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BioPharm INTERNATIONAL

October 2015

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Volume 28 Number 10 October 2015

BioPharm International integrates the science and business of biopharmaceutical research, development, and manufacturing. We provide practical, peer-reviewed technical solutions to enable biopharmaceutical professionals to perform their jobs more effectively.

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BioPharm International ISSN 1542-166X (print); ISSN 1939-1862 (digital) is published monthly by UBM Life Sciences 131 W. First Street, Duluth, MN 55802-2065. Subscription rates: $76 for one year in the United States and Possessions; $103 for one year in Canada and Mexico; all other countries $146 for one year. Single copies (prepaid only): $8 in the United States; $10 all other countries. Back issues, if available: $21 in the United States, $26 all other countries. Add $6.75 per order for shipping and handling. Periodicals postage paid at Duluth, MN 55806, and additional mailing offices. **Postmaster** Please send address changes to *BioPharm International*, PO Box 6128, Duluth, MN 55806-6128, USA. PUBLICATIONS MAIL AGREEMENT NO. 40612608, Return Undeliverable Canadian Addresses to: IMEX Global Solutions, P. O. Box 25542, London, ON N6C 6B2, CANADA. Canadian GST number: R-124213133RT001. Printed in U.S.A.

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Alice Till, PhD, is the 2015 president of the American Association of Pharmaceutical Scientists.

In the development of biopharmaceuticals and pharmaceuticals, the line is blurring.

Evolving to Meet Industry Changes

Like any other discipline, the pharmaceutical science industry is not immune to change. In recent years it has seen company consolidations, expansions, and mergers; limited/declining research funding; and a decreasing pool of workforce talent. The nature of our industry has shifted, and the growing interest in biotechnology has led to a metamorphosis. Pharmaceutical companies continue to diversify into biologics through acquisitions of biotechnology companies, in-licensing of products, academic-industry partnerships, and R&D alliances.

A decade ago, a clear distinction was made between biopharmaceuticals and pharmaceuticals based on their origin and method of manufacture. Since then, however, various industry business reports, including those supported by the Pharmaceutical Research and Manufacturers Association (PhRMA), have asserted that with the metamorphosis of the industry noted above, driven in part by the adoption of significant technological advances, "pharmaceutical" and "biopharmaceutical" are essentially synonymous, signaling that the lines between large and small molecules, chemical entities and biologically derived therapeutic drug products, and also between large and small companies are rapidly disappearing, which is—in fact—reflected in and across the PhRMA and the Biotechnology Industry Organization memberships.

As of 2011, consistent with these industry trends, nearly 50% of American Association of Pharmaceutical Scientists (AAPS) members are now affiliated with small biopharmaceutical/pharmaceutical companies, contract research organizations, or consultancies, with many of these pharmaceutical scientists having, or expected to have in the future, overlapping responsibilities for the discovery, development, and manufacturing of both small and large chemical and biologically derived molecules, as do those members affiliated with large biopharmaceutical/pharmaceutical companies, regulatory agencies, and universities. With the acceptance of biosimilars, pharmaceutical scientists affiliated with both brand and generic-drug companies, large and small, are likely to have overlapping responsibilities for large and small molecules. Though details may differ, the overarching principles, processes, and challenges of academic pharmaceutical research, industrial drug development and manufacturing, and regulatory review, approval, and oversight of new therapeutic products are not significantly different for large and small molecules.

AAPS meetings evolve to meet industry changes

AAPS is working to merge its National Biotechnology Conference and its Annual Meeting beginning in 2018 to provide a forum for all pharmaceutical scientists to work together to advance the field and facilitate the discovery, development, and approval of new medicines. The programming for this single meeting will be designed to leverage the diverse expertise of its members to provide opportunities for sharing of cutting-edge science, building on the commonalities between large and small molecules, as well as for furthering the understanding of unique differences.

As the pharmaceutical industry changes, the association must change as well if it is to remain relevant. The new combined meeting is a response to one key aspect of the evolving industry and consequently the professional needs of our members; other changing needs have been and will continue to be identified and addressed through the dynamic strategic planning and management processes initiated this past year. AAPS is playing an important part in advancing the capacity of pharmaceutical scientists to develop products and therapies that improve global health. ◆

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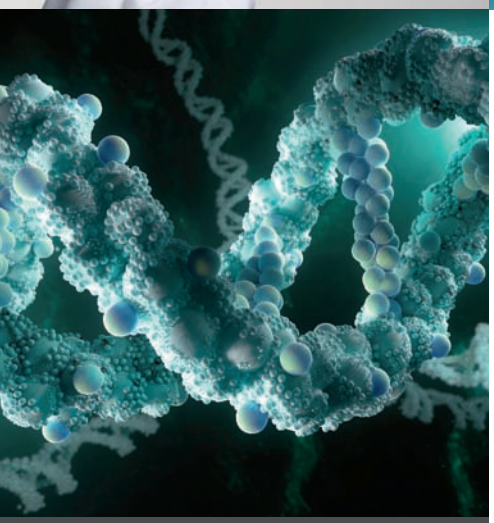

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FDA Faces Controversy Over Quality Metrics and Biosimilars

Manufacturers challenge details in new policies designed to promote access to important therapies.

FDA officials have been busy addressing some difficult drug regulatory issues important to biopharma manufacturing, marketing, and R&D. In July 2015, the regulators rolled out a much-discussed proposal for how companies should collect and submit data to measure the quality and reliability of manufacturing systems, only to meet strong objections from industry (1, 2). FDA followed the metrics program with a highly controversial plan for naming biosimilars and innovator biotech therapies, which continues to divide innovator and generic firms (3, 4).

A stated goal of these and related FDA policies is to facilitate patient access to needed medicines, a process that involves preventing and reducing critical drug shortages. The FDA Safety & Innovation Act (FDASIA) of 2012 addressed shortages—in addition to providing FDA authority to collect additional manufacturing data for its metrics program—by enabling the agency to require early manufacturer notification of expected supply disruptions for life-saving medicines. FDA issued a final rule in July 2015 that addresses shortages by requiring a broad range of companies to provide advance (six months) notification of an event likely to cause a "meaningful disruption" in the supply of critical medicines (5). Biotech manufacturers had protested extending the initiative to vaccines and other biologics, and generic-drug makers complained that even a five-day notification requirement may be a burden, but those concerns did not stop FDA from implementing what it considers a practical early notification policy.

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SLIGHTLY DIFFERENT BIOSIMILAR NAMES

Much more contentious is FDA's proposal for addressing the hot-button issue of how to identify biosimilars related to innovator products. Biosimilar makers and payers want their new products to carry the same proprietary names as reference drugs to encourage prescribing and reimbursement; brand companies argue that different names are necessary to prevent inadvertent substitution and confusion regarding adverse events.

FDA appears to lean towards the "ensure safety" camp by establishing a new biosimilar naming policy that adds a unique, four-digit suffix to a "core" name for all biotech therapies (3).

This approach aims to prevent erroneous prescribing and dispensing of biosimilar and reference products and to facilitate tracking of postmarketing safety issues, a process that FDA says can't rely on national drug code numbers (NDC) because many biologics are administered in hospitals and clinics. Biosimilar advocates fear that even slightly different names will discourage product uptake, but analysts note that the similar core names will permit brands and biosimilars to be grouped together on health system databases, which will encourage their use.

While innovators may support FDA's approach for differentiating biosimilar names, they are up in arms about the agency's unexpected related proposal for adding suffixes to all biotech therapies, including those already on the market. To start what is sure to be a lengthy process of revising product names retroactively, FDA issued a proposed rule that specifically applies the new naming policy to six licensed biologics facing near-term competition from biosimilars (6). Requiring new names for old products has never occurred before, says Gillian Woollett of Avalere Health, noting that significant database and software changes may be needed to accommodate the new system.

Still unclear is how FDA will apply the new naming policy to biosimilars that achieve "interchangeable" status, which applies to a

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drug that can be substituted by the pharmacist without prescriber permission; while this may be the case for most conventional generics, biosimilars may be approved as similar. One option is to permit the same suffixes for these products, and FDA seeks comments on this issue, as well as its broader naming policy.

GO SLOW WITH METRICS

Both brand and generic-drug firms are troubled by FDA's plan for collecting data on a range of measures for the reliability and quality of drug-production operations and resulting products. After three years of workshops and white papers on developing quality metrics, FDA finally spelled out its program in a draft *Request for Quality Metrics* guidance document (1). Janet Woodcock, director of the Center for Drug Evaluation and Research (CDER), opened an Aug. 24, 2015 public meeting to discuss the plan by suggesting that the proposed metrics are what "any manufacturer would want to know" and that FDA had worked hard to keep the new data collection initiative manageable and useful. The guidance outlines a number of data points that CDER and the Center for Biologics Evaluation and Research (CBER) believe will help field inspectors assess the ability of an operation to reliably produce high-quality medicines; firms with good reports may merit less frequent plant inspections and reduced reporting of post-approval manufacturing changes.

Somewhat surprising after such extensive FDA-industry collaboration were the many objections raised by manufacturers about the metrics proposal being too broad, unclear, and moving towards mandatory implementation too quickly. Industry reps stated at the August 2015 meeting that gathering and reporting the data will be costly and time-consuming and voiced fears about the program generating "report-card" listings and superficial comparisons open to misinterpretation by patients and payers.

Genentech Vice-President Diane Hagerty, representing the International Society for Pharmaceutical Engineering (ISPE), advised FDA to phase in the initiative, starting with higher risk facilities and products, and to drop for now a measure for on-time completion of annual product reviews. Camille Jackson, director for science & regulatory advocacy at the Pharmaceutical Research and Manufacturers of America (PhRMA), questioned FDA's use of guidance, as opposed to more formal notice-and-comment rulemaking, to provide sufficient authority for the agency to require metrics reporting in advance of inspections. Similarly, David Gaugh, senior vice-president of the Generic Pharmaceutical Association (GPhA), speculated whether FDA can require metrics reporting by foreign companies, a limitation that he said could encourage US firms to shift drug production overseas.

Excipient makers objected to the idea of collecting metrics on "high risk" excipients, while API producers raised a host of questions about providing quality measures on products made for many drug companies. Non-prescription drug firms want to limit initial metrics to high-risk medicines, as opposed to hand creams. And Gil Roth, president of the Pharma & Biopharma Outsourcing Association, voiced uncertainties about how contract manufacturers can submit data on a facility making drugs for multiple clients. Richard Johnson, president of the Parenteral Drug Association (PDA), optimistically described the FDA proposal as "a good place to start," but cited challenges in assessing the "quality culture" at companies—potentially the next phase for the program, but now apparently on the back burner.

All these objections clearly disappointed FDA officials, who said they sought an objective list of measures that companies already collect internally and that could be assessed easily by field inspectors. FDA believes the program will assist in inspection scheduling and in efforts to avoid supply disruptions. But staffers acknowledged the need to clarify terms, how data will be used, and reporting relationships for contractors and suppliers. Issuing final guidance "is a high priority," said Russell Wesdyk, acting director of the Office of Surveillance in CDER's Office of Pharmaceutical Quality, but FDA extended its comment period through November 2015, and no one expects any revisions until 2016, at the earliest.

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Small-Molecule API CMOs Are Thriving

Despite emergence of biologics, small-molecule APIs benefit from industry growth.

It has been the general consensus among bio/pharmaceutical industry participants, investors, and observers that small-molecule drugs are becoming obsolete as the industry focuses on biologics. To paraphrase what Mark Twain allegedly said about his own situation, reports of the death of small-molecule drugs are greatly exaggerated.

Small-molecule pharmaceuticals accounted for 82% of all new drug application (NDA) approvals in 2014 and 60% of all new molecular entities. Further, they represent two-thirds of the drug-development pipeline. While there can be no doubt that the number and share of large-molecule therapeutics is increasing, small-molecule drugs are an important and highly effective component of the bio/pharmaceutical portfolio. Many of the most important drugs introduced in recent years, including kinase inhibitors such as Gleevec and anti-retroviral products such as Sovaldi, are small molecules.

Small molecules have some considerable advantages over large molecules. They can be engineered to deliver a strong therapeutic effect with a small dose, often below 10 mgs and even into micrograms. The smaller amounts of API, combined with the maturity of chemical manufacturing technology, typically translate into a lower cost of goods relative to efficacy versus large-molecule therapeutics. Analytical technology for small molecules is highly refined, ensuring quality, efficacy, and reproducibility.

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Further, small molecules can be formulated into orally delivered dose forms, which offer better compliance and a lower cost of administration than injectables, the principal delivery route for large molecules. There is a vast amount of formulation expertise and experience with small-molecule drugs, enabling highly controlled formulations that can be delivered to specific sites and released over specific time periods.

> Clinical trials involving small-molecule therapeutics are often simpler and less expensive.

Clinical trials involving small-molecule therapeutics are often simpler and less expensive than those involving large-molecule drugs. Process development and manufacture of clinical-trial materials for small molecules are typically much less expensive for small-molecule candidates. Clinical supplies for small molecules must be handled carefully, but they often don't require the cold chain assurance of large molecules, which makes shipment and storage of clinical supplies costly. Further, procuring comparators for biopharmaceutical candidates (i.e., drugs already on the market) can be extremely expensive.

OUTSTANDING PERFORMANCE

Small-molecule therapeutics have participated fully in the explosion of drug development activity of the past few years, which has been fueled by record amounts of fundraising and spending for research and development.

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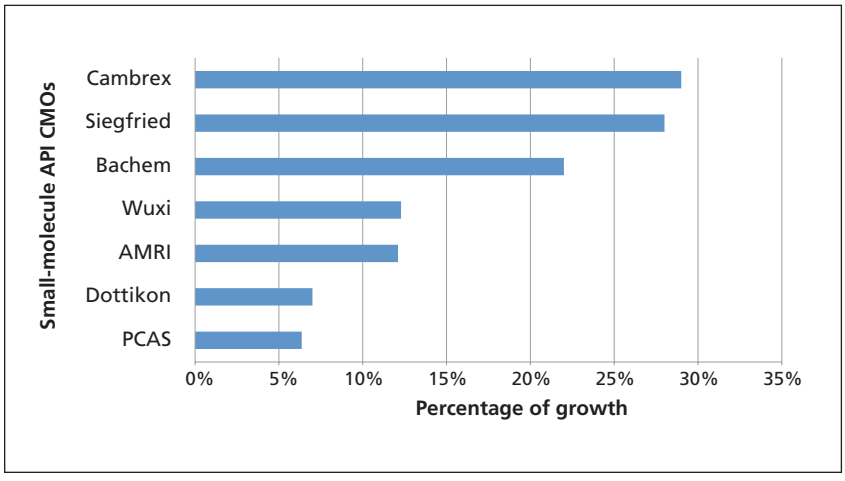
Figure 1: Growth in custom manufacturing revenues of small-molecule API contract manufacturing organizations (CMOs) in the first half of 2015.



Contract manufacturing revenues of publicly-traded "pure-play" small-molecule contract manufacturing organizations (CMOs) grew 15% in the first half of 2015, with some companies enjoying growth in excess of 20% (see **Figure 1**).

Within the small-molecule API world, certain segments appear to be especially strong, notably controlled drugs and highly potent APIs (i.e., cytotoxics, hormones, and very low-dose compounds). These products require specialized facilities that protect operators from exposure to the chemical and, as with controlled drugs, may have to meet special regulatory requirements.

Demand for capacity to manufacture high-potency products has been so strong that CMOs with the capability have been expanding it while those lacking the capability have been adding it. CMOs that have recently completed or announced expansions include SAFC, Johnson-Matthey, Cambrex, Carbogen-Amcis, and Novasep.

On Sept. 1, 2015, Fareva (Luxembourg) announced that it is acquiring an API manufacturing site in La Vallée, France from Merck and will invest €25 million ($28.3 million) to build two units at the site with occupational exposure band 4 (OEB 4) containment. Fareva already has high-containment API manufacturing capability at its facility in Feucht, Germany, but needs to expand its capacity to meet market demand.

Demand for capacity to manufacture high-potency products has been strong.

M&A ACTIVITY

Further speaking to the attractiveness of the small-molecule API space has been acquisition activity. There have been a number of significant deals with prices equating to multiples of two or more times the acquired company's revenues.

In March 2015, Patheon acquired Irix (Florence, SC), a mid-size API manufacturer with development and commercial capabilities. The acquisition added small-molecule capabilities to Patheon's offerings in large-molecule API and dosage forms. In July 2015, AMRI announced it would acquire Gadea Pharmaceutical Group (Valladolid, Spain), a specialist in hormone and steroid APIs. That followed its 2014 acquisition of Cedarburg Laboratories (Grafton, WI), which has controlled and high-potency drug capabilities. Also, Siegfried (Zofingen, Switzerland) announced in May 2015 that it will acquire the API business of BASF, giving it three additional manufacturing sites and considerable greater scale and presence in the industry.

The level of business, acquisition, and capital investment activity in the small-molecule API market shows that the industry is taking full advantage of all of the technologies in its tool box to address the opportunities being revealed by the increased understanding of disease processes. New therapies like antibody drug conjugates are marrying the specific capabilities of large and small molecules to delivery highly effective drugs to specific sites, and the technology is expanding beyond its initial focus on delivering cytotoxic compounds to broader applications.

In times like these, when investment activity reaches frenzied levels, investors and executives can be distracted by fads or the latest technologies no matter how untested they might be. Small-molecule APIs have performed well and cost effectively for patients and pharmaceutical companies for decades and there is every reason to believe that small-molecule API CMOs will participate in the bio/pharmaceutical industry's success for decades to come. ◆

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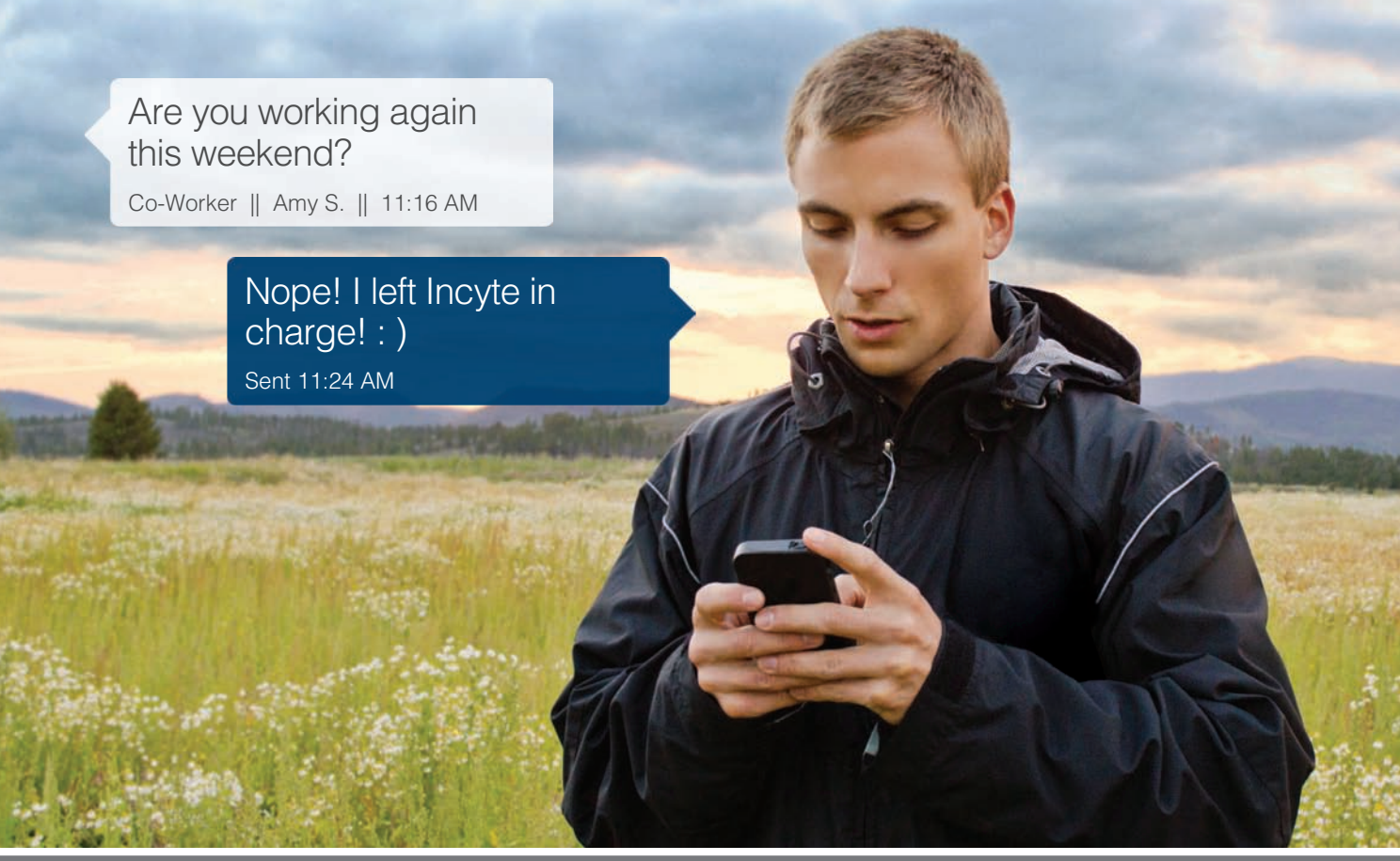

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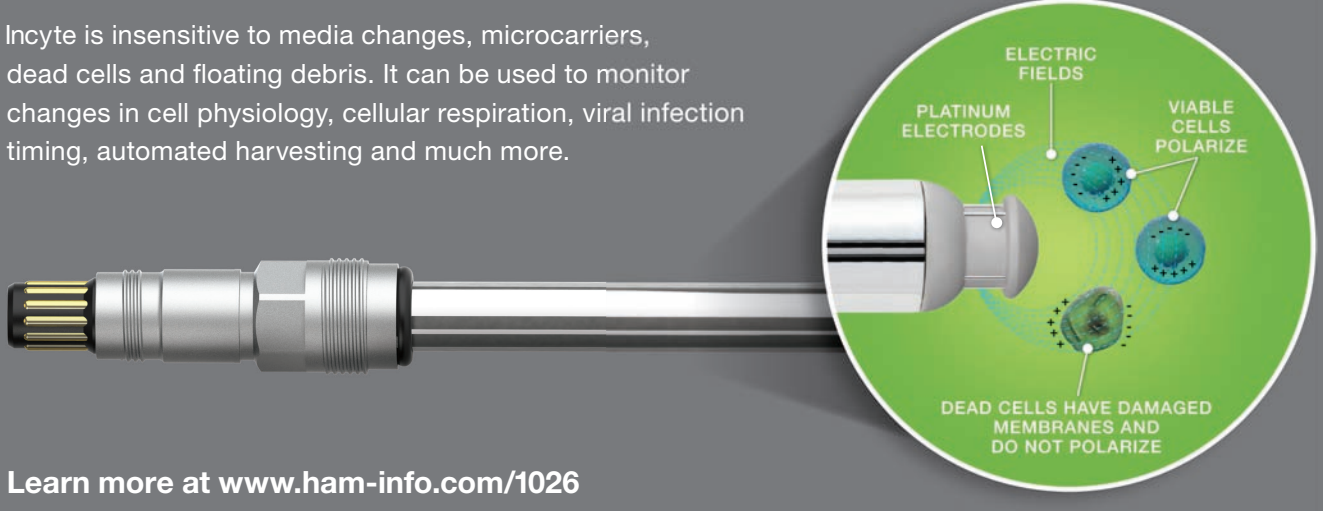

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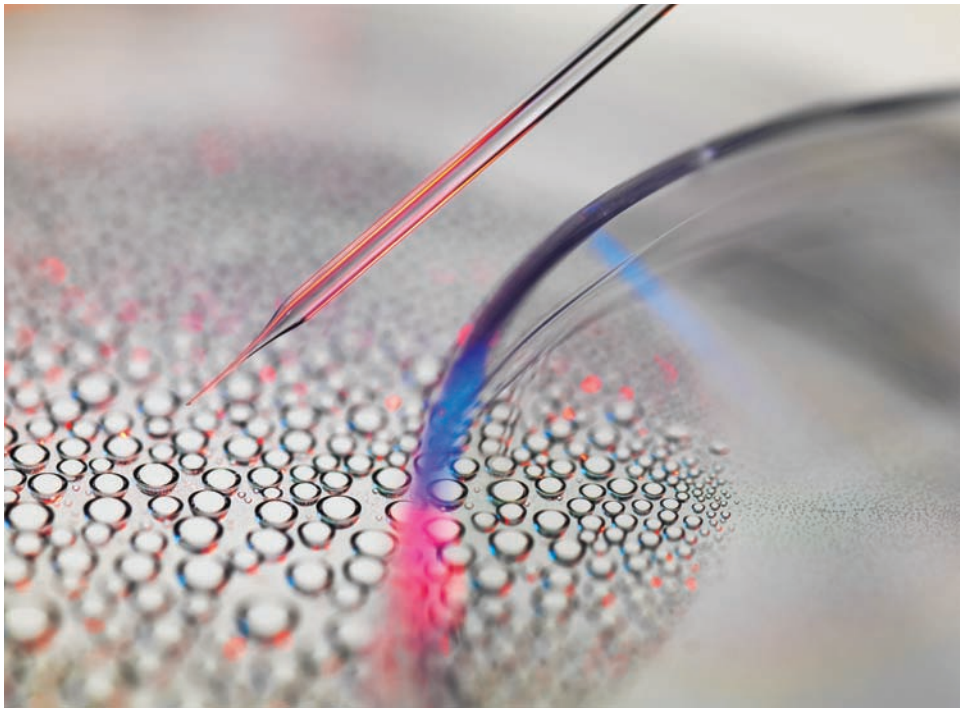
Leonard P. Freedman, Mark C. Gibson, and Richard M. Neve

Taking immediate steps to address misidentified and contaminated cultured cell lines will improve the reproducibility of preclinical research.



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Irreproducible preclinical research is a global, expensive, and well-recognized problem that contributes to delays and increased costs of drug discovery (1, 2). One published study conservatively estimated the total prevalence of irreproducible preclinical research to exceed 50% with a cost of $28 billion per year in the United States alone (3), while other researchers have estimated that an astounding 85% of biomedical research is wasted as a result of correctable problems (4). Excluding scientific misconduct, which is not a major source of irreproducibility (5), lack of reproducibility typically results from cumulative errors or flaws in one or more of the following, non-discrete categories of the research and publication process: biological reagents and reference materials, study design, laboratory protocols, and data analysis and reporting (3). Although each contributes to a systemic problem that requires extensive changes in the overall scientific culture milieu, taking immediate steps to address biological reagent issues—specifically the use of cultured cell lines—is a relatively straightforward fix that will improve the credibility, reproducibil-

Leonard P. Freedman is president, Global Biological Standards Institute; **Mark C. Gibson** is policy research analyst, Global Biological Standards Institute; and **Richard M. Neve** is senior research scientist, Gilead Sciences, Inc.

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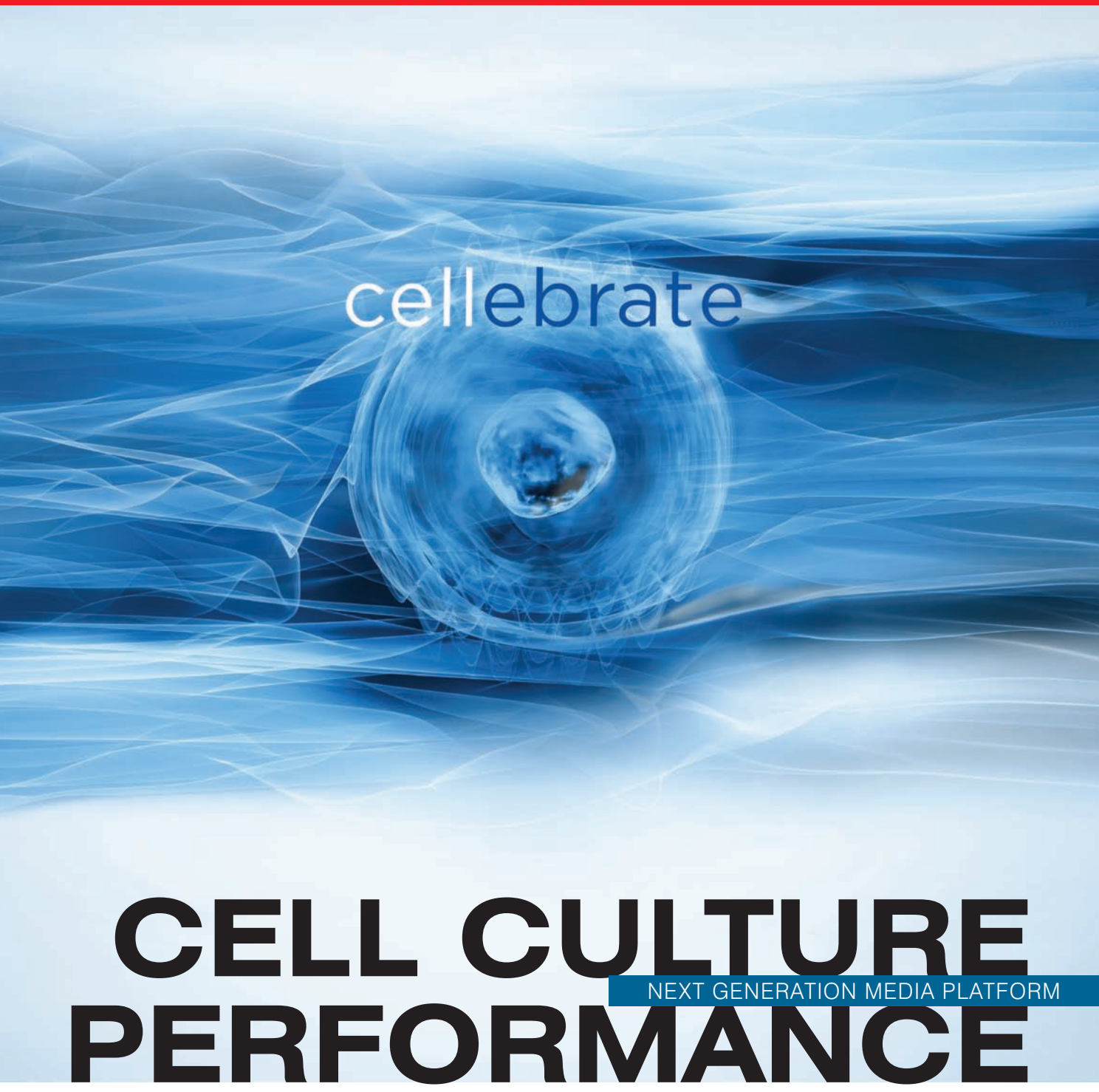

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Table I: Factors affecting reproducibility of cell-based research.

Observation	Possible Cause(s)
Changes in cell viability	• Cells were stored or cryopreserved incorrectly • Cells thawed incorrectly • Freezing/thawing media incorrect • Freezing media components expired • Cells overstressed during preservation • Too few cells frozen • Faulty incubator (water, temperature, etc.)
• Changes in cell growth rates • Changes in morphology/attachment	• Growth media and components incorrect • Media components expired • Poor quality serum • Poor culture technique (e.g., over-trypsinization) • Cells allowed to grow to confluency • Cells passaged too long • Cells contaminated (e.g., mycoplasma) • Cells cross-contaminated or misidentified • Poor quality culture vessels • Faulty incubator (water, temperature, etc.)
Altered response to therapeutics	• Growth media and components incorrect • Cells passaged too long • Cells contaminated (e.g., mycoplasma) • Cells cross-contaminated or misidentified • Poor quality therapeutic reagents
Genomic changes	• Cells passaged too long • Cells contaminated (e.g., mycoplasma) • Cells cross-contaminated or misidentified
Contamination by microorganisms	• Poor cell culture technique • Contaminated laminar flow hood • Contaminated incubator • Contaminated cell culture reagents or consumables
Cross-contamination by other cell lines	• Poor cell culture technique • Human error

ity, and translation of preclinical research. It will also make more efficient use of scarce biomedical research resources.

Immortalized cancer cell lines isolated from various human and other mammalian tissues have been used for decades across multiple areas of biomedical research (6). Their use is central to most drug-discovery projects, from initial target validation studies, through clinical candidate selection, to subsequent translational studies (7). It is essential for drug-discovery scientists to have routine access to a wide variety of high-quality, well-characterized, and contaminant-free mammalian cell lines. For these applications, accurate determination of species, sex, and tissue of origin (i.e., identity) is crucial to interpretation, validity, and translation of research results (8). Cell lines are cultured, passaged, and processed in and among laboratories with widely varying quality control (QC) procedures, while sharing cell lines is endemic—particularly in academia. For these reasons, misidentification errors, including intraspecies (most commonly by HeLa cells [9]) and interspecies (nonhuman) cross-contamination, as well as labeling and cell naming errors, occur frequently and can persist for years (10). Changes in the genotype and phenotype of cells (i.e., drift) as a result of over-passaging of cell lines or poor culture technique continue to be persistent problems (11).

Microbial contamination of continuous cell cultures by a wide variety of microorganisms is also problematic in cell culture laboratories worldwide, particularly by mycoplasma. The latter can be difficult to detect, grow to high densities without adverse effect on cell morphology, and can impact a wide variety of cell functions, including changing response to

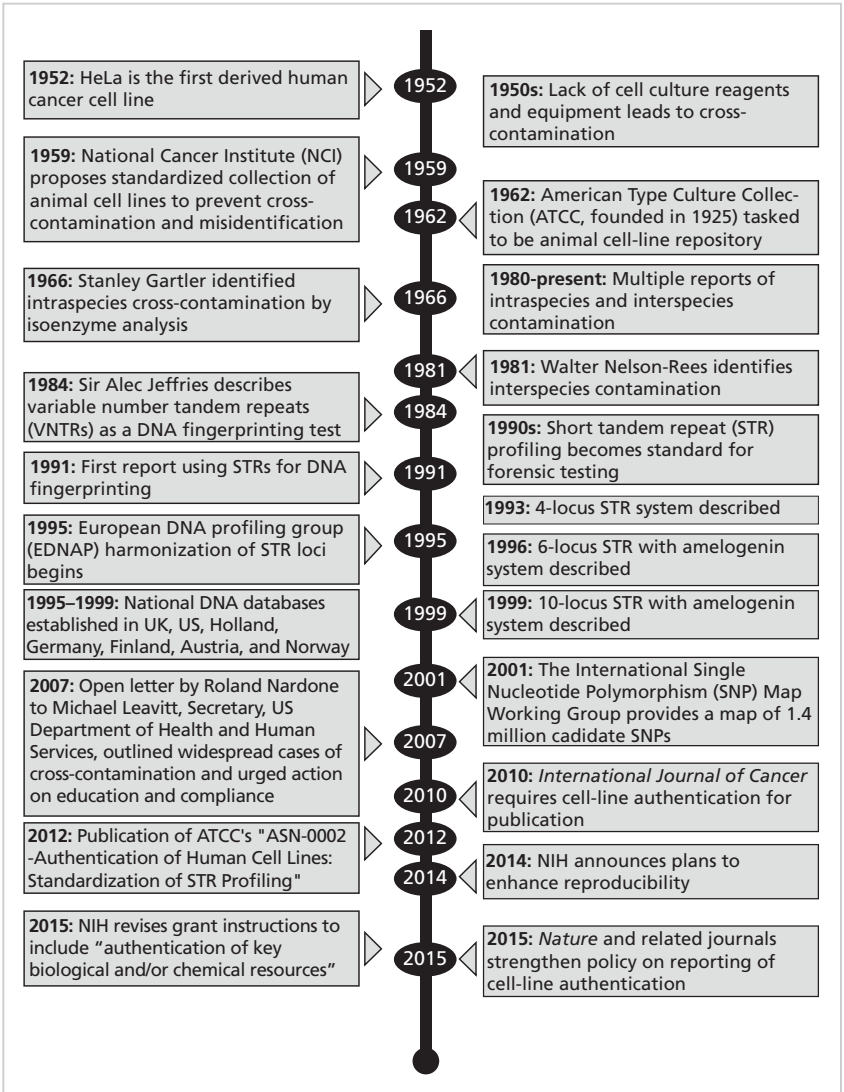
therapeutics (12). Commercial tests are available to detect the most common forms of mycoplasma, but no affordable test exists to detect all Mollicute species.

Although multiple organizations (e.g., National Institutes of Health [NIH]) promote or recommend best practices for handling biospecimens and other biologicals, including cell lines (7, 13), none are universally followed by a majority of biomedical researchers. Expanding the development and use of best practices and consensus-based standards for obtaining and maintaining authenticated and contaminant-free cell lines should also include smaller repositories, otherwise biological materials in the public domain will likely become compromised over time (8). **Table I** summarizes factors that can contribute to irreproducible cell-based research.

USE AND COST OF MISIDENTIFIED AND CONTAMINATED CELL LINES

How widespread is the problem? One key review examined the prevalence of contaminated cell lines from 1968 to 2007 and reported combined cell line misidentification and contamination rates ranging from 18% to 36%, with only a small improvement over time (14). A more recent estimate places the cross-contamination rate at 20% (15). While intraspecies contamination receives the majority of attention, approximately 6% of cell cultures are thought to be affected by interspecies cross-contamination (16). To complicate matters, a study of more than 200 biomedical papers found that only 43% of cell lines could be unambiguously identified by their description (e.g., authors provided a name and source for the line such as a repository) (17). This type of problem—coupled with journal-imposed space limitations—is symptomatic of a widespread lack of consensus on the level of detail required to allow adequate documentation of materials and methods in the literature to facilitate external replication of study results.

Figure 1: Timeline of key events in cell line-based research and authentication.



A search of NIH RePORTER identified 9000 projects and sub-projects that use cell lines at a total estimated taxpayer expenditure of $3.7 billion. If 18–36% of these research projects use misidentified or contaminated cell lines, potentially $660 million to $1.33 billion in research dollars could be affected. Based on two well-known misidentified cell lines, HEp-2 and INT 407, more than 7000 articles have been published that may have inappropriately used one or both cell lines at a total estimated cost of more than $700 million (15). Estimates of the prevalence of mycoplasma contamination of cell cultures vary widely, from 15% to as high as 35% (12, 18). An assessment of mycoplasma contamination in the National Center for Biotechnology Information Sequence Read Archive conservatively found that 11% of projects were contaminated (19).

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Table II: Comparison of short tandem repeat (STR) and single nucleotide polymorphism (SNP) techniques as methods for DNA profiling. Adapted from (8).

Attribute	STR	SNP
Application	Assess sample identity	Assess sample identity
Level of discrimination [a,b]	2.82×10^{-19}	1.0×10^{-18}
Number of loci for discrimination	16	48
Number of alleles per locus	Multiple	Biallelic
Detection of cross-contamination	Yes (2–10%)	Yes (2–10%)
Detection with degraded DNA	Yes	Yes
Sex determination	Yes	Yes
Ethnicity determination	No	Yes
Multiplexing	Yes	Yes
Commercially available reagents	Yes	Yes
Fee-for-service testing	Yes	Yes
Databases of cell line profiles	Yes	Yes
Cost per sample (in laboratory)	$15–30	$6

[a] STR: www.promega.com/products/pm/genetic-identity/population-statistics/power-of-discrimination/.
[b] SNP: http://agenabio.com/products/panels/sample-id/.

The authors estimated that hundreds of millions of dollars of NIH-funded research using continuous cell lines had been potentially affected.

Although cell lines contaminated with mycoplasma or other microorganism constitute an ongoing and expensive concern in cell banks and cell culture labs around the world, the remainder of this paper focuses primarily on misidentification and intraspecies cross-contamination of human cell lines used in preclinical biomedical research.

AUTHENTICATION IS THE SOLUTION

The identity of a cell line (i.e., authentication) can be determined by comparing the genetic signature (profiling or fingerprinting) with established databases (e.g., American Type Culture Collection [ATCC] in USA, Japanese Collection of Research Bioresources [JCRB] in Japan, Deutsche Sammlung von Mikroorganismen und Zellkulturen [DSMZ] in Germany) to discover misidentified cells (20). It is important to emphasize, however, that profiling comprises only one component of understanding the complex molecular and phenotypic properties of a cell line, which is not a uniform, clonal population (8). To fully characterize a cell line requires detailed genomic, proteomic, and phenotypic analyses, which remains implausible and costly for most cell banks, let alone typical research laboratories. For this reason, cell line authentication and QC measures such as mycoplasma detection constitute an essential first step to establish and maintain the integrity of cell cultures and to enhance reproducibility of results using cultured cells. An American National Standards Institute (ANSI)-accredited, low-cost (approximately $150 fee for service or $15–30 in-house) standard for cell line authentication based on short tandem repeat (STR) profiling has been available for several years (21). **Figure 1** provides a timeline of key events in cell line-based research and authentication.

Another DNA profiling test of cell-line identity uses single nucleotide polymorphism (SNP) variations between members of the same species within a specific locus, which are conserved during evolution (10, 22). Although commercial kits are becoming available, at present there is no ANSI-approved standard or cen-

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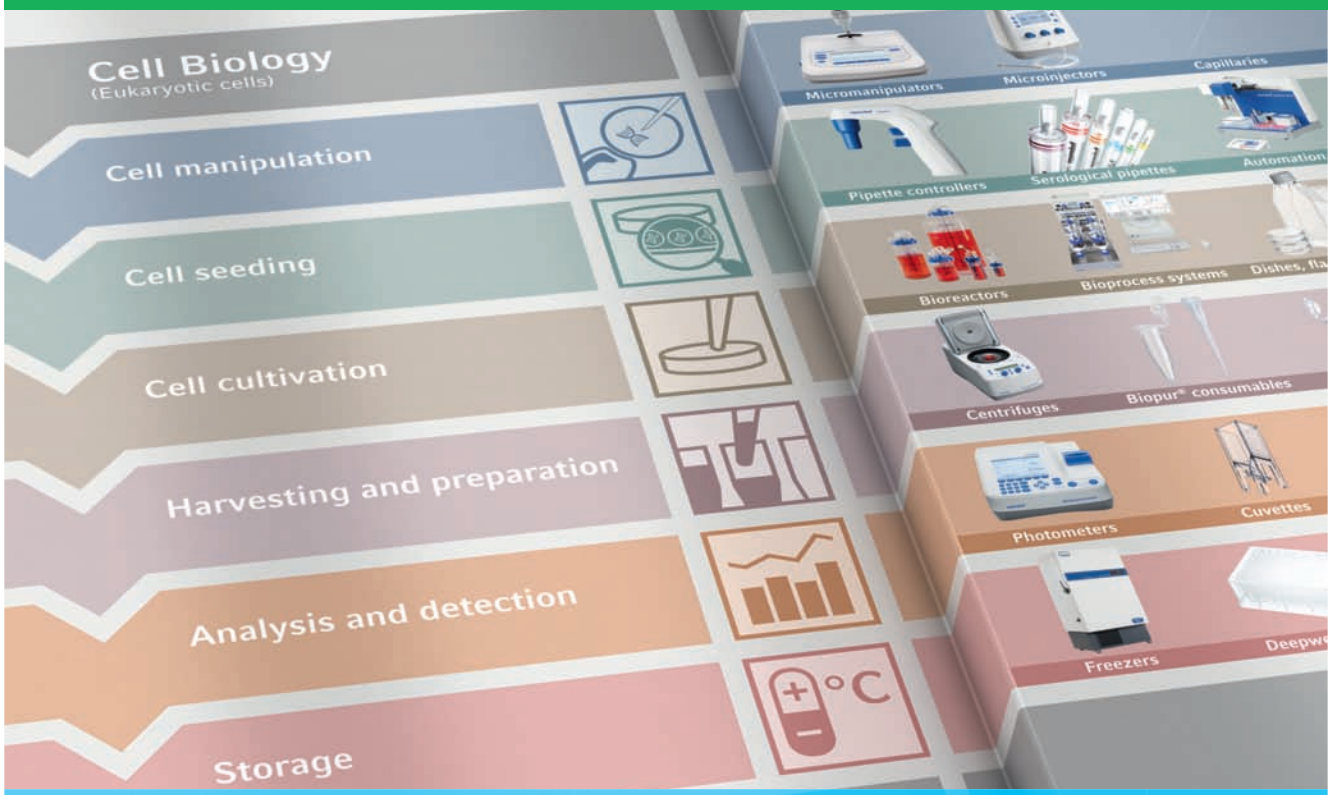

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tralized, online database for SNP-based cell line authentication. **Table II** compares and contrasts the pros and cons of STR and SNP profiling assays.

Despite the widespread availability of the STR standard and its low cost, there is little evidence that authentication is routinely used in the life sciences—particularly among academic researchers (23). One widely cited survey reported that only one-third of laboratories tested their cell lines for identity (24). A *Nature Cell Biology* editorial reported that only 19% of papers using cell lines published in the latter months of 2013 conducted (or at least reported conducting) cell-line authentication (25). Although the International Cell Line Authentication Committee (ICLAC) online database of Cross-contaminated or Misidentified Cell Lines is widely considered to be the "go-to" reference in the field (26), less than half of the respondents from a 2014 Sigma-Aldrich survey were familiar with the database, and only 11% searched the database during 2013 (27).

Many scientists remain unaware or unconvinced of the need to carefully establish and maintain cell cultures, and many do not authenticate their cell lines often enough or at all (8). More worrying is a lack of understanding of how to interpret DNA profiling results. The current *status quo* entails a *de facto* honor system that assumes all scientists use proper cell culture practices and authenticate their cells lines, as well as a pervasive presumption that misidentified or contaminated cells is a problem "for others" or is inconsequential for the final conclusions. This culture persists because most scientific journals—with few exceptions—do not require authentication as a condition of acceptance of research for publication (8). Merely reporting or attesting that cell lines were authenticated or checked against a database of misidentified or cross-contaminated database is not sufficient. To date, compliance levels and the impact of reporting guidelines to improve study reproducibility have been disappointing (28), but multidisciplinary efforts continue to promote transparency, openness, self-correction, and reproducibility in science reporting (29, 30).

Despite the widespread availability of short tandem repeat profiling and its low cost, there is little evidence that authentication is routinely used in the life sciences.

Although expanding the commercial availability of inexpensive assays and fee-for-service providers will help make authentication more universal, a systematic approach with commitment by all key stakeholders that embraces the importance of targeted training and education is needed. At present, there is little or no standardized training on cell-culture best practices and authentication in basic biological research groups, although these do exist in GLP and GMP labs.

IMPROVING AWARENESS AND TRAINING

To effect meaningful change, enhancing the reproducibility and translation of biomedical research using best practices for cultured cell lines and authentication must build upon ongoing multi-stakeholder efforts to raise awareness of the issues and solutions (6). The Global Biological Standards Institute (GBSI) #authenticate campaign (www.gbsi.org/authenticate) facilitates this kind of engagement (31). NIH's proposed Principles and Guidelines for Reporting Preclinical Research, which were developed and are endorsed by many journals and research societies, recommend establishing best practice guidelines for cell lines, such as the need to authenticate cell lines, report the source of the cell lines, and communicate their mycoplasma contamination status (32). More recently, NIH announced clarifications to their expectations from the scientific community regarding the rigor of research proposed in grant applications, as well as additions to the review criteria used to evaluate proposals; these clarifications were developed in an attempt to enhance reproducibility (33). Notably, the latter changes, which will take effect in early 2016, include expectations for authentication of key biological and/or chemical resources, such as cell lines, antibodies, and other biologics.

Additional systematic changes are needed beyond raising awareness, expanding use of reporting guidelines, and revising proposal preparation and review criteria. Development, implementation, and dedicated funding to support

targeted training and education is absolutely essential. Toward that end, in 2014 NIH launched an extramural grant initiative, "Training Modules to Enhance Data Reproducibility (R25)" (34).

Many scientists remain unaware or unconvinced of the need to carefully establish and maintain cell cultures, and many do not authenticate their cell lines often enough or at all.

Understanding existing barriers that prevent implementation of universal cell authentication is central to changing this state of affairs. GBSI conducted an online survey to determine why cell authentication and the STR standard specifically is not used more broadly, the results of which will be shared in 2015. As a central component of a broader educational program to improve the credibility, reproducibility, and translation of the life-science research, GBSI is developing an exportable "active learning" training module to reduce cell line misidentification, mislabeling, and contamination errors.

CONCLUSION

Targeted training, education, and access to reliable and affordable assays are crucial to change the culture of cell authentication. In conjunction with effective policies and expanded use of standards and best practices for cell culturing and authentication, knowledge of why and how often to perform cell authentication will improve; hundreds of millions of dollars in annual research expenditures will be used more efficiently; and the translation of discoveries from bench to clinical trial to bedside diagnostics and therapies will be accelerated. Considering the billions of dollars spent on cell-based research each year, expanded awareness and adoption of authentication protocols through targeted training is a relatively inexpensive way to considerably increase our annual return on biomedical research investment.

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Analytical Method Transfer Conditions Used by Global Biologics Manufacturers

Stephan O. Krause

The author presents the results of a survey of biologics manufacturers to evaluate how these manufacturers transfer analytical methods.

Although guidance can be found in available best practice documents (1–3), limited information exists about how exactly larger biological manufacturers are transferring analytical methods. The author conducted this brief survey of how manufacturers transfer analytical methods as it was expected that a significant variation exists. Specifically, the preferred pre- and post-licensure analytical method transfer (AMT) options for the execution model, sample size, and acceptance criteria were surveyed. A total of eight large pharma/biotech manufacturers, representing the three major regulatory regions of the United States, the European Union (EU), and Asia, provided answers. All eight manufacturers have global operations, multiple commercial biological products, and more than 3000 employees. This survey was intended to evaluate how these manufacturers handle internal versus external AMTs as well as pre- and post-licensure AMTs.

The manufacturers' representatives were contacted directly by the author via a detailed email survey request and instructions. At total of 15 manufacturers were contacted and eight responded with complete answers. Subject matter experts, responsible for AMTs, typically answered the survey questions. Only one email response was received from each of the eight participants.

DESCRIPTION OF AMTs

AMTs may occur at any point in a method and product lifecycle. An AMT transfer may be associated with transfer of the entire manufacturing process during product development, may occur after product licensure, and/or may be a portion of a larger technology transfer process. Or, an AMT may be required to implement the use of a new laboratory for quality-control release and/or stability testing, either within or outside the company (e.g., contract laboratory). Using Parenteral Drug Association (PDA) *Technical Report (TR) 57* as the reference point for AMT models, execution matrices, sample size calculations, and acceptance criteria, the following options and conditions were provided so that each manufacturer's information could be part of the evaluation (1). Most of the surveyed manufacturers follow *TR 57* for AMT execution matrices and sample-size calculations. The strategy used for AMTs can vary, and several possible options, per PDA *TR 57*, are described as follows:

A. Co-validation—Sending and receiving laboratories participate in the analytical method validation study execution. This may be used early in the lifecycle of a test method when appropriate.
B. Comparative study—AMT study performed concurrently by sending and receiving laboratories. Acceptance criteria determine the equivalence of the two laboratories. Historical and validation data may be used when appropriate for parts of the method transfer study. The sending laboratory typically has collected a significant amount of historical data for test method performance results in addition to test results for the samples to be tested at the receiving laboratory. Acceptance criteria are typically set based on relevant

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Table I: Which typical AMT model do you use (A, B, C, or D)? DS is drug substance. DP is drug product. AMT is analytical method transfer.

	Clinical trial DS/DP		Commercial DS/DP			
Survey participant number: 1–8	Internal AMT	To/from CMO AMT	Internal AMT	To/from CMO AMT	To in-country release lab AMT	Comments
1	All (A–D) are allowed	All (A–D) are allowed	All (A–D) are allowed	All (A–D) are allowed	All (A–D) are allowed	AMT model used depends on receiving units (RU) capability and experience, whether method has been previously validated, number of RUs and other project requirements.
2	B	B	B and C	B	B	Most AMTs use B. Some individual co-validation (Model C) AMTs have been used.
3	A and B	B	A and B	B	B	
4	A and B	A and B	A and B	A and B	A and B	A and B are most common approaches.
5	B	B	B	B	B	
6	A or B	B or C	A or B	A or B	B	
7	A, B, C	C, D	A, B, C and D	C, D	C, D	All transfer models are practiced as applicable.
8	B	B	B	B	B	

product or material specifications with consideration of the previous validation/qualification studies and/or recent routine quality control (QC) testing data. See the following B-1 and B-2 options:

B-1. Fixed AMT execution matrix—The fixed AMT execution matrix does not integrate known test method result variation and has therefore an identical set of comparative data generated between both laboratories for each method transfer executed. A fixed execution matrix can be more advantageous when transferring multiple products to/from multiple locations.

B-2. Variable execution matrix—The variable execution matrix does consider test method result variation and may require a larger data comparison set for highly variable test methods. A variable execution matrix may be advantageous when transferring bioassays with a relatively high degree of test-result variation.

C. Performance verification—The receiving laboratory may already perform the method for a similar product or for another type of sample for the same product. In this case, a formal method transfer may not be required. Any reduced prospective study considered should be properly justified.

D. Waiver—The receiving laboratory may already perform the method for a similar product or for another type of sample for the same product. In this case, a transfer may be waived. Any waived study should be properly justified using available data.

Besides AMT options A–D, PDA *TR 57* provides recommendations to use an intermediate precision-type AMT execution matrix and detailed instructions for risk-based acceptance criteria. In addition, for the option of using a variable execution matrix, sample-size calculations and a detailed AMT example are given. Although the AMT process is conceptually similar for pre- and post-validation transfers as well as for internal vs. external transfers, PDA *TR 57*, as well as other available guidance by the International Society for Pharmaceutical Engineering (ISPE) and the US Pharmacopeial

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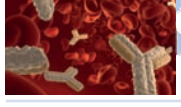

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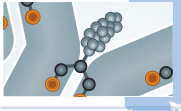

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Table II: Which typical execution matrix do you use when using model B (B-1 or B-2)? DS is drug substance. DP is drug product. AMT is analytical method transfer.

Survey participant number: 1–8	Clinical trial DS/DP		Commercial DS/DP			Comments
	Internal AMT	To/from CMO AMT	Internal AMT	To/from CMO AMT	To in-country release lab AMT	
1	B-1	B-1	B-1 and B-2	B-1 and B-2	B-1 and B-2	Procedure allows for use variable execution matrix.
2	B-1	B-1	B-1	B-1	B-1	Bioassay can use B1 (6, 18 for pre-, post-validation, respectively) or B2 (variable).
3	B-1	B-1	B-1 and B-2	B-1 and B-2	B-1 and B-2	
4	B-1	B-1	B-2	B-2	B-2	
5	B-2	B-2	B-2	B-2	B-2	
6	B-1	B-1	B-1	B-1	B-1	
7	B-1 and B-2	B-1 and B-2	B-1 and B-2	B-1 and B-2	B-1 and B-2	B-1 for all AMTs, B-2 for bioassay and ELISA methods. The number of replicates is based on method complexity and variation.
8	B-1 and B-2	B-1 and B-2	B-1 and B-2	B-1 and B-2	B-1 and B-2	Fixed AMT sample size for a particular method but variable AMT sample size among different methods

Convention (USP), is more focused on post-validation AMT.

SURVEY QUESTIONS

Because limited specific guidance exists for pre-validation AMTs, and they may not be visible to the chemistry, manufacturing, and controls (CMC) assessors, the survey questions were posed to manufacturers to understand the variation among manufacturers frequently transferring analytical methods. A total of five possible AMT cases that include pre- and post-validation transfer stages and possible sending units (SU) and receiving units (RU) were evaluated for the preferred AMT model use. To ensure that the data can be fully evaluated and compared, the questions were also provided in tabular format. Survey results for the four questions are summarized in **Tables I–V**.

The questions were as follows:

1. Which different model, sample size(s), and/or acceptance criteria do you use for:
   a. Clinical vs. commercial AMT studies?
   b. Internal vs. external (to/from contract manufacturing organization [CMO]) AMT studies?
   c. To/from CMO vs. in-country batch release lab AMT studies? (In-country batch release lab = non-US regulatory-approved labs to release commercial product into their respective countries/regions).
2. What are the total number of samples/replicates tested by both labs, SU and RU? Are you using a fixed AMT execution matrix (e.g., n=6, n=12, n=18, n=24, or n=36 at each site, SU and RU) or a variable execution matrix (calculated sample size which considers test method variation?
3. Was an intermediate precision-type comparison used (at least two critical variation factors selected)?
4. What is the acceptance criterion for RU used: fixed or risk based (e.g., fixed: ≤ 1.0 SD difference between SU and RU; risk based: calculated primarily by considering specification and process capability and maximum acceptable result drift from SU to RU and result variation at RU)?

Results for preferred use of AMT model(s) were somewhat surprising in that two of the eight manufacturers make use of all or most of the available models regardless of the stage of product development. Six of the

Table III: Which typical sample size(s) do you use (6, 12, 18, 24…)? DS is drug substance. DP is drug product. AMT is analytical method transfer.

Survey participant: 1–8	Clinical trial DS/DP		Commercial DS/DP			Comments
	Internal AMT	To/from CMO AMT	Internal AMT	To/from CMO AMT	To in-country release lab AMT	
1	Phase-appropriate AMT design	Phase-appropriate AMT design	Minimum of n= 6	Minimum of n=6	Minimum of n=6	
2	n=6	n=6	n=18	n=18	n=18	Bioassay can use B1 (n=18) or B2 (variable n=6-18+).
3	A: n=24 B: n=12	B: n=12	A: n=24 B: n=12	B: n=12	B: n=12	For model A, n=24 is used. For model B, n=12 is used.
4	n=6	n=6	n=24	n=24	n=24	Typically, n=6 for clinical and n=24 (4x6) for commercial are used but n can also be variable and risk based.
5	n=12	n=12	n=12-24	n = 12-24	n = 12-24	A fixed sample size of n=12 is used for clinical DS/DP. For commercial DS/DP AMT, sample size is calculated based on analytical and process capability.
6	n=6	n=6	n=24	n=24	n=24	
7	n=6–12	n=3–6	n=6	n=3–6	n=3–6	
8	n = 12-24	n = 12-24	n = 12-24	n = 12-24	n = 12-24	n is calculated based on method and process capability.

eight limit the model options to only two, with two of the six using only model B for any of the five possible AMT cases. All manufacturers use model B, and this option is by far the most often used model. As the author expected that option B would be the most frequently used option, the question of whether the fixed (B-1) or variable (B-2) comparison models were used was therefore further evaluated, and the results are shown in **Table II**.

Six of the eight manufacturers stated that they use fixed execution matrices for pre-validation AMTs, which offer the advantage that the SU and RU always know how much testing is to be planned and executed regardless of product-type and/or product experience. A lot of the master plan's and AMT protocol's content can thus be readily copied to reduce the master plan and protocol generation time. For post-validation AMTs, this ratio changes in favor of using a variable execution matrix for three manufacturers. This change to a more rigorous AMT planning and execution process can be justified, as the investment of more rigorous AMT process steps is commensurable with the product development stage(s). The biological product is more valuable at the commercialization stage(s) as both successful clinical and product/process validation studies were completed. Overall, the results show significant variation among the eight survey participants with all four cases (only B-1; only B-2; only B-1 and B-2; and B-1 changing to B-2 for post-validation AMTs) represented.

An appropriate sample size can be determined using the risk-based approach outlined in PDA *TR 57*. Based on the sample size n, the study plan should ideally be designed so that at least two independent factors (e.g., analysts and/or days) known to (potentially) impact test method results are investigated during the transfer. Statistical equivalence testing is usually performed to confirm that the transfer study results are acceptable and fall within preset limits.

Results for sample size varied significantly in that the typical AMT sample sizes can range from a minimum of n=3 to as much as n=24. Two of the manufacturers surveyed use n=24 for all post-validation AMTs regardless of whether methods are transferred internally or externally.

Table IV: Which intermediate precision matrix do you use for AMT model *B*? DS is drug substance. DP is drug product. AMT is analytical method transfer.

Survey participant number: 1–8	Clinical trial DS/DP		Commercial DS/DP			Comments
	Internal AMT	To/from CMO AMT	Internal AMT	To/from CMO AMT	To in-country release lab AMT	
1	Phase-appropriate AMT design	Phase-appropriate AMT design	Yes	Yes	Yes	Intermediate precision design is used for quantitative, product-specific methods.
2	Yes	Yes	Yes	Yes	Yes	Intermediate precision design is used.
3	Yes	Yes	Yes	Yes	Yes	
4	Yes	Yes	Yes	Yes	Yes	Direct transfer is also allowed (repeatability study only).
5	Yes	Yes	Yes	Yes	Yes	Minimum of 2 factors are used.
6	Yes	Yes	Yes	Yes	Yes	
7	Yes	Yes	Yes	Yes	Yes	Acceptance criteria are based on sending units intermediate precision and method development history.
8	Yes	Yes	Yes	Yes	Yes	

Most of the manufacturers surveyed tend to increase the sample size for post-validation AMTs, which again can be explained by the increase in "product value" and the level of confidence in the AMT results for late-stage/commercial products.

In an intermediate precision matrix study, at least two independent factors (e.g., analysts and/or days) are deliberately varied to better estimate routine testing conditions. Intermediate precision at the RU could be evaluated from this data set; however, when a more detailed result interpretation is desired at the RU, a more extensive set-up may produce test result variation that is more representative of the variation that typically occurs with routine testing. Only one manufacturer surveyed indicated that it might use repeatability-type precision for pre-validation AMTs. All manufacturers surveyed use at least two independent variation factors for post-validation AMTs.

It is the author's experience that a large data set for the product- and method-specific performance capabilities may not exist prior to method validation.

For post-validation AMTs, more than half of the AMT acceptance criteria are risk based and calculated primarily by considering specifications and process capability and the maximum acceptable result drift (from SU to RU) and result variation at RU. The survey suggests further that some manufacturers integrate more risk-based acceptance criteria for post-validation AMTs.

It is the author's experience that a large data set for the product- and method-specific performance capabilities may not exist prior to method validation. The potentially large level of uncertainty in the required method performance as well as product/process capability in early product development stages can lead to setting fixed acceptance criteria. In late-stage, post-validation stages, ideally, the maximum acceptable differences between laboratories for the method performance characteristics of quantitative methods such as accuracy

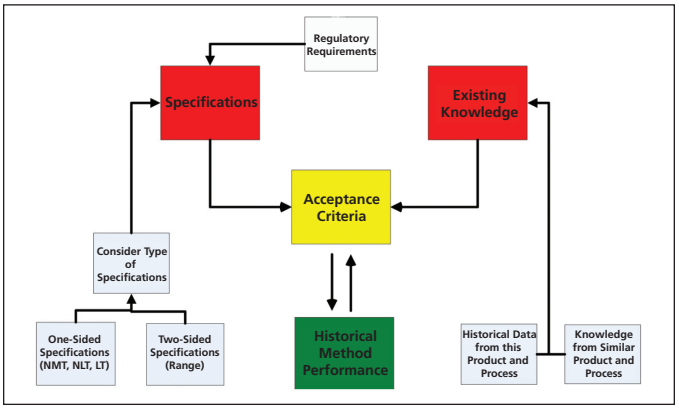
and intermediate precision are estimated based on product-specific historical data with respect to the specifications. Other approaches—such as setting the acceptance criteria based on previous validation/qualification studies and/or recent routine QC testing data with respect to the relevant product or material specifications—may also be used. When using fixed acceptance criteria such as a standard deviation-based limit for the maximum allowed difference between SU and RU, recent SU historical, product-specific data are usually not considered. Each approach has its advantage(s). The risk-based approach in PDA *TR 57* evaluates risks to both patient and manufacturer.

Figure 1 highlights the two primary sources to be considered to set risk-based acceptance criteria for analytical method performance. To be truly risk-based, from a patient and data-continuity perspective, investigators should use the product specifications and existing knowledge of product and process capabilities (1). Typically, in early-stage product development, the specifications and product/process performance can be estimated from historical data of similar products. In late-/commercial-stage product development, more product-specific historical data exist so that product-specific, risk-based acceptance criteria can be set using product-specific specifications and process/product capabilities.

The AMT acceptance criteria should be set to balance patient and manufacturer's expectations. First, if a high level of method capability is desired within the given specifications and expected process capability, setting tight acceptance criteria may be appropriate. Fulfilling the second driver—which involves setting protocol acceptance criteria ranges wide enough to ensure successful AMT completion—may be in direct opposition with the expectations of the patient. Acceptance criteria may be set unsuitably wide to assure that all criteria are readily passed. The method performance may therefore be considered validated, compliant, and acceptable, although the actual method performance may not be suitable with respect to specifications and/or overall process capability expectations. It is therefore important to set risk-based and balanced acceptance criteria intended to satisfy both drivers as much as possible (1).

Uncertainty and test-result variation result in risks to patient and firm. This relationship should be understood and used to set acceptance criteria to ensure the continued suitability for use of the analytical method following an AMT. Simplified, the relationship of the primary variation sources are shown in **Equation 1**. As specifications are for the observed manufacturing process variation, we should therefore control result drifting and variation at RU through risk-based acceptance criteria.

Figure 1: Risk-based AMT protocol acceptance criteria (1).



ALL FIGURES ARE COURTESY OF THE AUTHOR

Table V: Which acceptance criteria do you use (i.e., fixed or risk based)? DS is drug substance. DP is drug product. AMT is analytical method transfer. ACs is acceptance criteria.

Survey participant number: 1–8	Clinical trial DS/DP		Commercial DS/DP			Comments
	Internal AMT	To/from CMO AMT	Internal AMT	To/from CMO AMT	To in-country release lab AMT	
1	Generally fixed	Generally fixed	Both	Both	Both	Procedures allow for use of both. Justification for use of fixed or risk based is required.
2	Fixed	Fixed	Risk based	Risk based	Risk based	
3	Both	Both	Both	Both	Both	Bioassay is only AMT which has risk based ACs.
4	Fixed	Fixed	Risk based	Risk based	Risk based	For clinical, fixed (but based on the number of n used) in the study is most common approach. For commercial, ACs based on specification or tightest release limit, method variability.
5	Risk based	Risk based	Risk based	Risk based	Risk based	Risk-based ACs with respect to specification, known variability of both process and test method.
6	Fixed	Fixed	Fixed	Fixed	Fixed	Fixed acceptance criterion (NMT 1.0 SD) for maximum difference between SU-RU is set from SU performance.
7	Fixed	Fixed	Fixed	Fixed	Fixed	Fixed acceptance criteria are used for most of the AMTs.
8	Risk based	Risk based	Risk based	Risk based	Risk based	

$$[\,\sigma \text{ mfg process observed}\,]^2 = [\,\sigma \text{ analytical method}\,]^2 + [\,\sigma \text{ mfg process actual}\,]^2 \quad \textbf{(Eq. 1)}$$

The demonstration of equivalence in average test results (accuracy and/or matching) and similar precision (intermediate precision) performance between the laboratories is of primary interest in the evaluation of quantitative methods. A comparison of additional validation characteristics (such as quantitation or detection limits) may be considered for particular method types. Statistical tests can be used to demonstrate equivalence between laboratories. Equivalence testing by two one-sided t-tests (TOST) is generally applicable in most cases (1). The TOST results are statistically satisfactory and AMT results pass if the confidence interval for the difference in means between the two laboratories falls within an acceptable interval [-Θ, + Θ]. The interval should define the largest difference that could be accepted between the laboratories while not significantly impacting the suitability of the transfered analytical method (1).

CONCLUSION

In summary, the survey results provide valuable insight into how large biologics manufacturers are transferring analytical methods in different situations. Some significant variation was observed among the eight manufacturers, specifically in the use of AMT models, sample size, and setting of acceptance criteria. Analytical method transfers are becoming more important as biological products transfers are occurring more frequently and increasingly more globally. More practical global guidance could help to harmonize current method transfer practices. This again would ensure that product approval could occur faster which is in the interest of all stakeholders.

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Automated De Novo Identification and Profiling of Disulfide Bonds in Biotherapeutics Including Analysis of Disulfide Bond Scrambling in Monoclonal Antibodies

LIVE WEBCAST: Tuesday, November 3, 2015 at 11am – 12pm EST

Register for free at www.biopharminternational.com/bp/Automated

EVENT OVERVIEW:

This webinar will highlight the latest instrumentation and software used to automatically detect and analyze disulfide bonds in biotherapeutics, including monoclonal antibodies. Application of a new liquid chromatography–matrix-assisted laser desorption/ionization time of flight (TOF)/TOF (LC–MALDI-TOF/TOF) approach, coupled with the latest advances in software, will automatically detect, analyze, and report—in a de novo fashion—the presence of disulfide bonds within a monoclonal antibody. This workflow:

- Is less time consuming; only one LC run is necessary, not two with comparison
- Can detect, analyze, and report disulfide bonds with no previous knowledge of their whereabouts within the biotherapeutics.
- Can detect, analyze, and report disulfide bond scrambling in a fast, efficient and automated fashion.

A case study showing automated detection of native and scrambled disulfide bonds in a monoclonal antibody will be presented.

Who Should Attend:

- Lab Directors, lab managers, and lab personnel involved in antibody and recombinant protein analysis/characterization at pharmaceutical, biotech, and CRO/CMO companies.

Presenter

JASON S. WOOD. PhD
Market Area Manager
Biopharmaceuticals

Moderator

KRISTEN MOORE
Multimedia Producer
BioPharm International

Key Learning Objectives:

- How the latest high resolution MALDI-TOF/TOF systems can be used to analyze disulfide bonds in your biotherapeutics including monoclonal antibodies.
- Learn about implementing the latest workflow for MALDI-TOF/TOF MS for reducing analysis time when analyzing disulfide bonds in therapeutic proteins including easier access to information about scrambled bonds.
- See the latest software for automated, de novo, detection of disulfide bonds in biotherapeutics..

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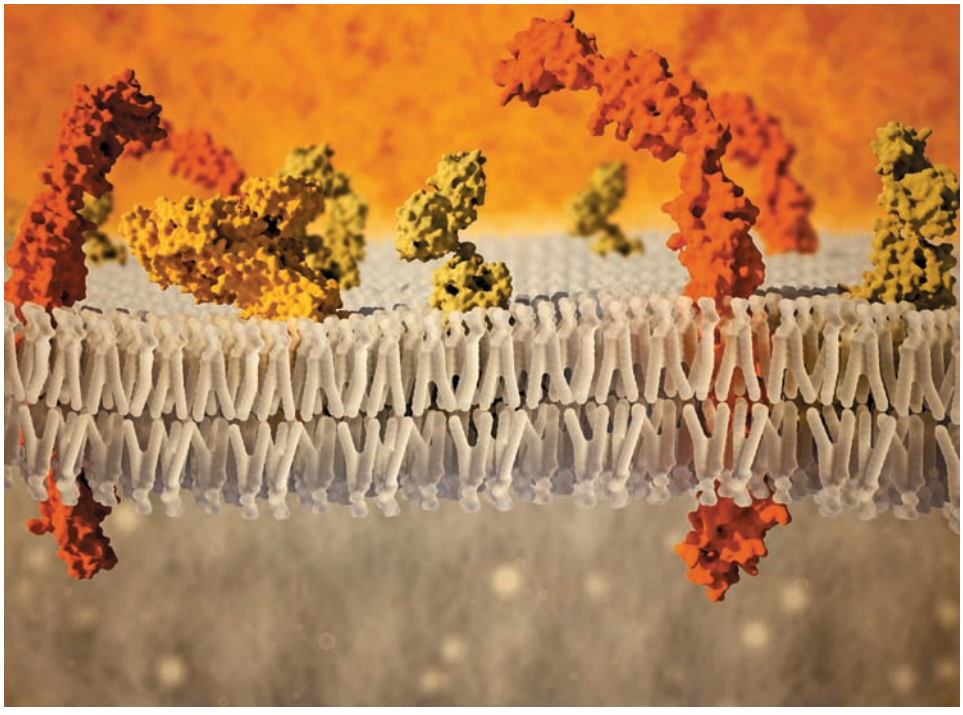
For questions contact Kristen Moore at Kmoore@advanstar.com

A Review of Glycan Analysis Requirements

Jennifer Fournier



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The author explores the basic rationale and requirements for standardized glycan analysis.

More than two-thirds of recombinant biopharmaceutical products on the market are glycoproteins, and every stage of their manufacture is carefully monitored and tested to ensure consistency in quality, safety, and effectiveness (1). Of the various aspects of biopharmaceutical production (such as yield, protein folding, and post-translational modifications), the host cell's biosynthesis of attached oligosaccharides (glycans) is often the most difficult to control. Selected expression systems and even slight changes in process conditions can alter the synthesis of glycans and as a consequence, the physicochemical properties (e.g., serum half-life), safety, efficacy, and immunogenicity of the end product. Regulatory agencies worldwide require state-of-the-art glycan analyses and the demands placed on these methods have steadily increased as better technologies have been developed. Ultimately, robust, information-rich, and reproducible methods for glycan analysis must be included in regulatory filings for glycoprotein-based biotherapeutics to ensure accuracy and consistency. Method simplification and standardization will provide additional assurance that the glycan-analysis methods used are transferrable between testing sites both within and outside (e.g., contract research organizations) of the organization, ensuring better quality and efficiency in manufacturing.

GLYCANS FACE NEW SCRUTINY

By 2008, the biotechnology company Genzyme had developed and marketed

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Table I: Common sample preparation methods in glycan analysis.

Sample preparation		Description	Application
PNGase F	Peptide-N-glycosidase F	Release of N-glycan chain except those with (∝1,3)-linked core fucose	Release of complex, hybrid, and oligomannose N-glycans
PNGase A	Peptide-N-glycosidase A	Release of N-glycan chain containing (∝1,3)-linked core fucose	Release of complex, hybrid, and oligomannose N-glycans
Proteolysis		The use of a protease to generate peptides (including glycopeptides) from a glycoprotein	The peptides are often analyzed to investigate glycosylation sites and occupancy
Alkaline beta elimination/ hydrazinolysis		Chemical cleavage of O-linked glycans from polypeptide chains	Primarily used in the analysis of O-linked glycans
Permethylation		The methylation of oligosaccharide hydroxyl groups to make glycans more amenable to mass spectrometric (MS) analysis	MS-based characterization of glycans including linkage analysis
Amine/ glycosylamine labeling		Modification of glycans to facilitate fluorescence detection	Detection of glycans and glycopeptides when native detection is not available. Increases options for chromatographic separation methods. May also enhance MS analysis

the drug Myozyme (alglucosidase alfa) for the treatment of Pompe disease, a rare and progressively debilitating disorder characterized by deficiency of lysosomal enzyme alpha-glucosidase (GAA). The company was preparing to expand the targeted treatment population from primarily children to adults. Its 160-L production facility was working at capacity, so $53 million was invested to build a 2000-L facility for Myozyme in Allston, MA (2). The company was ready to launch, but FDA rejected Genzyme's application to sell the drug from the 2000-L plant. According to regulators, the version made in the 2000-L tank was no longer the same drug as the one produced in the 160-L tank. FDA argued that the differences in glycosylation—specifically in this case, the composition of mannose-6-phosphate—meant that the drug was no longer the biological equivalent of the original material produced in the 160-L bioreactor, and may in fact introduce unknown clinical variables. Genzyme argued that it had already conducted a clinical trial on the larger batch material, demonstrating safety and effectiveness. Ultimately, Genzyme had to market the product from the larger bioreactor under a different name.

The incident was a watershed moment in the biopharmaceutical industry, marking the emergence of new challenges (1). First, regulatory authorities were beginning to scrutinize the glycan structures of biopharmaceutical products more carefully based on established technical guidelines (e.g., ICH Q5E, ICH Q6B, and FDA's *Guidance for Industry, PAT—A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance*), yet there remained inconsistencies in how FDA, the European Medicines Agency (EMA), and Japanese regulators determined what is "biosimilar". Second, products with complex glycosylation patterns have the potential to easily fall out of specification with changes in biomanufacturing processes and scale-up, so to meet the new regulatory demands, manufacturers had to start carefully characterizing product glycosylation and its relation to the biological and clinical activity of a medication, and begin monitoring relevant glycan characteristics during production (3, 4, 5).

In the years following FDA's decision on Myozyme, the attention given to glycan structure in biopharmaceuticals has only increased, reflecting improvement in analytical technology and a greater understanding of the role these structures play in the physical characteristics, stability, biological activity, and the clinical safety and effectiveness of a drug (6, 7). The technical guidelines for characterizing and monitoring glycans have changed little since 2008; manufacturers refer mainly to International Conference on Harmonization (ICH) documents Q5E and Q6B (3, 4). These documents list the following recommendations on characterizing glycans:

"For glycoproteins, the carbohydrate content (neutral sugars, amino sugars, and sialic acids) is determined. In addition, the structure of the carbohydrate chains, the oligosaccharide pattern (antennary profile), and the glycosylation site(s) of the polypeptide chain is analyzed, to the extent possible."

Other guidelines exist, setting expectations for glycan analysis, such as FDA's *Guidance for Industry, Immunogenicity Assessment for Therapeutic Protein Products*, and EMA's 2007 monograph on the characterization of

Table II: Common separation methods in glycan analysis.

Separation method		Description	Application
HPAEC	High-pH anion-exchange chromatography	Liquid chromatographic separation of negatively charged (acidic) molecules carried out at high pH	Separation, identification, and quantification of glycans and glycopeptides. Analysis of monosaccharide and/or sialic acid composition. Often coupled with pulsed amperometric detection (PAD) for detection of underivatized molecules
HPCE/CZE	High-performance capillary electrophoresis/capillary-zone electrophoresis	Separation of molecules by charge using an electric field in a narrow capillary channel	Separation, identification, and quantification of charged glycans. Analysis and quantification of sialylation
HILIC	Hydrophilic-interaction, high-performance liquid chromatography	A variation of high-performance liquid chromatography (HPLC) that separates molecules using a hydrophilic stationary phase and an organic-yet-water-miscible liquid phase	Separation, identification, and quantification of glycans and glycopeptides
WAX–HPLC	Weak-anion exchange–high-performance liquid chromatography	Separates anionic molecules based on their degree of charge	Separation, identification, and quantification of glycans and glycopeptides
RP–HPLC	Reverse-phase–high-performance liquid chromatography	Separates molecules on the basis of differences in the strength of their interaction with a hydrophobic stationary phase	Separation, identification, and quantification of glycans and glycopeptides

monoclonal antibodies (8). The monograph says the following on glycans:

"Glycan structures should be characterized, and particular attention should be paid to their degree of mannosylation, galactosylation, fucosylation, and sialylation. The distribution of the main glycan structures present (often G0, G1, and G2) should be determined."

These documents, however, present few details on how to set specification limits on glycans, or recommend technologies and procedures for consistent analytical results. The consequences for this long-standing ambiguity are that manufacturers and regulators sometimes end up with different ideas as to what constitutes a necessary specification for a glycan structure. Furthermore, companies submit reports to regulatory authorities with widely different analytical approaches. Procedures may vary even within the same organization, potentially leading to inconsistent results, analytical testing failures, and ultimately, regulatory delays.

QUALITY BY DESIGN VS. QUALITY IN PRACTICE

In 2002, in response to an increasing burden on FDA of regulating product manufacturing, and a perception among companies that regulatory requirements were limiting flexibility in process optimization, FDA implemented changes through its Pharmaceutical cGMP 21st Century Initiative and the release of FDA's process analytical technology guidance (PAT) (5). The new approach placed greater responsibility on the manufacturers to monitor quality control through timely measurements and corrections during processing.

Around the same time, ICH published two guidance documents: ICH Q8 *Pharmaceutical Development* (7), ICH Q9 *Quality Risk Management* (8), and ICH Q10 *Quality Systems Approach to Pharmaceutical cGMP Regulations* (9, 10, 11). These documents helped to further define current scientific and risk-based approaches to pharmaceutical quality control.

The concept of quality by design (QbD) was incorporated into FDA review in 2004, which together with the aforementioned guidelines, emphasized a greater understanding of the product and its manufacturing process, and designing quality control into the process, rather than testing it after the fact (12). This approach is particularly well-suited to glycan analysis, which is typically associated with a complex set of critical quality attributes (CQAs) (such as sialylation, antennary structure, or glycan structure heterogeneity) that are important to the biological or clinical activity of the drug. The CQAs must be identified, measured during process development, and maintained within required parameters (i.e., the design space) during production.

In the case of glycans, the measurement itself may introduce uncertainty and risk, due to a high variability of outcomes when characterizing oligosaccharide chains. An interlaboratory study presenting 11 industrial, regulatory, and academic labs with the same set of four released N-glycans demonstrated that results were not consistent between the laboratories when

Controlling Contamination in Biopharmaceutical Manufacturing

ON-DEMAND WEBCAST (originally aired September 15, 2015)

Register for free at www.biopharminternational.com/bp/contamination

EVENT OVERVIEW:

Contaminants can have serious impact on in a biopharmaceutical manufacturing processes, facilities, drug products, and ultimately, patients. These contaminants can include prions, viruses, mycoplasma, and bacteria, as well as those utilized in production such media additives. Manufacturers must assess the risk of contamination in raw materials, the potential for introduction during the manufacturing process, the implications of a contamination event on drug products and patients, and potential for drug shortages.

In this webcast, experts will review the sources of contamination, regulations and guidance documents on the control of potential contaminants in raw materials, and best practices to guard against the introduction of contaminants in the manufacturing process. The challenges of detecting contaminants, including mycoplasma, assay development, and testing practices will be discussed.

Key Learning Objectives:

- Review sources of contamination in biopharmaceutical raw materials and manufacturing
- Understand regulatory and technical documents that provide guidance for raw materials quality
- Learn about the challenges of detecting mycoplasma

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SGS Life Science Services
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Moderator:

Rita Peters
Editorial Director
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Who Should Attend:

- Regulatory personnel
- Quality control/Quality assurance
- Product development scientists
- Process Development scientists
- Manufacturing engineers

For questions contact Sara Barschdorf at sbarschdorf@advanstar.com

Table III: Common detection methods in glycan analysis.

Detection method		Description	Application
PAD	Pulsed amperometric detection	Permits detection without fouling electrodes	Detection of non-derivatized glycans (most sugars do not absorb UV). Frequently linked to high-pH anion-exchange chromatography (HPAEC)
FD	Fluoresence detection	Selective fluorescent labeling and detection, which may use derivatizing agents	For the analysis of derivatized glycans and glycopeptides when native detection does not offer sufficient sensitivity. Increases options for chromatographic separation methods. May also enhance mass spectrometric (MS) analysis
ESI–MS	Electrospray ionization–mass spectrometry	Mass measurement of gas-phase ionized molecular species, where ions are generated by applying a high voltage to a liquid to create an aerosol, with little fragmentation of molecules. Can be directly integrated with liquid chromatography	Mass mapping of glycans and glycopeptides (including non-derivatized) for identification of sequence, antennary pattern, modifications, and heterogeneity, etc.
MALDI–TOF MS	Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry	Mass measurement of gas-phase ionized molecular species, where ions are generated by embedding molecules in a solid matrix, and releasing them as ions via laser ablation	Mass mapping of glycans and glycopeptides (including non-derivatized) for identification of sequence, antennary pattern, modifications, and heterogeneity, etc.

comparing analyses of sialylation and antennary structure (13). This particular study did not address the potentially added variability caused by sample preparation. The variability in outcomes may be due in part to the availability of numerous analytical approaches and differences between labs as to the selection of approach and limitations of available equipment. Inconsistent levels of training and expertise in glycan analysis may also have had an impact.

BASIC REQUIREMENTS FOR STANDARDIZED PROTOCOLS OF GLYCAN ANALYSIS

The establishment of a robust protocol for glycan analysis can help extract the maximum benefit from QbD practice; give manufacturers greater control over product quality and comparability between batches and process modifications; and ensure consistency and quality in regulatory submissions. Such a protocol should have the following features.

Well-characterized reference standards

A selection of known glycoproteins, glycopeptides, released glycans, and monosaccharides will help calibrate and validate any system of glycoprofiling used in the initial characterization of the product or monitoring of the manufacturing process.

Well-characterized sample standards

Isolated product with a known clinical safety and efficacy profile provides a reference point for comparing glycan structure of batch products under different process conditions and times.

Comprehensive identification of critical glycan attributes

Structural features of glycans have been linked to circulating half-life of the glycoprotein in the blood (sialylation); placental transport (galactosylation); antibody-dependent cell-mediated cytotoxicity (core fucosylation); and a wide range of effector functions, bioavailability, and safety characteristics (14, 15). Critical attributes may include:

- Antennary profile
- Sialylation state, including degree and linkage type (∝2-3 vs. ∝2-6)
- Site-specific glycosylation profiles and occupancy
- Fucosylation
- Galactosylation
- N-acetyl-lactosamine repeats
- High mannose residues composition
- Absence of immunogenic elements such as *N*-glycolylneuraminic acid (Neu5Gc), deacetylated *N*-acetylneuraminic acid (Neu5Ac), and Gal∝(1-3)Gal.

Variations in these CQAs introduced by manufacturing can originate from selection of cell line, bioreactor conditions such as nutrient levels, pH or oxygen content, as well as inadvertent modifications during downstream purification.

Establishment of ranges of acceptable variation in complex glycosylation patterns

Many glycoproteins, particularly those with multiple glycosylation sites, do not exist as a single species, but as a mixture of glycoforms. The natural complexity and heterogeneity of glycan structures can have important functional relevance for a protein, and even minor, low-abundance glycoform species can be crucial. For clinical purposes, each product may have a different tolerance or requirement for glycoform distribution. In particular, clarity on the extent to which low-abundance glycoforms should be identified and monitored is essential.

Adherence to best practices in sample preparation

Selecting the most appropriate method from the wide range of published and commercial sample preparation methods can be daunting. For example, purification of glycans after release from protein may be performed by solvent precipitation, solid-phase extraction, or size-exclusion, hydrophobic-interaction, or hydrophilic-interaction chromatography. Some methods may lead to a non-stoichiometric recovery of oligosaccharides, skewing the results of glycan profiling. Recent developments in sample preparation have allowed for a reduction in preparation times and improved quantitative yields of both high- and low-abundance glycoforms (16).

Selection of glycoanalysis technologies, methods, and strategy

There is a wide array of technologies that can be applied to glycan analysis (see **Tables I–III**). A series of detailed optimal workflows and best practices would help to harmonize analytical procedures between and within organizations that submit regulatory reports. Workflows would cover initial characterization through to routine monitoring and quality control. Considerations should be made with respect to the simplicity and time of analysis, as long as the required levels of accuracy and reproducibility are not compromised.

The use of orthogonal and complementary methods of analysis help compensate for systematic errors in measurement. These methods typically isolate molecules and their fragments based on different physical properties (e.g., high-performance capillary electrophoresis [HPCE] vs. hydrophilic interaction liquid chromatography [HILIC]) or analytical treatment (e.g., electrospray ionization–mass spectrometry [ESI–MS] vs. matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry [MALDI–TOF–MS]), and are compared to compensate for potential bias introduced by each analytical method.

CONCLUSION

Pharmaceutical regulatory agencies worldwide have laid out the general principles of quality control and risk management in biopharmaceutical manufacturing. Of the many CQAs that require consideration, the variation of the N-linked and O-linked glycosylation profiles of biotherapeutic glycoproteins is one of the most complex to assess. Currently, there are numerous methods used to elucidate these structures with varying degrees of accuracy and precision. In addition, the use of these somewhat disparate methodologies makes it not always possible to directly compare results between laboratories. To meet regulatory requirements for consistent process and quality control, it would be beneficial to establish more specific and standardized guidelines for glycan analysis performance with respect to reproducibility, accuracy, and sensitivity for the characterization and routine monitoring of critical glycoforms, including those of low abundance. While such guidelines are within purview of national regulatory bodies and international consensus organizations (such as ICH), no such guidelines have been released to date. The requirements for glycan analysis described in this article could address many of the issues related to process and quality control in glycoprotein manufacturing.

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Applications of Surface Plasmon Resonance for Detection of Bispecific Antibody Activity

Robert Karlsson

ABSTRACT

Biotherapeutic antibodies are still the largest growing class of medicines and are used globally to treat a wide range of diseases such as cancer, asthma, and rheumatoid arthritis. Compared to traditional small-molecule drugs, their structures are complex, and they may assert their effects by binding to more than one target molecule. To date, the majority of monoclonal antibodies (mAbs) have generally targeted a particular antigen; however, there are several additional antibody formats currently in the development pipelines of pharmaceutical and biotech companies. One of these promising groups of antibodies is called bispecifics, where one antibody can simultaneously bind to two targets.

Advanced antibody engineering has resulted in the ability to manufacture new recombinant bispecific antibodies suitable for therapeutic use. FDA has approved a bispecific antibody—Amgen's Blincyto (blinatumomab)—for the treatment of refractory B-cell precursor acute lymphoblastic leukemia (ALL). Blincyto targets cell surface proteins CD19 and CD3 simultaneously, helping to put T cells within reach of the targeted cancer cell with the goal of allowing the T cells to inject toxins into cancer cells, prompting cell death (1). Bispecific antibodies, thus, have the potential to improve cytolytic effects in cancer therapy. Bispecific antibodies are also considered an effective platform for the delivery of therapeutic antibodies across the blood-brain barrier (2). This suggests that in certain cases, bispecific antibodies may have a clinical advantage over monospecific antibodies.

During development and quality control, a range of analytical technologies are used to characterize biotherapeutic drugs, including methods to analyze their structural integrity and activity. Target/ligand binding is an essential critical quality attribute (CQA) that needs to be monitored. The enzyme-linked immunosorbent assay (ELISA) is often used to assess ligand binding;

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PEER-REVIEWED

Article submitted: May 21, 2015.
Article accepted: July 27, 2015

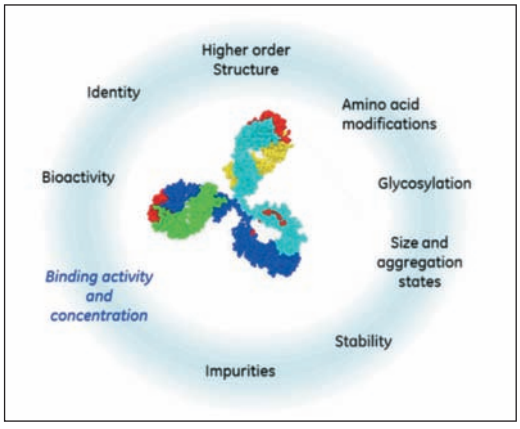
however, it is an indirect method and the output is limited to end-point data. By contrast, label-free technologies such as surface plasmon resonance (SPR) and biolayer interferometry allow users to follow the dynamics of the interactions so that binding and dissociation events can be directly measured. SPR is a direct binding technique that can measure sequential binding events and is a key part of any analytical toolbox, enabling the examination of dual-target specificities in a bispecific antibody within a single assay.

In the early phases of development (3), the focus is on the drug substance and the assumed mechanism of action. Early biotherapeutic development is typically target based (4), and for a therapeutic antibody, the mechanism of action includes target binding, but may also include Fc receptor and complement binding. A lead substance shall elicit the desired functional response of the target molecule, have adequate bioavailability and biodistribution, and is evaluated from a toxicity perspective.

As the lead candidate enters late-stage development, several CQAs have been established (i.e., properties essential for clinical safety and efficacy), including data on how it interacts with target proteins. See **Figure 1** for a sample of the interaction properties that may affect binding activity and concentration of a bispecific antibody.

Important manufacturing CQAs related to protein integrity, homogeneity, presence of host-cell proteins, host-cell DNA, and/or substances released from process or package material, can be identified using risk assessments. Risk can be assessed by comparing test results with previous experience, knowledge, and through the use of control procedures. While CQA analysis is typically performed in a core analytical lab, there are also other in-process controls that allow a manufacturer to steer the process toward a product with known quality. For each step in the manufacturing process, critical process parameters (CPPs) that can affect CQAs are identified (5). Temperature, pH, cell density, and concentrations of nutrients and metabolites are typical CPPs during cell culture, while other CPPs are important during purification. Controlling these parameters can define the quality of the product. Test procedures and acceptance criteria for CQA analysis have been described in regulatory guidelines for biotechnological/biological products (6, 7).

Figure 1: Changes of critical quality attributes, such as high-order structure, amino acid modifications, glycosylation, and stability might impact binding activity and concentration.



Late-stage development starts with set up and validation of analytical methods and with securing reagents (8–10) for the analytical program. A broad range (50–60 variants) of different analytical technologies may be used for CQA analysis (11). Mass spectrometry (MS) can be used to establish the identity of the drug (primary sequence) and for detection of size-distribution profiles linked to post-translational modifications such as glycosylation (12). MS is further used in combination with high-performance liquid chromatography (HPLC) and other chromatographic methods to detect and localize amino acid modifications (13). Chromatography techniques are broadly used for detection and isolation of charge and size variants (14). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting demonstrate protein integrity and in the case of antibodies, the presence of heavy-heavy-light, heavy-light, or light (HHL-, HL- or L-, respectively) chains can easily be detected (15). ELISA and SPR are commonly used for *in-vitro* confirmation of biological activity by measuring interactions with antigens, receptor, Fc-receptors, or other binding proteins (11, 13). Cell- or

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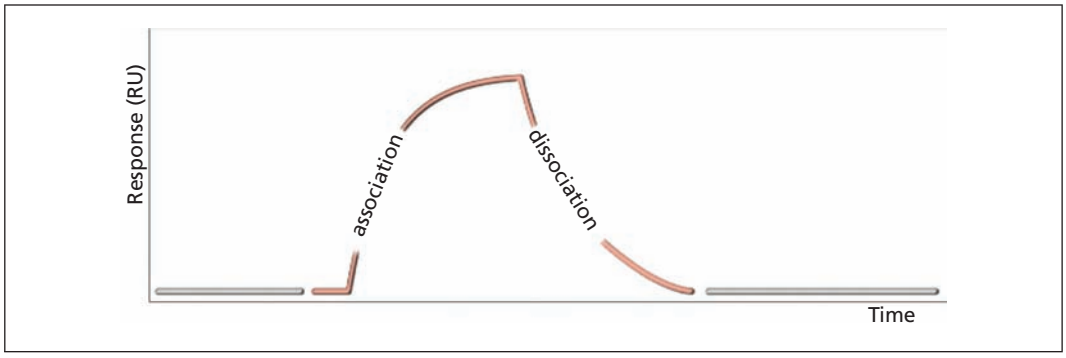
Figure 2: Sample surface plasmon resonance (SPR) readout ("sensorgram") that shows binding (association) and dissociation of an analyte interacting with a surface-bound molecule.



animal-based bioassays can also be used to provide information about the complete function of a biotherapeutic drug.

Process development for chemistry, manufacturing, and controls (CMC) (15) includes selection of a cell line for protein expression, development of the culture process, and purification of the drug substance, while formulation includes selection of excipients for the final drug product. During process development, the goals are to obtain high product yield and a good process economy; to ensure that properties related to initial CQAs are maintained; and to secure that the process itself is well controlled and has low impurity levels. Formulations ensure that the final product has proper stability, administration properties, and pharmacokinetic profile. Throughout all steps of late development, initial CQAs are monitored and possible new CQAs are evaluated. Before transfer to manufacturing, the complete list of CQAs will be established, along with methods for their control. Manufacturing and quality control helps ensure the supply of a consistent quality product to the market. CQAs are determined using the "best" knowledge at a given time; they may later have to be revised based on the results of accumulated clinical data.

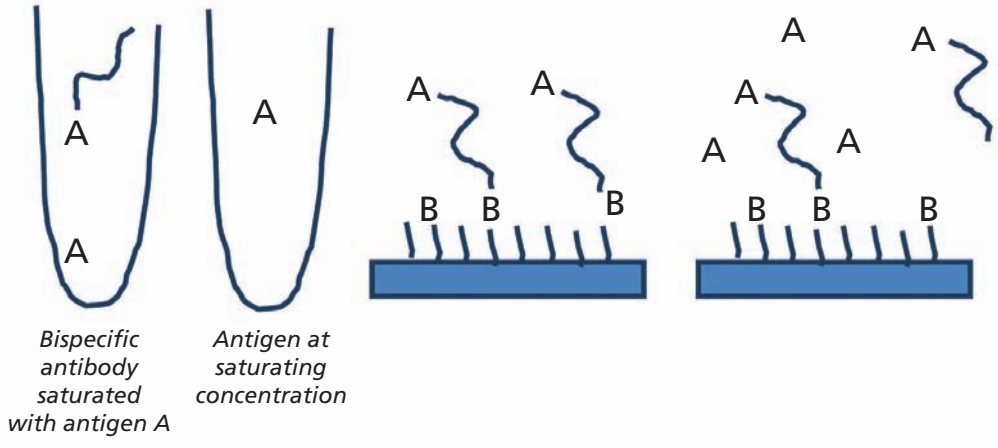
Manufacturing timelines extending over decades present both challenges and opportunities. A major challenge is to maintain a high and consistent product quality, as manufacturing and analytical technologies evolve, and material and reagents become obsolete. The opportunity lies in improvements that simplify processes and make them more reliable and/or more economic. The demonstration of comparability does not necessarily mean that the quality attributes of the pre-change and post-change products are identical, but based on results and existing product knowledge, it should be possible to predict that any differences in quality attributes have no adverse impact on the safety or efficacy of the drug product (16). Manufacturing changes may be frequent and approval times for changed products can be lengthy (17).

In this article, the use of SPR in the characterization of bispecific antibodies—including understanding mode of action; defining specific target binding and overall antibody activity; and control of their therapeutic development—are discussed. The applicability of SPR in validating potential bispecific molecules for quality and in monitoring multiple binding sites is also discussed.

SPR ANALYSIS IS FOCUSED ON LIGAND BINDING

Ligand-binding assays are key for characterization of biotherapeutic medicines. SPR and ELISA are extensively used in ligand-binding assays. SPR analysis has been used for antibody characterization for more than 20 years. The sample readout from an SPR system such as Biacore (GE Healthcare) is related to molecular mass and any binding event can be detected without the use of labels (**Figure 2**). The readout is continuous, which

Figure 3: Assay setup for kinetic analysis of a bispecific construct that has been pre-incubated with antigen A. A = Antigen 1, B = Antigen 2, = Bispecific antibody.



allows for the quality control of the entire binding event and provides opportunities for data analysis based on binding responses obtained at one or several specific time points (report point analysis) or by comparing and even fitting entire binding curves for determination of kinetic and affinity parameters. SPR systems can deliver a direct binding assay focused on relevant interactions and can provide information on binding activities and detect even very weak interactions (18, 19).

Characterization of binding events is essential for confirmation of CQAs, plays a vital role in forced degradation studies, and can either complement or form the basis for potency assays. Binding assays reflect the molecular mechanism of action and can be developed into such potency assays.

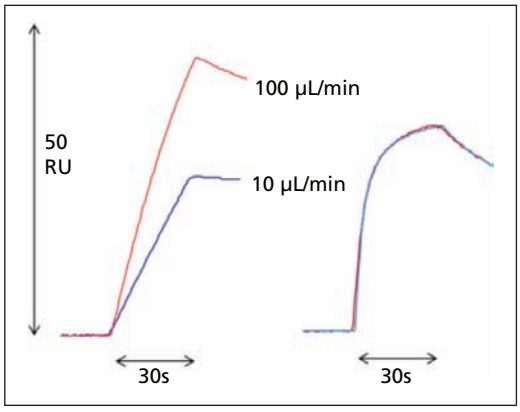
TARGET BINDING

Antibodies, cytokines, and hormones typically interact with their receptors. While cytokines and hormones retain their natural sequence and folding, antibody therapeutics are engineered to interact with target molecules (including antigens, Fc receptors, and complement factors) based on their intended mechanism of action. The focus in this section is on the primary target molecule.

Binding properties can vary considerably with estimated dissociation half-lives from 50 seconds (including interferon 2α to IFNAR [20]) to 12 hours (VEGF to bevacizumab [21]). There are currently several antibody formats in development, with a particular focus on bispecific antibodies, where two distinct target functionalities are combined in one molecule. This provides a single biotherapeutic with the ability to target more than one effector function, improving the chance of overcoming or slowing the progression of the disease. More than 20 bispecific antibodies are currently in clinical trials (22). There are two main categories of bispecifics, immunoglobulin G (IgG)-like bispecific antibodies, and small bispecifics. IgG-like bispecifics have a conserved immunoglobulin-constant domain that demonstrate properties of Fc, while smaller bispecifics often lack Fc functionality.

Small bispecifics can potentially reach more hidden targets, but the lack of Fc functionality may result in shorter half-lives and thus, introduce a new balance between half-life and efficacy. In certain cases, pegylation may be used to increase the half-life of these smaller contructs (23). SPR has been used for successful characterization of a number of different bispecifics, including in the following examples:

Figure 4: Calibration-free concentration analysis (CFCA) is based on initial binding rates. If binding rates are flow-rate-dependent (left pane), then CFCA is possible. When binding rates are flow-rate-independent (right panel), CFCA is not possible. RU=response.



- Bridge T-cell and target-cell receptors (24)
- Bridge Factor IXa and Factor X (FIXa and FX) binding to mimic the natural function of factor FVIII (25)
- Combine VEGF and Ang-2 functionalities to reduce the formation of blood vessels in cancer tissue (26).

Target binding is clearly an essential critical attribute and has to be controlled during development and later in quality control (QC) for batch-to-batch consistency. While release assays traditionally are based on bioassays, ligand-binding assays (21, 27) can be considered when the mechanism(s) of action is/are defined. Although kinetic data are useful for in-process characterization and batch-to-batch comparisons, release assays require that the ligand-binding assays produce a product concentration that reflects the pharmacological activity (potency assay). Release assays are often based on relative comparisons and parallel line/parallel logistic analyses with defined conditions for equivalence (28).

In one example, the dual specificity assay for Roche's VEGF-Ang2 CrossMab (19) was developed into a potency assay (26). VEGF was immobilized to the sensor surface, followed by CrossMab and angiotensin injections. In this way, individual binding events of VEGF-CrossMab and CrossMab-angiotensin were measured, as well as the change in binding of angiotension with increasing concentrations of CrossMab. Two response values related to the individual binding events of VEGF-CrossMab (R1) and CrossMab-angiotensin (R2) were obtained. The R2 value that reflects the entire interaction was plotted versus the logarithm of the concentration of the CrossMab. The assay was tested using CrossMabs with known deviation in concentration values including stressed samples, and was shown to have excellent linearity, precision, and accuracy in the range of 60–140% of the nominal concentration.

In another example, a zybody with five specificities was investigated (23). Multivalent molecules, with additional peptides providing functional binding sites to the C- and N-terminii of both the heavy and light chains, were added to adalimumab, trastuzumab, and cetuximab. These zybodies were shown to have increased tumor inhibition and showed improved efficacy in a tumor xenograft model. When different "modular recognition domains" (MRDs) were fused to different chain positions of trastuzumab and analyzed for their binding efficiency using SPR, it was found that fusion of the C-terminus of the light chain resulted in lower binding than at any other potential fusion position analyzed in the study.

ANALYTICAL CHALLENGES WITH BISPECIFICS

CrossMab and zybody analyses demonstrate that several specificities in one molecule can be measured in sequence. However, the first binding event may result in a biased selection of antibodies that are available for the second interaction, as antibody molecules with impaired activity may not bind or else bind with lower stability. Therefore, it is important to determine the fraction of active molecules available for interactions. Calibration-free concentration analysis can potentially be used to determine the activity of the separate binding sites. This involves the immobilization or capture of the different antigens, followed by calibration-free concentration analysis (CFCA) analysis. Because bispecific molecules are used as analytes, the ratio of CFCA concen-

trations can directly provide information about the integrity of the bispecific molecule.

Another assay set up that may prove useful for analysis of bispecifics is illustrated in **Figure 3**. The bispecific in this example was exposed to two different antigens using a dual-injection protocol. In the first step, the solution from the left tube containing the bispecific antibody saturated with antigen A was injected. Once exposed to surface-bound antigen B, the bispecific antibody bound via the corresponding binding site. Antigen A at high concentration was then injected from the second tube, and dissociation of the antibody from antigen B was monitored in the presence of excess antigen A. This ensures that the observed dissociation is related to the antibody's interaction with antigen B. This type of experiment can be used to understand if and how the binding of one antigen affects the binding of a second antigen.

Fc-RECEPTOR BINDING

Fc-γ receptors are expressed on different cell types and can be activating (FcγRI, FcγRIIa and FcγRIIIa), inhibitory (FcγRIIb), or without effect (FcγRIIIb) in antibody-dependent cellular cytotoxicity (ADCC). The mechanism of action for several anti-cancer antibodies involves ADCC, where the interaction with the FcγRIIIa receptor present on natural killer cells may be of particular importance. SPR analysis is widely used in research where antibodies are either designed for improved interaction with Fc-γ receptors (29) or for the elimination of immune effector functions when non-immunostimulatory mAbs are developed (30).

Bispecifics with combined target and Fc receptor functionalities to improve effector functions include HER2/neu antibodies that were combined with F(ab') s directed to Fcγ receptors (31) or with FcαRI to trigger an F(ab') directed towards the IgA Fc receptor CD89 (32).

CALIBRATION-FREE CONCENTRATION ANALYSIS FOR RAPID, ABSOLUTE, AND RELATIVE CONCENTRATION MEASUREMENTS

Concentration analysis based on ligand binding typically requires a standard preparation with known active concentration. A standard preparation may not always be available, however, as is the case when a protein is expressed for the first time or when concentration data for the standard reflects the total protein concentration and not the active concentration. In such circumstances, SPR analysis can be used for the direct assessment of the active concentration. This technique was first described in 1993, but has been further refined with modern numerical integration tools for data analysis (33, 34). The CFCA method illustrated in **Figure 4** provides a good estimate of the absolute binding concentration, provided that the diffusion coefficient of the analyte is known and the observed binding rate is flow-rate-dependent. There are small remaining uncertainties related to the conversion of the SPR signal to surface concentration units. All of these uncertainties will cancel out when CFCA is considered as a relative concentration method rather than an absolute concentration method.

Using CFCA as a relative concentration method is an excellent tool for comparing concentration data, and may be particularly useful for reagent characterization and as a complement to kinetic analysis. This is especially true when changes in the interaction can be related to changes in the active concentration. The use of CFCA for analyzing chromatography fractions and to guide purification efforts have been described (35). Considering that relative concentration data are easy to obtain with CFCA, it may also be used to complement and support potency assays.

SUMMARY

The lifespan of an approved, branded biotherapeutic agent can reach more than 20–30 years. During this time, a manufacturer must develop and deliver a product with consistent quality. To support this process, regulatory authorities such as FDA and the European Medicines Agency have issued guidelines aimed at securing safety and efficacy of biotherapeutic medicines. Antibodies, cytokines, and hormones assert their actions through interactions with their target molecules, and ligand-binding assays are highly relevant for the characterization of these drugs.

Label-free technologies such as SPR can be used in cell culture, purification, and formulation workflows for the determination of antibody concentration and for kinetic analysis of drug-target interactions. SPR measures interactions directly and is capable of measuring sequential binding events. This enables the analysis of dual-target specificities for a bispecific antibody in a single assay set up. SPR has already been applied as a potency assay for bispecific antibodies, and the use of CFCA introduces novel opportunities to assess the activity of separate binding sites for bispecific and multispecific antibodies.

Bispecific and multispecific antibodies constitute a growing area of biotherapeutics. Improving the assessment and understanding of multiple binding sites will be important to understanding their mechanisms of action, to determine their optimal configuration, and for quality control in manufacturing. SPR, therefore, has an important role in the ongoing development of bispecific antibodies.

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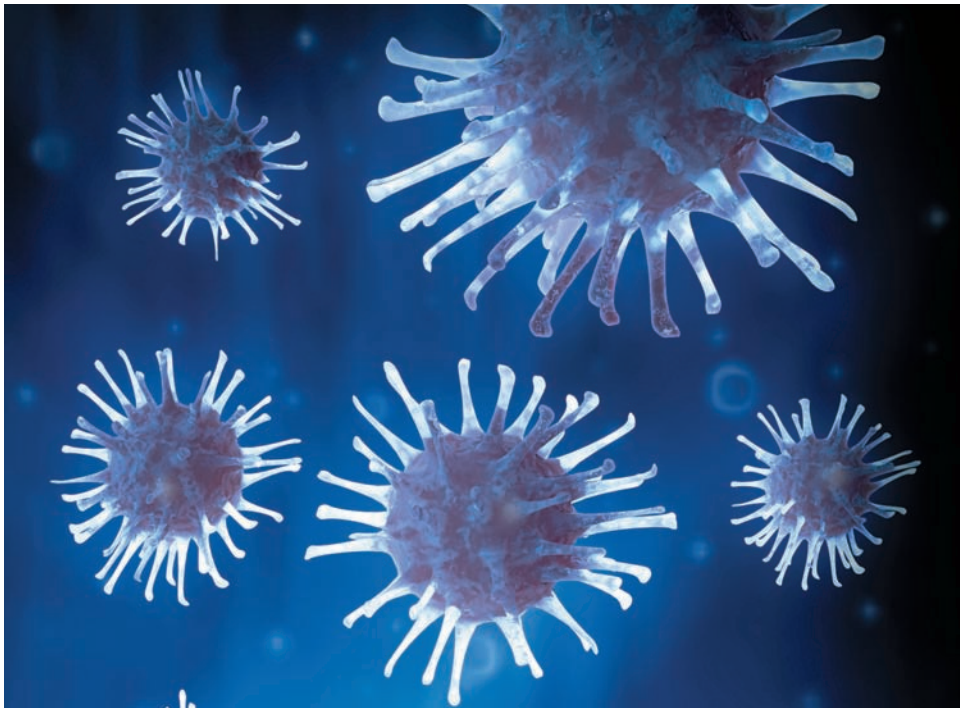
Dynamic Light Scattering for Non-Destructive, Rapid Analysis of Virus-Like Particles

Cynthia A. Challener



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Dynamic light scattering techniques can monitor viruses and virus-like particles in their native state.

Effective analysis of viruses and virus-like particles (VLPs) is crucial for the development of vaccines and drugs. VLPs are designed to mimic the activity of viruses via viral surface proteins, but they lack the infectious genetic material of viruses. Detection of VLPs can be a challenging task, and any detection method must be able to differentiate virus and VLP particles—which range from tens to hundreds of nm—from proteins. In addition, many sample preparation techniques have the potential to impact the structure and properties of virus particles and VLPs.

As a non-invasive method, dynamic light scattering (DLS) offers many advantages over more traditional approaches such as microscopy, which often require manipulations that can alter the particles, according to Sophia Kenrick, an application scientist with Wyatt Technology. "DLS provides the hydrodynamic size of molecules and particles in solution and consequently can measure the size of viruses and VLPs in their native state," she adds.

Cynthia A.Challener, PhD, is a contributing editor to *BioPharm International*.

THE CHALLENGE OF THE NATIVE ENVIRONMENT

Characterizing viruses and virus-like particles in their native environments is difficult, particularly when it comes to identification of crucial traits that are relevant to API development, production, and targeted applications. Particle size and morphology and the interactions that influence their self-assembly behavior, reactivity, and stability must be evaluated in the presence of many other types of molecules, according to Yuanming Zhang, chief application scientist for Brookhaven Instruments.

Determining the structures of virus particles and VLPs includes quantifying the total particle size, heterogeneity, and aggregate content in a final formulation solution, according to Kenrick, and all these aspects can be investigated with DLS. In batch mode (i.e., with an unfractionated sample) DLS provides an average hydrodynamic size for a given sample and some information about the size distribution. The DLS data can also be analyzed to determine if other species are present in solution in addition to the VLPs. DLS reveals the sizes of multiple species in the solution as long as there is approximately a five-fold difference in the hydrodynamic radii of the species. "Larger species may be contaminants or aggregates formed during the purification or formulation of the VLP. Researchers are very concerned about these aggregate species and want to find the formulation that minimizes their appearance and promotes the overall stability of the VLP," Kenrick says.

"Non-invasive and label-free analytical techniques are highly preferable in order to minimize the disturbances/stresses induced on the particles by sample preparation and analysis," observes Zhang. The best methods are also suitable for routine analysis of virus particles and VLPs for quality control (QC) purposes at various stages of the manufacturing process (upstream and downstream) and can be implemented in flexible platforms suitable for diverse field requirements.

> As a non-invasive method, dynamic light scattering (DLS) offers many advantages over more traditional approaches such as microscopy, which often require manipulations that can alter the particle.

NON-DESTRUCTIVE AND FLEXIBLE

The non-invasive nature of DLS enables it to be flexibly implemented either in flow-through mode for in-line characterization or in batch-mode for offline analysis. "By taking advantage of the technological advances in lasers, photon-detectors, and fiber optics, DLS analyses can be performed even with a hand-held device, a versatile measurement format for monitoring manufacturing processes and performing product quality control analyses," says Zhang.

In addition, DLS measurements are rapid—typically requiring less than 30 seconds—which allows researchers to measure the effects of a large number of formulation, processing, and environmental conditions on the size, conformation, and stability of VLPs quickly and efficiently, according to Kenrick. In particular, she notes that with a plate-based DLS instrument, all of these conditions may be arrayed in a 96-, 384-, or 1536-well plate and scanned in a single, automated experiment. The amount of sample needed for a typical DLS analysis is also quite small, 20–50 µL for typical 384-well plates or as low as 1 µL for cuvette-based instruments.

USEFUL INFORMATION

"DLS is appealing as an analytical technique because it covers a very wide range of particle sizes in a single measurement (~0.1 nm–1 µm). In the size range of virus and virus-like particles, DLS is very sensitive and can detect particles at weight fractions in the parts-per-million (ppm) range," Zhang comments.

DLS measures the time-dependent fluctuation in scattered light intensity caused by the Brownian motion of particles in solution, such as virus and virus-like particles. The pattern of fluctuations is quantified using an autocorrelation analysis. This autocorrelation function is then fitted to provide the quantitative information on particle diffusivity (diffusion coefficient).

At a sufficiently low-particle concentration, particles diffuse independently from each other, and each particle's hydrodynamic radius (R_h) can be calculated from its diffusivity using

the Stokes–Einstein equation. At higher particle concentrations, particle-particle interactions (electrostatic, van de Waals, hydrogen bonding, hydrophobic forces, etc.) influence the diffusion behavior of the particles. The sum of these effects can be determined from the concentration dependence of the particle diffusivity and is quantified by the diffusion interaction parameter k_D. A positive value for k_D reflects net repulsive interactions between particles, which are generally favorable for a formulation, while a negative value indicates a net attractive force, which can lead to aggregation.

Batch DLS can also be used to quantify trends as a function of time, temperature, and concentration. Researchers often quantify the thermal stability of proteins and VLPs by measuring the temperature at which they unfold or aggregate, and then try to identify conditions to delay this behavior, according to Kenrick. Through use of DLS, these behaviors can be observed as an overall size change, a change in the polydispersity of the sample, or the appearance of a second large species.

To complement batch analysis, DLS can also be combined with a fractionation technique, such as size-exclusion chromatography or field-flow fractionation, and multi-angle light scattering (SEC–MALS or FFF–MALS) to provide information about the conformation of a VLP. In this situation, the MALS analysis can provide the molar mass and root mean square radius (RMS radius or R_g) of the VLP, and DLS can simultaneously provide the hydrodynamic size. The combination of R_h and R_g can then be used to determine the shape of the VLP (i.e., rod-like, hollow sphere, filled sphere).

MULTIPLE APPLICATIONS

Because virus and VLPs are typically much larger than the rest of the assay ingredients, it is possible through use of DLS to pick up signals arising from very small populations of virus and VLPs among highly concentrated assay ingredients, including buffer chemicals and nutrient proteins, according to Zhang. He notes, however, that the diffusion characteristics of virus and VLPs can be significantly skewed and even completely overshadowed in the presence of a few impurities of larger sizes.

During sample preparation, proper care must be exercised to remove impurities like cells/cell fragments and dust contaminants to perform a valid DLS assay of virus and VLPs. Such sample preparation can typically be readily achieved with centrifugation and filtration tools available in an analytical lab, Zhang explains.

With proper sample preparation, DLS is appropriate for measuring the size, conformation, and thermal and colloidal stability of purified VLPs, and is particularly useful for quality assessment. Some examples include the comparison of multiple lots of a VLP production, understanding changes in size and aggregate content after stress testing, and ensuring a uniform sample distribution prior to analysis with another technique, according to Kenrick. "DLS is most appropriate for this type of analysis because the measurement is fast, non-destructive, and can be multiplexed using a plate-based instrument," she adds.

DLS cannot, however, quantify the sizes of aggregated particles with radii greater than ~5 µm; these large sizes require a different characterization technique. DLS also cannot be used to count particles, although complementary light scattering techniques, such as SEC–MALS and FFF–MALS, can provide particle density values.

ENHANCEMENTS IN DLS

New developments in DLS technology are expanding its applicability. The availability of multiple DLS detection angles on a single measurement platform—forward (~15°) and backscatter (~173°), in addition to the standard 90°—allows practitioners to choose a desired detection angle with the click of a mouse. "Enabling exploration of the angular dependence of scattering from typical Mie scatterers, such as viruses and VLPs, makes it much more convenient to optimize DLS-detection conditions for targeted particles," says Zhang.

Microrheology, in which probe particles of known sizes are used to determine the viscoelastic behavior of liquids containing virus and virus-like particles, is a technique now possible using DLS. "Compared to conventional viscometry and rheometry techniques, microrheology covers a much broader range of dynamic frequencies/shear rates (~10^{-3} to ~10^{7} s^{-1}), and only a very small amount of sample is needed. As a result, microrheology enables rheological studies on biological samples that previously were not possible due to the limited material availability," Zhang observes.

For Kenrick, the adoption of DLS for high-throughput analysis using plate readers is an important development that expands the capabilities of this method for viruses and VLPs. The latest device from Wyatt, for example, can use standard 96-, 384-, or 1536-well plates, does not require any liquid handling during the measurement, and provides no opportunity for cross-contamination once the plate has been

loaded into the detector. With the capability to heat samples to 85 °C, the system also expands the temperature range for which VLP unfolding and aggregation can be evaluated using DLS, according to Kenrick. The ability to take pictures of each well after completion of a DLS analysis also allows users to observe any bubble formation or precipitation that may have occurred during long time-course or high-temperature experiments and eliminate those test results.

ORTHOGONAL METHODS COMPLEMENT DLS

The essential limitation of DLS, or light scattering in general, is that it does not distinguish the chemical nature of the scatterers. In addition, particle-size distribution calculated from the measured particles diffusivity determined using DLS reflects only a scattering intensity-weighted ensemble average over particles in the detection volume of a DLS measurement, according to Zhang. "From time to time, these limitations present challenges in terms of the interpretation of DLS results. Therefore, cross-validation of DLS assays is strongly recommended using analytical techniques that are orthogonal to DLS, such as spectroscopy, microscopy, or chromatography," he comments.

Kenrick agrees that DLS alone is not sufficient to quantify the range of sizes and molecular weights present in a VLP solution. For example, the presence of doublets and triplets will increase the average radius measured by batch DLS, but these aggregates will not be resolved as separate populations. For this type of analysis, a fractionation technique, such as SEC or FFF, is required to separate the different sizes and quantify them individually. This separation process is then coupled with MALS to provide molar mass, root-mean-square radius, and particle-number density, as applicable, in addition to the hydrodynamic size from DLS.

"For these analyses, we recommend that DLS detection occur in the same measurement volume as the MALS detection so that the sample is not diluted by traveling from one detector to another, which can lead to broadening of eluting peaks," Kenrick notes. "Dilution can break up reversible associations, so by avoiding dilution, researchers ensure that the size they obtain using DLS corresponds to the exact species for which they determined the molar mass and size using MALS," she adds. Limiting the dilution effect is also crucial for ensuring an adequate DLS signal during an SEC or FFF separation because DLS is generally less sensitive than MALS. ◆

Contract Testing Laboratories Update

Contract testing laboratories serving the biopharmaceutical industry have reported facility and service upgrades. The following information was reported in company news releases.

SGS Facility Achieves Biosafety Level 2 Compliance

SGS Life Science Services announced on Sept. 9, 2015 that the company's facility in Fairfield, NJ, has been upgraded to be Biosafety Level 2 compliant, according to the Centers for Disease Control and Prevention (CDC) guidelines. SGS reports that the company invested in facilities and training of the laboratory staff to handle virulent organisms for the purposes of microbiological testing, chemical and raw material testing, and stability testing of pharmaceutical materials. The facility can support development programs in vaccines against pathogens such as poliovirus, rotavirus, influenza A, and hepatitis A, B and C, also drugs against bacteria, which are increasingly becoming antibiotic resistant, such as methicillin-resistant *Staphylococcus aureus* (MRSA), NDM-1 *Escherichia coli* and *Clostridium difficile*. SGS reports that it has seen an increase in demand for services related to pathogens covered by the CDC guidelines, such as *Legionella* and *Burkholderia cepacia*.

ABR Launches Bovine Polyomavirus Testing Service

ABR, the services division of VMRD, announced in August 2015 the availability of a testing service that can detect the presence of bovine polyomavirus (BPyV) in animal origin products, master virus seeds, and other sample types. The infectivity assay can detect viable BPyV in three weeks or less, with increased specificity and sensitivity compared with other assays on the market, without using non-infectivity assays such as PCR, according to a company press statement. ABR reports that its BPyV assay employs proprietary testing methods and an antibody based detection strategy that results in sensitivity of 1 virion. The virus can be detected by immunofluorescence, and cytopathic effect. ABR also offers many other virus assays, as well as testing for mycoplasma, bacteria, fungi, and endotoxins.

Market Access in China

Michael J. Kuchenreuther

China's emergence as a significant commercial market is forcing manufacturers to re-evaluate their overall business model.

SHI ZHENG / EYEEM/GETTY IMAGES

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Many pharmaceutical manufacturers consider China as one of the most important strategic markets for future growth. It's the second largest market in the world after the United States. More importantly, China currently accounts for less than 3% of global revenue for most major pharmaceutical companies, leaving significant opportunity for expansion and growth (1). China is more willing to support biomedical research than other countries, and its healthcare system is characterized by expanding coverage and access, as well as a higher prevalence of chronic disease. China's emergence as a significant commercial market coincides with changes in the global landscape that are forcing manufacturers to re-evaluate their overall business model.

Manufacturers, however, have struggled to realize this market's true potential to date, because China's market is highly complex and fragmented. Multiple, conflicting regulations concerning market approval requirements, market access/drug procurement, and pricing have undermined the growth opportunity China presents.

In recent years, the Chinese government has been focused on simultaneously improving the quality of healthcare, relaxing certain regulatory policies, and allowing market forces to drive healthcare and pricing reform. This article presents an overview of some of these recent market dynamics and discusses their potential impact on global pharmaceutical manufacturers, exploring new and existing opportunities for growth in China.

CHINA'S HEALTH AND PHARMACEUTICAL MARKET OVERVIEW

By the turn of the century, Chinese citizens had become increasingly dissatisfied with a healthcare system that

suffered from chronic government underfunding, urban and rural inequalities, and overpriced, low-quality products and services. Much of the population was without access to medical care (2). Spurred by these social and economic challenges, China began planning for healthcare reform. Plans for reform have largely focused on healthcare coverage, access, and pharmaceutical expenditure (**Table I**).

Due in part to these reform efforts, China's healthcare market is seeing strong growth as health spending continues to advance. With rising per capita incomes, China's increasingly affluent consumers are demanding the latest in medical treatment and services (5). At the same time, changing diets and an ageing population are increasing the incidence of cancer, heart, diabetes and other chronic diseases. For instance, according to the World Health Organization (WHO), there are an estimated 3.07 million new cancer cases annually in China—21.8% of the global total (6). WHO also estimates that approximately 230 million and 114 million Chinese currently suffer from cardiovascular disease and diabetes, respectively (7, 8).

Against this backdrop, it's not surprising that the Chinese government expects healthcare spending to surpass $1.3 trillion by the end of this decade (9). The aforementioned market dynamics—the emergence of China's middle class, increased coverage and access, and the chronic disease burden of the country's rapidly aging workforce—clearly create significant opportunities for pharmaceutical and medical device manufacturers. In fact, China, already the world's second largest pharmaceutical market, is projected to reach spending levels of $155–185 billion by 2018 (10).

Table I: China's plans for healthcare reform.

Focus	Impact
Broadening basic healthcare coverage	By 2013, basic insurance programs covered more than 95% of the population (3).
Providing urban and rural populations with equal access to basic public healthcare services	Increased subsidies taken together with a system of price controls on doctor visits, surgery, and other procedures have widened access to basic care for rural and urban populations alike (4).
Improving the quality, accessibility, and regulation of pharmaceuticals	Interventions have sought to cut pharmaceutical prices for consumers, either through government subsidies or regionally fixed drug prices that target common infectious and chronic diseases (4).

In addition to opportunities for increasing product sales, the recent growth and future prospects of China's healthcare market have also attracted infrastructure investment from major multinational pharmaceutical companies. More than half of the top 20 global pharmaceutical firms have built R&D facilities in China and, together with smaller firms, are taking advantage of China's sizeable skilled workforce (4). Manufacturers' interest in making these infrastructure investments is spurred by the country's requirement for at least some domestic clinical testing before drug approval, as well as China's willingness to invest in life and medical sciences research. China is currently the world's second-highest investor in R&D and is poised to overtake the US in R&D spending by 2023 (11).

While pharmaceutical and biotech companies continue to view China as an attractive market, regulatory issues and other hurdles continue to serve as obstacles. China offers poor intellectual property (IP) protection, and the drug application timeline is the longest of all Asian countries. Consequently, innovation continues to stagger in China compared to other parts of the world, and more than 80% of the market is comprised of generic drugs (12). There are also a number of challenges to setting up clinical trials and performing biopharmaceutical development, including language and cultural barriers as well as infrastructure, regulatory, and quality issues.

KEY TRENDS AND DEVELOPMENTS

Regulatory Approval

Gaining regulatory approval has been and continues to represent a significant roadblock for manufacturers trying to bring a new drug to the Chinese market, particularly multinational companies. The China Food and Drug Administration (CFDA) has been conservative, slow, and extremely risk averse, causing the waiting list for approvals to exceed more than 18,500 drugs at the end of 2014 (13). For an imported new drug, it generally takes two to three years or more for approval (14).

At the same time, quality and integrity have come under the microscope in China amid recent allegations of bribery and corruption, as well as a number of reported cases where implementers of clinical trials have manipulated results. Against this backdrop, the CFDA finds itself in a delicate position of looking for ways to expedite drug approval processes while simultaneously upholding quality in the

application process, implementation, and scrutiny of clinical trials.

On one hand, China recently vowed to slash its approval backlog for foreign drugs within the next two to three years (15). China's efforts will reportedly include the increased use of clinical trial waivers under certain conditions, outsourcing some of its approvals, and eliminating the time spent by government officials in dealing with the intermediaries manufacturers often hire to take their products through the approval process (16).

One the other hand, in 2015 the CFDA issued guidance on international multi-center clinical trials that adds new levels of regulation and could significantly lengthen manufacturers' road to market approval. According to the guidance, international multi-center clinical trial data used for the application of drug registration must be derived from two countries, including China (17). Because these regulations are rather new, manufacturers are still trying to figure out how to interpret them and what changes need to be made to address them. At the same time, the impact of these regulations on the overall attractiveness of the Chinese market still remains to be seen.

Drug Pricing

In China, branded pharmaceuticals and generics that earn placement on the National Reimbursement Drug List (NRDL) are partially (10%–90%) or fully reimbursed. Since 2000, China's National Development and Reform Commission (NDRC) has played a key role in pricing drugs on the NRDL by setting price caps based on the manufacturer-reported costs of production. This system, however, gradually created concerns that focusing purely on price would sacrifice drug safety and quality, as well as provide opportunities for manufacturers to inflate costs to obtain a higher price. At the same time, pricing restrictions squeezed certain drugs out of the market as manufacturers stopped production over profitability concerns (18). In the end, this system failed to achieve the intended reduction in drug prices.

Recognizing that a government-controlled pricing system was not working, the NDRC announced that price caps for all drugs aside from anesthetics and grade-one psychiatric medications would be removed (19). While at first glance this new policy may appear to benefit manufacturers, pricing pressures remain.

Drugs that receive regulatory approval in China must be purchased on a provincial procurement platform through a competitive bidding process before they can be used in hospitals. Until recently, bidding systems at the provincial level placed imported branded drugs in a separate group where they didn't compete directly with low-cost local alternatives. Under the new system, these premium products must now compete with Chinese generic drugs. In fact, foreign manufacturers dropped out of approximately 61% of bids in the wealthy eastern province of Zhejiang in 2015 and thus won't be able to sell their products to public hospitals (20).

In addition to facing more stringent drug procurement processes, manufacturers will also need to prepare for greater transparency and scrutiny from the government regarding pricing activities. Specifically, pharmaceutical manufacturers will be required to provide information on drug production and distribution costs. Also, the NDRC will launch a six-month campaign against price-related violations such as excessive pricing, price fraud, and collusion to manipulate market prices (21).

Online Pharmaceutical Sales

Drug sales to hospitals currently make up approximately 75% of the Chinese pharmaceutical market (22). Some of the market access challenges manufacturers may encounter due to more heavily scrutinized procurement and competitive bidding procedures, however, could be partially offset by the government's recent decision to lift bans on online prescription drug sales. The country continues to wait for the CFDA to draw up regulations that are expected to be robust at first, in light of growing concerns over quality, safety, and corruption (e.g., counterfeit drugs). While manufacturers may have to wait some time before the true impact of this policy change on market penetration and pharmaceutical sales can be realized, even opening a slice of the huge prescription drugs market could be a big boost to the industry.

IMPLICATIONS FOR MANUFACTURERS

China's government has verbalized a commitment to making its regulatory landscape for new and innovative pharmaceuticals more stable, predictable, and efficient (23). It has also taken multiple steps to create a more market-driven pharmaceutical industry, particularly with respect to pricing and access. Concurrently, China's economy continues to develop at an unparalleled pace. The country is more focused on genuine innovation than ever before, and its healthcare landscape is characterized by an aging population with greater access to providers and medicines. While manufacturers across the globe cannot discount the aforementioned challenges

and should anticipate policy changes to take some time, there are plenty of reasons why these organizations should be bullish on China's potential for providing future growth and expansion opportunities.

The dynamic nature of global markets continues to apply significant pressure on manufacturers' business models, from product design and drug development through commercialization. China is no exception. To maintain competitiveness, manufacturers are encouraged to develop an accurate and up-to-date understanding of the Chinese regulatory framework and an adaptive market access strategy.

Until there is more clarity around how the CFDA will reduce the length of the drug approval process and until these policy changes are fully implemented, multinational manufacturers looking to launch drugs already marketed in other countries will likely continue to face delays. By expanding their presence in China, either through partnerships with domestic companies or infrastructure investments, manufacturers may be able to reduce the extent of these delays. In fact, leading manufacturers such as Eli Lilly and Novartis are already shifting from late-stage drug development and R&D outsourcing to building facilities that will house more fully integrated R&D capabilities (1).

China's domestic pharmaceutical industry has been dominated by generic-drug manufacturers that have not heavily invested in R&D or manufacturing excellence. Global manufacturers remain well positioned to leverage quality and innovation as competitive advantages. Lilly is among the pharmaceutical companies to have capitalized on this by forging a strategic partnership with a domestic pharmaceutical company to create a platform for Lilly-branded generic medicines (24).

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Small-Scale and At-Scale Model Development and Optimization

An approach to small-model generation and calibrating small-scale models to reliably predict performance at scale is presented.

During development, there is a need to characterize and optimize processes. In vaccines and biologics development, there are often scale and system differences between shaker flasks, 0.5 L, 2 L, 5 L, 10 L, 200 L, and 2000 L environments. Often the difference in scale makes scale up and prediction uniquely challenging. This article presents an approach to small-model generation and how to calibrate the small-scale models to reliably predict performance at scale. This assumes that the small-scale model development has been scientifically and thoughtfully designed to represent as many of the functions and environments that will be present at scale. No amount of secondary model tuning and correction will make up for a failure to select and reasonably range the parameters and build a good model of the process at small scale.

SMALL-SCALE DEVELOPMENT AND STRATEGY

For most product development, characterization, and control strategies, there is a need to develop small-scale and at-scale process models to aid in product knowledge and promote process understanding, prediction, and control.

Advantages of developing small-scale models include:

- Materials use and availability
- Equipment availability
- Cost of development
- Time to development solution
- Ability to generate product and process models at both scales
- Facilities availability
- Efficiently explore control strategies
- Experimental strategy.

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No amount of secondary model tuning and correction will make up for a failure to select and reasonably range the parameters and build a good model of the process at small scale.

Problems with small-scale models include:

- They may not be representative of the product or process at scale.
- They may not accurately represent the mean response at scale.
- They may not accurately represent the variation at scale.
- They may not accurately represent out-of-specification (OOS) and failure rates/modes.

Regulatory guidance documents provide the following comments on process development requirements:

- International Conference on Harmonization (ICH) Q8 *Pharmaceutical Development* (1) states, "an assessment of the ability of the process to reliably produce a product of the intended quality (e.g., the performance of the manufacturing process under different operating conditions, at different scales, or with different equipment) can be provided."

IMAGE: PASIEKA/SCIENCE PHOTO LIBRARY/GETTY.

Figure 1: Whole model for the small-scale purification experiment.

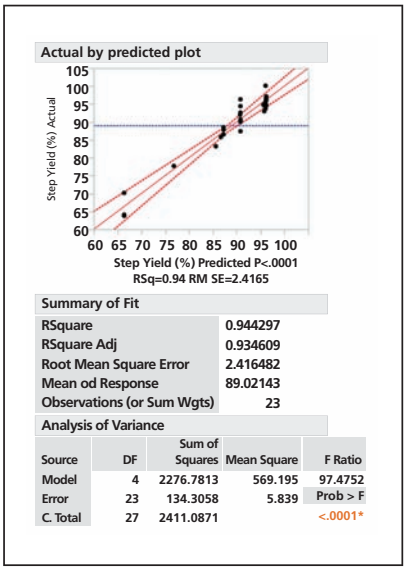


Figure 2: Scaled estimates or half effect of the factors in the small-scale

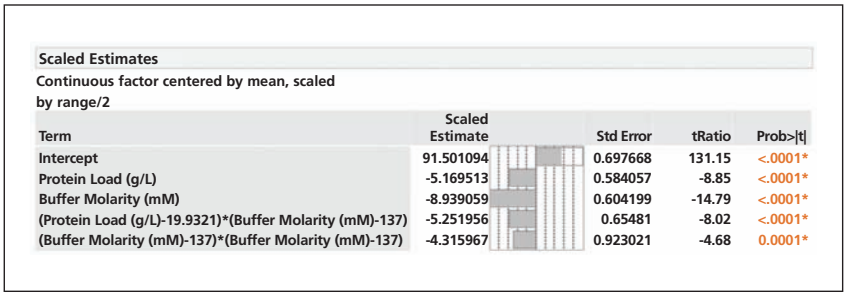


Figure 3: Small-scale model equation for step yield in titer.

245.973340434413

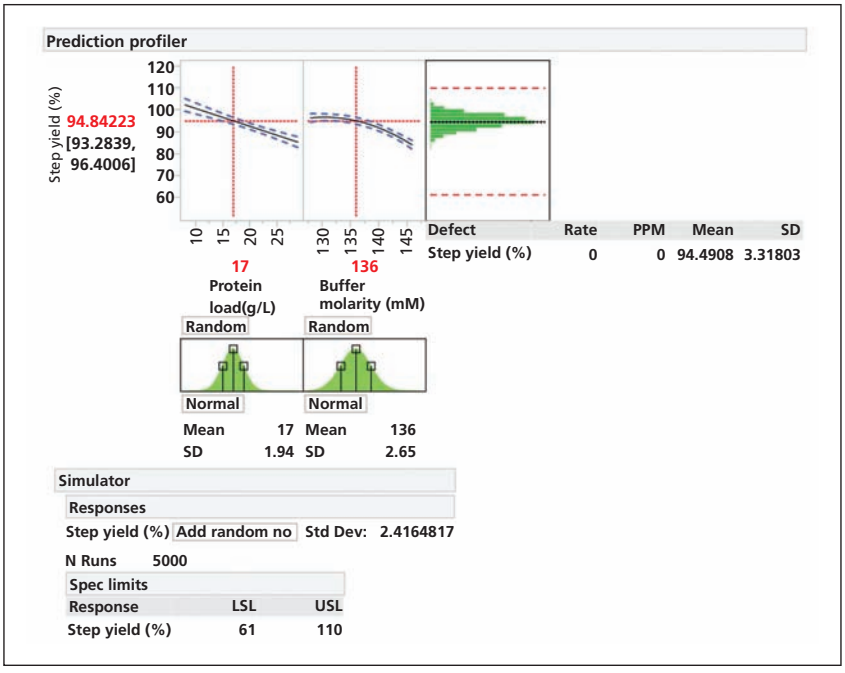
+ -0.9231273167598 * *Protein load (g/L)*

+ -0.9932287691945 * *Buffer molarity (mM)*

+ [*Protein load (g/L)* - 19.9321428571429] * [[*Buffer molarity (mM)* - 137] * -0.1042054835185]

+ [*Buffer molarity (mM)* - 137] * [[*Buffer molarity (mM)* - 137] * -0.053283548078]

Figure 4: Simulation of full-scale results using the small-scale model.



A low-level risk assessment and experimental design should be generated and the process model generated and saved.

- ICH Q11 *Development and Manufacture of Drug Substance* (2) states, "small-scale models can be developed and used to support process development studies. The development of a model should account for scale effects and be representative of the proposed commercial process. A scientifically justified model can enable a prediction of product quality, and can be used to support the extrapolation of operating conditions across multiple scales and equipment."

- ICH Q9 *Quality Risk Management* (3) states the following regarding development and scale up: "To establish appropriate specifications, identify critical process parameters, and establish manufacturing controls (e.g., using information from pharmaceutical development studies regarding the clinical significance of quality attributes and the ability to control them during processing) ... To assess the need for additional studies (e.g., bioequiv-

ALL FIGURES ARE SUPPLIED BY THE AUTHOR.

Figure 5: Small-scale and full-scale results.

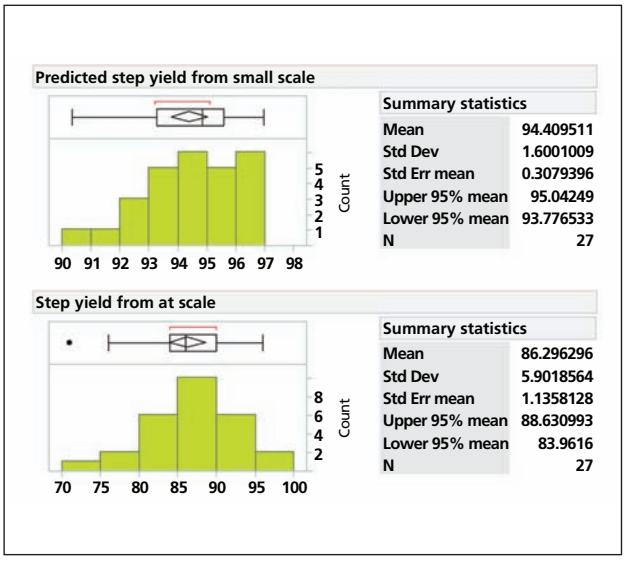
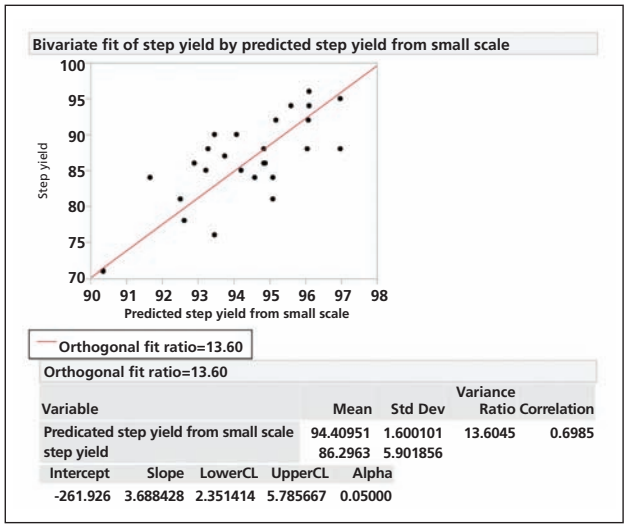


Figure 6: Full-scale and small-scale model calibration using orthogonal regression.



alence, stability) relating to scale up and technology transfer."

- FDA's process validation guidance (4) states, "before any batch from the process is commercially distributed for use by consumers, a manufacturer should have gained a high degree of assurance in the performance of the manufacturing process such that it will consistently produce APIs and drug products meeting those attributes relating to identity, strength, quality, purity, and potency. The assurance should be obtained from objective information and data from laboratory-, pilot-, and/or commercial-scale studies. Information and data should demonstrate that the commercial manufacturing process is capable of consistently producing acceptable quality products within commercial manufacturing conditions."

SMALL-SCALE MODEL DEVELOPMENT AND DESIGN OF EXPERIMENTS

Small-scale experimental models must be first generated. Care needs to be made that operational ranges represent conditions that occur in the full-scale process. For example, fill times may be 20 minutes in a small-scale process; however, in the full-scale process, it may take two hours. Small-scale models should be generated to represent conditions at scale as much as is practicable.

A low-level risk assessment and experimental design should be generated and the process model generated and saved. The following purification process example is presented.

Two significant factors are influencing the step yield: protein load on the column and wash molarity. The model explains more than 94.4% of the variation (R^2) in titer step yield. The following are the steps in generating the small-scale model:

1. Low level risk assessment
2. Design of experiment
3. Model refinement for significant factors (**Figure 1** and **Figure 2**)
4. Save the formula (transfer function) (**Figure 3**)
5. Simulate the process set point at scale (model, variation at set point, noise) (**Figure 4**)
6. Determine the variation in X at scale
7. Add the root mean squared error (RMSE) into the noise for the simulation
8. Simulate process conditions to predict the mean and standard deviation at scale.

GMP OR ENGINEERING PRODUCTION RUNS AT SCALE

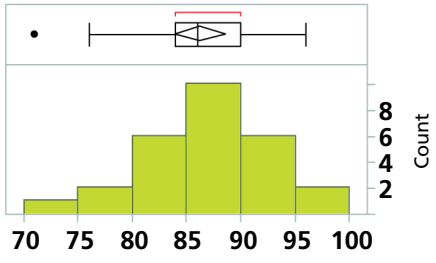
At some point in the development, full-scale GMP or engineering runs will be performed. Care needs to be taken to record all of the run conditions that were present in the small-scale model. Using the equation from the small-scale experiment, plug in the full-scale measures into the small-scale model and compare them to the full-scale results. Notice the means and standard deviations are not well matched (calibrated) to the full-scale results. The difference in means and standard deviations (**Figure 5**) indicates some scale effects, and the small-scale model needs to be calibrated to predict the step yield titer more reliably at scale.

Figure 7: Full-scale calibrated model.

$$-261.92636066547 + 3.68842769808727 * \left[\begin{array}{l} 245.973340434413 \\ + -0.9231273167598 * \textit{Protein load (g/L)} \\ + -0.9932287691945 * \textit{Wash molarity} \\ + [\ \textit{Protein load (g/L)} - 19.9321428571429\ ] * [[\textit{Wash molarity} - \textit{137}] * -0.1042054835185] \\ + [\ \textit{Wash molarity} - \textit{137}\ ] * [[\textit{Wash molarity} - 137] * -0.053283548078] \end{array}\right]$$

Figure 8: Calibrated full-scale model and actual measurement data.

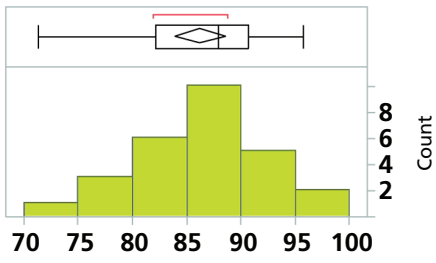
Step yield actual at scale



Quantiles		
100.0%	maximum	96
99.5%		96
97.5%		96
90.0%		94.2
75.0%	quartile	90
50.0%	median	86
25.0%	quartile	84
10.0%		77.6
2.5%		71
0.5%		71
0.0%	minimum	71

Summary statistics	
Mean	86.296296
Std Dev	5.9018564
Std Err mean	1.1358128
Upper 95% mean	88.630993
Lower 95% mean	83.9616
N	27

Calibrated small scale model



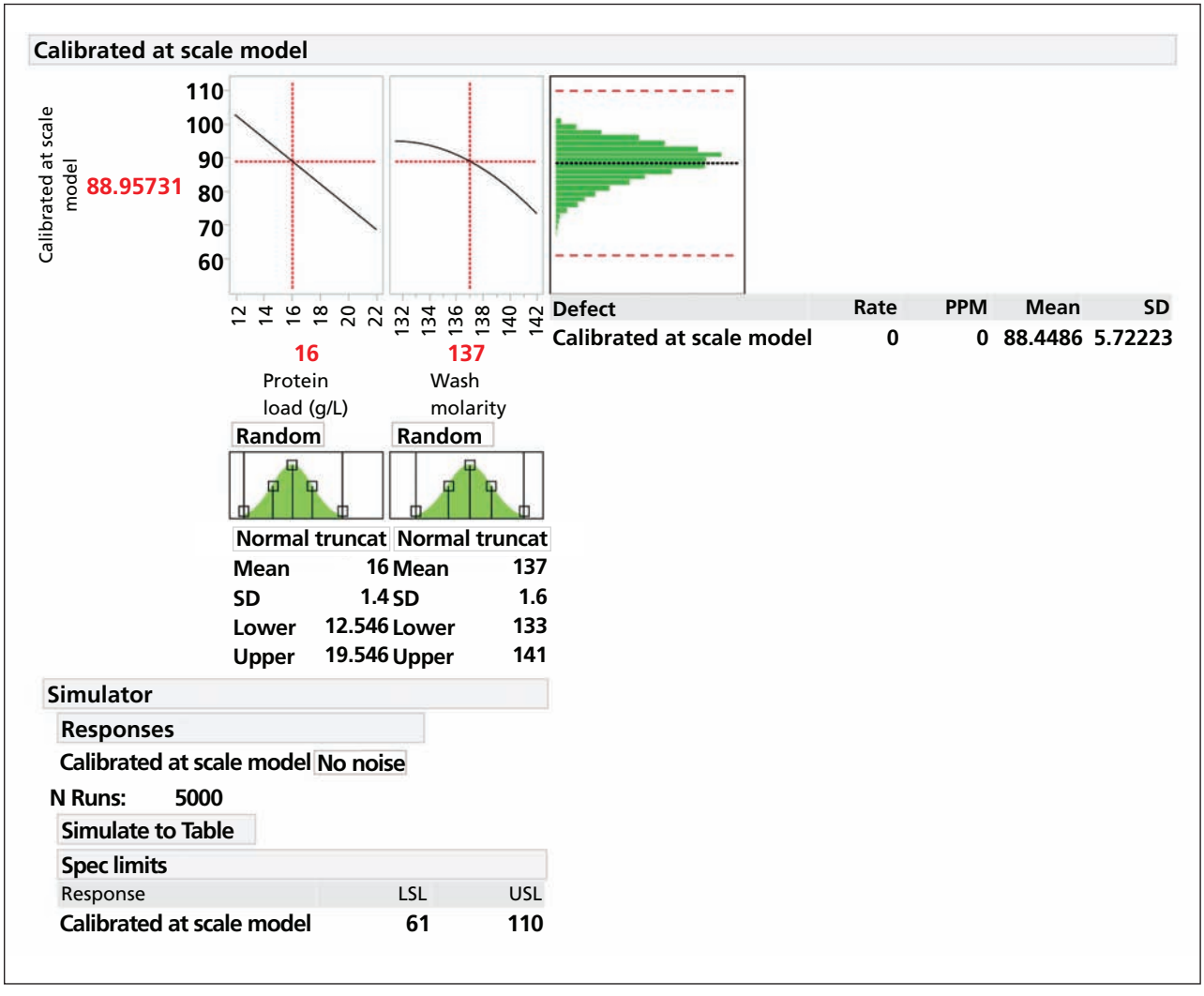
Quantiles		
100.0%	maximum	95.7670912
99.5%		95.7670912
97.5%		95.7670912
90.0%		93.196448599
75.0%	quartile	90.686317151
50.0%	median	87.892364112
25.0%	quartile	82.146635662
10.0%		78.670459395
2.5%		71.346160717
0.5%		71.346160717
0.0%	minimum	71.346160717

Summary statistics	
Mean	86.296296
Std Dev	5.9018564
Std Err mean	1.1358128
Upper 95% mean	88.630993
Lower 95% mean	83.9616
N	27

SMALL-SCALE MODEL CALIBRATION

Generally, regression is used to determine how to calibrate the model. When calibrating the small-scale model to the full scale, the Y response is the full-scale measurements, and the X factor is the predicted small-scale model results (see **Figure 6**). An orthogonal principle components fit was used in fitting the line. The intercept corrects for the mean, and slope corrects for the standard deviation. Linear regression using a least-squares error method of line calculation is used when the correlation is high (70%+R^2); however, when the correlation is poor, an orthogonal method of regression is typically more reliable. If a least-squares line were used in this example, it would not correctly calibrate the small-scale model. Other regression fits are possible to calibrate a small-scale model; however, it is beyond the scope of this paper.

Figure 9: Full-scale simulation.



To correct the small-scale model (**Figure 7**), an intercept of -261.926 and a slope of 3.688 must be applied to the small-scale model. Once this is complete, the results should match the at-scale model (**Figure 8**). In most cases, there is a small-scale to full-scale correction to make the models match both mean and standard deviation.

At scale mean and standard deviation MFG data 86.3, 5.9

Pre-calibration small scale mean and standard deviation 94.4, 1.6

Post-calibration full scale mean and standard deviation 86.3, 5.9

USING THE FULL-SCALE CALIBRATED MODEL

Using the current process settings of 137 and 16, simulations can be run to determine the failure rates and OOS and operational ranges (**Figure 9**). The power of generating a calibrated full-scale model is how the model can be used to reliably predict the influence of variation in the input parameters, set operational ranges, and evaluate the design space (**Figure 10**) and edge of failure (**Figure 11**).

The full-scale simulation is used to determine design margin and to visualize and evaluate failure rates. The design space helps to visualize design margin and process centering. The edge of failure plot helps to predict design margin relative to specifications and acceptance criteria.

FULL-SCALE MODEL AND PROCESS VALIDATION

Once predictions are made from the full-scale model, they can be compared to actual GMP production lots. Typically, a 99% interval is used to see if every

batch is falling within the full-model predictions. If so, it indicates the validity of the full-scale model. Model simulation and scale-up results should be part of the development report and Stage I process validation reports.

Once predictions are made from the full-scale model, they can be compared to actual GMP production lots.

CONCLUSION

Model development at various scale and variation understanding and modeling is a core component of modern drug development. It is a required element of Stage I validation and increases product and process knowledge and reduces risk. Generating reliable small-scale models and calibrating the models to full-scale results are essential steps in generating process understanding and communicating that understanding to development and GMP team members. Using a valid and well-defined process model to simulate, predict, and control drug substance and drug product is core to every drug development team globally.

Learning how to reliably predict performance at multiple scales is an essential skill for all CMC team members. Failure to build reliable process models will likely generate higher variation and is likely to result in OOS measures and lot failures.

Figure 10: Purification design space from full-scale model.

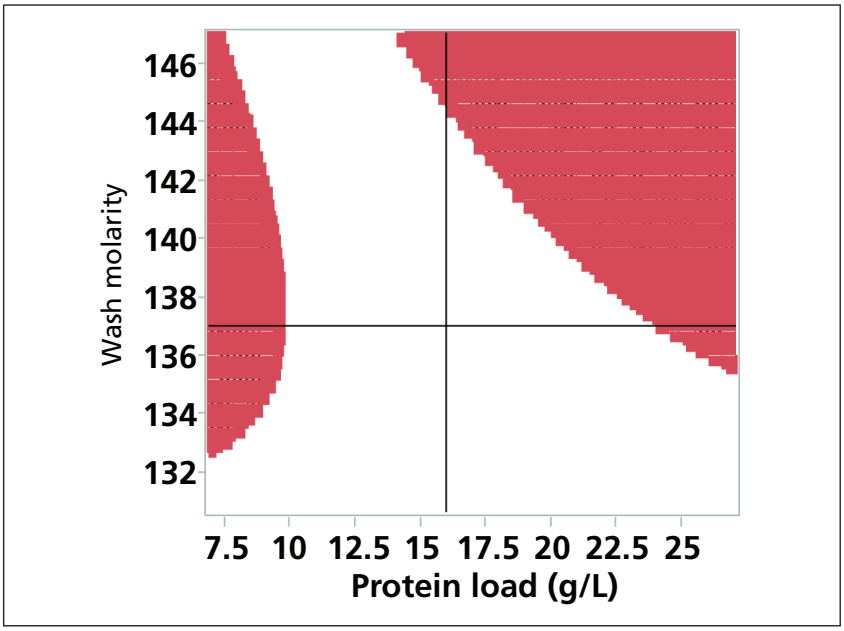
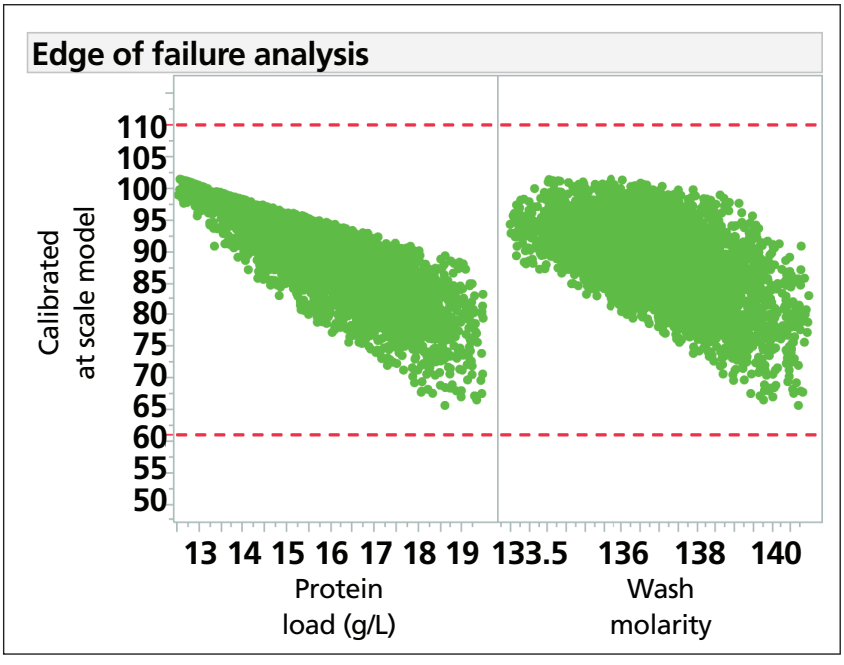


Figure 11: Purification design space from full-scale model.



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A Risk-Based Strategy for Implementing Disposables in a Commercial Manufacturing Process

The author explores a dual-supplier sourcing strategy for single-use products and its ability to mitigate business continuity risk.

Today's single-use technology (SUT) is more than just bioprocess bags and the silicone tubing to connect them. Most companies now use SUT bags for at least one other application, usually storage of small-volume buffers or for in-process sampling. For sample handling in particular, bags provide obvious advantages over traditional sample bottle assemblies that must be steamed in place to obtain an aseptic sample of a cell-culture vessel. However, single use now reaches into many more phases of the modern biologics manufacturing process. Bags are available with internal mixing systems from several suppliers. Disposable filter capsules containing more than 2.3 m^2 of membrane area are available. Aseptic connection devices in a variety of sizes and shapes allow tubing connections in seconds. Single-use formats are available for many sensors, including those that measure pH, conductivity, and dissolved oxygen. Together, these advances in SUT have enabled wide adoption of single-use bioreactors (SUBs) across the industry. Even chromatography columns up to 60 cm in diameter are now available pre-packed and fully disposable.

The author evaluated the potential to reduce operating costs by switching to largely disposable process equipment during the technology transfer of a commercial purification manufacturing process. The design considered replacing most of the steel tanks, filter housings, and transfer lines with SUT. Detailed design planning identified more than 80 SUT components that would be required, including roughly a dozen static bag designs from 1 L to 2000 L, mixing systems from 100 L to 1000 L, and more than 20 separate tubing assemblies, manifolds, and hoses. To support routine operations, more than 500 individual pieces of SUT would be required per batch. With only 20 batches a year, the number of SUT items that need to move annually through procurement, warehousing, release, use, and disposal quickly exceeds 10,000.

Selecting which SUT to use is only the beginning of the implementation process. Implementation of SUT at any stage of a commercial manufacturing process requires bringing the SUT components into GMP systems as a raw material. This means generation of new part numbers, material specification documents, manufacturing procedures, release testing procedures, supply agreements, quality technical agreements, quality audits of the supplier manufacturing sites, and assessment of potential SUT extractables and leachables (E/L). The E/L testing is one of the longer duration pre-requisite activities to GMP implementation. For process development teams supporting post-approval processes, the scope and duration of this work can be easily underestimated. This workload will grow exponentially when considering the technology transfer of processes currently being developed that heavily leverage SUT in multiple steps.

RISK ASSESSMENT

The potential for polymeric components to leach unwanted chemicals into drug products must be evaluated (1, 2). E/L data are used to assess potential risks to patients from the use of SUT in manufacturing processes (3). The scope of the assessment should include everything from small surface area polymers such as valve diaphragms to large surface area materials such as chromatography resins. A process based largely on SUT will

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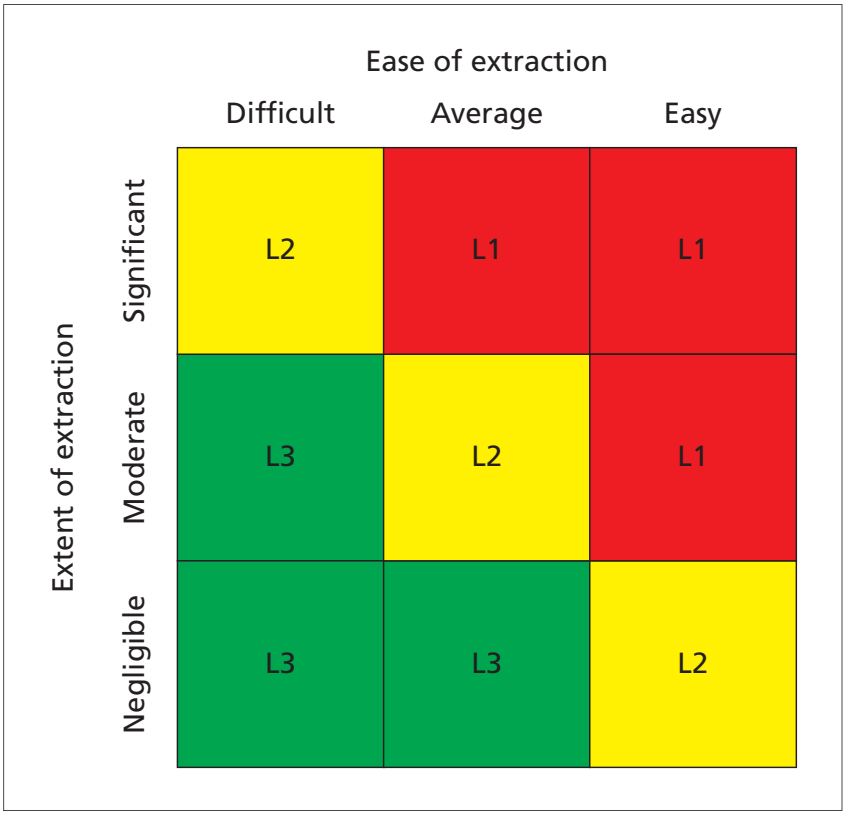
have hundreds of SUT polymeric components to be evaluated and potentially tested for E/L. It is not feasible to expose every process component to every process solution to confirm chemical compatibility in all situations. However, risk assessment tools can be used to provide thorough assessments and prioritize high-risk component solution combinations to result in a reduced E/L study scope.

The risk assessment tool used to design the E/L studies is a three-step process streamlined from the numerical method described by the Biopharmaceutical Process Extractables Core Team in 2002 (4). Quantitative evaluation criteria are first defined. The capability of extraction for each solution and component pair is then determined based on the criteria for both ease and extent of the extraction. Finally, the proximity to final product is determined. These three steps yield a risk action level for each SUT component solution combination. The ratings should be agreed upon by a cross-functional team, including members from the manufacturing users, technical experts, and quality assurance. Definitions and category criteria should be defined prior to making any rankings to ensure a precise, unbiased, data-driven ranking process.

The capability of extraction is determined by rating the ease and extent of the potential extraction. The ease of extraction is rated either as difficult, average, or easy. Use of organic solvents or operating a material at the extremes of the supplier-supported chemical compatibility conditions, for example, can be considered easy to extract, whereas an aqueous solution used within the component's recommended temperature range can be considered difficult to extract. The extent of extraction is rated either as negligible, moderate, or significant. For example, components with small surface area or short exposure duration can be considered to have a negligible extent of extraction; whereas material exposed to steam or extended durations, such as liquid storage bags, can be considered as having a significant extent of extraction. The two ratings are applied to the extraction capability matrix shown in **Figure 1**, to identify an extraction capability level of L1, L2, or L3.

Figure 1: Extraction capability risk-assessment matrix. L1 has a higher capability of extraction than L3. The extraction capability level is applied to the proximity risk-assessment matrix in **Figure 2**.



This "L" rating is applied to the y-axis of the action level matrix shown in **Figure 2**. The x-axis represents the proximity to finished goods and is categorized into three zones. Zone 1 represents materials used in a process step such as the final drug substance filtration or formulation step where there are no clearance steps (e.g., diafiltration step, bind and elute chromatography step) before the finished product. Zone 3 consists of component solution combinations that are substantially upstream in the process, typically separated from the drug substance by two or more clearance steps before the finished product. From this second matrix, the final risk action level is defined. SUT component solution combinations rated as risk action level 3—low risk—would not require additional E/L data to be generated. In all cases of product contact, the materials should be confirmed to meet the minimum United States Pharmacopeial

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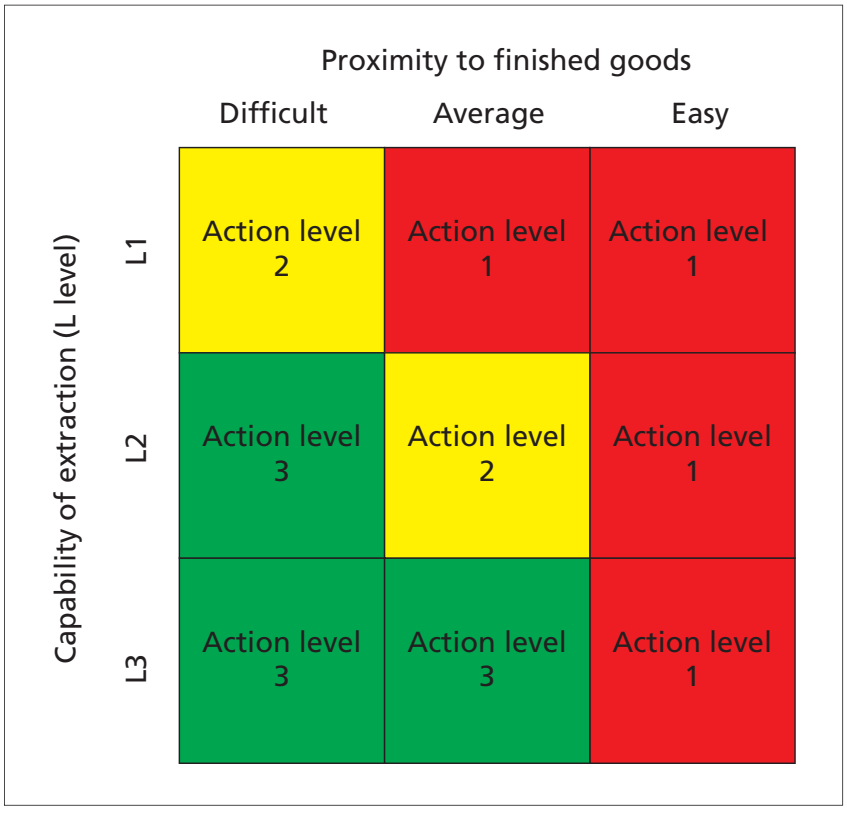
Figure 2: Proximity risk-assessment matrix. Solution component combinations determined to be action level 1, or high risk, require extractable/leachable (E/L) data to be generated. Combinations determined to be action level 3, or low risk, would not need additional E/L data.



Convention (USP) class VI or other compendial expectations or else additional risk mitigation steps may be appropriate. For components rated as risk action level 1—high risk—E/L data must be obtained and assessed. E/L data may not be necessary for materials rated as risk action level 2—medium risk— based on the justifications provided by the cross-functional risk assessment team. Many upstream components can typically be excluded from the E/L studies based on low-risk action levels.

SUPPLIER SELECTION AND DUAL SOURCING AS RISK MITIGATION

A manufacturing process that relies heavily on SUT requires the manufacturer to depend on the SUT suppliers. There are ways to reduce the risks resulting from this dependence. Supplier selections and the quality of your supplier relationships become crucial to ensuring consistent supply of the manufactured drug products. When identifying new SUT components to implement, leverage existing supplier relationships to expedite implementation by eliminating the need for new quality technical agreements (QTAs), supply agreements, or supplier audits. When a new supplier is required, the strength of the supplier's quality system and their reputation in the industry are as important as the technical process needs. Understand the supplier's validation program and lot-release process. Define requirements for endotoxin and sterility assurance. Risk assessment tools can also be used to determine the level of sterility assurance required for each component. The impact of potential bioburden ingress is not the same for all process steps, so not all components require the same level of sterility assurance. This may represent an opportunity to reduce the overall cost of goods (COGs).

High consumable volumes necessitate consideration of both capital investment and operational expenditure costs together over a fixed period before selecting SUT technologies or suppliers. The comparative analysis of capital costs and operational expenses will facilitate important process decisions such as technology and supplier selections. Establish business agreements for ≥3 years to lock in important assumptions that impact COGs at least through the initial phases of commercial production.

As with all raw materials, supply-chain risk can be reduced by qualifying a secondary source for SUT components. Occasionally, technology selection prevents dual sourcing. For example, several currently available disposable mixing systems utilize patented mixing technologies that are not interchangeable and require purchase of the compatible mixing bags from one supplier alone. Many SUT components, however, can be dual sourced.

There are essentially three ways to implement dual sourcing for SUT. The first is true redundancy for 100% of the SUT components, but this presents several challenges. It is simple enough to select two qualified suppliers and approve SUT design drawings with both. However, if the SUT parts are considered critical process raw materials and the fluid handling systems are qualified

as part of the process validation (PV), it may not be feasible to validate equivalence for all SUT components. Each SUT component will require creation of two material specifications, one for each source. Enterprise inventory management systems and quality control material release systems will also have to manage the two source options for each process component. The warehouse may also need a strategy to segregate the two sources and determine when each option should be delivered to the manufacturing floor. In general, it will be necessary to generate twice the number of GMP documents to incorporate all of the duplicate SUT components into GMP systems.

A second option is to identify one supplier as primary and purchase all SUT components from that supplier for normal operations. The secondary supplier would only need to be engaged in the event of a supply-chain interruption or quality problem with the primary supplier. This strategy minimizes the number of items to be incorporated into GMP document systems. The secondary supplier, however, would need to build and maintain the templates and documentation required to produce these materials without receiving a commitment from the process owner for any significant volume purchases. This could lead to long start-up times when the material is requested. The lead time to satisfy a production request when demand is infrequent is a crucial consideration in this case.

The third, recommended option is to make each supplier the primary source for some materials and secondary for others. This option allows both suppliers to be engaged by a constant stream of revenue and dedicate some portion of their manufacturing capacity to supporting a process. E/L testing can be performed on both films to accelerate timelines. This provides an inherent alternative if any SUT components are found to have unacceptable leachable levels late in the schedule, because it is unlikely that both tested source materials will experience the same failure. Approve design drawings for most components with both suppliers for future use. The advantage of this option compared to 100% redundancy is the reduced number of new GMP documents required. Material specifications, QC material release documents, and inventory management systems require only single examples, not one for each supplier. Manufacturing procedures can also be specific to the expected source component and will not need to incorporate provisions for two possible components being used.

As part of the QTAs and business agreements, expectations for customer change notification and demonstration of component equivalency should be considered. An extractables study was performed by the SUT-integrator ASI comparing two platinum-cured silicone-tubing materials with similar specifications. The study found the extraction profiles were similar enough to conclude they were comparable within the variability of the analytical techniques used (5). Such data could allow the two raw materials to be deemed interchangeable so the SUT supplier could use either one without notifying the end user of a change in raw material source. This is a shift away from the traditional quality definition of supplier change notification expectations, but it may become a necessary flexibility as industry use of SUT increases. Questions around dual sourcing are being discussed by industry groups such as the BioPhorum Operations Group (BPOG) and the Bio-Process Systems Alliance (BPSA). SUT suppliers have the same interests in dual sourcing of raw materials as drug substance manufacturers, so expectations for supplier-change notification should be contractually defined to avoid future unwanted surprises.

SUT now exists to support all phases of drug substance manufacturing. Implementation of a commercial GMP manufacturing process that heavily leverages SUT components requires a large scope of work, including legal and business agreements; procurement and supply chain system designs; GMP documentation; and E/L studies. High numbers of SUT components can also mean a significant potential for in-process leachables, but good risk-assessment tools can prioritize the potential risks and reduce the scope of the E/L testing. The SUT-dependent process also means relying heavily on the quality systems of SUT suppliers. Dual sourcing and strong QTAs can mitigate some of that risk.

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1. **Publication Title:** *BioPharm International*
2. **Publication Number:** 1542-166X
3. **Filing Date:** 9/30/15
4. **Issue Frequency:** Monthly
5. **Number of Issues Published Annually:** 12
6. **Annual Subscription Price (if any):** $79.00
7. **Complete Mailing Address of Known Office of Publication:** 131 West First Street, Duluth, St. Louis County, Minnesota 55802-2065

   Contact Person: Rochelle Ballou

   Telephone: 218-740-7005
8. **Complete Mailing Address of Headquarters or General Business Office of Publisher:** 2450 Colorado Avenue, Suite 300, Santa Monica, CA 90404
9. **Full Names and Complete Mailing Addresses of**

   Publisher: Mike Tracey, 485F US Highway 1 S, Suite 210, Iselin, NJ 08830-3009

   Editorial Director: Rita Peters, 485F US Highway 1 S, Suite 210, Iselin, NJ 08830-3009

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15. **Extent and Nature of Circulation**

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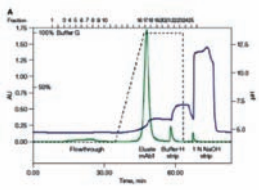

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In the Pipeline

NIH Awards Grants for Genomics Research

Six new grants from the National Institutes of Health (NIH) will support researchers to develop new computational approaches for searching among millions of genomic variants to find those that make a difference in disease susceptibility or in other traits. The awards are for three years each and total approximately $13 million, pending the availability of funds, NIH announced in a Sept. 21, 2015 press release. The awards are administered by the National Human Genome Research Institute (NHGRI) and the National Cancer Institute, both parts of NIH.

Comparing the genomes of many people suggests that there are tens of millions of genetic variants, or DNA spelling differences. For the past decade, scientists have used genome-wide association studies (GWAS) to find regions of the genome associated with diseases and traits. In GWAS, the genomes of thousands of people with and without a disease are compared to find the genomic regions containing variants that affect disease risk. Although GWAS may find hundreds of variants that appear to be associated with a disease, it remains a challenge to find out which variants actually have a role in the disease process, and what that role might be.

Most variants are in genomic regions that do not code for proteins. These variants usually affect the regulation of genes, residing within "switches" in the genome that determine when and where proteins are made.

The researchers are developing computational approaches to combine many different sets of data to identify disease-causing variants or narrow down the set of candidate variants. They will use data from experiments to determine the accuracy of the computational predictions.

MedImmune and 3M Collaborate on TLR Agonist Cancer Therapies

MedImmune, the global biologics research and development arm of AstraZeneca, and 3M Drug Delivery Systems, announced a research collaboration focused on developing next-generation toll-like receptor (TLR) agonists, the companies announced on Sept. 25, 2015. As part of the agreement, MedImmune has in-licensed from 3M MEDI9197 (formerly 3M-052), a novel TLR7/TLR8 dual agonist. FDA recently accepted an investigational new drug application (IND) for a Phase I study to explore the safety and tolerability of MEDI9197 as a potential treatment for patients with solid tumors.

TLR agonists are promising agents that activate antigen-presenting cells such as dendritic cells, enhancing the visibility of a tumor to the immune system. MEDI9197 has been designed to activate a broad range of innate immune cells through targeting of both TLR7 and TLR8, leading to a more robust, adaptive immune response. A TLR7/TLR8 dual agonist can additionally convert immune suppressive cells in the tumor to those with anti-tumor properties, allowing the generation of an effective anti-tumor response. MEDI9197 will also be the first dual TLR7/TLR8 agonist administered directly into a tumor in a clinical setting.

Preclinical studies demonstrate that intratumoral dosing of MEDI9197 may inhibit tumor growth of both the injected and distant lesions in multiple types of cancer, including melanoma. MEDI9197 is uniquely designed for intratumoral injection, allowing the compound to be retained in the tumor and provide specific immune activation, enhancing its safety and tolerability profile.

Under the agreement, MedImmune is responsible for the clinical development and strategy for MEDI9197. 3M will continue to develop additional TLR agonists in oncology and other therapy areas, with MedImmune holding exclusive rights to conduct research on new molecules resulting from the collaboration and to determine which to progress to clinical development. The terms of the agreement include an upfront payment and development-related milestone payments for MEDI9197 in addition to research funding paid by MedImmune to 3M. 3M retains the rights to 3M-052 in certain topical applications and use in vaccine admixtures.

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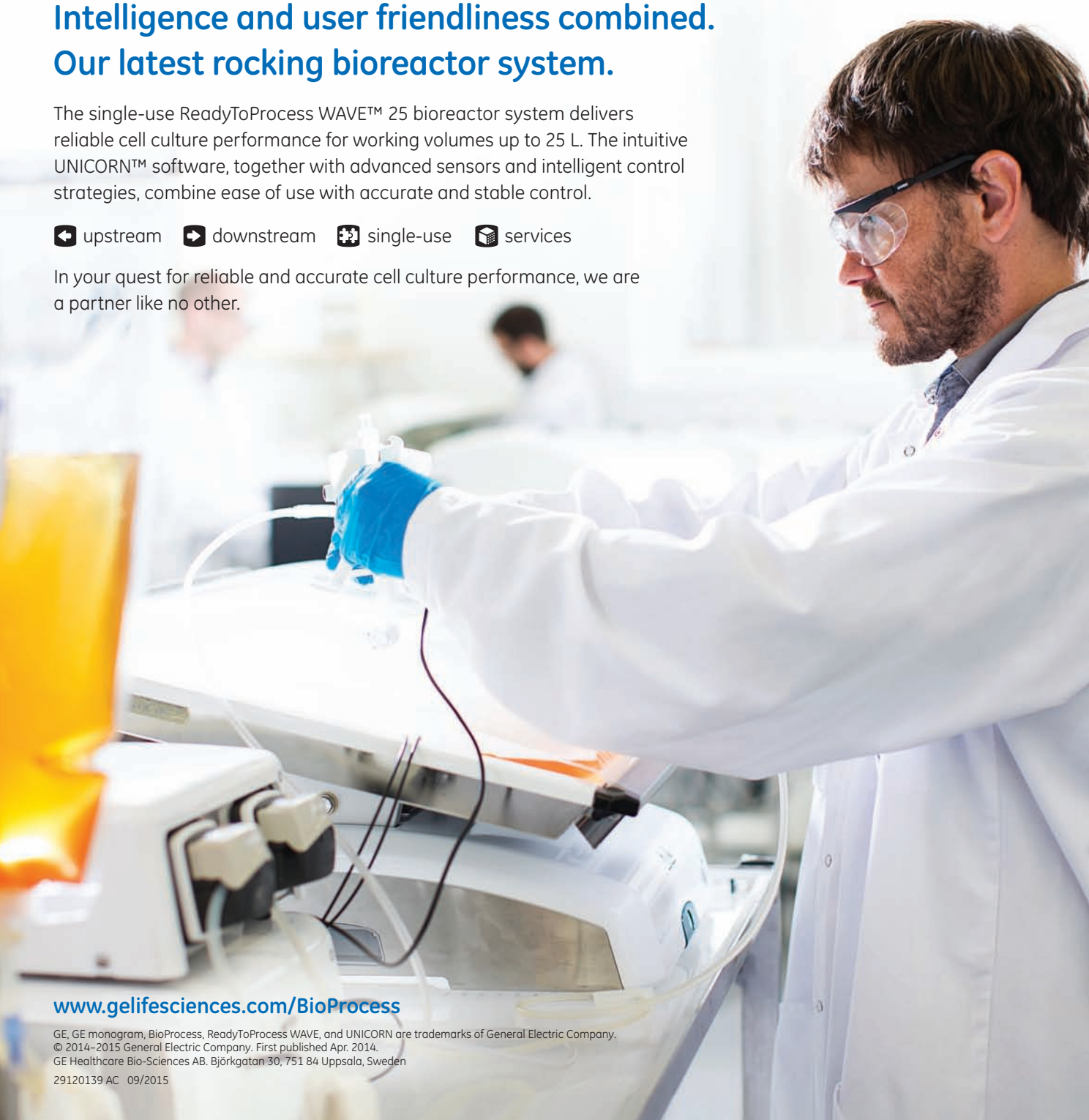

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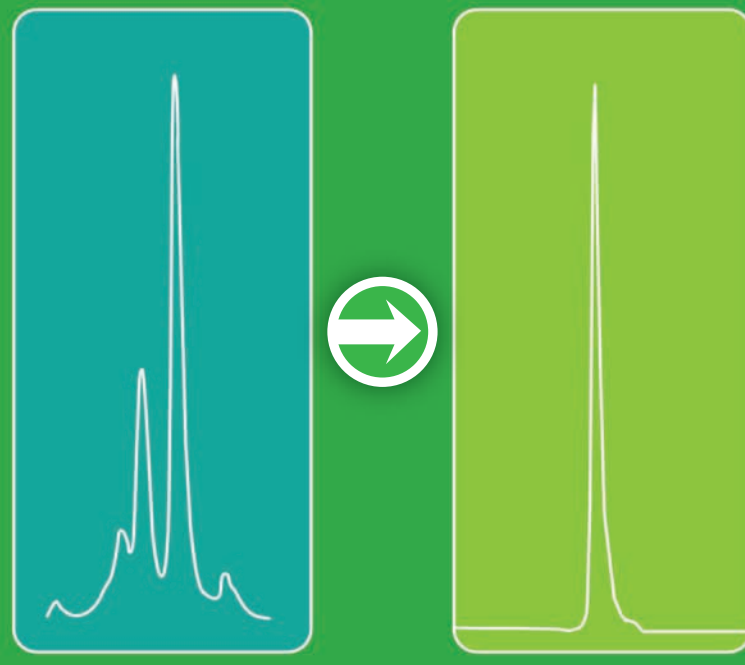

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Upstream improvements in expression systems continue to impact the level of downstream product- and process-related impurities. This includes aggregate formation, which influences biotherapeutic efficacy and immunogenicity. Bio-Rad offers a range of robust, easy-to-use, and scalable chromatography resins — CHT™ Ceramic Hydroxyapatite and Nuvia™ HR-S — to address your aggregate clearance challenges.

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- **Secure Supply** — uninterrupted supply of process chromatography resins
- **Global Support** — process development expertise and consulting offered worldwide

Visit bio-rad.com/info/cht to receive a resin sample.

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