The Analyst's Perspective

Assuring and Controlling Quality of Well-Characterized Biologics

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Analytical methods are the foundation of all biopharmaceutical work: from product discovery, characterization, formulation, and testing to process development and optimization, raw materials analysis and equipment qualification, and quality assurance and control. Over the past 15 years, technological advancements have improved precision, robustness, reproducibility, linearity, and control over the vast array of assays and analytical instrumentation available for biochemical, microbiological, and cell biology studies. Single-use technologies have introduced organic chemistry and materials science into the mix. Authors, reader-survey respondents, and editorial advisors helped us identify and examine trends in comparability testing, multivariate analysis, automation, statistics, validation, and more.

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Survey Results

In our reader survey of about 300 readers (self-selected), we asked analysts about automation and big data, process analytical technology (PAT), environmental sustainability, and validation. Perhaps to be expected: Analytical scientists are somewhat cautious about jumping to conclusions and demand empirical evidence before they make up their minds about something.

Automation: For example, even though over two-thirds admitted that automation (of molecular analyzers, sample preparation, and so on) improves their laboratories' workflow efficiency, one reader pointed out emphatically that we should have listed the associated "negatives" as well. It's true that automation is not something to undertake casually, and related technology does not offer the plug-and-play simplicity of consumer electronics. Integrated software often needs to be customized, and scientists need to learn how to interface with even the most user-friendly applications. Instruments require care and maintenance, and physical limitations come into play for viscous samples and those involving certain solvents.

There's also a learning curve, although a third of our respondents believe that today's automation-friendly laboratories require less time to train analysts (e.g., in remaining manual processes). Half said that variability in analytical results associated with operator differences has been greatly reduced. And nearly half again agreed that automation yields faster and more accurate results overall. Laboratory staff may have opportunities to enhance their knowledge by working with new instrumentation.

Process Analytical Technology: Automation has allowed some testing to bypass laboratories entirely. Over the past 15 years, PAT has transformed from a nice idea to a reality, at least for some types of measurements. Our readers said that flow, pressure, and temperature sensors are well established and trustworthy. Emerging are in-line, at-line, or near in-line solutions for measuring cell density, carbon dioxide (CO_2) and dissolved oxygen (DO), conductivity, osmolality, pH, glucose and total organic carbon (TOC), UV absorption, and viscosity. Still needed are technologies for cell viability, titer, and near-infrared (NIR) absorption.

Sustainability: Although environmental considerations are taking more of a foothold in biopharmaceutical laboratories now than they could 15 years ago, they have yet to become established. Sure, we all recycle paper and plastic — even metal and glass. But green chemistry isn't so easy when you're talking about biochemistry. Survey respondents' companies are trying to reduce their labs' environmental footprints, but some options are not yet scalable, on par with industry standards, or cost efficient. Corporate managers seem to be receptive to suggested changes — even if they transfer costs to other departments (e.g., single-use technologies increasing disposal needs while decreasing cleaning costs) — when scientists can back requests up with real data. Even so, technological platforms can lock many laboratories into using familiar chemicals and components rather than substituting other materials.

Validation: Regulatory guidance for process validation and assay qualification has changed substantially over the past 15 years. Most of our

surveyed analysts say that quality by design (QbD) and increased access to statistical tools have improved their confidence in results overall. With several decades of published results and industry literature, most quality laboratories are adequately conversant with the requirements of and differences between good manufacturing practices (GMPs) and good laboratory practices (GLPs). And many respondents are pleased to report that analytical methods now offer acceptable sensitivity, accuracy/precision, and range for use even with new product modalities (e.g., cell therapies). Meanwhile, new product classes are requiring more specialization among analysts and contract laboratories. And as mentioned above, process analytical technologies are becoming better understood and actively incorporated in manufacturing process lines.

I talked to Maureen Costello (Costello Consulting) about the relationship between quality assurance (QA) and quality control (QC), about assessment of single-use technologies, and about product characterization and comparability.

QA/QC: QA and QC are so often lumped in together that many people think of them as one thing. But they have very different responsibilities and approaches. "QC now must have more QA aspects," Costello says, "particularly in the acquisition, analysis, and storage/retrieval aspects — data integrity. In addition, the appropriate investigation of errors or invalid results has taken on more of a QA approach. From the quality system perspective, I see them effectively merging more in their approaches."

Single-Use Technology Assessment: Biopharmaceutical laboratories are well versed in the techniques of microbiology, cellular biology, and biochemistry — often involving methods based on liquid-chromatography. But with the advent of disposables, they have found themselves using gas chromatography and dealing with materials-science and organic chemistry. I wondered whether companies are hiring new specialists, training their own analysts in those new methods, or contracting such work out. "I am finding that the cost and maintenance of the equipment pushes most companies toward the outsourcing mode," Costello said. "That, and the idea that such analyses are considered 'boring.'"

Product Characterization/Comparability: The concept of the "well-characterized biologic" was enabled by advancing analytical technologies that made it possible to define proteins and other biomolecules structurally rather than simply as the products of documented processes. That led us inevitably to biosimilars. "As [BPI editorial advisor and principal consultant at Global Biotech Experts LLC] Nadine Ritter says," Costello said, "the protein analysts' time has finally come. Efforts in analytical development are paramount to the success of regulatory submissions and maintaining products on the market. It was always so — but now, it is recognized as an absolute necessity. It has not necessarily been a boon to the creation of more jobs; rather, analysts are required to constantly challenge themselves to learn more and do more in less time. This has pushed requirements for data acquisition, analysis, and storage systems that comply with 21 CFR Part 11."

Analytical Trending

I also asked some of our authors to help me look back over the past 15 years and identify trends and issues shaping the work-lives of analytical scientists in biopharmaceutical QA/QC and other laboratories. We talked about host-cell proteins and nucleic acids, endotoxins and pyrogens, comparability and product concentration testing, multivariate analysis and high-throughput screening, statistics, and analysis of single-use technologies. Here's where technology pushes science forward — and in turn drives the capabilities and expectations of the biopharmaceutical industry.

Host-Cell Contaminants: Bing Hu (principal scientist in CMC analytical services and operations at Teva Biologics) has been involved in two BPI articles over the years: one on detection of residual host-cell nucleic acids in biopharmaceuticals (**1**), the other on development of an enzyme-linked immunosorbent assay (ELISA) for testing host-cell proteins (HCPs) (**2**). "In the past seven years," he told me, "regulatory agencies and the biopharmaceutical industry have significantly advanced the regulatory pathway (e.g., US Pharmacopeia <1132>) and the technical approach for understanding HCP assays and characterization of critical assay components. It's well accepted by the industry to develop a phase-appropriate HCP assay for the support of the biopharmaceutical development."

I wondered whether that's made generic assay kits valuable only for comparative purposes. "Through 15 years of collective efforts of regulatory agencies and the industry," Hu said, "commercial HCP assay kits are believed to be valuable only for early phase biopharmaceutical development and filing of investigational new drug (IND) applications. When products advance into phase 3 and process validation, information generated from a commercial kit doesn't meet demands for a full risk assessment." Each company must develop its own process-specific HCP assay with demonstrated suitability (e.g., antibody coverage of specific HCPs). However, Hu pointed out that "commercial HCP antibodies in kits still could be used for process-specific late-phase and commercial HCP assays if those antibodies react well with the process-specific HCPs and are well characterized by 2D Western blotting with a satisfactory coverage." That hybrid approach would be valuable for any company that hasn't yet generated its own satisfactory in-house HCP antibodies.

Next, I asked him to compare challenges of HCP assays with those to detect host-cell nucleic acids. "Looking back," he said, "the HCP and DNA assays remain largely manual in many companies, with high assay variability and low productivity." Automation and high-throughput methodologies should change how companies test for such impurities. "Compared with HCP assays," Hu said, "host-cell DNA testing is less selective, and assay sensitivity (limit of

quantitation, LoQ) is far lower than regulatory limits. DNA impurity data are less questioned, and long-term clinical risks seem low."

HCPs are immunogenic, and their protein profiles vary among companies and products. Early industry efforts to discover common, consistent, and universal HCPs present in every process or product were unsuccessful. Each company therefore must develop HCP assays specific to each manufacture process and product for late-stage products. "Often from a well-established program," Hu says, "an in-house HCP antibody is generated and characterized from a pool of null cells specific to the manufacturing process. Commercial HCP antibodies could present an alternative for new programs if they react well with process-specific HCPs and are well characterized themselves."

I wondered what technological/regulatory advances have affected bioassay development over the past 15 years. "Even though ELISAs remain as a standard methodology for bioassay development," Hu said, "many new technologies have emerged, with different degrees of implementation throughout the industry." Examples include mesoscale discovery (MSD) in combination with robotics (e.g., from Tecan) and time-resolved fluorescent energy transfer (TR-FRET) assays, which have replaced some traditional ELISAs for product release. Immune-based polymerase chain reaction (PCR) kits and instruments from Thermo Scientific have shown early automation success, as has microfluidic cartridge technology from Gyros and Protein Simple. "It's important for each organization to choose a technology suitable for its intended use," Hu stated. "I am optimistic that collective efforts from regulatory, industry, and research will fundamentally revolutionize bioassay method development and testing over the next 5–10 years, if not earlier."

Pyrogens and Endotoxin Testing: Even the lay public is pushing for change related to animal testing and test methods that require animal-sourced reagents. Thomas Hartung (Doerenkamp-Zbinden professor and chair for evidence-based toxicology at Johns Hopkins University's Bloomberg School of Public Health) has developed an alternative for pyrogen testing (**3**). He described three options: rabbit pyrogen tests, bacterial endotoxin tests (e.g., the *Limulus* amebocyte lysate, LAL assay), and his own monocyte activation test using human cells. Historically, they chart a course from a live-animal test to a reagent derived from wild animals to a cell-based assay. The older methods bring up ethical questions of animal testing, not to mention issues of variability. "Having led the European validation body for alternative methods and centers for alternatives to animal testing since 2009 in the United States and 2010 in Europe, I see an ongoing paradigm change," he agreed. "The scientific community is embracing novel mechanistic approaches not only for animal welfare reasons: We are not 70-kg rats!" He also pointed to issues with reproducibility of animal experiments as well as their costs and duration. "At the same time, our mechanistic understanding is advancing in all life sciences. A pyrogen test based on the human fever reaction is a perfect example."

From 2008 to 2011, rabbit use for pyrogen testing in Europe "increased by about 10,000 to more than 170,000 rabbits per year. That occurred despite the new EU Directive 2010/63/EU on the use of animals for scientific purposes" (**3**). It will be difficult to compare further data, however. "In line with that directive," Hartung said, "the Commission is required to publish compiled EU data following new reporting obligations for the first time in November 2019. Unfortunately, the reporting scheme will change, so old and new reports will not be easy to compare."

I wondered about the potential for cell-based assays eventually to replace animal testing entirely. "For pyrogen testing," affirmed Hartung, "I have not seen a single product in 21 years that could not be tested with the novel tests, at least with some adaptations." For other areas, however, the question is more complex. "It is difficult to imagine behavioral effects to be studied in cells, and the development of animal drugs will require animal testing just as human drugs need patient testing. However, the better we know what we are looking for (the mechanism), the more we can design tailored tests to measure it exactly." People used to say that fever could not be measured in a test tube. But once scientists knew which cells produce what signal to induce fever, then tests could be designed. Such mechanistic tests are the reason why the European pharmaceutical industry — even while increasing research spending — dropped overall animal use by >30% from 2005 to 2011. "This shows that the most advanced industry is using new nonanimal approaches to make many development decisions."

And in just the year since his article, there appears to be momentum on the new test method. The European Pharmacopoeia now wants to make that its default method (companies would have to justify why they still use rabbits). The US Pharmacopeial Convention accepted the new test last year and includes it in its medical-device testing standards. "We also are in discussion with the US FDA and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)," Hartung reported, "about a possible validation study for medical devices. It is still a slow process."

Concentration Measurements: In a 2014 article, Joe Ferraiolo (senior product specialist at C Technologies) and coauthors from Bristol-Myers Squibb discussed the application of UV-vis spectroscopy to determination of protein concentration (**4**). The SoloVPE instrument they used is primarily intended for protein potency/concentration determinations, but it has been applied to other uses such as color analysis, DNA/RNA and polysorbate 80 concentration measurements, virus filter integrity testing, and analysis of antibody–drug conjugates (ADCs).

I asked what advances have been made with the system since the article was published. He pointed to a new fiberoptic coupler added to minimize errors and a disposable vessel

introduced to minimize cleaning. And Amgen authors published their own application of the technology with BPI in 2015 (**5**).

The article focuses on validation parameters (precision, accuracy, linearity, reproducibility, and robustness), so I asked about speed and PAT potential. "Speed often plays a significant role in justifying the acquisition of technology and determining its efficiency and return on investment," Ferraiolo said. "A SoloVPE measurement of a sample takes less than 60 seconds." His company's new FlowVPE instrument allows for real-time, inline measurements of protein concentration. "Because of its similarly broad linear range, it can be used to better understand column loading in continuous processes or for PAT in batch or continuous downstream operations. In chromatography, the system can be used to quantify protein mass in real-time or, using its spectral capabilities, for multi-variate analysis. In UF/DF and further downstream, it provides real-time process control for determining protein concentration."

Next I asked him about automation. "We've been looking at ways to import data from the instrument directly into things like our electronic laboratory notebooks and LIMS systems," Ferraiolo said. "There has been a significant drive within the industry over at least the last 10 years to be as paperless as possible, with benefits in both speed/efficiency and regulatory compliance. Any time you can automate a process — in this case data transference between systems — you can eliminate the possibility of errors and missing data/information. That can pay off in a big way through a reduction in the number of investigations and audit findings."

We hear a lot lately about the increasing power and importance of mass spectrometry (MS) in biopharmaceutical characterization. So I wondered whether UV-vis spectroscopy could play just as crucial a role in concentration measurements. "MS always has been considered the gold standard among spectroscopic applications," Ferraiolo pointed out, "but in many cases using it is like killing a fly with a bazooka. Certain types of samples or analyses require that level of rigor and confidence in results, but others don't." In the latter, he said, other technologies can answer questions (with high confidence in their accurate results) using esaier and less expensive tools to qualify, run, and maintain. "We believe that in past seven, years the SoloVPE system has virtually eliminated traditional UV-vis technology for measuring protein concentration." Its slope spectroscpoy technology employed has reduced sample preparation and dilution requirements (including serial dilution), buffer correction, cuvette washing and cleaning, and pipetting tedium — all saving time and resources.

Michael Johnson (business development engineering manager for Entegris) has written for BPI several times (**6–9**). In one article, he focused on the importance of measuring product concentration upstream, downstream, and even in cleaning procedures. I asked him about formulations. "The number of highly concentrated protein formulations has risen rapidly over the past several years," he said. "As they do, new manufacturing challenges are created. Some include product stability and protein aggregation. It becomes critical to develop an accurate, reliable, and precise analytical technique to measure solution concentration. Some current concentration measurement methods may not be reliable for such applications because of limited range, low precision, and drift. Many drug manufacturers are evaluating nonconductivity methods to meet their measurement performance needs." He also highlighted the trend toward continuous processing. "Successful implementation depends highly on robust on-line analytical techniques. Conventional measurement systems may not suffice, creating a potential technical gap in the market."

Single-Use Technologies: Next, we turned to contaminants, especially related to single-use components. Detection and measurement of particle contaminants has been a big part of drug-product and container–closure testing for decades. Now it's become more of an issue with single-use technologies. "The need for better particle mitigation in single-use technologies continues to be a hot topic," Johnson confirmed. Most single-use bag and system suppliers state compliance with USP <788>, a standard that establishes limits for particles in injections. But the industry has yet to establish rigid particle criteria for disposables. Johnson pointed to a best-practices guide published by the Bio Process Systems Alliance in 2014 and noted that other groups such as the BioPhorum Operations Group (BPOG) and the bioprocessing equipment committee of the American Society of Mechanical Engineers (ASME-BPE) are discussing opportunities to develop particle size and quantity criteria just for disposables

Editorial Advisors Speak Up

On QA/QC: "Quality assurance and quality control are very different functions in a biopharmaceutical company. QC is a service group that counts both QA and manufacturing as it customers, which is why in some organizations QC reports to manufacturing — and yes, that is acceptable to regulators, although some 'empire-builders' in QA might claim otherwise. The biggest challenge faced by QA in recent years is dealing with risk. Part of the issue in many QA groups is a lack of technical expertise, which leads some QA staff to be extremely conservative when faced with the need for change. That can lead to conflict with manufacturing when the manufacturing staff has deeper technical knowledge. For QA to be successful with current challenges, the staff needs to be as technically adept as manufacturing. Some companies have gained advantage by transferring employees from manufacturing to QA and from QA to manufacturing — to broaden the capabilities of both groups and strengthen ties between them. QA cannot successfully evaluate manufacturing risk without input from manufacturing; manufacturing cannot effect change without input from QA. Both groups must work together, bringing products to market for the benefit of the company." — Scott Wheelwright (Strategic Manufacturing Worldwide Inc.)

(**10**). "Along with the need for better particle standards and mitigation techniques," Johnson said, "end-users have suggested a particle reference library. It would use morphology such as size, shape, color, and hardness to categorize particles into groups such as intrinsic and extrinsic along with potential particle source identification." Such a library would expedite root-cause analysis and product disposition when particles are detected.

Leachables and extractables (L&E) are another topic with which container–closure experts have more history. But process applications seem to be more liable to "extract" more potential contaminants than do simple storage conditions. And as Johnson has pointed out, the range of disposable materials is much broader than those of elastomers and vials/ampules/syringes (**8**). He wrote that "the type and quantity of compounds detected in an extractable study are not only process dependent, but also material dependent." I asked him about the general progression in materials and how L&E are studied with them as disposables have advanced and biopharmaceutical companies have implemented them. "A majority of the progression has come in the form of recommended test protocols," he said. To address polymeric components and systems used in biomanufacturing, USP <665> and <1665> recently were made available for public review. "Although test protocols and risk assessments are beneficial, they are only tools used to identify potential problems. I believe the industry would benefit more from addressing the source of the risk, which includes fillers, additives, adhesives, and processing agents used in the formulation and manufacturing of many polymeric materials. Other industries that are highly concerned about contaminants from their process systems have implemented advanced polymers in which additives are minimized or nonexistent. In such applications, the extractable evaluation has been simplified while at the same time providing end users with reliable data to perform a thorough risk assessment."

Just last month, in fact, we worked together on an "Ask the Expert" webinar/write-up about fluoropolymer film for disposables (**9**). "Use of polymeric materials in bioprocessing has rapidly increased over the past 10 years or more," Johnson said, "but there hasn't been a great deal of material advancement in that time." The overall consumption of polymers for bioprocess applications is much smaller than that of some other industries. Resin manufacturers and compounders thus haven't invested much in new polymer formulations to address L&E concerns or broad temperature ranges (e.g., for frozen drug-substance storage or cell therapies). BioPlan Associates' *Twelfth Annual Report and Survey of Biopharmaceutical Manufacturing and Production* pointed to a need for new and improved plastics that enable design innovation. "This is where fluoropolymers come in," Johnson said. "They are advanced materials that provide the performance attributes needed for many critical bioprocess applications requiring very low extractables and broad temperature ranges. Sometimes, to successfully innovate into the future, it's necessary to look at what's worked in the past for similar applications."

Characterization and Comparability: Yves Bobinnec (director of global regulatory affairs at DBV Technologies) wrote on comparability protocols in 2013 (**11**). Comparability began as an exercise primarily used in scale-up and technology transfer; now it has become an essential part of biosimilar development. "Our strategy for designing comparability protocols is based mostly on comparison of post-changes batches with historical batches from a previous process version," he explains. "The goal is to confirm that clinical results obtained with previous batches are still relevant for the next development stages. This is a long process, and for such innovator products comparability can be seen as a path going away from initial development batches and leading (hopefully) to better control and quality." For biosimilars, whatever their stage of development, the reference is an innovator product. "The biosimilar should always match the innovator," Bobinnec says. "I envision comparability of biosimilars as a product profile moving around the innovator, never very far from it."

We talked about submitting comparability protocols to the FDA and the European Medicines Agency (EMA). "We do not submit comparability protocols for review unless we want to address critical issues with the authorities. The FDA requests submission of a complete report of the comparability exercise, and that can trigger comments if reviewers do not agree. In Europe, a summary of key findings is considered sufficient at the clinical stage." Over the past 15 years, many highly sensitive analytical methods (e.g., based on mass spectrometry, MS) have become more robust and more accessible for use in these protocols. "These methods can be very useful to address more subtle changes," Bobinnec advises, so that today's characterization efforts take a larger place in comparability protocols.

Whether working on biosimilars or innovator projects, developers need to keep track of sufficient samples for future comparison. "The main issue when addressing comparability for a biosimilar is to build a library of innovator batches and keep it updated," Bobinnec explains. "This library should include a reasonable number of batches (eight to 10) issued from different production runs to understand the intrinsic variability of the innovator's process. The innovator itself may perform process updates and generate subtle product changes. Thorough characterization of innovator batches, repeated over time to detect changes, is key when preparing comparability exercises for a biosimilar."

Xing Wang (president of Array Bridge Inc.) has addressed comparability of biosimilar monoclonal antibodies (MAbs) with a focus on higher-order structure (HOS) (**12**), which is closely related to their potential for immunogenicity. We hear a lot these days about the power of MS technologies in protein characterization. But Wang says there are limitations to what it can do. "One type of MS

technology used for MAb HOS analysis is based on hydrogen–deuterium exchange (HDX-MS), which can provide information at molecular level just like protein conformational array (PCA) technology (although they are based on different principles). But its major limitation is in reproducibility. The technology involves protein digestion, quenching, chromatography, and MS analysis — so it is very difficult to achieve the level of accuracy and precision often needed for MAb characterization. PCA-based ELISAs can easily achieve good accuracy and precision.

Wang's company invented PCA technology in 2011. "In the past six years, innovator companies (such as Genentech, Johnson & Johnson, Bristol-Myers Squibb, and Biogen) are using this technology for novel MAb development. Meanwhile, biosimilar developers (such as CellTrion, Samsung Bioepics, and Lupin) are using it for their development work."

In biosimilar studies over the years, Array Bridge has discovered a broad range of conformational profiles, even when all the molecules tested came from the same type of expression system. "Not all Chinese hamster ovary (CHO) cells are created equal," he wrote, and innovator processes have not been duplicated as easily as biosimilar developers had hoped early on. "From our analysis of more biosimilars since then," he told me, "that situation remains the same. Some show high similarity in HOS compared with the innovator MAbs, whereas others have showed minor to significant differences in HOS."

Statistics and Multivariate Analysis: Protein arrays are a type of high-throughput analysis, a general trend that is transforming biopharmaceutical laboratories. Last year, Ronan O'Kennedy (ROK Bioconsulting) wrote for BPI on multivariate analysis (MVA), an approach that such technologies have enabled (**13**). Statistics and design-of-experiments (DoE) work are vital to making it work.

"Over the past 15 years," he explains, "the value of process statisticians has been recognized in addition to their clinical colleagues. The quality by design (QbD) initiative, increased use of DoE techniques, and lean/six-sigma improvement programs have been important drivers to justify the benefits of process statisticians. Basic statistics and DoE are essential skills for all process scientists. I firmly believe that it is important for process development scientists to develop their statistical understanding and meet process statisticians in the middle. Ideally, scientists will gain confidence and competency in setting up routine experiments with statisticians providing support for more difficult experimental designs. I aim to support this process by providing biopharmaceutical-specific examples online (www.rokbioconsulting.com). The learning curve is steeper for MVA, and it is likely to remain a niche skill for the foreseeable future. I'm also planning to develop more examples from biopharmaceutical applications to support that as well."

His article focused specifically on testing growth media/feed formulations. So I asked how that's changed over the past 15 years. O'Kennedy said that such development was carried out in house 15–20 years ago, when DoE was a niche skill. "DoE use has become widespread since then," he reported, "and there has been rapid expansion of contract media/feed development services. The industry in general has risen to the challenge, resulting in product yield improvements and addressing manufacturing variability. Media manufacturers also have improved formulation techniques that help large-scale media formulations and improves process safety. Media and feed development had been focused on increasing product concentration and moving toward chemically defined formulas. However, increased interest in biosimilars also has focused media development on product quality attributes as targets. It is likely that both in-house and outsourced development will remain important because of this."

Next we talked about the value of in silico modeling, a general term for computer-based modeling based on either statistical or empirical models. "My interest in process modeling started with the 'windows of operation' concept published by UCL in 1996 (**14**). This work demonstrated the potential to predict yield and quality over multiple unit operations. It predated the design-space modeling now regularly applied for QbD. Since then, statistical and empirical modeling techniques have delivered increasing benefit to commercial bioprocess development and manufacturing. Hybrid modeling that merges statistical and empirical methods is likely to make important contributions in the future."

O'Kennedy points out that a model is only as good as its latest predictions. Thus, it will be important in the future to begin with rich data sets when setting up and verifying model predictions. Scientists also will need to consider how data are collected and compiled to allow continuous model verification. "I think the role of 'big data' should not be underestimated," he says. "Collection and annotation of experimental context and unit operations genealogy from upstream, downstream, and product analytical data will be essential for data mining of process development and production data, particularly to combine learning from platform process development with hybrid modeling."

Lee C. Smith (principal consultant and managing director of GreyRigge Associates Ltd.) also has written on statistics (**15**). I asked him whether companies are hiring statisticians or contracting with them — or simply expecting analytical scientists to get statistical experience/training. "Companies are hiring statisticians and consultants," he said. Statistics related to chemistry, manufacturing, and controls (CMC) can be quite complex, however, so a statistician "can't just walk straight from college or another area of statistics and hit the ground running in biopharmaceutical development or CMC. It takes years to build the experience for what is relatively a niche area." Drug companies often have many statisticians experienced in clinical studies — which is very different from process statistics. Echoing O'Kennedy,

Smith says it's "a bit like comparing a biochemist and a chemist. Both are scientists, but they have very different skill sets. They can cross over, but it takes time and training to do so."

Relatively few scientists have sufficient experience to perform the complex statistics required, "but they do exist," Smith affirmed, "and companies are lucky if they find one." The frequency of calculations varies, he explained, so certain statistics will be performed frequently and can be trained for. But more complex statistical activities are not as common. "From what I see, companies use some internal statisticians and scientists to do more routine analysis and support them with expert consultant statisticians for more complex activities or those with higher business risk."

Good statistical support is an important activity, Smith states, both for businesses and regulators. "This situation has always been the case, and I think it is becoming increasingly recognized over the past 15 years. Regulators expect sound statistics to be used, and they have made that clear in guidance. But what is often less appreciated is that good understanding of a process or assay can save a company a fortune simply through prevention of costly errors." Those can manifest as failed batches (each one costing thousands of dollars) or regulatory issues that delay licensure.

"QbD is providing some push," he says, "but it isn't easy to convince budget-holders and senior management that it is a necessary and a worthwhile investment. It is complex to understand and they are time poor, so an appreciation for QbD doesn't necessarily hit home." Regulators are pushing companies to apply QbD approaches, however, which provides a stick incentive (rather than a carrot) to convince them to apply it.

Regarding bioassays, Smith wrote that "although statistics won't improve an assay itself, it should help you understand assay performance" (**15**). I asked him for some examples. "Since the article, I have worked on a number of cell-based assays for biopharmaceuticals — both licensed therapeutics — and in product development of cell therapies and vaccines. The absolute truth remains that statistics will not transform a bad assay into a good one. However, they can extract maximum performance from an assay, no matter what state it is in. Often by understanding the sources of noise in an assay and quantifying them through variance-component analysis, operators can direct their replications more efficiently and reduce confidence intervals from a perspective of ruggedness. This gives a lot more confidence in the results and prevents unnecessary failures by reducing noise."

Smith has worked over the past few years in applying QbD approaches to screening and identifying critical components of assays to build in robustness. "This not only maximized the assays' reliability," he reports, "but it made technology transfer to a contract manufacturer straightforward because we knew what mattered and what didn't, what levels of precision to expect during transfer. We applied two one-sided *t* (TOST) tests that reflect bioassay variability and led to successful transfer on the first attempt. I have applied these approaches to a cell therapeutic, two vaccines, a protein therapeutic, and a diagnostic assay — all with good outcomes."

I wondered whether recently updated US and EU guidances have changed the five-step approach to validation that he described in his paper. "The approach of prioritize and scope, screen, optimize, validate and verify, and routinely monitor is as relevant and valid as it's ever been," Smith said. "Current guidances on process validation are entirely consistent with this approach." Furthermore, Q7–Q11 documents from the International Council on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) reflect the same approach with risk-based assessments, quality systems, product lifecycles, DoE, and continuous process verification.

Clearly, multivariate analysis and high-throughput screening have increased in importance for biopharmaceutical laboratories over the past 15 years. "A number of drivers have increased industry's focus on multivariate tools," said O'Kennedy. "The QbD initiative has been a major catalyst. Although multivariate tools were developed out of small-molecule applications, MVA is an ideal tool to gain greater understanding of biological systems because of the inherent multivariate complexity of biological production systems and biopharmaceutical products." He pointed to emerging opportunities for MVA equipment and software suppliers to increase the value of data from high-throughput screening data from systems such as parallel multibioreactor systems. "Key developments in the future will be better integration of high- (and low-) throughput tools and software with multivariate tools. Better integration should remove data-preparation bottlenecks and allow for seamless data transfer and more routine application of multivariate analysis."

Enabling Technologies, Increasing Understanding

At biopharmaceutical companies around the world, analytical scientists truly are coming into their own. Technological advancements enable greater precision in their analyses, making the "well-characterized biologic" a solid reality. The past 15 years have brought new analytical and bioanalytical methods into their laboratories and new questions for them to answer with those shiny new instruments. More and better information allows for improved modeling, prediction, control, and decision making. The vital scientific work that has always formed the foundation of bioprocessing and product development has continued to grow in importance and recognition over the years — and the foreseeable future looks bright enough to make sunglasses a prerequisite laboratory accessory.

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