much more comprehensive view of potential molecule degradation pathways and subsequent adjustments to critical quality attribute (CQA) control strategies,” he says.
The demand for biopharmaceuticals has risen dramatically over the last two decades, and so has the demand for contract development and manufacturing of those biopharmaceuticals. Between existing and newly emerging CDMOs, how do you decide which one to partner with?

Considering the long-term nature of CDMO partnerships in general and the intensely regulated and complex nature of biologics manufacturing in particular, selecting a robust and resilient service provider that fits your strategy is vital.

FROM FIRST MEETING TO FIRST MILESTONE
A CDMO partner must cover the rapidly accelerating demand for biopharmaceutical development and manufacturing as well as have the flexible capabilities and agility for emerging biopharma companies to secure the long-term success of their drug strategies. Here are four common attributes to look for in a potential CDMO for a successful partnership:

1. QUALITY. A solid regulatory track record is a very basic trait a CDMO must have. Nowadays, CDMOs must add value beyond that and should be able to support customers in quality through the adoption of a digital quality system, which not only offers improved visibility on quality documents across the entire process, but also reduces compliance risk.

Samsung Biologics has its own Enterprise Quality Unified Information System (EQUIS), which gives clients access to the project in real-time, no matter where they are, to request, review, and approve documents on-demand.

2. ACCESSIBILITY. When partners have common access to a secure platform that enables clearer and more open communication in real-time, collaborations are more successful.

Samsung Biologics’ Live Virtual Tour solutions offer a high-definition virtual connectivity platform where clients are able to view the company’s manufacturing facilities virtually as if they are on-site. It also allows regulatory auditors to inspect and evaluate the level of cGMP compliance from any location around the world, ultimately creating a more efficient process.

3. FLEXIBILITY. Any CDMO can meet your short-term needs if they have enough time, manufacturing capacity, and certain level of competency with the technology. But if you’re looking for a long-term reliable CDMO, look for one that can read the market’s potential future and be ready to respond dynamically in your best interest.

Samsung Biologics’ facilities are designed to scale to the varying needs of clients from large-scale commercial manufacturing to small-scale CMO. The company currently holds 364,000L of biomanufacturing capacity and will attain up to 640,000L in total upon completion of Plant 4 in 2023. The company is able to provide a full range of CDO, CMO, and analytical testing services to offer a complete one-stop capability.

4. SPEED. Expediting the development timeline is directly related to obtaining IND clearance for initiating Phase 1 study faster. To speed things up, Samsung Biologics offers a high-performing proprietary cell line, S-CHOice capable of titers above 7g/L for standard monoclonal antibodies, and enhanced cell viability of 90% at day-21 in fed-batch studies.

Samsung Biologics also offers clients reduced timelines, combined with well-validated systems and open communication. Making subsequent tech transfers and scale-ups less complex with fewer hurdles, all of which accelerate the commercialization of drugs.

THE BEST CHOICE: SAMSUNG BIOLOGICS
With remarkable manufacturing agility plus state-of-the-art digital quality systems, Samsung Biologics delivers quality-driven development and manufacturing services, as demonstrated with more than 85 global approvals within less than a decade.

Its innovation and commitment to quality, willingness to be open, readiness to be flexible, and unparalleled speed make Samsung Biologics your perfect CDMO partner.
### Biophysical Analysis Developments Important

Gang Huang, senior vice-president of analytical sciences and clinical quality control for WuXi Biologics, emphasizes the importance of the development of methods for in-vitro bioactivity analysis that better reflect the clinical effect of biologics products in humans. “Through the study of 70+ specific cellular models of pathologies, WuXi Biologics has developed proprietary technologies enabling the best linking of in-vitro cellular response and ex-vivo biomarkers to in-vivo responses for biologics. These technologies allow effective reduction of complicated clinical effects into simple, clear CQAs at the cellular or molecular levels, and are therefore becoming an integral quality tool for us,” he comments.

Considerable advances have also been made in biophysical instruments, according to Berangere Tissot, director of biochem method establishment and biologics characterization with Eurofins BioPharma Product Testing. “Manufacturers are striving to produce new models that are more versatile, more resolute, and more efficient from a sample-volume-requirement standpoint,” he says. The latest analytical ultracentrifugation (AUC) instruments, for example, allow analysis using up to 20 discrete wavelengths. “This advancement has the potential to revolutionize the field of AUC by enabling more precise analysis of complex systems,” Tissot asserts.

### Data Analysis Developments Are Noteworthy

Some of the biggest developments have occurred with data processing capabilities. “Although advanced MS techniques are attracting researchers’ attention, what is advancing the refinement of analytical techniques more recently is the introduction of increasingly smart information and data analysis and sharing systems and automation solutions that speed up things like sample prep and analysis,” says Bill McDowell, group leader and head of analytics at Abzena.

“The refinement of both the specific techniques and their supporting technologies, as well as the refined and focused application of the methodologies themselves, has evolved to a point where the industry’s confidence in these studies is extremely high because the data adds such tremendous value,” says McDowell.

### Resistance to Change

Innovation and its adoption are linked to confidence in the measurement, asserts Colette Quinn, marketing manager for biopharmaceuticals at Waters Corporation. One way to build that confidence, she says, is to introduce new methods to various places within the workflow. There tends, Tissot agrees, to be less opposition to new characterization techniques for new quality control methods. “Because characterization relies on scientifically sound principles, it is up to the applicant (and its providers) to demonstrate that the method used or that the technology used is suitable for use and scientifically sound,” she notes.

Fortunately, regulators realize that protein characterization as quality control is an emerging field and, therefore, are flexible when it comes to the development of new tools and methods, according to Jones. “Drug manufacturers are often conservative in their approach to new methods because of the uncertainty it might cause in approval delays. We have found, however, that regulators are open to novel approaches so long as they are scientifically sound and meet validation and/or qualification criteria,” he comments.

The key to successful adoption of new analytical methods by regulators, according to Huang, is demonstrating that they are not only scientifically sound and robust, but also capable of generating reliable results and useful for defining control strategies. New methods should, he adds, be developed to analyze certain CQAs that are not covered by the existing panel of methods or otherwise be superior with respect to performance (accuracy, sensitivity, reliability, etc.). Ideally, says Khimani, they should also offer a broader applicability from discovery through development, including downstream process monitoring and quality assurance/quality control.

For more novel approaches such as multi-attribute monitoring (MAM), also known as quantitative peptide mapping, the emerging technologies teams within regulatory bodies often act as bridges, enabling companies to collaborate with regulators to build confidence and best practices for employing those methods, according to Berger. This approach has, he says, allowed many companies using MAM approaches to work toward deploying them beyond development and into clinical lot release.

There is also, however, Darling adds, the need to build consensus within the industry as a whole before any new method is widely adopted. First the consensus must be established between scientific experts at various biopharma companies; wider consensus can then only be built through back-and-forth with different groups within the various regulatory authorities. “Achieving critical mass is a slow process. Most companies vet a new
technique or method for at least a year or two before moving it into quality control," she says.

For regulators to accept new technologies for CMC-based characterization analysis, they need to see demonstration of equivalency or superiority of the new method for its intended purpose, Berger says. “Significant crossover studies may be required to demonstrate these outcomes, as assays in characterization not only document normal product variation, but are often employed for stability, forced degradation, and process development studies that give rise to a wider variety of product variants, often at low levels,” he comments.

Contributing factors leading to slower adoption of new analysis and characterization techniques include the need to invest in new equipment and build up necessary competencies, and the time delay for projects compared to what is possible using conventional methods, most notably due to the need to demonstrate comparability to current methods, according to Rahbek.

In addition, Darling points out that if an existing method is part of a regulatory filing for a global product, then both the old and new methods will need to be performed until all of the regulatory agencies approve the new method, which can take years. “Not surprisingly, there have to be compelling reasons and significant benefits for a drug maker to switch to a new method for a commercial product,” she says.

The resistance to adopting new analytical techniques for development candidates is often tied to the need to adhere to strict program timelines, according to Robert Vaughan, associate principal scientist in Catalent’s analytical development group. He also notes that there is additional risk associated with adopting less-established technologies given the added cost and potential for unanticipated method-specific challenges that might be encountered during development.

“Furthermore,” Vaughan adds, “adoption of any new method that requires specialized equipment would require training analysts in both process development and quality control, acquiring systems for both groups, integration into a regulatory-compliant data management system and subsequent validation. Redundancy of equipment is also important to maintaining timelines should one system go down, which can significantly increase the cost. When benchmarked against established platform methods that have a history of success within a regulatory environment (albeit lower resolution), the added time and cost can be a significant barrier.”

Rogers agrees. “Many companies already have investments in proprietary and/or established methods that would require significant commitment and investment to replace. There also may be questions regarding the validation requirements, and often there is a fundamental resistance to change that must be overcome as well,” he says.

Adding to the challenge is the fact that the benefits of new and improved methods may not initially be obvious prior to testing in one’s own laboratory, according to Hanneman. “In a fast-paced industry where experience is highly-valued, experienced developers may need to choose familiar approaches for expediency, presenting a barrier to adoption,” he observes.

INCENTIVES FOR ADOPTION OF NEW METHODS, TOO
It should be noted, though, that because the increasing number and diversity of new modalities is creating the need for new analytical methods, there is a growing level of incentive to adopt new techniques for these new biologics in particular. “All of thesebiotherapeutics would benefit from the use of orthogonal approaches that can help to reduce bias toward one method or one result,” Quinn asserts.

“Scientists tend to gravitate towards solutions with which they are most comfortable, and although data might be collected along the way, inserting a number into a cell on a spreadsheet can be a lost opportunity,” says Quinn. “Instead, the goal should be to increase confidence and look for trends that could save time when future studies are conducted. In addition to asking what has changed, it is important to consider whether that change matters. To answer both questions truly requires an orthogonal approach,” she asserts.

In addition, Quinn notes that by linking outcomes, the values from these systems can possibly become predictors of the next set of tests. As an example, she points to linking chemical outcomes like those determined via LC separation with native ion mobility or biophysical data such as differential scanning calorimetry and ultimately extending this information into interpretation of binding assays.

Berger adds that with the recent progress in International Council for Harmonisation guidelines Q12 and Q14 there is greater potential for more flexibility in analytical methods used for product analysis, which is aligning well with the growing interest in pursuing more formal analytical quality-by-design (AQbD) method development approaches. “The power of platform methods to accelerate COVID-19 molecule development and the benefits of systematically optimizing these methods is becoming very clear,” he says.

INNOVATORS LEAD THE CHARGE
All stakeholders, such as biologics manufacturers, regulators, technology providers, and contract research organization (CROs), need to consider the properties and stability of protein drugs. A concerted collaboration and partnership between biologics developers, technology/service providers, and regulatory agencies greatly facilitates bringing a safe and efficacious protein drug to market, says Khimani.

“Collaborations between industry, regulatory agencies, instrument companies, and standards organizations during the development of new methods [are] critical,” agrees Rogers. It is key to developing technologies and methods that are robust and fit-for-purpose, adds Berger.

“Organized industry meetings bring these
groups together to explore technology, methodology, and its impact on regulatory science. Other more focused collaborative organizations such as the National Institute for Innovation in Manufacturing Biopharmaceuticals and the MAM Consortium dive deeper and look to establish consensus performance expectations and best practices to bring new methods into routine use,” he explains.

The R&D/analytical development departments of biologics manufacturing companies are in the unique position to bridge established, legacy-testing methods with newer technologies emerging from academic research programs and innovative instrument companies, Kehoe asserts.

“Whether it is an improved reagent, a new assay, or a more efficient instrument, these departments can show equivalency to current CQA assays, demonstrate improved test performance, or provide additional orthogonal testing options,” she says.

“Instrument companies may be looking for new applications for their existing technology that have not yet migrated to the protein biologics field, or they may partner with a biologics manufacturing company or CRO who tests biologics to develop something completely new to solve an existing problem,” Tishmack comments.

Instrument vendors, meanwhile, will often introduce brand new technologies to the entire community including regulators, building a sense of urgency, according to Rahbek. In addition, Kehoe observes that as instrument companies continue to include installation qualification and operational qualification in their analysis programs, methods developed on these instruments can be transferred readily to quality control laboratories for the release of manufactured drug products.

Most CROs, biosimilar developers, and contract manufacturers often have low inducement to develop new analytical and characterization methods because they are mostly required to follow already established methods, according to Rahbek. However, Jones notes that some CROs and contract development and manufacturing organizations (CDMOs) do pursue the development of cutting-edge methods for protein characterization as a means of differentiation and to attract pharmaceutical companies seeking outsourcing partners that specialize in specific assays.

CROs with biologics expertise will know the existing challenges andcontinuously look for appropriate and efficient solutions, adds Tishmack. “Many times a CRO will advance a unique application of a common technology because of the wide variety of problems they encounter and solve for their clients,” he says.

Overall, Huang observes, instrument companies manufacture analytical instruments and periodically incorporate new technologies into these analytical tools for biologics manufacturers and CDMOs/CROs. Drug developers are willing to explore new technologies and turn them into new analytical methods. Regulators ensure these new analytical techniques can serve the intended purpose while ensuring patient safety is not compromised. If a regulatory agency finds a new method for biologics characterization acceptable in a filing for one developer, this increases the likelihood of propagation of the method for use with other biologics, Tishmack observes.

While the fact that large biopharmaceutical manufacturers set the tone for biophysical characterization helps get cutting-edge techniques publicized, recognized, and exposed to regulators, there is a downside, according to Tissot: smaller companies are sometimes left in a difficult position because not all can afford highly expensive, specialty instruments nor the time to develop an optimized method internally or at an external provider. In addition, she notes that when instrument vendors collaborate with large biopharmaceutical manufacturers, they often tailor their software to the needs of those companies. Once the software is released, smaller drug developers are sometimes forced to follow the process that the software was optimized for.

Quinn argues, though, that most of the time the molecules drive innovation in the analytical instrumentation sector. “Strong collaborations between manufacturers, CROs, and analytical instrumentation companies leads to hyper-personalization of equipment, chemistry, and methods and enables faster progression of the science,” she asserts.

“What is exciting,” claims Fabio Rossi, scientific lead at Abzena, “is the increasingly collaborative role the industry is taking collectively to shape and refine protein characterization methods and study protocols. Abzena is able to improve on its clients’ methods to achieve more efficient and effective outcomes. Instrument companies are also in a cycle of continuous improvement, and over the past five years or more we have seen a tremendous improvement in study cost efficiency, speed, and the efficacy of the data analytics,” he says.

**A FEW UNIQUE CHALLENGES**

Despite all of the advances in mass spectrometry and other methods, there is still opportunity for further improvement. For instance, there is a significant need for alternative approaches to HCP characterization and clearance during purification, according to Vaughan. “HCPs are a unique challenge given their complexity and diversity, and they pose a significant risk to any protein therapeutic given the potential of certain HCPs to impact product stability and immunogenicity of patients,” he says.

Enzyme-linked immunosorbent assay-based approaches used currently have high sensitivity against a single target, but there are thousands of HCPs present in the initial harvest, which have the potential to vary between lots. Mass spectrometry, Vaughan believes, is well-poised to fill this gap given its ability to both identify and directly quantify individual HCPs with both high sensitivity and accuracy.

Quantification of polysorbates (PS20 or PS80) in protein therapeutic formulations is another area in which advances
in analytical techniques are still needed, according to Kehoe. “Regulators have recently started to increase the detail required of drug formulations, and the analytics for characterization of polysorbates is lagging behind,” she observes.

Glycosylation analysis remains a challenge as well. “Analysis of the N- and O-glycan profiles of any molecules but mAbs is an arduous task,” Tissot remarks. “Most of the methods are either highly quantitative or highly qualitative and there is no real happy medium where you can safely assign structures and report relative percentages in one go without major bias,” she explains. The ability to use LC-MS with electron capture dissociation/electron transfer dissociation (ECD/ETD) is an improvement, but most of the workflows and techniques remain in the academic realm to date.

The bi/multi-valent nature of multi-specifics bears the risk of self-association that can result in poor high-concentration solution behavior, according to Fischer. She believes high-throughput predictive assays to assess colloidal stability of biotherapeutics early on are a key advantage in the discovery of multispecific drugs. Similarly, the analytics of mispairing, in particular when compound numbers are high—in discovery but also in cell line development—is a challenge. Here again, Fischer notes that compatible MS approaches are important for effective analyses of samples with increasing compound numbers.

Rogers also points to the need for tools and workflows for efficient protein variant screening and structure determination across a broad molecular weight range, amino acid misincorporation, characterization of intact proteins and protein complexes, characterization of low-abundance PTMs (natural or introduced by the manufacturing process), and the characterization and quantitation of specific proteoforms. “Workflows utilizing CryoEM, [ultra-high mass range] (UHMR)-MS, and complementary tools and software are of great interest to structural biology researchers in academia and industry because they offer great potential for improved screening, characterization, and understanding of protein structure and proteoforms,” he states.

The rapid emergence of viral and nanoparticle-based delivery has created the need for methods that address questions of composition (e.g., lipid profiling), as well as measures for proper assembly of these macrostructures (e.g., empty/partial/full analysis for the biotherapeutic molecule), according to Berger. The acceleration of gene and cell therapies reinforces and extends these needs.

Waters is working with emerging technology companies such as MegaDalton Solutions to bring new technologies like charge detection-MS (CD-MS) into the commercial realm. The company is also, Berger notes, investing in new generations of HPLC and ultra-performance liquid chromatography/ultra-high performance liquid chromatography chemistries and consumables for the next generation of size- and charge-based separations for application to new modalities because these methods will also play a key role in routine analysis.
Overcoming Vaccine Development Challenges

Vaccine development is inherently challenging; however, in light of the COVID-19 pandemic, innovations have been prioritized, leading to accelerated development processes.

FELICITY THOMAS

Vaccines are known to be hugely important in maintaining global public health and minimizing the impact of infectious diseases worldwide. Despite their worth, vaccines are subject to intense scrutiny, and, due to the fact that they are intended for large patient populations—including healthy patients and infants—the assurance of their safety, prior to approval and administration, is of paramount importance.

According to recent market research, the vaccines market is expected to grow at a compound annual rate of 14.7% for the forecast period of 2020–2026 (1), the growth of which has been accelerated by the recent COVID-19 pandemic. “Heightened by the recent COVID-19 outbreak, vaccine innovation has become a top priority worldwide,” confirms Elham Blouet, strategic portfolio manager, Roquette—specialist in plant-based ingredients and provider of pharmaceutical excipients.

“In recent years, a paradigm shift has been observed in the way vaccines are being developed,” says Smruti Phadnis, biopharma marketing manager at Agilent. “The focus on disease-causing pathogens has been well integrated with intensive studies on human immunity. This research has opened doors to allow advances against complex noncommunicable diseases as well as cancers. However, inherent challenges, such as antigen selection and genetic diversity, need to have a broader immune response that includes T-cell protection, and alternative routes of vaccine delivery still exist during vaccine development.”

DEVELOPMENT CHALLENGES

A constant challenge facing pharma companies developing vaccines is the wide range of potential pathogens that could cause serious illness, explains Kai Lipinski, chief scientific officer of Vibalogics. “Great strides have been made over the years to develop and commercialize effective vaccines for a wide range of infectious diseases,” he adds. “However, there are still particular illnesses that remain difficult to prevent or treat, such as HIV, malaria, norovirus, tuberculosis, and Zika virus.”

Particularly when trying to develop vaccines for difficult to treat illnesses, development obstacles can arise due to the inherent
properties of the responsible organism, Lipinski continues. “Organisms displaying high rates of antigenic shift, such as RNA viruses including influenza and even the recent coronavirus (COVID-19), pose one such challenge,” he notes. “Antigenic shift of organisms yields frequent new variants, which develop very quickly, subsequently requiring surveillance, identification, and recomposition of the modified vaccine antigen or strain, often with a seasonal cyclicality.”

As vaccines are derived from pathogens, there are myriad antigen or target types to identify and select for development, emphasizes Phadnis. “Each type has its own degree of safety and efficacy,” she says. “To add to the complexity, non-live vaccines need to be combined with adjuvants (carriers) to induce immune response and slow release. Target/antigen selection is complicated further by genetic diversity across strains, including post-infection evolution and geographic variability.”

A key challenge in vaccine development for Anissa Boumlic, head of global vaccine and plasma segments, bioprocessing, within the life science business at Merck KGaA, is the fact that there is not a singular manufacturing template available to be widely used. “Manufacturers need to adapt constantly to tackle new pathogens and, sometimes, under tight deadlines,” she says. “A number of processes need to be optimized to increase scalability, robustness, efficiency, and cost. Additionally, formulations of certain vaccines need to be improved to increase efficacy and stability while ensuring patient safety, and facilitation of distribution and access.”

Lipinski concurs that, in addition to the challenges associated with vaccine candidate discovery, companies need to develop vaccines that are stable, can be stored, and administered in a variety of climates to ensure widespread distribution and use. “For example, emerging countries often lack appropriate facilities for vaccine storage at sustained sub-zero temperatures at or near the point of treatment, which can pose issues in launching vaccination programs that require cold-chain storage,” he says. “With this in mind, developers are challenged to devise vaccine formulations capable of being stored and transported at temperatures that don’t restrict their widespread use. Formulations must also allow for in-use stability, in the brief window between removal from storage and administration. They must be able to remain stable in tropical environments, where temperatures might exceed 30°C.”

“Lyophilization or spray drying of vaccines can provide stability during transport and introduce alternate vaccine administration routes,” continues Phadnis. “Packaging is also heavily impacted by liquid vaccines. Durability of glass vials at very low temperatures and permeability of plastic vials has complicated the packaging decisions as well.”

Since the beginning of the pandemic, the bio/pharma industry has been under pressure to produce stable formulations for effective vaccines in accelerated timescales, Blouet asserts. Moreover, the drive for a COVID-19 vaccine has occurred during a period of increased basic scientific understanding, such as in genomics and structural biology, supporting a new wave of vaccine development and production, she says.

“As well as bringing about much needed innovation in a historically conservative field, [COVID-19] has also raised questions about whether conventional vaccines—developed by attenuating or inactivating the respective pathogen—are enough in today’s landscape. Although mostly successful in the past, these established methods may not always be suitable or even feasible in outbreak situations because they simply don’t allow for the fast response required,” Blouet reveals.

OVERCOMING BARRIERS

There are numerous tools that have been developed to help accelerate vaccine development, and novel technologies and platforms are emerging. “Manufacturing platforms, such as viral vectors, virus like particles, and messenger RNA, allow a reduction in process development time once established,” Boumlic remarks. “Systematic methodologies such as design of experiments (DoEs) and quality-by-design (QbD) have demonstrated the ability to increase process robustness and scalability.”

Processing has been simplified through the implementation and availability of single-use and filtration technologies, Boumlic continues. These tools have enabled an increase in speed of development and are easy to use. Boumlic also notes, however, that flexibility in scale, technologies to manufacture different modalities, more process simplification, and modernization are areas that require improvement still.

According to Phadnis, in addition to single-use technologies, automation for high throughput and robust analytical assays are necessary for rapid turnover during development and manufacturing of vaccines. “Automation technologies can speed up vaccine research as well as the manufacturing process by efficiently reducing manual errors. Automation also gives the flexibility of scaling up or down depending on the external demand. Simultaneous high-throughput workflows supported by robotic automation can swiftly generate consistent data across samples and users,” she says.

Using robust analytical assays for the characterization and validation of vaccine components, and to measure potency, are critical for inducing immunogenicity, Phadnis emphasizes. “Right from antigen selection and functionality testing at laboratory level to its final manufacturing, an integrated workflow of several analytical methods is required to ensure efficacy and safety of the vaccine,” she notes. “Innovations in mass spectrometry (MS) and capillary electrophoresis (CE) combined with in-line detection methods, such as high-performance liquid chromatography (HPLC), provide high sensitivity, selectivity, and accuracy for multidimensional analysis of samples. This innovation aids robust monitoring and control of key attributes of...
vaccines during development and enables a quick and reliable decision-making process during product development and manufacturing.”

For Blouet, the critical role of functional excipients, specifically cyclodextrins, is gaining interest for those in vaccine development seeking to overcome formulation challenges and produce a safe and efficacious vaccine quickly. “Cyclodextrins are a family of oligosaccharides produced from starch using enzymatic conversion,” she states. “2-hydroxypropyl-beta-cyclodextrin (HPβCD), in particular, is reaching into therapeutic biologic processes, final formulations, and delivery of vaccines, and is being used in some promising new areas where work is still at a very early stage.”

“To shorten formulation development timelines, accelerated temperature predictive modeling is key,” adds Lipinski. “However, this requires robust and well-designed analytical assays to assess a formulation’s performance.”

**STABILITY ISSUES**

Both physical and chemical stability of vaccines are ongoing formulation challenges, notes Blouet, particularly given the novel technologies being employed and demand for increased shelf-life for convenience and cost-efficiency. “Physical and chemical instabilities may lead not only to the loss of biological activity, but also potentially to the formation of products that detrimentally affect toxicity and immunogenicity,” she says.

Using adeno viruses as an example, Blouet highlights that a “robust formulation, with precise optimization of buffer type, pH, and specialized excipients, are critical to ensure stability over a wide range of conditions. But options for addressing stability challenges in adeno virus vector formulations are limited to those few excipients approved for parenteral use.” Here, she emphasizes the utility of HPβCD, which is already approved for existing commercial formulations.

“At the same time, a key challenge in recombinant subunit vaccines is their stabilization for delivery,” Blouet continues. “These are typically protein-based and very few approved excipients are available for formulators working in this area.” Again, she emphasizes HPβCD as a potentially useful excipient to stabilize subunit vaccine proteins.

“Certain vaccines are less stable than others depending on their inherent properties,” specifies Boumlic. “For example, viruses or mRNA can be degraded through the process and a number of approaches need to be considered to minimize product loss—gentle purification, specific reagents, degradation inhibitors, stabilizers, delivery vehicles, and so on.”

It is imperative that the stability of process intermediates, or the product matrix as it moves through the manufacturing process, be understood and addressed, emphasizes Lipinski. “As downstream purification processes can span multiple days, a vaccine that is sensitive to the process itself, or cannot be subjected to freezing due to freeze-thaw processes, will present complications or limitations,” he says. “Developers must link drug substance and drug product manufacture, often within one facility, or within the limitations of the stability of the fresh (unfrozen) product, which can pose issues with often desired manufacturing flexibility.”

Combinatorial platform excipient banks can be used to speed up formulation development while overcoming stability issues, Lipinski states, which involves developing each vaccine in the same design space to optimize stability while reducing time-to-market. “Vaccine technology platforms (materials, processes, formulations) can also be used to minimize the development time for new products, as this approach offers considerable advantages where one universal formulation creates efficiencies that can be utilized across new products minimizing changes and allowing for a more predictive development paradigm,” he adds.

QbD and DoE-based principles, supported by orthogonal analytical methods, are significant when optimizing and streamlining the assessment of formulation stability, Lipinski remarks. “As an example, using physicochemical analytical methods to assess formulation performance as an indicator for infectious titer in the case of live-attenuated vaccine development can potentially reduce development timelines,” he says. “If sufficient knowledge is not available in-house to perform formulation stability studies to a high standard and with minimal delay, collaboration with experienced [outsourced service providers] is vital.”

The majority of commercially available vaccines are formulated as liquids, which, if kept in cold temperatures, can maintain their potency for about three years, asserts Phadnis. “However, freeze-thaw cycles can lead to immediate loss of potency,” she stresses. “Alternate formulations such as lyophilization and spray drying are being considered for alternative administrative routes as well as for ease of distribution and stability of the vaccines. However, dehydration needs antigen-protective excipients such as a combination of amino acids and glucose derivatives to replace the water molecules.”

**ROUTE OF ADMINISTRATION**

“The route of administration has a significant impact on the development pathway of a vaccine,” Lipinski asserts. “Oral vaccines, such as those utilized for poliomyelitis or rotavirus, offer a number of advantages when it comes to development compared with parenteral products.” However, oral administration is not suitable for all vaccine candidates.

Administering vaccines nasally can also provide advantages over the more traditional parenteral approach, such as the avoidance of needles, ease of administration, and convenience for respiratory diseases, Lipinski continues. “One further delivery device being utilized is the micro-needle-based vaccine,” he says. “This [route] overcomes patients’ syringe needle anxieties while providing further benefits in its ease of use, and lack of cold chain distribution and storage need, due to its solid-state nature. This technology
can also be self-administered by patients. However, sufficient production capabilities and capacities for such products are not available yet.”

The majority of vaccines are delivered intramuscularly with other routes including subcutaneous, intradermal, oral, or nasal, confirms Boumlic. The administration route of choice is determined by the type of vaccine being developed (i.e., whether the vaccine is adjuvanted or not) and whether the efficacy versus side effects can be balanced, she adds.

“The route of administration will influence the process to meet the associated safety requirements or to trigger sufficient immune response. For instance, injected vaccines will need to be sterile, and this can impact process design and cost efficiency,” Boumlic says. “Certain vaccines will need to be injected in high quantities to be efficacious and, therefore, this will impact the dimensions of the process (more volumes to handle, more scalability challenges). Messenger RNA vaccines need to be delivered in lipid nanoparticles to remain protected and be translated to antigens.”

The route of vaccine administration is dictated by the formulation and potency requirements, notes Phadnis. “Technologies that enable lyophilization, spray drying, and use of inert stabilizers in the form of lipids and nanoparticles are being developed for ease of administration and distribution of the vaccines,” she reveals. “A careful optimization of stabilizers as well as different formulation parameters during the first stage of vaccine product development should be done along with antigen screening and validation.”

“A glaring constraint with injectable delivery systems for large-scale vaccine programs is a complete reliance on cold storage, which directly escalates operational costs and diminishes market reach,” emphasizes Blouet. “For example, to ensure that each patient benefits from the injectable vaccine, meticulous logistical planning is required to successfully maintain the cold chain at every step, often involving collaborations with supply chain partners.”

**A PARADIGM SHIFT**

The COVID-19 pandemic has presented industry with significant and unprecedented challenges in terms of vaccine development; however, opportunities have also arisen. “Historically, vaccines took about 10 years of development to reach the market and were mostly restricted to affected areas,” states Phadnis. “With the COVID pandemic, we have seen a paradigm shift in shortening the vaccine development process to less than a year. mRNA vaccine technology to make highly potent and safe vaccines was a breakthrough during this pandemic. This technology was implemented for the very first time in commercial vaccines.”

Additionally, the fact that the pandemic has raised questions about whether or not conventional vaccines are sufficient in today’s landscape has challenged developers to push the boundaries of vaccine development, Blouet notes. “Rapid development and accelerated large scale vaccine manufacture are both critical factors in outbreak situations, key to combatting pandemics and protecting individuals worldwide by getting solutions to market faster,” she says. “To get vaccines to market quicker and overcome complex formulation challenges, more developers than ever are moving away from traditional vaccine development models.”

For Boumlic, the pandemic has demonstrated the requirement of standardized vaccine manufacturing and has highlighted the need for expanded capacity to ensure biopreparedness. “A clear trend is the development of platforms that can be easily adjusted for new targets. Using recent technologies, such as viral vectors and mRNA, single-use technologies and tapping into contract manufacturing organization capacity has also been beneficial to increase productivity and will shift vaccine development and manufacturing in the future,” she says.

“One key development that has proven useful during the pandemic and will change the way we operate in the future is the creation of vaccine platform technologies,” concurs Lipinski. “The advantage of such a platform approach, in the example of adenoviral vector platforms, is that the different vaccine products are, with the exception of the disease-specific transgene, the same. This shortens the production timeline for a number of reasons, as the producer cell bank and expression technology are already in place and the vaccine design tools and manufacturing infrastructure are already established. Moreover, formulation and stability data are available from products previously developed with the same platform technology.”

Furthermore, collaboration by all stakeholders has been highlighted as crucial during the COVID-19 pandemic, Lipinski emphasizes. “Greater collaboration should continue into the future. Not only will it support national governments in preparing for future outbreaks, but it will also allow knowledge-sharing between businesses and academia to devise the technology and infrastructure needed to develop treatments more quickly,” he says.

“As mentioned before, the inherent challenges would remain as different pathogens emerge,” Phadnis concludes. “However, the rapid development of the COVID vaccines and their usefulness to control the pandemic has given us hope for a better future in vaccine development.”

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**REFERENCE**

Recombinant Supplements Offer Lower Risk of Cell Culture Contamination

Demand for recombinant microorganism-based cell culture supplements is rising on the back of lower contamination risk.

FELIZA MIRASOL

The global market for recombinant cell culture supplements is forecast to grow more than 6% from 2021–2031 as demand rises, and specific demand for microorganism-based recombinant cell culture supplements is expected to grow at an even higher pace, driven by rising demand for animal-free cell culture supplements (1). Microorganism-based recombinant cell culture supplements offer the benefit of eliminating the risk of contamination from cell culture media and are animal-free derived material.

CHALLENGES IN TRADITIONAL MEDIA AND SUPPLEMENTS

Among the ongoing challenges in commercial-scale cell culture using traditional media and supplements has been a reliance on further supplementing traditional media with material such as human AB serum, human serum albumin, or platelet lysate, says Alexis Bossie, director of Media R&D, Lonza. Often, these undefined supplements are bound to various cytokines, hormones, and other growth factors necessary for primary cell growth and function, but, unfortunately, lot-to-lot variation of these components often leads to differential responses from cells and can produce unwanted side effects, she states.

“Additionally,” Bossie continues, “as the number of cell and gene therapy clinical trials and commercial applications increases, the supply of these critical raw materials is becoming increasingly limited and can cause delays in patient treatments.”

Trissa Borgschulte, head of Bioprocessing Upstream R&D at MilliporeSigma, also points out that having enough capacity to supply critical, high-quality raw materials that are needed for traditional cell culture media and supplements (e.g., animal sera) continues to be a major ongoing challenge. Specifically, COVID-19 has negatively impacted many critical supply chains. “There just aren’t enough materials to supply manufacturers with what they need for their bioprocesses,” she says.

Meanwhile, one of the longer-lasting challenges surrounding traditional cell culture media and supplements is related to patient safety and stems from the potential risk of adventitious agents potentially introduced into bioprocesses from
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animal–derived raw materials. “To reduce this risk, there is a strong push to remove animal–derived components from modern bioprocesses whenever possible. As a result, today’s standard production processes for recombinant therapeutic proteins are chemically defined and non–animal origin (NAO),” Borgschulte says.

Sonjoy Mukerjee, PhD, principal application scientist, Cell Culture and Cell Therapy, Thermo Fisher Scientific, adds that scale–up, manufacturability, reproducibility, and achieving consistent performance are also among the challenges encountered with using traditional cell culture media and supplements. He explains that this variability in performance can result in sudden spikes in titer, variability in product quality, and difficulties with developing a consistent manufacturing process.

“Additionally,” Mukerjee continues, “there is an increased need to prevent mycoplasma contaminations through the use of 0.2–micron or even 0.1–micron filtration steps at the end of the final formulation. This poses a challenge because it adds additional steps to the process that could be avoided with the use of raw materials that are validated to more stringent bioburden specs.” Finally, variations in glycosylation profiles can limit the ability of quality control (QC) teams to release final batches because it is difficult to set these kinds of limits.

“These challenges highlight the importance of manufacturing supplements and media specifically for biomanufacturing to provide better and more consistent performance,” Mukerjee says.

**BENEFITS OF MICROORGANISM–BASED RECOMBINANT MATERIAL**

Several recombinant proteins and peptides are already used in many cell culture media, including, for example, transferrin and insulin, says Bossie, who explains that using defined components in place of the alternative form that is isolated from serum leads to less lot–to–lot variability and can produce more robust cellular responses. “Elimination of human or animal sources for media components reduces the risk of virus or other contaminant and eases the regulatory burden on the final therapeutic product,” she says.

**Specific demand for microorganism–based recombinant cell culture supplements is expected to grow at a higher pace.**

“As mentioned above,” adds Borgschulte, “today’s standard bioprocesses for the production of recombinant therapeutic proteins are chemically defined, NAO, and, in some cases, protein–free. Optimized media formulations and robust bioprocesses have resulted in manufacturing templates that only require low–molecular weight, recombinant peptide growth factors for the cell to thrive.” By contrast, however, the rapidly growing field of novel gene and cell therapies still requires more sophisticated media formulations owing to the diverse nature of cells (i.e., primary cells, embryonic and tissue–specific, regenerative stem cells, induced pluripotent stem cells) used therein, she points out.

Thus, for these close–to–the–patient cell expansions and/or differentiation media, sophisticated supplementation will need to closely mimic the bodily environment from which they are taken, Borgschulte further explains. “Not surprisingly, high–quality bovine and clinical–grade human sera, or fractionated components thereof, are still at the core of these protocols. Replacing sera with supplement cocktails containing only NAO recombinant growth factors and transport proteins is an achievable goal,” she states.

**COMMERCIAL CONSIDERATIONS**

Recombinant supplements are already used in commercial cell culture media. Recombinant insulin and transferrin are two examples of widely used recombinant supplements in many cell culture media, Bossie says. She also points out that cytokines and interleukins (e.g., IL–2, IL–7, IL–15) as well as other growth factors (e.g., Rho kinase inhibitor, TGF–β) are common recombinant proteins used in cell culture media.

While microorganism–based substitutes for undefined serum–derived components could alleviate some of the challenges associated with commercial–scale cell culture, care must be taken, however, to also supply the additional growth factors that are non–specifically associated with the native forms of these components, Bossie cautions. For instance, trace elements, lipids, interleukins, and as–yet–unidentified other proteins might also need to be added to the cell culture medium when recombinant supplements are used in place of the native form isolated from human serum.

“Determining the optimal concentrations of all of these ingredients can be a time–consuming discovery effort,” Bossie says. Moreover, the costs associated with additional components can result in an extremely expensive medium, resulting in an additional cost burden to the therapeutic product.

Demand for recombinant animal–free components in cell culture media is growing especially on the back of concerns regarding contamination in raw materials. Borgschulte points to the threat to the supply of porcine trypsin (African Swine Fever) and general animal–origin concerns, as well as the current need to accelerate COVID–19 vaccine programs, for example, as driving factors for increased recombinant animal–free cell culture components.
Meanwhile, peptone hydrolysates, specifically manufactured for biomanufacturing, are being widely used in both pre-clinical and all phases of clinical trials as well as many marketed molecules, emphasizes Mukerjee. “Successful applications include monoclonal antibodies, microbial fermentation applications, and various vaccines for both human and animal health,” he says.

In the area of cell therapies, there is need for greater consistency as well as higher regulatory standards, which is also driving the increased use of recombinant growth factors wherever possible, says Bossie. “This trend should be expected to continue,” she continues, adding that “hurdles to greater utilization of recombinant proteins revolve around the need to more completely understand the metabolic demands of the cells to be able to provide all of the necessary ancillary factors bound to or associated with the serum-derived components.”

Once there is greater utilization of the recombinant proteins in place of serum-derived ingredients, however, the economies of scale in their manufacture and increased competition among manufacturers should serve to drive down costs of these materials, Bossie adds. Until then, the high cost of some recombinant proteins might limit their adoption, she says.

Furthermore, NAO alternatives reduce the risk of introducing adventitious agents to the biomanufacturing process, Borgschulte confirms. Additionally, recombinant cell culture supplements can offer more consistent purity and activity levels, she explains. “In the growing field of cell and gene therapy, high-quality animal derived supplements are still commonly used. However, replacing these supplements with NAO recombinant growth factors and transport proteins is the direction of the future,” she states.

“Many companies in this space are looking for lot-to-lot consistency for the media and supplements that they use in their processes,” adds Mukerjee. These companies also want full traceability of the components that make up the media and supplements. “These are a couple of the drivers towards microorganism-based recombinant cell culture supplements and media. It’s possible that these type of supplements and media could replace traditional supplements and media, but it would come at a higher cost. The incremental added benefits of these newer supplements might not outweigh this increased cost and given that there are many high-quality options on the market right now, it’s not likely that companies will be making the switch anytime soon,” he concludes.

REFERENCE

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Moving Closer to End-to-End Continuous Bioprocessing

As downstream improvements continue, efforts are underway to integrate downstream processes into continuous operations.

AGNES SHANLEY

The past few years have brought significant cost and competitive pressures to biopharmaceutical manufacturers. Process intensification and integration have allowed manufacturers to address these challenges, improving yields and reducing timelines.

Conditions are now set for change. “For monoclonal antibodies, outputs continue to increase, kilograms per batch are getting lower, and we’re getting higher titers out of cell cultures. In addition, there is a need for more flexible, adaptable manufacturing plants, particularly multiproduct plants, that can be run more flexibly,” says Darren Verlenden, head of bioprocessing at MilliporeSigma.

More companies are moving beyond intensification and evaluating continuous processing, which would usher in a future of lower manufacturing costs, real-time product release, and predictive control. “Continuous bioprocessing promises to transform biomanufacturing by reducing inter-campaign downtime, removing time- and labor-consuming steps, and reducing risk by chaining or combining serial steps in current processes,” says Phil Vanek, chief technology officer at Gamma Biosciences, which specializes in tools and technologies for advanced biotherapeutics development and manufacturing.

However, Vanek says, “in order for continuous biomanufacturing to work, we need to think about each manufacturing process in its entirety and seek areas where product can be harvested in real time without disrupting the ongoing process.” He visualizes highly productive expression systems under tight process control, chained to efficient cell harvesting and subsequent downstream technologies, which can be developed into modular continuous biomanufacturing platforms, all within a highly efficient and compact footprint. An example of this, he says, is Univercells Technologies’ NevoLine biomanufacturing platform for intensified and automated viral production.

FOCUS ON PERFUSION

Currently, most companies are applying continuous processes upstream, particularly to perfusion. Within 10 years, such efforts are expected to drive reactor productivities from 0.05–1 g/L per day to 0.5–10 g/L per day (1). Technology providers, including Cytiva, Sartorius, and MilliporeSigma have all released new automated perfusion systems and
bioreactors. However, the continuous concept is also being developed downstream, enabled by new and improved alternatives to traditional chromatography. WuXi Biologics is using continuous perfusion and downstream processing in its WuxiUp platform.

ELIMINATING CLARIFICATION AND PRODUCT HOLD TIME

Transcenta Holdings is working to improve continuous processing both upstream and downstream functions. Based in China and the United States, the company was formed in early 2019 when two drug development companies merged. MabSpace brought a focus on novel technology in discovery and research, while Hangzhou Just Biotherapeutics contributed strengths in chemistry, manufacturing, and controls development and bioprocessing platform. “The idea was to combine two companies with complementary cutting-edge technologies in R&D and biomanufacturing. The goal is to use innovative approaches to develop and deliver affordable innovative drugs to patients with unmet medical needs across the globe,” says Chris Hwang, chief technology officer of Transcenta, and a 25-year veteran of Genzyme and Sanofi. Although there is still much work ahead, continuous bioprocessing offers a number of advantages, he says.

Transcenta’s manufacturing platform, Integrated Continuous Bioprocessing (ICB), uses continuous perfusion that can achieve very high productivities by maintaining cells at high densities in a productive state for the duration of each run to reduce downtime, he says. The company plans to rely on process integration with intensified and connected/continuous downstream to remove process bottlenecks where production takes place.

By implementing ICB, Transcenta will also be able to eliminate harvest clarification and product hold steps, says Hwang. Those changes, combined with robust process control and automation, promise to result in significant reductions in footprint, labor, materials, consumables, and operator errors and overall cycle time.

Transcenta envisions a future based on small, closed, and highly automated single-use flexible facilities that use continuous processes and can be set up quickly and inexpensively, much like its facility T-BLOC in Hangzhou China. T-BLOC was built in January 2018, using G-CON’s prefabricated modular clean room technology, the first of its kind for protein production. Also, the facility will fully leverage single-use technologies, allowing it to support multi-product and scale-out as necessary to support clinical and commercial production needs.

“Since we opened our T-BLOC facility, we have invested significant efforts in developing our own cell lines, chemically defined and low-cost culture media (<1/10 of commercial media), and as well as our own continuous perfusion platform. Since then, we have demonstrated a more than 10-fold increase in process output when compared to conventional fed-batch processes for multiple cell lines and molecules,” says Hwang. He estimates that it would take 22 2000-L single-use or four 12,500-L stainless-steel bioreactors in fed-batch mode to match the output of four 1000-L single-use bioreactors using the same cell line in their plug-and-play perfusion platform.

To handle such a high cell-culture output efficiently without large-scale equipment, Transcenta is also developing continuous downstream bioprocesses in parallel. To accelerate implementation of ICB for good manufacturing practice production in its T-BLOC facility, Transcenta established a strategic technology collaboration with MilliporeSigma in mid-2020, leveraging its BioContinuum platform, as well as its expertise in continuous processing (2). However, rather than go all in to end-to-end continuous, the team decided to implement in a step-wise manner, to minimize any risk to manufacturing operations.

FLOW-THROUGH POLISHING

The first phase of this multi-year partnership, the team will co-develop a first-of-its-kind single-use flow-through polishing system, says Hwang, and the second phase will focus on expanding the boundary of continuous to the rest of the process, as well as digital technologies to optimize ICB. “To further minimize manufacturing risks, the technologies we are developing and implementing need to strike a right balance of risks and benefits with strong preference for simplicity as oppose to complex systems,” he added. “Significant progress has already been made and I expect our end-to-end continuous and automated ICB technology will be ready for operation in two years in our T-BLOC facility and ready to support clinical and commercial production,” Hwang says.

Transcenta’s work reflects greater industry interest in continuous processing across the board. Pilot-scale continuous operations are showing progress, says Verlenden. A recent report projected demand for continuous bioprocessing to increase by nearly 23% per year through 2027, reaching $198 million by 2027 (3).

At this point, the only dampening factor appears to be concern about regulatory acceptability of continuous, Verlenden says, noting a recent company survey on BioPharma 4.0, in which respondents found regulatory concerns to be the largest hurdle for companies to overcome. “This may prevent some companies from moving beyond pilot level,” he says.

At this point, savings are attracting more companies to continuous manufacturing approaches. “Continuous processing has been shown to demonstrate cost-of-goods reductions for monoclonal antibody purification, especially for orphan drugs,” says Dmitrii Sorokin, drug product development engineer at ExoThera, a contract development and manufacturing organization affiliated with Univercells that specializes in viral vector manufacturing. “In these cases, the cost of Protein A resins is pushing developers and manufacturers to explore alter-
At the same time, advances continue to be made in downstream bioprocessing technology itself. In January 2021, Sartorius Biotech acquired Novasep’s chromatography business, while, in April, the company opened a new center of excellence for downstream bioprocessing in the United Kingdom.

Improvements are also being seen in traditional products such as Protein A resins. “Next generation medicines are complicated systems ranging from in-vitro transcribed RNA, purified extracellular vesicles, viral-based vectors, and vaccines, to genetically modified cells,” says Vanek.

Although these therapies promise transformational health outcomes for patients underserved by conventional medicines, Vanek explains, their complexity translates into manufacturing challenges that range from inefficient production steps to difficulties in packaging and distribution, he says.

**AFFINITY IMPROVEMENTS**

The introduction of affinity technologies, most notably Protein A in the monoclonal antibody workflow, has boosted productivity by simplifying the number of steps necessary to achieve functional purity. Affinity methods will continue to be applied to all forms of advanced therapies with the goal of improving recovery and quality of final product, as well as reducing cost through process simplification, Vanek says. In addition, he notes, identifying highly tunable ligands and conjugating them to appropriate chromatography substrates such as resins, magnetic ferrofluids, or electrospun non-woven membranes promises to open up a whole new dimension of bioseparation possibilities.

Nanopareil electrospun nanofibers, one of Gamma Biosciences’ newer bioseparation product offerings, combine extremely efficient chromatographical properties such as short residence time and high dynamic binding capacity with large pore sizes that are better suited to advanced therapy modalities such as viral vector and exosomes. “This platform is being developed with trauma
tives to big expensive chromatography columns, and continuous multi-column chromatography (MCC) instruments offer an opportunity to decrease investment during development, clinical trial material manufacturing, and commercialization of such products,” says Sorokin.

For products that are difficult to produce using traditional technology—often due to stability issues or cytotoxic/cytostatic effects on cell culture—a combination of upstream perfusion, continuous downstream processes using MCC, and in-line conditioning and tangential flow filtration (TFF), are being used in development, says Vasily Medvedev, head of development at Exothera. “In viral processes, more efforts are using continuous purification for sensitive viruses, to allow purification to take place more quickly, since target recovery remains one of the industry’s top challenges,” he adds.

**DOWNSTREAM ADVANCES**

At the same time, advances continue to be made in downstream bioprocessing. Suppliers have expanded production capacities to meet the demand for bioprocessing equipment and supplies. Sartorius opened a new manufacturing facility for downstream bioprocessing equipment in Havant, United Kingdom, in mid-April 2021. The 58,000-ft² facility will be used for the design and manufacture of systems for the biopharmaceutical market, including chromatography columns and filtration systems (1).

In addition to a 28,000-ft² production area, the center accommodates a 4000-ft² customer test lab to demonstrate the functionality of Sartorius equipment. More than 90 employees relocated to the new from the company’s former Portsmouth site. The company reports it will invest more than $481 million (€400 million) in 2021 to expand manufacturing capacities around the world including expansion at the Havant site. “The new facility in Havant will be an important part of our global operations network as it will enable our business to grow to meet the requirements of the expanding industry,” said Thorsten Peuker, head of operations, BPS Systems, at Sartorius in a press release. “The new facility increases Sartorius’ capacity to deliver support in all areas of our business and provide the highest quality equipment and services to our customers.”

Avantor is increasing its global capacity for single-use technologies, expanding the manufacturing footprint by 30% and doubling its cleanroom space in the United States and Europe. The expansion will support customer demand for monoclonal antibodies, cell and gene therapies for oncology and other diseases, and vaccines, including those for COVID-19 (2).

“The industry’s response to the COVID-19 pandemic has relied heavily on the integration of single-use products into biopharma production processes,” said Ger Brophy, executive vice-president of biopharma production at Avantor, in a press release. “These additions to our global single-use manufacturing ecosystem are critical steps in enabling our customers to get therapies to patients quickly.”

**References**


—The editors of BioPharm International
Additional chemistries as well as Astrea Bioseparation’s affinity ligands to address new applications,” Vanek says.

POLISHING APPLICATIONS
Nanofiber chromatography technology promises to improve cell and gene therapy capture and polishing applications, offering high binding capacities, fast cycle times, and scalable formats, says Fletcher Malcom, head of product at Nanopareil. As a result, this technology can help reduce processing time, chromatography facility footprint, buffer requirements, and waste significantly, shortening time to market while cutting costs and ensuring environmental sustainability, he says.

In viral-vector manufacturing, downstream processing is adapting chromatography and TFF steps to aseptic processing for viral particles that are too small for sterilizing-grade filters, says Sorokin. Currently, more chromatography materials are available as membrane capsules and monoliths, which can be gamma-irradiated and ready to use in closed processes. In addition, presterilized TFF hollow-fiber cartridges are available with aseptic connectors, he says.

“This trend is unlocking aseptic downstream processing at a large scale, which is critical for several categories of viral vector products (e.g., oncolytic viruses),” says Medvedev. “Another trend we are following closely is the availability of new modalities for separation of viruses (e.g., steric exclusion chromatography), which offers value in cases where conventional affinity or ion exchange resins would result in low recovery,” he adds.

MEMBRANES AND CAPSULES
“Many anion exchange diffusion-based media (e.g., resins based on Q-Sepharose) that are suitable for viral vector purification are being replaced by single-use convective media (e.g., anion exchange membranes or Q-adsorber capsules),” says Sorokin, “but a lot of polishing steps are required for monoliths to remove aggregates and residual DNA-VP complexes.”

More biopharma developers are also looking into the idea of combining unit operations, such as the midstream approach used in Univercells Technologies’ NevoLine or coming up with innovative approaches for sensitive targets such as Repligen’s tangential flow/depth filtration concept for lentivirus, says Medvedev.

REFERENCES
Advancing Cell and Gene Therapy Expansion Processes

Scale-up challenges can be addressed using the AppliFlex ST single-use bioreactor.

As the cell and gene therapy field continues to grow, the need for control, consistency, and process flexibility keeps growing along with it. Nowhere do we see this more than with the challenges that emerge when shifting from traditional static and shaken cultivation conditions to stirred controlled environments. As labs attempt this scale-up journey, they are learning that finding systems and processes that can grow—and adapt—with them is a real plus. Tom van Arragon, product manager at Applikon Biotechnology, is well aware of these challenges, and of the solutions that companies like his are innovating to address them. So we sat down to discuss advances in cell and gene therapy expansion processes, and how Applikon’s AppliFlex ST single-use bioreactor enables those advances in process development across applications.

**BIOPHARM:** Can you tell us why people should be using bioreactors for cell expansion?

**VAN ARRAGON:** A bioreactor offers a closed, standardized environment to cultivate cells. Typically, if we look at cell-line development, you start with cell expansion in an uncontrolled system that is not fully defined. By contrast, the key advantage of a bioreactor is obtaining reproducible results as a result of the constant cultivation conditions it offers. With the data that can be obtained from the controlled environment, further automation can be introduced into the process. Specifically for the cell and gene therapy field, we see single-use equipment being adopted rapidly.

**BIOPHARM:** How is the AppliFlex ST single-use bioreactor uniquely suited to support cell and gene therapy applications?

**VAN ARRAGON:** These applications are supported with a unique manufacturing process that makes use of 3D printing technology for the bioreactor headplate and fittings. This allows us to manufacture a bioreactor customized to the user requirements for that given cell type and process.

For instance, we can quickly design impellers that stir cells gently. We know from experience that cells can undergo shear stress when passing near the impeller, and that’s a challenge that’s prevented many scientists from moving into bioreactors when they need to scale up. For example, we worked with a scientist who first used a traditional-design marine impeller. But their experiments showed...
that this mixing was too aggressive for their stem cells, which grew in aggregates. For the next bioreactor solution, we included an upsized impeller, which lowered the sheer stress applied to the cell, and that gave them the desired control better to achieve uniformity of aggregate size. With that success, the scientist was able to improve quality and have consistency in their process for scale-up.

**BIOPHARM:** How does the AppliFlex ST address scale-up?

**VAN ARRAGON:** We talked with many cell and gene therapy companies that culture cells in plates and flasks, and they do indeed face issues associated with going from a static system to stirred tank bioreactors, particularly with the scale-up. And this is a hot topic, considering the fact that there are different scale-up paths that can be chosen—for example, strategies based on mixing time, shear forces on the cells or controlling oxygen levels in the bioreactor. The AppliFlex ST addresses scale-up needs because it provides an environment where the process can be characterized—and with that information, the tech transfer for the scale-up can be done. Applikon’s long experience in the field of bioreactors makes it easy for the company to support this tech transfer. And the benefits of working in a single-use bioreactor for expansion are also well understood in the larger field of bioprocess and scale-up.

**BIOPHARM:** Can you discuss these benefits further?

**VAN ARRAGON:** If we talk about a key benefit for single-use bioreactors, it’s that they introduce a reduction of risk into the overall process. A major risk for any bioprocess is contamination. Essentially, by working in a closed environment, there’s reduced risk of operator error or contamination from vessel preparation, media transfer events, or harvest.

We currently see that cell and gene therapy labs are already relying on single-use equipment for risk reduction in cross-contamination between cell lines—which is no surprise, as speed and simplicity are also offered by these products. The added value that we make to these benefits is that we can optimize for the specific cell type or process.

**BIOPHARM:** Does the AppliFlex ST offer any other unique benefits?

**VAN ARRAGON:** Yes, absolutely. The AppliFlex ST is a box-to-bench system. The philosophy behind box-to-bench is that you take the bioreactor from its packaging to the bench and are able to start right away. The recent addition of single-use sensors to the portfolio really embodies this: It enables users to start without the traditional sensor calibration routine, so they’re ready to get going right away.

Next to box-to-bench, we offer a completely custom bioreactor design. This means that the headplate, diptubes, impellers, and also the tubing kit can be customized as needed. Special connectors can be placed that might be required to make aseptic connections. We say that as long as our engineers can design it, we can provide it. Our “menu” is broad, and we can provide fast turn-around.

A great example of such custom design is an application where we customized a key component. This was a process where Vero cells on microcarriers needed aggressive mixing to remain in suspension. This mixing strategy led to damage to the Vero cells and microcarriers. So for this instance, a special helical impeller design was created and supplied, and we found that the novel impeller design showed better results for this process.

**BIOPHARM:** Can you explain why media exchanges and perfusion is important in cell and gene therapy applications?

**VAN ARRAGON:** To support successful cell expansion, modern media are developed that contain specific components. As the cells grow, these components are consumed, but need to be replenished for further cell expansion. And to keep the media fresh and get rid of potential toxic metabolic products, we need to do media exchange or additions. So most cell-expansion processes require refreshment of media to keep cells growing, or if cell differentiation is required, they require a complete media exchange.

We see many processes requiring media exchange or perfusion. We have supplied several bioreactors that enable automated media exchange. You can think of special harvest ports in combination with level sensors for a settle-and-harvest strategy. This is a strategy where the agitation is stopped for a short period and the cells or cell aggregates sink to the bottom. A drainpipe placed atop the media level pumps off the supernatant. Following this, new media is pumped into the bioreactor, agitation is started, and cells are re-suspended—all while supporting a controlled environment.
The Ins and Outs of Syringe Inspection
Consider a best practices approach to pharma’s most challenging-to-inspect container.

ANDREA SARDELLA

Syringes are arguably the most difficult containers to inspect. Among other obstacles, syringes require unique handling protocols, and their far-ranging shapes and sizes require customized, multi-area inspection that present impediments both to per-item accuracy and overall production speeds. This article explores the various parameters that define syringe inspection and discusses best practices for inspecting syringes with different closure types, as well as syringes holding a variety of drugs, including vaccines and viscous drugs.

HANDLING SYRINGES
While other containers such as vials, cartridges, and ampoules largely “stand still” and can therefore enter the inspection process independently, syringes typically are carried via conveyor and then turned upside-down. To inspect syringes accurately and efficiently, they usually must be rotated with the head pointed upward (i.e., inverted), so that any hidden particles in the funnel can sink down into the liquid for increased inspection detectability. If the syringes are sterilized, the containers are typically in a “nest and tub” arrangement, which requires they be denested by a robotic unit communicating with the inspection machine. This intricate process requires gentle handling and should avoid glass-to-glass contact to mitigate the likelihood of cracks or breakage.

Once in the inspection carousel, the syringes are inverted for inspection. The syringes can be handled by holding them on the upper and lower part using mandrels with customized cups, or below the plunger by a two-finger adaptive gripper. The method of holding syringes by both upper and lower portions allows higher rotation speeds. Syringes with needle shields can be held in this manner, and rigid needle shield formats can be held if the shield has a smaller diameter than the syringe body. Unfortunately, this handling system is not compatible with all closure systems. Gripper systems typically operate at slower speeds, but are well suited for more delicate closure systems such as luer-lock cone. Grippers don’t touch the closure mechanism and therefore present a lower risk of affecting the closure integrity.

INSPECTION PROCESS
As noted, syringes’ inherent multi-component nature makes them complicated to inspect. One of the more challenging parts is the flange because they are typically not exactly flat. This factor frequently foments deceptive shadows and reflections, both of

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which make it more difficult for inspection stations to determine whether the glass is scratched or otherwise damaged. The solution typically lies in employing appropriate optical and illumination setups to minimize false rejects, in conjunction with more advanced image processing functions.

Another multi-faceted component, the plunger, is an inspection pain point for similar reasons. In fact, plungers can be so tricky that it is often necessary to employ a dual control system to avoid a high level of false rejects. A dual-view optics approach allows for analysis of two spatially coherent views in one image, without the need for duplicate inspection stations. Angled views tasked with inspecting plunger tops are combined with a frontal view monitoring the plunger top’s lateral section. In general, this strategy of combining two complementary views leads to improved inspection performance and lower false rejects. In Figure 1, the region of interest (outlined in green) is shown in a frontal view (right) and an angled top view (left). If an element is detected as an air bubble in one view but not the other, as shown in this figure, it does not need to be rejected.

The comparably small diameters of syringes also present an inspection headache because of the limited space in which particles can move and therefore be noticed. To inspect an injectable drug, the particles in the formulation might be made to move—a process that requires the syringes to be rotated at speeds up to 9000 RPM. Single rotation units on the carousel mandrels meet this need and ensure flexibility in rotating a variety of drugs at speeds best suited to their individual inspection. Here, though, another issue emerges: while high-speed rotation is sufficient for liquid drugs, what if the container can’t be rotated at high speeds for stability reasons? For example, subjecting many biopharmaceutical drugs to such turbulence could impact their integrity. Furthermore, with highly viscous drugs, particles generally don’t move and therefore cannot be distinguished via this common tactic. In these scenarios, the answer often lies in three-dimensional inspection, which allows a module to infer whether a suspected contaminant is inside or outside the container by analyzing its trajectory; specifically, the radius will be shorter if the foreign matter is inside and longer if it is outside.

Another technique when inspecting syringes is using line-scan cameras. A method especially effective when inspecting turbid liquids, line-scan cameras continuously capture images line by line, then stitch together a comprehensive image from upwards of 10,000 exposures. By rotating the container at high speeds, the particles move toward the container barrel. Line scans are fast and accurate, resulting in a nearly 100% rate of particle detection with very few false rejects (1). Such setups are ideal for inspecting cylindrical surfaces, especially when it comes to cosmetic lateral side inspection, as there are no distortions.

Proper lighting conditions are a prerequisite for identifying anomalies within liquid drugs since, due to a lack of con-

![Figure 1. Top plunger inspection through two complementary views, a frontal view on the right and a top view on the left. A: Region of interest for particle detection. B: Air bubble in y-coordinate position, transfer from frontal to top view. C: Elements detected as air bubbles. D (top): Frontal view shows air bubble in same y-coordinate position. D (bottom): Frontal view shows no air bubble or plunger bump in this y position.](https://www.biopharminternational.com)
trast, their identification under natural lighting conditions is infeasible via the human eye or current automatic visual inspection technologies. Light intensity should be sufficient for illuminating the container while providing a moving contrast to identify the smallest particles. Reliable detection must combine the advantages of various lighting methods to detect the widest range of contaminants, as different contaminants react to light in different ways. Figure 2 illustrates using back and lateral lighting to detect both light-absorbing particles and light-reflecting particles.

CHALLENGING FORMULAS

Some formulations are more challenging to inspect than others. For example, in water-like liquids or light suspensions, dynamic trajectory analysis increases detection probabilities, since particles behave in a statistically measurable way. When drugs present as heavier suspensions, however, other strategies are needed.

For example, a vaccine, presenting as a heavy suspension in a syringe, could not be mixed up completely. While particles that stick to the syringe’s inner wall are inspected, noise in the image related to the spin made it difficult to detect small particulates in the liquid portion. It was also difficult to differentiate clear particles (e.g., white fiber and glass). To solve for this, particle inspection was performed using continuous rotation. Each position in the carousel had an individual servo rotation unit with a specific rotation schedule. The position of some particles were correlated and found to be different relative to their location at the first station. From this position change, it was concluded that these were moving particles.

With gels or highly viscous products such as hyaluronic acid, inspection processes can’t rely on spinning the liquid to provide particle motion differentiation. In this case, the container is kept in rotation to cover the full 360° facade and track all visible potential contaminants, as portrayed in the red trajectories in Figure 3. The potential contaminants’ apparent speed is used to determine if they are inside or outside the container. They move on “different radiuses” at the same angular rate covering different displacements.

With these drugs, a proper lighting scheme becomes particularly important because certain contaminants, such as white fibers, are more easily visible against a dark background. Similarly, many cosmetic defects, such as thin scratches, are also easier to find against a dark backdrop.

Biopharmaceutical drugs, such as monoclonal antibodies, can be more difficult to inspect than other liquids because of their increased density, higher turbidity, and the greater need to protect product integrity, which limits the available inspection methods. The trend toward higher protein concentration and the resulting higher viscosities creates another challenge for inspection, because “spin and stop” inspection technology becomes ineffective for particle detection above viscosities of 4–5 centipoise.

Turbidity also increases in parallel with protein concentration, which makes discerning acceptable particles from unacceptable ones a real challenge. Process-related impurities are not necessarily harmful if they fall within the compendial guidelines for size and count. But when inspected with current auto-

Figure 2. Simultaneous back and lateral light inspection to detect absorbing particles (left) and reflecting particles or fibers (right).

Figure 3. Objects in blue circles have been identified as external, while those circled in red are contaminants inside the container—in this case, fiber. In the right-most container, inside contaminants are signified by their red trajectory, with outside dirt given grey trajectories.
matic vision inspection systems, high rejection rates can occur due to the elevated difficulty in sizing and counting particles with precision. For this reason, manual inspection of biotech products has been the norm. A more efficient approach would be an automated inspection system that can both identify, size, and count each particle within a container and use historical particle data in clinical lots to make a patient safety-based assessment to determine whether it warrants rejection.

Protein instability and the generation of agglomerates can be minimized by eliminating mechanical shock during handling. The main driver for protein aggregation is the cavitation effect induced in the liquid that increases the agitation at the air-liquid interface and increases the liquid’s local temperature and pressure. Inspection equipment must have smooth product handling while considering overall throughput.

A secondary driver for protein instability is the shear force exerted in the liquid by high-speed spinning, notably when acceleration and deceleration cause the transition from laminar to turbulent flow, destabilizing the meniscus and generating bubbles in the liquid. To reduce the applied shear force, it is advisable to incorporate a cross-correlation inspection system between particle stations, which allows for sequence comparisons that can determine whether or not a particle is inside or outside the syringe. With this method, the container is kept in rotation at a steady speed, and the liquid slowly accelerates to segregate bubbles on the top and bottom in a controlled manner while particles are located at different heights and can be detected during inspection.

CONCLUSION

Syringe inspection continues to be among the most challenging facets of the post-production pharma quality control process. In a scenario where both containers and their ingredients must be thoroughly inspected, both syringes and the varying drugs they deliver pose unique pain points concerning handling, visual detection, and minimizing false rejects. A best-practices approach to syringe inspection means careful consideration of both the type of syringe and the type of ingredient it houses, and the optimal use of lighting, rotation speed, and inspection technologies and methods.

REFERENCE


For biologic drug products that require frozen storage—even down to cryogenic temperatures for some cell and gene therapies—the primary packaging and closure materials and their effect on container closure integrity (CCI) risks should be considered in the system design. Evaluating CCI of the system is crucial because the system needs to maintain integrity throughout the shelf life of the product, which could be two or three years, notes Jennifer Riter, senior director of Business and Technical Operations, Services, and Solutions at West Pharmaceutical Services, which manufactures delivery systems for injectable drugs.

Evaluating CCI

Specifically for low temperatures, CCI testing should evaluate any ingress or egress of gases in the headspace of a container, which could affect product quality by putting the product at risk or affect product stability. “For example, carbon dioxide ingress over time could result in product loss or degradation,” explains Riter. “The next step is to choose a test for how to examine the MALL and the associated risks. For example, laser-based headspace analysis can check for carbon dioxide ingress.”

A best practice is to evaluate CCI on the complete container and closure system during development. Laboratories should perform tests under the cold-storage temperature conditions. Riter says that it is even possible to do testing of systems at cryogenic temperatures, which is important for packaging of cell and gene therapies.

Evaluating materials and systems

Material properties are another aspect to consider when designing a container system for low-temperature storage conditions. “Causes for failure could be the material itself or the way the closure is processed or sealed,” says Riter. “It is important to look at how the materials fit together (a ‘stack-up’ analysis) and to identify the appropriate compression of the stopper with the vial, and the seal quality. Different material hardnesses or coatings can affect compression and sealing.”

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—Jennifer Markarian
A top-down approach can identify GxP-relevant processes and ensures the integrity of data exchange.

Correct data is fundamental in a GxP quality system, and a modern lab instrument can ensure data integrity compliance. BioPharm International recently spoke with Sebastian Weber, product manager for lab weighing at Sartorius, about how to achieve data integrity, minimize risk and the potential for FDA violations, and how digital workflows can be supported by technology like the Cubis II lab balance.

**BIOPHARM:** Why is data integrity important for a biopharmaceutical company?

**WEBER:** Biotechnology companies are investing in digital solutions such as electronic lab notebooks (ELNs), laboratory information management system (LIMS), and connected instruments to support their activities and work paperless. Maintaining data integrity of electronic lab data is important for several reasons. All decisions in the lab, such as batch release, are based on data. We must be sure that the data is correct to make the right decisions. Otherwise, this can have a negative effect because we risk patients’ health and the success of a therapy when a drug does not have quality and efficiency. It can also lead to financial consequences from FDA’s enforcement actions, such as facility shutdown, product recalls, delayed drug approvals, and loss of customers as the result of a damaged reputation.

**BIOPHARM:** Based on FDA warning letters, what are the most frequent violations made by pharma companies?

**WEBER:** Most warning letters are related to role and access management, data recording, and data transfer. A common example is a company not implementing controls to ensure that only authorized users have access to an instrument. Anyone can use the instrument without traceability. An example is role management where everyone works as an administrator on an instrument or in a software program. A lab analyst could change configuration settings or delete data, which can lead to manipulation. Another common problem is that the critical GxP-relevant data is not properly backed up, or there is an incomplete transcription of the GxP-relevant data from the instrument into LIMS or ELN software. Finally, when it comes to the audit trail, typical violations are when the lab instrument did not have an audit trail, or the audit trail was incomplete, disabled, or not reviewed.
**BIOPHARM:** How can an organization minimize risk and ensure data integrity?

**WEBER:** Data integrity starts with a quality culture within the organization. Some organizations focus only on particular software or a specific instrument, but this brings the risk of forgetting other important systems or causing integrity problems with data exchanged between different systems. Therefore, I recommend a top-down approach to identify GxP-relevant processes and then dive deeper into subprocesses and individual activities with standalone systems and instruments. Make sure that the instrument has all the features to meet the requirements of FDA 21 CFR Part 11 or EU Annex 11 for trusted electronic records. Instruments should include audit trail, user management, electronic signature, and safe data transfer options for integration into other software systems. Furthermore, automate work processes as much as possible to avoid manual errors.

**BIOPHARM:** Are there modern lab instruments that provide all the necessary technical features to automate processes and still avoid data integrity and compliance issues? Can you give an example of such an instrument?

**WEBER:** A good example is the Cubis II balance. This state-of-the-art instrument enhances connectivity, data integrity, and compliance data handling. The Cubis II balance series was designed to follow ALCOA+ principles—the key concept of a good documentation practice—and contains all the features to support regulation compliance.

**BIOPHARM:** How does the Cubis II lab balance support for pharma compliance?

**WEBER:** The Cubis II balance contains all the technical controls to support compliance without the need for additional hardware or PC-based software. All functions are integrated in the balance software and can be easily accessed with the 7-in. balance touch screen. Features include a comprehensive audit trail with functions for filtering and exporting for convenient reviewing. User and role management can be integrated into your company domain for easy management and to support electronic to support electronic signatures. Additionally, there are network and time synchronization functions. For secure and compliant data transfer, the Cubis II balance can be integrated into lab software via a variety of different standard IT interfaces without the need for additional middleware. This simplifies qualification and reduces operating costs. One of the connectivity options featured in the Cubis II is web services, a state-of-the-art technology for bidirectional integration into a LIMS or ELN system that complies with the highest standards of data integrity and IT security.

**BIOPHARM:** How do software applications with guided workflows help achieve trustworthy, compliant results? Are there other balances on the market today that offer full pharma compliance and digital workflow support without requiring additional software?

**WEBER:** Automated guided workflows eliminate many steps that can lead to mistakes. The user is guided step-by-step through the workflow as the process is specified in the standard operating procedure (SOP). The workflow application ensures that specific parameters, such as limits or tolerances, are followed. This not only improves the quality of the data, but it also brings more efficiency to the entire lab process and can reduce and can reduce lab costs per sample. Cubis II brings workflows onto the balance as small applications called QApps. Having the QApp on the balance means there is no need for additional PC-based software. This allows the user to concentrate on the weighing process and save space in the lab. The Cubis II balance also has more than 50 QApps that can be parameterized according to your SOP and cover all typical weighing applications. QApp solutions can also be customized to fit your workflow and integrate seamlessly with existing lab software systems.

At this point in time, it is hard to find another balance on the market that offers the comprehensive compliance functionality, flexible workflows, and future-proof connectivity options of the Cubis II.
Getting to the Root of the Matter

Bio/pharma can learn ways to prevent recurring events and ineffective CAPA from the nuclear power sector.

NUALA CALNAN AND ROB DE LA ESPRIELLA

In government-regulated industries—which include, not only bio/pharmaceutical operations, but aeronautics, automotive, and nuclear power—root-cause analysis (RCA) is integral to effective risk management and mandated by Federal laws. When regulatory failures occur, companies must identify the deepest-seated causes of the non-conformances, commensurate with the significance of those events. If the causes are not properly identified, any corrective actions that are taken will not prevent problems from recurring in the future.

One key to developing an effective approach to corrective and preventive actions (CAPAs) and RCA is having mature quality management systems in place that help reduce the total cost of quality. This requires the right mindset to support effective RCA so that the causes of problems can be identified and addressed. For bio/pharmaceutical facilities, expectations are outlined in the International Council on Harmonisation’s (ICH) Q10 (1), which specifies the use of methods found in ICH Q9 (2).

Although it is less expensive to do something right the first time, the authors continue to see rework, rejects, recurring deviations, and recalls within the bio/pharma sector, which lags other regulated industries in evaluating and targeting improvements in the total cost of quality by getting to the root of issues. The bio/pharmaceutical industry’s approach to RCA has also been fraught with misunderstandings (3). Best practices from the nuclear power industry demonstrate how bio/pharmaceutical manufacturers can leverage lessons learned and apply them to operations to create a stronger, more transparent quality culture.

RELYING ON ADMINISTRATIVE CONTROLS

One consistent problem in bio/pharmaceutical circles has been an over-reliance on administrative controls to address compliance and quality failures. This was clearly seen in the industry’s response to nitrosamine contamination, including the presence of N-nitrosodimethylamine and N-nitrosodiethylamine, probable human carcinogens, in small-molecule APIs and finished drugs, as well as biopharmaceuticals. A recent report on lessons learned from the contaminations (4) showed that corrective actions proposed by the industry were, in large part, administrative in nature. Although there is no disputing that these actions would have some measurable positive effect, an overabundance

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of these administrative controls do not address root cause, and instead rely on the following:
• Reminding personnel of their responsibilities
• Encouraging better performance
• Clarifying existing control
• Ensuring that there is clarity
• Considering new requirements
• Considering additional recommendations
• Considering amendments to existing guidance
• Reviewing existing guidance
• Revising existing guidance
• Developing new guidance
• Drafting a new document
• Improving existing strategies
• Providing more training
• Enhancing oversight
• Exchanging more information.

As shown by the Hierarchy of Hazard Controls (Figure 1), administrative controls are among the least effective means to address identified hazards. Bio/pharmaceutical CAPAs can use this hierarchy to screen corrective actions so that, wherever possible, hazards are eliminated, replaced, or isolated. Other strategies include the following:
• An integrated causal analysis to achieve a deeper understanding of human-centric problems
• Integrating causal analysis into risk management systems
• Developing more advanced methodologies for generating and pursuing lines of inquiry when performing RCAs.
• Improving the bio/pharmaceutical industry’s basic definition of RCA.

A company’s quality management maturity (QMM)—first described in 2019 by the FDA’s Drug Shortage Task Force (5)—is central to the International Society of Pharmaceutical Engineers’ (ISPE’s) January 2021 Advancing Pharmaceutical Quality guide (6), and should be considered.

Companies with low QMM take a reactive rather than a scientific, probing approach to compliance or quality failure events, risks, or hazardous situations. At the senior management level, this culture results in what author and researcher Brené Brown calls “armored leadership,” in which business leaders and subject-matter experts are more concerned with being right than getting it right (7). Even scientists, who have dedicated their careers to discovering the truth, can become zealous defenders of the status quo, as Wharton organizational psychologist Adam Grant has found in his research (8). Where QMM is high, leaders and subject-matter experts (SMEs) take the lead in getting to the root cause of adverse events based on available knowledge, intelligence and analysis.

RCA AND THE NUCLEAR POWER INDUSTRY

All regulated industries struggle to meet regulations that call for effective RCA and CAPA programs. Driven by catastrophic failures—including the Three-Mile Island core meltdown (1979), Chernobyl disaster (1986), and the Fukushima Daishi reactor core damage (2011)—the commercial nuclear power industry leaped ahead of other industries in approaching this challenge. Its survival depended on it.

These and other incidents have driven dramatic improvements in programs, processes, and procedures. Reflecting the industry’s progress is its nuclear power plant capacity factors (CF). A CF is the ratio of actual energy produced by a power plant in a given period to the hypothetical maximum possible (i.e., energy produced from continuous operation at full rated power).

As causal analysis, CAPA, and other programs have improved, so too have nuclear plant CFs (8). In 1980, following the Three Mile Island incident, the US nuclear CF national average was 55%. By 2019, it had risen to 93.5%, reflecting the real benefits that can be gained by continuous improvement.

BEYOND HUMAN FACTORS

Human-centric problems involve a complex combination of human factors (i.e., human performance issues including at-risk behaviors); equipment/tool/material interface issues because humans design, fabricate, manufacture, transport, install, operate, and maintain equipment, tools, and materials; and latent cultural/
Integrated Causal Analysis (HCA), Power Operations (INPO) found that which works by gathering, organizing, human factors, equipment issues, and the human-centric factors were caused by deeper-seated organizational and programmatic weaknesses. The nuclear industry has been learning how to solve these kinds of problems ever since (9).

In the 1980s, the Institute of Nuclear Power Operations (INPO) found that up to 80% of events were caused by human-centric factors (Figure 2), and the majority (approximately 70%) of those human-centric factors were caused by deeper-seated organizational and programmatic weaknesses. The nuclear industry has been learning how to solve these kinds of problems ever since (9).

Traditional root-cause methodologies invoke different tools for analyzing human factors, equipment issues, programmatic weaknesses, and in some cases, default to a process of elimination rather than cause-and-effect analysis. When using a process of elimination, investigators start out by identifying a finite set of possible causes and then test/evaluate each one until they rule out what they can and identify the most probable causes.

One method that is finding use in the nuclear power industry is Hyper-Integrated Causal Analysis (HCA), which works by gathering, organizing, and analyzing available evidence and data on a single chart. This approach integrates traditional methods—human factors analysis, barrier analysis, task analysis, change analysis, comparative timeline analysis, event and causal factors charting, and the fishbone—into one approach that generates focused, evidence-based lines of inquiry (LOI).

Pharma and bio/pharma companies could also benefit by applying two approaches that are widely used in the nuclear power industry: probabilistic risk assessment (PRA), which is used to evaluate and mitigate the consequences of potential nuclear accidents, and the Bowtie model (Figure 3), which is used to analyze and communicate how high-risk scenarios develop (10,11). In both PRA and the Bowtie approach, a finite list of potential risks is created and steps are taken to ensure that defenses are in place to prevent the risks from causing events. In addition, actions and other mitigation strategies are developed to mitigate the consequences if an event were to occur.

In bio/pharma operations, RCA is traditionally used after a consequential event has taken place. In the nuclear industry, however, causal analysis is often used proactively, to analyze lower-level events by tying causal analysis programs to the risk prevention side of the Bowtie model. Using key performance indicators and trending and analysis programs, negative trends of low level events can be identified and analyzed, and their deepest-seated causes can be identified and eliminated before they allow the next hazardous event to occur.

REDEFINING BIOPHARMA RCA

Traditional definitions of the term “root cause” have been in place for over 50 years in regulated industries, but decades of work in the nuclear industry has shown that RCA efforts often stop prematurely, based on an inadequate definition of the concept.

Using the traditional definition of a “root causes,” organizations often declare victory too soon, and corrective actions often fail to keep problems from recurring because the deepest-seated causes are still active. Traditional root causes are best characterized as the “event root causes,” and the true, deepest-seated causes for any serious compliance or quality event lie much further down cause-and-effect pathways. The deepest-seated causes will not only account for the event or issue being investigated, but also for many other events that have taken place in the past, and will continue to cause events in the future until they are identified and addressed.

One way to get to the deepest-seated causes is by taking focused, evidence-based lines of inquiry. Companies in the nuclear power industry take a rigorous approach that generates lines of inquiry from multiple perspectives, including the following concepts:

- Analysis of defenses (i.e., evaluating applicable regulatory requirements and implementing procedures to determine the effectiveness of existing controls)
- Simultaneous completion of comparative timeline analysis and task and change analyses
- Insights generated from the analysis of additional data, including testing, sampling, and other methods used to evaluate the evidence
- Additional reviews that come from a clear understanding of the anatomy of an event, including human factors; other applicable programs, processes, and procedures; other equipment, tools, or materials that were not previously evaluated; the evaluation of

![Figure 2. As the nuclear power sector learned, most human-centric factors are due to organizational weaknesses, rather than individual error.](ww.biopharminternational.com)
error-likely situations that may have set up the organization for failure; and the impact of management and oversight involvement.

Rather than conducting individual interviews, facilitated causal analysis sessions are held with small representative samples or focus groups of SMEs from affected teams or organizations, who are asked to provide their expertise. Instead of taking interview notes, their answers are captured directly on a cause-and-effect chart. In addition, SMEs are guided through cause-and-effect analysis as they answer each question, harnessing their expertise in getting to the root causes. Using this approach, an investigation can harness the expertise of hundreds of SMEs in a very short period of time. In one recent HCA investigation, which looked into recurring procurement program failures at a US nuclear laboratory, 14 facilitated causal analysis sessions were held with more than 120 subject-matter experts in just one week (12).

This advanced approach to conducting interviews is more transparent, it eliminates bias, it prevents those being interviewed from feeling like they are being interrogated, it elicits much more cooperation from all parties, and it reinforces to all participants that the investigators are not seeking someone to blame but are seeking to learn.

Bio/pharmaceutical companies can adopt the nuclear industry’s best practices for RCA and CAPA. In return, they stand to gain the following:

- A better understanding of the fundamentals of causal analysis, continuous improvement, and risk prevention
- A better understanding of how to analyze complex, human-centric problems using more modern, lean, agile, flexible, accurate, and effective RCA methodologies that identify causes with speed and accuracy
- More effective CAPAs that focus on the elimination, replacement, or isolation of hazards, and prevent the recurrence of similar problems or events because they address deepest-seated causes of failure
- A transformation of the organizational culture, from compliance, towards a culture of continuous improvement, proactive problem solving, and learning.

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Packaging Considerations for Clinical Trial Materials
Packaging materials, kit design, and cold-chain handling should be optimized for each study.

JENNIFER MARKARIAN

Contract packaging organizations for clinical trial materials are increasingly handling biologic drugs, which are typically delivered by injection and have more stringent packaging requirements than other drug forms. In addition to the shift to biologics, the trend to direct-to-patient shipping affects how materials are packaged and makes tracking even more important. Careful consideration of primary packaging materials, kit design, and distribution is crucial for optimizing packages that balance user requirements, protection of drug product quality, and efficiency.

“The shift to parenteral delivery impacts both primary and secondary packaging systems for clinical trials,” says Claudia Berrón, senior vice-president of Clinical Services at Avantor. Parenteral drugs are shipped as liquid doses that require cold-chain distribution or as lyophilized dosage forms, which require packaging for optimal reconstitution at point of care, explains Berrón. She explains that primary parenteral packaging (e.g., prefilled syringes, autoinjector pens, or vials) should be protected by the secondary packaging to prevent breakage during shipping. Packaging should also incorporate patient-friendly and child-resistant features.

KIT DESIGN
When designing a kit, it is crucial to understand how it will be used by investigators, says Fiona Geiger, vice-president of client services at Yourway. “The more complete understanding that the kitting provider has about what materials will be necessary and in what order they will be used, the better the solution. Communication with sponsors and ideally the sites themselves can make a huge difference.”

“Items must be stored within the kit in a way that minimizes the risk of breakage or contamination,” adds Pieter Vercruysse, commercial director at Tjoapack. “They must also be designed in such a way that the entire product [such as a syringe or autoinjector] can be combined quickly and effectively with minimal manual input.”

Other factors to consider include package dimensions and cold-chain requirements. “Kits ultimately have to be packaged in shipping containers, and these have fixed, standard dimensions. So, when you are preparing kits that will need to end up in a 12-in. x 12-in. shipper, you have to make sure that you are sourcing ancillary supplies (e.g., syringes) with an eye on these dimensions and repack-
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ing materials to fit these constraints, or costs can skyrocket," notes Geiger. She adds, "Many drug products require temperature-controlled shipping and storage, but ancillary materials do not." If a kit’s ancillary increase the size of a unit, it may be better to ship a separate ancillary kit that doesn’t need to be kept cold. When shipping direct to a patient’s home, convenience might be more important, however.

Decentralized clinical trials occur in a participant’s home rather than at a central clinical site. “Direct-to-patient (DTP) and direct-from-patient (DFP) services have really transformed what materials are needed for a study. We’ve seen more demand for more patient-centric kits,” says Geiger. “In trials at clinical sites, ancillary items are stocked, and drug product materials can be kept in storage for a patient for multiple visits, but for visits at the home, you need to package not just the drug product but also the means to administer it, as well as ancillary materials or medical equipment. In addition, return packages need to hold patient samples and other items such as used wearable tracking devices or completed questionnaires. DTP/DFP is new, and the packaging is specific for each unique study.”

“The trend of moving the center of clinical trials to patient homes drives requirements for primary packaging in the form of patient-friendly administration systems,” adds Berrón. “For secondary packaging, this shift increases clinical kit complexity: inserts and dividers in top-loaded cartons provide a more logical presentation for the end-users. Personalized therapies, such as CAR-T and gene therapy, will only push this trend further, increasing the requirement for user-friendly clinical kits.”

SMART PACKAGING
“The push in 2020 for hybrid and remote clinical trials provided fertile ground for home-based technologies,” says Berrón. She suggests that use of patient-monitoring technologies, such as barcodes that can be scanned by patients with a smart phone, will continue to increase.

In addition to barcodes used with smart phones, “smart” packaging uses digital technologies such as labels with radio frequency identification or near-field communication (NFC) tags, to track the package and how the patient uses it.

For cold-chain packaging, consider the effects of low temperatures on container system, seal, and label materials.

“Patient non-adherence is a prevalent concern in clinical trials and one reason why clinical trial costs are on the rise. Companies are turning to digital solutions to overcome challenges and ensure patient adherence,” notes Deepak Thassu, vice-president, R&D and regulatory submission at LGM. “Smart packages provide a means of capturing unbiased information on a patient’s dosing history. The data can be analyzed to help clinical trial teams better understand patient adherence and exposure to the drug. This dosing information can also be used in counseling patients to stay compliant with the protocol.”

“NFC technology integrated with secondary packaging can wirelessly communicate with a patient’s NFC-enabled smartphone to provide updates for the patient on when and how much of their medication to take,” adds Vercruysse.

In addition to monitoring patient adherence, smart packaging technologies help track the storage conditions of a package to ensure product safety and stability. “NFC technology can also transmit information about the package such as ‘factory sealed’ or ‘opened,’ and can contain unique identifiers for authentication and tracking,” says Vercruysse. Time-temperature indicators in labels can provide clear evidence if a temperature-sensitive medication has been stored in conditions outside the set parameters of the trial. Other chemical or biosensors can be used to determine the chemical and biological integrity of the product and to alert doctors and patients if the drug is unsafe to administer, says Vercruysse.

COLD-CHAIN AND PACKAGE INTEGRITY
For biologic drugs, cold-chain storage and transportation are typically crucial parts of the packaging design. Although indicators could warn a patient of potential damage to a product, the aim is to maintain the cold chain with a package that arrives undamaged. Technologies for real-time tracking can monitor conditions such as temperature, humidity, and location and can alert distributors to take action before a package’s conditions are compromised (1). In addition, packaging should be carefully designed for cold temperatures. For example, it is important to consider how the materials and adhesives used in packaging as well as any tamper-evident seals will behave in cold temperatures, reports Almac Clinical Services (2).

“The packaging materials must be able to maintain their structural integrity at low temperatures, protecting the product within from damage,” adds Vercruysse. “Labeling must also be able to withstand low temperatures and moisture to ensure the information they convey remains
Hydrogen–Deuterium Exchange Coupled with Cyclic Ion Mobility for Higher Quality Analyses

ON-DEMAND WEBCAST
Aired: Thursday, March 25, 2021

Event Overview
Hydrogen–deuterium exchange (HDX) is increasingly employed in the study of larger proteins and protein complexes and in screening applications. These more challenging experiments drive a need for improved performance in the capacity of the liquid chromatography–mass spectrometry (LC–MS) system. Cyclic ion-mobility mass spectrometry (IMS), which provides higher ion mobility and mass resolution, offers improved peak capacity to address these challenges. In this webcast, the performance of cyclic IMS for hydrogen deuterium exchange is demonstrated relative to other types of high-resolution mass spectrometry, for sample-limited applications and for higher throughput data collection.

Key Learning Objectives
• See how HDX-MS helps address challenges with screening protein complexes
• Find out how to improve sequence coverage with limited sample volumes
• Learn how multiple ion mobility passes provide higher throughput experiments to truncate chromatographic gradients

Who Should Watch
• Biopharm discovery and development, BMR discovery, CXO, structural biology, higher-order structure
• Job Roles: Academic, lab director, R&D, bench scientists; lab managers, directors, Vice Presidents in charge of protein characterization, structural biologist
• Biopharma organizations, CXO, and research institutes
• Analytical scientists supporting biologics development

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legible. Innovation is happening all the time to enhance the properties of packaging materials and inks to achieve these goals.

The growing pipeline of biologics drugs has increased the need for cold chain capabilities. “We see this trend continuing, not only because of vaccines, but also because of more large-molecule and personalized medicines coming to market,” says Brian Keesee, vice-president and general manager of global clinical operations and supply at PCI Pharma Services.

OTHER PACKAGING TRENDS
Another trend is late-stage customization. “Keeping late-stage customization strategies in mind when designing patient kits and labeling helps ensure the end process is successful,” says Keesee.

Late-stage customization strategies are supported by just-in-time manufacturing concepts applied to packaging, which allow materials to be packaged and labeled just before shipping (3). Labeling is a crucial part of packaging clinical trial materials. “Labels for clinical trial packaging require the same degree of batch-to-batch consistency demanded from drug raw materials,” says Berrón. “Label consistency needs to be maintained across all batches, which could be printed over time and across multiple facilities. Lack of consistency, even in secondary packaging, carries the risk of bias that could inadvertently affect the trial.”

FDA requires serialization of labeling for clinical trial materials, adds Thassu. “The most important consideration for serialization is protecting dose anonymity in a blinded or double-blinded clinical trial. To be truly blinded, a single label lot would be printed with serialization, then individual labels would be randomly assigned from that lot to ‘reference (R)’ or ‘test (T)’ [material]. Only the labeler would hold the unblinding key between a serialized label and R/T treatment.” LGM Pharma uses an automated vision system to ensure conformance of the label to specification.

Thassu notes that automated inspection has been valuable for labeling and for other parts of packaging. “Technical advances in camera vision and verification systems over the past decade have allowed companies to replace manual inspection with an automated equivalent to ensure the uniform presentation of packaging and labeling, at a faster pace,” says Thassu. He says automation can create efficiencies in clinical trial management that can reduce the overall length of clinical trials.

**There are opportunities for automation in the packaging process.**

In addition to automating inspection, there are some opportunities for automation in the packaging process. Clinical kitting remains a generally manual process, although automation is used where its benefits can be justified, says Berron. Automation requires some level of standardization, but clinical kitting has a wide variety of kits and requires flexibility in volumes, which can vary widely at different clinical trial phases, she explains. Benefits of automation include increased productivity and consistency and eliminating ergonomic concerns for workers performing highly repetitive kitting activities. “In 2020, the use of automation certainly picked up another driver when high assembly absenteeism impacted productivity,” notes Berrón. “Efficiency driven by automation provides many benefits, but ultimately it is dependent on the size of the study and standardization of clinical trial kits across multiple studies, [which] limits feeder reconfiguration and retooling [on automated lines].”

**SOURCING**

Another key consideration for packaging of clinical trial materials is the availability of packaging supplies. Due to the pandemic, the supply chain has been tight across most materials needed for clinical trials, particularly for ancillary items such as swabs, needles, and even gloves, notes Geiger. Existing relationships with suppliers are crucial to being able to source materials when allocations are limited, she points out.

Vercruysse notes that biopharmaceutical manufacturers can consider alternative materials to the traditional glass vials and syringes. Polymers such as polypropylene and cyclic olefin polymers are options. “Careful consideration should be given to the selection of these materials, but also the sourcing to ensure high quality,” he notes. “Speed and agility became even more evident during the pandemic as we needed to package treatments and vaccines quickly and get them out the door. This meant being creative when sourcing materials as supply route and general availability of supplies was challenged with global shutdowns and reduced travel and logistics routes,” says Keesee.

Material supply partners should have the flexibility to quickly meet changing demand, says Keesee. He also sees a trend toward global, integrated logistics and materials suppliers “Partners that can manage logistics, storage, materials, temperature monitors, and integrated proactive communication globally provide tremendous value to the supply chain ecosystem,” concludes Keesee.

**REFERENCES**

2. N. Balanovsky and B. Thompson, Pharm. Tech., Partnering for Bio/Pharma Success Supplement s26-s28 (February 2021).
acceptable to provide the relevant links. A system description could therefore be structured like this:

- **System description.** In a few sentences describe the name, origin, and intended use of the system (e.g., by installed system module [in your case documentation, change, and deviation management]).
- **System history.** Describe the key milestones in the lifecycle of this automated system.
- **Technical information and system validation.** In a few sentences describe what system version is installed where and when it was last validated.
- **References.** Here you provide the links to all the relevant documentation.

Following this advice, you should be able to prepare your system description, and I hope you will also find it a useful exercise as it will help you find all relevant information faster in future.

**REFERENCE**

Q: Some 10 years ago, we implemented an electronic quality management system. Over the years, we have added some modules (e.g., for change control and deviation management). The system documentation was amended as changes happened. In a recent audit, we were given an observation: “Your system description does not meet regulatory expectations.” We are still unsure what is expected from us as we presented the system description given to us by the vendor, plus the validation documentation.

A: The regulatory authorities understand the importance of computerized systems in a modern pharmaceutical environment. They also understand the complexity of such systems and that these systems are likely to change over time. For that reason, companies must maintain an inventory of their automated systems, detail whether these systems are GxP-relevant, and if so, if these are validated. This is the very basis expected by the regulators.

To understand the intended use of each system, a system description is also required. Your auditors found your system description deficient. You mention that you provided the system description written by the vendor. This, I believe, is the key issue of concern.

Annex 11 of the European Union’s regulations state in paragraph 4.3: “An up-to-date listing of all relevant systems and their [good manufacturing practice] GMP functionality (inventory) should be available.

“For critical systems an up-to-date system description detailing the physical and logical arrangements, data flows and interfaces with other systems or processes, any hardware and software pre-requisites, and security measures should be available” (1).

Every company using the system will have different physical and logical arrangements, for example:
- You may have 50 users, whereas another company may have 5000 users.
- You may not have implemented any interfaces to other systems, whereas other users may have linked their system to other software packages.
- You may use the system for GxP relevant documentation, whereas another company may only use it for non-GxP purposes.

To understand the intended use of each system, a system description is also required.

Therefore, each company’s system description will be unique. The manufacturer can only provide a generic description, which can of course be the basis for your individual one.

You also mention that you do have additional pieces of information about the system, which can be found in the validation documents. That may be so, but these pieces of information are not consolidated, interlinked, or otherwise logically connected to fulfill the requirements of the regulations, which call for an up-to-date system description for critical systems. Your system is obviously critical as you use it for GxP-critical processes.

It doesn’t mean that you should copy/paste all the information that can be found in your validation documentation or elsewhere, into a document called “system description”. It is perfectly
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