

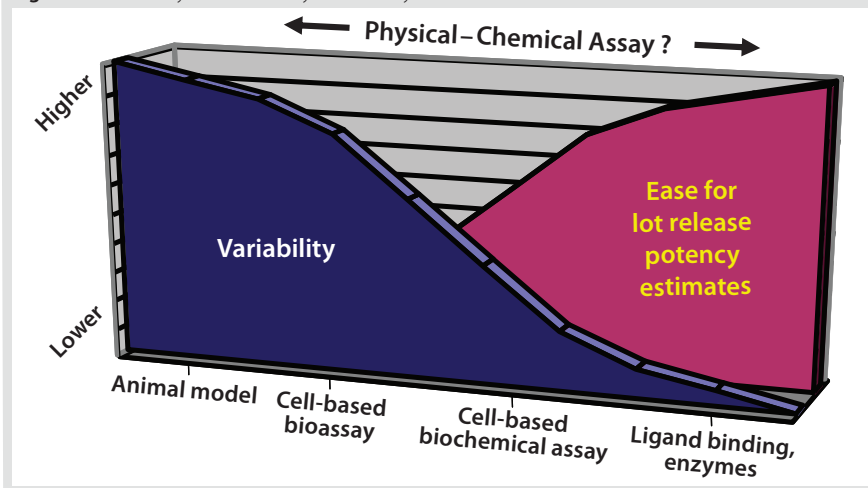
# The Roles of Bioactivity Assays in Lot Release and Stability Testing

by Noel Rieder, Hélène Gazzano-Santoro, Mark Schenerman, Robert Strause, Chana Fuchs, and Anthony Mire-Sluis, with Lorna D. McLeod

As defined in the *US Code of Federal Regulations*, *potency* is the specific ability or capacity of a product to affect a given result. Potency is a critical quality attribute of biological products that has been historically determined using some form of bioassay. A biological system is generally used to report on the potency of the product. The system may be animal, organ, tissue, or cell culture based. The concept of potency denotes an important feature of complex biologics: the biological activity produced as a direct result of the tertiary/quaternary structure of a molecule. Although a product may be well characterized by physicochemical (e.g., HPLC) assays, such tests are typically unable to confirm its higher-order structure. This molecular structure results in the mechanism of action (MoA) of a drug, which is the link between clinical response and activity measured in a bioassay. Because a bioassay or bioactivity assay can reflect the MoA, it is an important component of the complete analytical profile accumulated before a product is released for commercial use.

As a result of using a living system, bioassays present some challenges. First, maintenance of animals or cell lines may be difficult and expensive. Second, bioassays can be costly to perform, in part because they often require specialized equipment and lengthy training. Third, bioassays frequently lack the precision and robustness of other analytical methodologies, such as high-performance liquid chromatography (HPLC).

Figure 1: Variability and the utility of bioassays for lot release



For all those reasons, technology transfers of bioassays can be especially difficult. In fact, bioassays are generally the most challenging analytical technique to transfer to another laboratory, be it in-house or to a contract laboratory. Given the challenges, it is natural that alternative strategies to potency testing would be investigated. The potential use of binding and physicochemical assays in addition to (or in lieu of) cell-based bioassays is of growing interest. Unfortunately, the regulatory and scientific pathways under which such methods may be appropriately used are not clearly defined at present.

## STRATEGY FORUMS

A Chemistry and Manufacturing Controls (CMC) Strategy Forum on the roles of bioactivity assays in lot release and stability testing was held in January 2007 in Washington, DC. Its purpose

was to promote an understanding of the design and utility of bioassays throughout product development — and to delineate the conditions under which noncell-based surrogate assays could be used to determine product potency. Topics discussed included appropriate assay selection at each stage of product development, the potential use of a binding assay for potency testing, and the conditions under which multiple surrogate assays may be needed. A second CMC forum was held in Paris in April 2008 to follow up on the success of that first one and to expand the discussion on many of the same topics.

Case studies were presented both by biopharmaceutical companies and regulatory agencies on the role of the potency assay in correlating product biological activity to structure and MoA, the development and use of surrogate assays for monoclonal antibody (MAb) testing, and the

replacement of bioassays with binding or physicochemical assays for complex biological products. Perspectives were provided on the necessary characteristics of a reliable biological assay and the required level of correlation between the bioassay and surrogate assays. Open forums were held to discuss and gain consensus on the following topics:

- How is a potency assay defined and developed throughout the course of a product's life cycle?
- What characterization studies should be done to show that a surrogate to biological activity is valid?
- Under what circumstances might more than one surrogate assay be needed for potency testing?
- What role might physicochemical assays have in lot release and stability testing, given that they are generally less variable and more sensitive to change?

Those and other relevant questions were discussed at both forums. The Washington, DC forum consisted of two sessions; the Paris forum was a single session. Each session included three presentations followed by an interactive, moderated discussion with questions and comments from the audience. Although this review includes topics and opinions presented at both forums, the format follows that of the first meeting because the two meetings featured the same speakers and similar panel discussions.

### DESIGN AND UTILITY OF BIOASSAYS

On the morning of the first forum, Chana Fuchs from the FDA's Center for Drug Evaluation and Research (CDER) discussed the role of the potency assay in reflecting the biological activity, MoA, and the structural attributes of a product including the types of assays that may accomplish this at different phases of development and the possibility that a single assay may not be sufficient. Denise Gavin of the FDA's Center for Biologics Evaluation and Research (CBER) presented perspectives on potency measurements for complex biologics with special consideration on method selection and challenges for multiple product classes. Hélène Gazzano-Santoro of Genentech, Inc.

outlined an industry perspective on method selection, design, and validation, as well as considerations for the use of a surrogate binding assay for the determination of biological activity. These three presenters then participated in a panel discussion moderated by Anthony Mire-Sluis of Amgen, Inc.

Much of the first session focused on the proper selection, development, validation, and use of a potency assay (Figure 1). When considering the use of surrogate assays for potency testing, it is first necessary to understand the roles and capabilities of bioassays in product development and for commercial release. As understanding of the product MoA increases through development and commercialization it is likely, perhaps necessary, that the potency assay changes. In the absence of such information, the role of the potency assay in routine lot release and stability testing would remain ambiguous.

**How is a potency assay defined and developed throughout the course of a product's life cycle?** Because potency is a product specific measurement, assays must be evaluated for each product individually. Because knowledge of the MoA generally increases through the product lifecycle, a potency assay used early in development may not be suitable at later stages. So it is unlikely that a single assay platform could be used between products or even between development phases for a single product. It is generally expected that during early clinical phases the critical biological activity of a product will be investigated and a related potency assay developed. As products or assays are better understood, assays may be added, removed, or refined to best demonstrate product quality, consistency, and potency. As product development proceeds, an improved or more relevant assay should be developed.

It is difficult to select a bioassay for early phase development that is scientifically relevant, that reflects the MoA, and that is both robust and well behaved. It is unlikely that such an assay is in place as early as phase 1 because the MoA of a product is often unknown, and time and resources are limited. Nonetheless, partnering with

## THE CMC STRATEGY FORUM SERIES

The purpose of the CMC Strategy Forum series is to provide a venue for biotechnology/biological product discussion. These meetings focus on relevant chemistry, manufacturing, and controls (CMC) issues throughout the lifecycle of such products and thereby foster collaborative technical and regulatory interaction. The forum committee strives to share information with regulatory agencies to assist them in merging good scientific and regulatory practices. Outcomes of the forum meetings are published in this peer-reviewed journal with the hope that they will help assure that biopharmaceutical products manufactured in a regulated environment will continue to be safe and efficacious. The CMC Strategy Forum is organized by CASSS, an International Separation Science Society (formerly the California Separation Science Society), and is cosponsored by the US Food and Drug Administration (FDA).

the research and development organization early can be valuable because it may have data concerning the product MoA, which may aid in designing the most appropriate binding or bioassay at an early stage.

Understanding the MoA and target product profile can help you develop an appropriate assay. Although the final mechanism(s) of action may not be fully elucidated early in development, input from the research organization should make it possible to determine if a noncell-based binding assay would represent the proposed mechanism of action until a functional potency assay could be developed. Although a binding assay may be developed at phase 1, it was pointed out that a developer needs to replace and/or correlate it to a cell-based assay in phase 2 or 3 to demonstrate the biological relevance of the binding assay.

Assays, whether binding or cell-based functional assays, should be run concurrently so that a developer can select the appropriate potency assay in later development and have a body of data to support that choice. For example, if a bioassay is deemed too

variable or not optimal for quality control testing the manufacturer should consider switching back to the binding assays. However, there was consensus that to replace a bioassay with a binding or other surrogate assay, data must be gathered to demonstrate a strong correlation between the assays. Therefore, you should develop a cell-based assay as soon as possible because of the time required to do so and to gain experience with the cell line. This also allows ample time to gather correlation data between the assays, which should mitigate the regulatory risk associated with a poorly justified method.

With multiple potency assays analyzed before selecting one for phase 1, a parallel path can be built between the bioassay and a binding assay that justifies the selection of the final method. Even though assays may be replaced, the importance of retaining key materials was emphasized because it may be necessary to return to an earlier potency method at later stages in development. An example provided was the use of a cell-based bioassay to show product comparability following a change in the manufacturing process.

Because a bioassay can provide information that is not always reflected in a binding assay such as subtle structural changes in the product as a result of a process change, it is advisable for a laboratory to maintain the ability to perform previous versions of the potency assay.

The final method should be “locked down” and in place at prephase 3, which presents some advantages. This provides a good deal of experience with the final potency method before submission, which offers a true estimate of method performance and success rate. Also, fewer validations and bridging studies must be performed if a small number of methods are used throughout the clinical phases. However, it may be necessary to use multiple potency assays until a clear understanding of the product attributes and MoA has been achieved. Ultimately, the most well-characterized, precise bioassay reflective of the mechanism of action is generally selected as the lot release potency assay to support commercialization of the

product. Regardless of the final assay format, appropriate design, validation, and analysis are necessary if an assay is to provide reproducible and meaningful data.

**What assay design schemes are necessary to successfully validate biological assays and allow for accurate quantification and interpretation of the results?** For a cell-based bioassay it is important to choose a cell line that responds well to the drug. The cell line should also be stable and characterized, meaning that cell growth and response to the drug are consistent over time. This requires an understanding of the cellular growth patterns and receptor expression kinetics. A developer should determine how cell responsiveness and receptor expression are affected by passage number, cell density, and days in culture. Establishing these cell traits during development can help ensure a consistent and robust cellular response to the drug. The output used to measure cellular activity (e.g., fluorescence, luminescence) should be quantitative and indicative of a robust cellular response. Therefore, a primary goal in cell line and output selection should be to maximize the signal-to-noise ratio of the response.

Proper assay design also integrates multiple strategies to minimize variability and bias. There should be as few handling steps and reagents as possible to minimize dilution or technical errors. Reagents that are deemed critical should be well characterized and tightly controlled, monitored, and qualified. Whenever feasible, critical reagents should not be single-sourced, meaning that they should be available from more than a single vendor. This precaution will prevent an inability to perform assays if one source is suddenly unable to provide the quantity or quality of the reagent required.

A reference standard and control should be established as early as possible for continuous trending of assay performance. The system suitability, or acceptance criteria, of a bioassay should be sufficient to ensure that the assay remains in control

between runs. System suitability criteria often include requirements for cell viability, cell count, passage number, the signal-to-noise ratio, internal control potency, and parallelism, but may include any parameter that is determined to be important in minimizing interassay variability. Additionally, there are several statistical tools that can be used to improve assay robustness.

First, a plate layout with some degree of randomization should be used to protect against potential plate location effects. If this is not practical for the assay, other steps should be taken to minimize a potential location bias, such as balancing sample location across multiple plates or minimizing assay incubation time. Second, system suitability and other assay data should always be trended and routinely evaluated. That allows for analysis of assay performance past validation, which represents a snapshot of method capability during a relatively brief period of time. Third, use a parallel line analysis or an equivalent statistical methodology to estimate relative potency. Last, statisticians should be consulted to design robustness/qualification studies and to aid in the determination of the final format of the assay.

These studies are similar to validation testing, but they are not as protocol driven and are performed at earlier phases in development to demonstrate that they are suitable for use. Proper robustness studies are also key to method transfer and performance trending because they establish the method variability that may exist between runs without detriment to the results. An experienced biostatistician can aid in experimental designs to determine the component variance analysis, or the factors in the assay that contribute most to variability. Assay performance can be improved significantly by understanding and controlling for these factors.

Proper analyst training is also of great importance in delivering consistent and reliable assay results. Because an analyst is generally one of the most significant sources of bioassay variability, the focus of training should be to limit this source

of variability to whatever extent possible. Implementation of these practices will yield a bioassay that is well controlled and usable as a quality control release assay.

What value is added to product quality by performing bioassays for lot release and stability if physicochemical assays have been demonstrated to be more sensitive to change? Although the general conception about physicochemical assays is that they are more precise and robust than functional bioassays, they do have some drawbacks. In addition to their complexity, their narrow focus may cause them to miss key changes in bioactivity that are elucidated by a functional bioassay. For example, in the case of a monoclonal antibody with known Fc effector function (ADCC or CDC as a mechanism of action), a physicochemical assay may not be able to detect subtle changes to the molecule that could modulate activity.

Additionally, products with multiple biological activities present yet another challenge to the use of surrogate assays. Because interferon-alpha has both antiproliferative and antiviral activities, multiple bioassays may be required for potency testing. Representing both activities with physicochemical methods would be a significant challenge. Thus, a primary advantage of bioassays is that they can still provide an accurate potency estimate even if a product is not well characterized and its exact MoA is unknown.

Nonetheless, it is possible and perhaps even more desirable to use a physicochemical assay for potency testing if a product has been extensively characterized. Growth hormone, for example, has been in use for decades. All its known product variants have been correlated to potency impact, which has allowed for release using well-defined physicochemical assays alone. Only by linking all biochemical attributes to potency can surrogate assays be reliably and routinely used to replace bioassays. In other words, if the extensive product knowledge required to implement a surrogate assay is lacking, then a bioassay may still be the most appropriate methodology for potency testing.

**Due to the inherent variability of biological assays, do they serve a useful purpose in product comparability in comparison with other less variable tests, especially for well characterized process changes? It**

is not accurate to assume that all bioassays are inherently variable. A biological assay may be as precise as other methodologies if it has been well developed and validated. Alternatively, the number of independent determinations required to report a result may be increased so that the necessary degree of accuracy and precision is achieved. These approaches, alone or in combination, should make for a bioassay with a relative standard deviation below 10%.

Complex products may require more than sensitive physicochemical assays to reliably determine product potency. In such cases, it may be difficult to correlate biological activity to physicochemical assays without the additional use of a bioassay. In fact, it may not be possible to develop a single assay that encompasses all the elements of an acceptable bioassay. Some products may require an assay matrix, or a combination of assays in which the combined results constitute an acceptable potency assay. Examples included a limited knowledge of the product and/or MoA, a product with multiple components and biological activities, testing time constraints due to limited product stability (e.g., cellular therapy), or a bioassay that is not quantitative. In such cases it is necessary to establish a correlation between the analytical assays and the biological activity of the product. Ultimately, a firm understanding of the MoA will be required to create a collection of assays that correlate strongly to product potency (e.g., Fc and Fab functionality of a MAb). Developers may also need to reverse correlate clinical efficacy to assay results to better understand the clinical relevance of potency results. An example of a potential potency assay matrix for a complex biologic may be the combination of a quantitative physical assay with a qualitative bioassay. However, the specifics of each product will

**PERMANENT ADVISORY COMMITTEE FOR THESE FORUMS**

- Siddharth Advant (Imclone)
  - John Dougherty (Eli Lilly and Company)
  - Christopher Joneckis (CBER, FDA)
  - Rohin Mhatre (Biogen Idec Inc.)
  - Anthony Mire-Sluis, chair (Amgen, Inc.)
  - Wassim Nashabeh (Genentech, Inc.)
  - Anthony Ridgway (Health Canada)
  - Nadine Ritter (Biologics Consulting Group, Inc.)
  - Mark Schenerman (MedImmune)
  - Keith Webber (CDER, FDA)
  - Chana Fuchs (CDER, FDA) Hélène Gazzano-Santoro (Genentech)
- Forum CoChairs this Meeting**
- Anthony Mire-Sluis (Amgen)
  - Mark Schenerman (MedImmune)

determine the final strategy to accomplish reliable potency testing.

That point was reinforced by a case study that was presented for a fusion protein with multiple active sites required for its MoA. Manufacturing changes resulted in a difference in the ratio of existing species, which was detected with a charged based assay but not the bioassay. However, clinical data identified differences between pre- and postmanufacturing change products. The FDA required that multiple assays be used for lot release and stability testing to fully reflect the multiple activities needed for protein function in vivo. This demonstrates that an assay matrix, perhaps even multiple bioassays, may be necessary to detect process changes for some products.

**DEVELOPING SURROGATES FOR BIOASSAYS**

The first forum session had focused primarily on the selection and development of the bioassay. The second leveraged that discussion in an attempt to establish the necessary conditions for the use of surrogate assays in potency testing. Elizabeth Shores from CDER discussed the necessary attributes of a potency assay, the relevance of various methodologies to clinical activity, and case studies using enzyme, binding, and physicochemical assays for potency

testing. Robert Strouse of MedImmune focused on the use of binding assays for high-throughput MAb screening during drug development as a means to expedite discovery while decreasing project costs. Bhavin Parekh of Lilly outlined the drivers and considerations for using physicochemical assays in lot release potency testing. The three presenters then participated in a panel discussion moderated by Mark Schenerman of MedImmune.

**How much correlation between a cell-based bioassay and a surrogate assay is required to justify the switch to the latter early in development?**

This depends to a great degree on the level of biological characterization and scientific understanding of a product's MoA. The consensus was that a correlation study must be used to confirm the relevance of a desired surrogate to the biological activity of a drug. The switch can occur during development if it is clear that an assay can be verified as a true indicator of potency.

However, it is advisable to maintain assay reagents and expertise because it may be necessary to use a bioassay to characterize the biological impact of later process changes. In making this decision, a developer should consider that the farther from an *in vivo* model a potency test is, the farther from true clinical relevance are its results. So there may be a high degree of risk in making this change in the absence of a strong body of evidence that supports the use of the surrogate. There was no clear consensus on the use of the experience gained from previous molecules to support the use of surrogate assays with new molecules earlier in development.

**If a surrogate assay could be used as a replacement for a cell-based bioassay, what level of correlation is necessary between the two assays? A thorough, scientifically justified plan that includes product variants, degradation products, and varied potency lots is required. The acceptable level of correlation must**

be based on a rationale that considers characteristics of the product and each assay as well as the role of product variants.

To design a meaningful study, a researcher needs a rigorous understanding of the performance capability of the bioassay. The required precision and accuracy of a surrogate cannot be determined if these qualities are not well established in the bioassay. When considering product variants, it is not feasible or necessary to assess all variants, just those that are likely to have an effect on potency. For that reason, it is necessary to extensively characterize the biological activity of a product.

The impact of glycosylation (e.g., for a MAb), either increased or decreased, can be studied by altering the glycosylation state of the molecule and exploring the impact on product potency through the use of *in vivo*, binding, antibody dependent cell-mediated cytotoxicity (ADCC), or complement dependent cytotoxicity (CDC) assays. The function of other structural characteristics of a product (e.g., size) should also be understood and included in the design of correlation studies.

The role of Fc function should be studied using similar assays to determine the effect of removal of the Fc fragment. Preclinical and clinical studies can be used to remove product from serum to understand if any variant is formed (e.g., deamidation) or clears differently than others. Alternatively, specific variants can be supplied to potency/efficacy models to determine their biological relevance. Knowledge of product variants must then be used to generate data that correlate potency results to physicochemical analysis, which is necessary to develop a control strategy for variants that have a deleterious effect on potency.

If a study is designed such that it leverages the knowledge of the assay and product attributes, then absolute correlation may not be as important as a parallel data regression between the assays. However, if a study is to determine dosing units, both should be used.

**Assuming correlative studies have been performed, is it an acceptable strategy to use a surrogate assay for routine lot release and stability testing and the bioassay solely as a characterization tool on a limited number of lots?** Although this may be an acceptable approach, several factors must be taken into account when considering potency testing strategy. The product type, MoA, and associated risk should all be used to determine the appropriate potency test(s) for each drug individually. It may also be necessary that the surrogate assay provide information concerning the tertiary structure of a molecule because changes in this characteristic can affect product potency.

That strategy should also be considered in light of the amount of manufacturing process knowledge and control. Precise physicochemical assays can be used for potency testing when there are no expected changes to an established process, such as the confirmation of batch-to-batch consistency. However, cell-based or *in vivo* bioassays should be used for comparability studies to confirm product potency when a process has changed. So it is unlikely that a manufacturer would be able to entirely eliminate bioassay testing because of the need to demonstrate equivalent biological activity following a process change.

For stability testing, a surrogate assay may not need to be the most sensitive assay for product change, but it must be able to detect all aspects of change that are important for potency. Of course, extensive characterization studies must be performed to determine what changes occur to a product over time and the impact of each on its potency. It may then be possible to combine this knowledge with risk assessments to ensure that the surrogate assay provides necessary coverage for accurate and reliable potency testing.

Finally, the product history is not without importance. It was suggested that regulators would be more likely to allow this testing strategy with considerable manufacturing history

and clinical use. Such an approach was also suggested as being most feasible with a MAb and less likely for other product types, such as cytokines.

### **BINDING ASSAYS AS BIOASSAYS FOR MONOCLONAL ANTIBODIES**

**If it is clearly demonstrated that binding is the sole functionality of a MAb's MoA, can a binding assay be used as a surrogate for a cell-based bioassay in potency testing?** Binding assays (e.g., enzyme-linked immunosorbent assays, ELISAs, or those based on surface plasmon resonance, SPR), may be used as a surrogate for MAb potency testing if you definitively know the MoA and if no effector functions (Fc) are involved in the antibody's biological effect or binding activity.

However, if a MAb's activity requires binding to and activating cells, then a cell-based assay may be needed to demonstrate that signaling occurs. When MAb binding to receptors is not activating (e.g., an inhibitory mechanism), binding affinity should be demonstrated to validate a plate-based assay. This is intended to confirm that receptor activity is similar whether located on cells or bound to plate wells. The same applies to binding assays performed in solution.

**If the MoA of a MAb is believed to be through both receptor binding and Fc function, would surrogate assays be acceptable in lieu of a bioassay?** Similar to the use of surrogate assays for potency testing in other product types, this strategy should be supported with correlative studies between Fc function and binding assays. This is necessary particularly because not all Fc function tests are easy to reproduce with binding assays. Such an approach may be useful for MAbs whose known MoA is through ADCC. The ADCC reaction requires both Fab and Fc functionality because the former must bind to a target ligand and the latter must then recruit an effector cell to kill the target cell.

A case study was presented for two MAb products that were selected

to target tumor cell markers and whose proposed MoA was through antibody-dependent cell-mediated cytotoxicity (ADCC). An ADCC bioassay can be highly variable because it requires peripheral blood mononuclear cells (PBMCs) isolated from freshly drawn human blood. During the course of development, SPR assays to measure the binding of recombinant Fc $\gamma$ R1IIIA variants were performed on the same samples assayed by ADCC. For both MAbs, ADCC bioactivity correlated well with the SPR surrogate, and both could detect changes to the stressed molecule. However, more work was required and more samples needed to be tested to support use of the surrogate assay.

When both Fab and Fc functionality are critical to the biological activity of a MAb, as is the case when ADCC is the primary MoA, assays that demonstrate the activity of both components should be performed. In the case study presented, Fab functionality was measured with a ligand binding ELISA or SPR assay, which compares MAb–ligand binding relative to a reference standard. Fc functionality was demonstrated with a Fc $\gamma$ R1IIIA binding SPR assay, which measures the binding of the MAb to Fc $\gamma$ R1IIIA receptor relative to reference standard. It is necessary to perform both surrogate assays for lot release and stability testing to ensure the function of the entire molecule is retained. Additionally, because the Fc portion of a molecule may affect product safety, perhaps through stimulation of complement binding, companies must consider retaining some aspect of Fc testing even if potency is solely a function of Fab activity.

**Are there MAb examples for which binding does not correlate with bioactivity?** Antibody binding has often been observed to be poorly reflected in a surrogate binding assay. In some cases, bivalent binding of antibody to target is required for biological activity. Such antibody interaction does not always translate well to a binding assay and thus is a poor representation of

biological activity. That underscores the need to thoroughly understand the MoA when defining the nature of a relevant binding assay.

It has also been observed that degradation in a MAb can lead to nonspecific binding in an assay plate. Such antibody “stickiness” leads to an increase in the assay background and a concomitant decrease in the signal-to-noise ratio, a response that is not generally seen in cell-based assays and does not accurately represent biological activity.

Last, some MAbs have been seen to bind to the target ligand in vitro but do not prevent ligand-receptor binding in vivo. Such products would appear to be biologically active in the former case but ineffective in the latter.

**Are there examples in which a physicochemical assay did not detect a stability change earlier than a bioassay?** An assertion commonly made in favor of using physicochemical assays for potency testing is that they are more sensitive to change than are cell-based bioassays. However, this advantage may not be true in all cases. Some lyophilized products have shown an unexpected change in photostability upon reconstitution that was detected with binding and bioassays before it was detected with physicochemical assays. Other instances in which the bioassay was more sensitive to product change relates to how the assays were used in potency testing. The use of physicochemical assays for poorly characterized products may keep researchers from recognizing product-specific signs of degradation. In other words, an inappropriate assay may be used if you do not know where to look for stability change in the analytical results.

The same is true of stability programs that do not analyze changes in the tertiary/quaternary structure of a molecule, which can be detected by bioassays. Alternatively, some bioassays may be too variable to indicate changes in stability sooner than physicochemical assays due to poor development and precision. Improved assay precision may reveal stability trends that were previously hidden by assay variability.

Those examples demonstrate that the ability to measure stability change may depend upon the level of product characterization and assay robustness. More simply, a proper stability program monitors change in a well characterized product using methods that have been specifically designed and selected to detect the change(s).

### **ESTABLISHING A FOUNDATION FOR SURROGATE ASSAYS**

The underlying question in the use of surrogate assays is whether the biological activity of a product must be tested for lot release. Although a definitive answer does not exist for every scenario (and likely never will), certain themes developed through the course of the CMC forum on the roles of bioactivity assays in lot release and stability testing.

Perhaps the most apparent is that the answer to that question is highly product dependent. Product history and type must be considered when evaluating the use of binding or physicochemical assays for potency testing. Those products with an extensive manufacturing and commercial history (e.g., insulin and growth hormone) may find a less complex pathway to surrogate potency assays than products just entering the market.

The same is true of MABs compared with complex biologics (e.g., gene therapy products). In the case of the former, the MoA may be well understood and represented in a binding assay, as for an inhibitory mechanism that requires no effector functions. Conversely, the MoA of a gene therapy product can be difficult to elucidate and represent in noncell-based assays. For gene therapies and most other product types, regulators seem to have confidence that the critical quality attributes are best represented by a bioassay.

Regardless of product type or history, the replacement of a bioassay in potency testing is not possible without a strong body of data that strongly correlates product activity between assays. It is advisable to start putting that together early in development, devising a parallel path

that provides extensive experience with all assays and includes testing of multiple lots and product variants. The data must be combined with a strong knowledge of the MoA to demonstrate that product potency is well represented by the surrogate assay(s).

An interesting aspect of the replacement of a bioassay in potency testing is the fact that a robust and precise bioassay is required for the effort to end in success. It is unrealistic to expect you could replace a poor bioassay with a quality surrogate assay. That is because the results of a surrogate assay will not be accurate and reliable unless they were correlated to a bioassay that possessed those features in the first place. Given the value of a quality bioassay, this could represent an interesting dilemma to any company considering the use of a noncell-based assay for potency testing of biological products. Ultimately, the question of whether a bioassay can be replaced is wisely partnered with the question of whether it should. 🌐

*Noel Rieder is quality control specialist with Amgen, **Hélène Gazzano-Santoro** is director, quality analytical development with Genentech, **Mark Schenerman** is vice president of analytical biochemistry at MedImmune, **Robert Strause** is sirector of analytical biochemistry at MedImmune, **Chana Fuchs** is team leader, division of monoclonal antibodies, office of biotechnology products with CDER, and **Anthony Mire-Sluis** is executive director, global product quality and quality compliance at Amgen Inc. **Lorna D. McLeod** is a contributing editor for BioProcess International.*

### **DISCLAIMER**

The content of this manuscript reflects discussions that occurred during the CMC Forum workshop in addition to the personal viewpoints and experiences of the authors. This document does not represent officially sanctioned FDA policy or opinions and should not be used in lieu of published FDA guidance documents, points-to-consider documents, or direct discussions with the agency.