

Reference Standards for Therapeutic Proteins

Current Regulatory and Scientific Best Practices and Remaining Needs, Part 2

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Sponsors developing and manufacturing protein therapeutic products use a number of analytical tests (e.g., cell-based potency and chromatographic assays) to assess quality attributes of their active ingredients and drug products. Tests are conducted for a number of purposes, including characterization, comparability, lot release, and confirmation of stability.

This two-part article includes the findings of the California Separation Science Society (CASSS) Chemistry, Manufacturing, and Controls (CMC) Strategy Forum titled “Reference Standards for Therapeutic Proteins: Current Regulatory and Scientific Best Practices and Remaining Needs,” held in Gaithersburg, MD, on 15–16 July 2013. The discussion also is based on findings of previous CASSS reference standard meetings, two workshops held during the WCBP conferences in 2012 and 2013, and the conference “Reference Standards for Therapeutic Proteins: Their Relevance, Development, Qualification and Replacement” (1–5). The latter was coorganized by the International Alliance for Biological Standardization (IABS), National Institute of Standards and Technology (NIST), National Institute of Allergy and Infectious Diseases (NIAID), and



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the US Food and Drug Administration (FDA) in September 2011. (Approaches other than those presented here might also be acceptable, so the content of this document is not binding regulatory guidance; consult with your regulatory agency for specific reference standard strategies.)

Part 1 focused on therapeutic protein reference standard lifecycle elements and practical implications of reference standards (6). Part 2 covers potency assignment for bioassay reference standards and the role of public reference standards in global harmonization of protein therapeutics. Together, these programs focused on selected reference standard topics, including the following:

- initial qualification and lifecycle strategies from product development to postapproval maintenance
- potency assignment and potency stability monitoring
- assignment of content (mass and specific activity)
- critical operational aspects such as source material selection, configuration, and storage conditions
- regulatory expectations and experiences
- use of publicly available protein therapeutic standards and their role in biosimilars development.

DEFINITIONS CLARIFICATION

The “Definitions” box highlights some standards terms. For simplicity in this summary, both manufacturers’ in-house reference materials and international or national standards (as defined in ICH Q6B and ICH Q7) (7, 8) are referred to as *reference standards*. It should be noted that the term *primary reference standard* used here is distinct from a *certified reference material* (9). The latter can have the specific metrological meaning of a standard calibrated in Système International d’Unités (SI) units and traceable to the SI through a primary reference method. Thus the use of *primary reference standard* herein is distinct from a metrologist’s definition.

DEFINITIONS

In-House Primary Reference Standard:

Per ICH Q7, *reference standard primary* is defined as “a substance that has been shown by an extensive set of analytical tests to be an authentic material that should be of high purity. This standard can be: (1) obtained from an officially recognized source, (2) prepared by independent synthesis, (3) obtained from existing production material of high purity, or (4) prepared by further purification of existing production material.”

Specifically for biologics, rather than using an ultrapurified material as reference, ICH 6B states that “an appropriately characterized in-house primary reference material [should be] prepared from lot(s) representative of the production and clinical materials.”

In-House Secondary Reference Standard (also referred to as a *Working Standard*):

Appropriately characterized material prepared from representative clinical or commercial lot(s) prepared to support routine testing of product lots for quality control purposes, such as biological assays and physicochemical testing. It is always calibrated against a primary reference standard (either official or in-house).

In-House Interim Reference Standard:

Appropriately characterized material prepared from representative clinical or used for quality control purposes during the development stage of a product. It is not compared to an official or primary reference standard, but it is established based on appropriate demonstration of its inherent characteristics.

Official Reference Standard: According to ICH Q7 definition, it is a primary reference standard obtained from an “officially-recognized source.” Typically it is established by a public agency (e.g., WHO), government (e.g., NIST, NIBSC), or compendium (e.g., USP, PhEur) and is officially recognized as a standard by individual regulatory authorities.

POTENCY ASSIGNMENT STRATEGY FOR BIOASSAY REFERENCE STANDARDS

Juhong Liu of the FDA Center for Drug Evaluation and Research (CDER) and Katie Parks of Amgen Inc. cochaired a session about strategies for assigning potency for bioassay reference standards during development and commercialization.

Matthew Borer of Eli Lilly and Company presented “Assigning Potency to Reference Standards.” He focused on biomolecule reference standards for calibrating cell-based potency assays such as for monoclonal antibodies (MAbs). Borer assessed terminology associated with potency and property values. Certified property values — as defined by the International Organization for Standardization (ISO) — represent an important way to communicate intended use. As such, they must be carefully worded and clearly presented, and a robust measurement strategy must be used to determine them.

In her presentation, Heather Runes of Genentech (a Member of the Roche Group) addressed the question, “Is an international reference standard needed to assign potency value to the in-house reference standard?” Runes focused on potential risks in assigning potency value to an in-house reference standard against international standard.

Yi Tsong (with contributors Xiaoyu Dong and Meiyu Shen) of the Division of Biometrics VI, Office of Biostatistics, FDA CDER, presented “Statistical Tools for Assigning Potency to Reference Standards.” They shared examples of statistical models proposed by sponsors for establishing the acceptance criteria in establishing and bridging reference standards.

Sally Seaver of Seaver Associates LLC presented “Reference Standards for Potency Assays: Selection, Replacement, and Stability Issues.” She reviewed how recruiting and assigning a potency value to a reference standard lot has evolved over the past three decades. Her talk focused on selection, replacement, and stability issues for reference standards. Seaver also discussed some pitfalls and some nontraditional ideas for selecting a candidate reference standard, assigning its potency, and monitoring its stability throughout use.

The session concluded with forum attendees’ questions and answers. The panel consisted of Evangelos Bakopanos of Health Canada,

Matthew Borer, Heather Runes, Sally Seaver, and Yi Tsong. Along with comments and questions presented below, audience members were asked to point out aspects in the presented best practices that needed further clarity from the speakers.

PANEL DISCUSSIONS: POTENCY ASSIGNMENT STRATEGY FOR BIOASSAY REFERENCE STANDARDS

The integrity of reference standards must be proven for products used in registration applications, quality control release tests and stability studies, or pharmacokinetic studies. Biotherapeutic proteins (which may be natural or recombinant products) are complex with respect to primary, secondary, and tertiary structure as well as posttranslational modifications. Such attributes vary depending on the manufacturing processes and thereby may affect a molecule’s biological activity.

For such products, biological activity (potency) is commonly determined through one or more specific *in vitro* bioassays that ideally mimic, or serve as surrogates for, the *in vivo* mechanism(s) of action (10). *In vitro* bioassays may use a wide variety of biological formats, each measuring a different functional mechanism or measuring one mechanism in different ways. Differences in bioassay formats can provide different activity outputs for the same molecule. A product’s potency value is directly correlated with the specific bioassay used to generate it. So both the manufacturing processes and the specific assays used for qualification of a potency reference standard are critical to its establishment and maintenance.

A reference standard is the primary means of maintaining drug doses consistent with those of original clinical studies. A potency reference standard is used in bioassays for assigning dose strength for each batch of product. To assure accuracy of product potency values, it is critical to prevent reference standard drift over time because subsequent reference standards are qualified during a

CMC FORUM SERIES

The CMC Strategy Forum series provides a venue for biotechnology and 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 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 to 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 supported by the US Food and Drug Administration (FDA).

product's life cycle. A key tool for drift prevention is to establish a two-tiered in-house reference standard system: a primary reference standard that is reserved for establishing secondary standards and a secondary working standard to be consumed during routine use. Chaining from one reference standard to another (e.g., A to B to C to D) can set up a pattern of serial drift, so such practice is not recommended. Using a two-tiered system of comparing the primary in-house standard (A) to each working in-house reference standard batch (B, C, D, and so on) is strongly encouraged (e.g., A to B, A to C, A to D). This strategy is described in greater detail in Part 1 of this article (6).

A too-common feature of biological assays is the degree of performance variability that can affect the accuracy and reliability of the potency values (10). Therefore, other critical tools in the establishment and bridging of potency reference standards are mathematical equations that link acceptable uncertainties in certified potency values to a number of independent replicates required to achieve desired levels of statistical confidence. High uncertainties in potency values increase the potential for large shifts in test results when reference standard batches are

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replaced. They also increase the likelihood for drifts in assigned values that may be detected only over time.

The Nature of Potency Reference

Standard Batches: The primary potency standard does not necessarily have to be the same batch and same formulation as the primary physicochemical standard, but both must have suitable biological activity and have undergone extended biomolecular analysis to demonstrate acceptably comparable characteristics. Prior discussions (6) brought up the concept of pooling multiple batches of product selected to represent the degree of process variability in the allowable clinical range. This approach could generate a reference standard lot that was intentionally “centered” in the target performance range of the prior reference standard. In general, such a strategy was considered a justifiable possibility, with certain caveats on how batches are selected and characterized.

When an international standard is available and applicable, the potency value of the in-house standard should be calibrated against it, also using the designated bioassay. If potency is defined based on relative potency (as calculated relative to the reference standard run concurrently in the bioassay), then the value of the new reference standard batch should be within a predefined limit when compared with the primary potency standard, and the limit should be statistically predefined and justified.

Absolute or Relative Potency

Values? Potency bioassays used with some products (e.g., enzymes) generate reportable results as absolute values (units of activity per milligram or milliliter of product). Other products use bioassays that generate potency values relative to the activity of the reference standard run concurrently the results of which are normalized to 100%. In relative potency assays, graphical curves of the reference standard versus test sample results should be assessed for similarity in shape and parallelism or equivalency before a relative potency is calculated for the sample. Appropriate data evaluation criteria are defined in pharmacopeial chapters on bioassays (11–13).

For the primary in-house potency standard, a 100% relative potency value or a defined absolute unit (in the absence of an applicable international standard) is assigned through a sufficiently rigorous testing scheme using the designated bioassay. With potency standards that yield empirical units of activity, especially when a bioassay is calibrated to an international unit standard, it is possible to directly track/trend reference standard results over time as the in-house standard ages in storage, or in assessing comparative activity ranges when bridging two in-house reference standards.

With products that have potency values generated relative to the reference standard, the first in-house reference standard that represents the clinical trial material is usually assigned a value that is defined as 100% relative potency in the bioassay. Replacement reference standard batches are physicochemically compared with the prior standard to provide orthogonal characterization data to support the use of the batch as a potency standard. In some cases, a value of 100% may be assigned if the assay results obtained from a statistically sound testing plan are within an acceptable predefined limit (assume validated process, well-characterized molecule, assay variability most likely source of variability, and so on).

Alternatively, an independent potency value can be assigned to the new in-house standard based on its performance relative to the prior standard (e.g., it yields a potency of 95% relative to the prior standard). However, you must ensure that the new value isn't simply a result of method variability, but rather a real difference in reference standard activity between the batches. To bridge — or track/trend over time — the type of potency reference standard that generates percent relative potency values, it is important to examine the bioassay readout obtained on the reference standard itself (e.g., IC_{50}). In this manner, you can directly assess the standard's performance when establishing the acceptance criteria for bridging a new standard or when assessing the drifting of reference standard performance over time as it ages.

Whether the potency values are empirical or relative, the specific activity of the reference standard batch should be carefully considered during equivalency evaluation (ensuring that the same amount of protein is used when assigning relative activities). Trying to link potency values to product mass may not be suitable (as it is for many chemical products) because the specific activity (activity/mass) can vary with conformational variations or changes in higher-order structure from batch to batch, as well as over time as a batch ages.

What are the most commonly accepted statistical analysis methods to demonstrate equivalency of potency values of new and previous in-house reference standards? Whether you define “100%” as a relative result that falls within predefined assay confidence limits or within an absolute assay numerical range, the approach used to assign potency should be scientifically sound and justified with sufficient supporting data. An equivalency approach also may be used. Therapeutic use of products helps determine acceptance criteria for potency (products that are particularly toxic or that have narrow efficacy windows could have stricter criteria). Choosing the number of

replicates for the potency assay involves assessment of intermediate assay precision, level of confidence, width of desired confidence interval, and the probability of data being outside that interval. Often, 20–40 reportable bioassay values are used in the assignment of potency to in-house reference standards. A recommended practice is to introduce the appropriate amount of variability that reflects real day-to-day use. For example, tests may use different bioassay cell lots or vials or involve different operators. Some prefer not to collect all reportable bioassay values in a short time frame to mitigate possible bias from using materials and reagents that represent a small subset of actual operational variations. However, there is no single way to carry out statistical analysis because it depends on the situation. If in doubt about the statistical strategy you plan to use, then it is important that you discuss it in advance with regulators.

How many potential sources of assay variability should be incorporated into potency determination assigned to reference standards? Some attendees felt that although one exceptional analyst performing an assay can give the most precise results for assigning potency value to a reference standard, those results may not represent the most robust values derived from the actual performance range bioassay. The work of many analysts across different sites and days would provide a better measure of overall assay variability and ensure that the collated reference standard potency values come from all operational conditions. Moreover, it can confirm that the bioassay is being reliably performed across a quality control (QC) network.

Attendees discussed the pros and cons of both approaches regarding study design. It was agreed that in the end there must be sufficient precision in the potency value assignment to ensure it provides a suitably accurate and reliable estimate of a reference standard's potency.

Are there ways to reduce variability and bias in bioassay potency assignment? Participants discussed their experiences in remediating

sources of bioassay operational variability that could influence the potency value assignment:

- Treat cells as gently as possible (e.g., no harsh centrifugation or pipetting).
- Understand positional effects of plate assays and randomize the plate layouts to mitigate or eliminate them.
- Rotate the position of new and old reference standards on a plate to prevent bias in the accuracy of an assay.

A previous CMC Strategy Forum detailed practical examples of bioassay operational variability and assay control (10).

Another common mechanism used to monitor bioassay performance independent of reference standards is to include an assay system suitability control (“QC control”) in each run. The QC control is typically a product batch that is newer than or in some way different from the working reference standard. Typically it is a portion of a different released-product lot. The QC control lot should be aliquoted and stored in the same way as the house potency reference standard. Some companies prefer to store them in a different locations.

For trending of relative reference standard results, you can execute statistical process control (SPC) monitoring of the performance of the QC control. To prevent confounded data, it is important not to change the QC control lot at the same time as the in-house working reference standard lot.

Impact of Aging Reference Standards on Potency Values: The link between physical and functional degradation of reference standards was discussed. Some participants felt that even if certain physical attributes may be degrading over time, there is no need to recalibrate a potency reference standard as long as bioassay data continue to prove it is fit for use. However, others felt that if physical degradation does occur, it would be less risky simply to replace the potency reference standard rather than undertake a potency recalibration exercise. In general, it was thought that setting potency acceptance

criterion for stability of the reference standard tighter than that used for the product's shelf life stability study can help minimize the chance of drift over time during storage of the potency reference standard. Data from accelerated stability studies can provide useful additional data for assessing the accuracy of relative potency values from aging standards. If a bioassay demonstrates the ability to detect altered potency results with intentionally degraded material, then it supports the accuracy of potency data that do not show changes over time when obtained from standards held at recommended temperatures. So it confirms that the standard is not degrading under those storage conditions.

What potency value do you assign if you use two different potency assays (e.g., chromogenic/clot assays)?

Depending on the modes of action (MoA) of a product and the biological mechanisms being measured by the two different bioassays, it is possible to assign two potency values if two different potency assays are used. For example, if one activity is antiviral and another is antiproliferative, or if one measures epitope binding and the other measures cellular receptor activation, then they will generate different outputs. Potency values should be linked to outputs of the bioassays used, with supporting information on the nature of each assay and sufficient data that justify the different potency values obtained.

When do I use an in-house primary reference standard rather than an international standard (IS)? The panel agreed with the recommendation in ICH Q6B (7) that when an international or national standard is available and appropriate, an in-house reference standard should be calibrated against it. By doing so, you will generate an internal reference standard potency value expressed in international units. An IS is not designed for day-to-day use as a working standard. But when an IS is not available or applicable to your product, it is best to use it only to calibrate your in-house primary standard. In-house primary and

working reference standards should be prepared from material that is reflective of your process and your product. Your own in-house primary reference standard provides the most direct physicochemical and functional link to the original clinical studies for your product.

What about reporting calibration against World Health Organization (WHO) standards? Where available and applicable to your product, official standards — such as compendial and World Health Organization (WHO) — should be used for calibrating your in-house primary potency reference standard. Then in the two-tiered system, each new in-house secondary potency reference standard is thereby qualified against the calibrated primary potency standard.

However, international or pharmacopeial reference standards for biomolecules are generally manufactured and qualified very differently from in-house reference standards. They may be from processes that are completely different from yours and often are formulated uniquely to support their use as reference standards, not in a manner intended to reflect therapeutic dosage forms. Finally, the bioassays used to generate the unitage assigned to IS are not necessarily the same as those used to test potency for release and stability of your product.

Consequently, potency values assigned to international standards can be significantly different from those obtained from your in-house reference standards generated using your bioassay. Thus, qualification of an in-house reference standard against an IS can lead to a significant shift of assigned values to in-house standard. That shift can be especially pronounced for potency standards, because the value is determined against an existing official reference standard the value of which was assigned using a unique bioassay that may measure different functional characteristics.

If a manufacturer must recalibrate an in-house standard against an IS or WHO replacement standard, then that should be done according to your

agency-approved recalibration protocol with appropriate acceptance criteria. In the United States, the resulting recalibration data generated from an approved protocol could then be provided in the annual report. If those data demonstrate that a shift in the calibrated value assigned to the new IS or WHO standard makes it necessary to adjust the prior assigned potency value for your in-house primary reference standard, then discuss your options with the appropriate regulatory agency. Issues associated with shifts in your in-house potency calibration values as assigned from a new IS should be described in your regulatory filing.

PUBLIC STANDARDS AND THEIR ROLE IN GLOBAL HARMONIZATION

The role of publicly available reference standards — such as those offered by the US Pharmacopeial Convention (USP), National Institute for Biological Standards and Control (NIBSC), or WHO — in establishing and maintaining in-house reference standard programs was also included in the program's agenda. Presentations and subsequent discussions engaged participants in addressing potential solutions to the challenges of maintaining traceability, harmonization of public standards, and relevance of first-generation biotherapeutic product standards to next-generation biotherapeutic products.

Tina Morris of USP presented "Use of USP Potency and Physicochemical Standards." She discussed the use of product-specific reference standards that are linked to pharmacopeial monographs. Morris provided case studies of two legacy products (heparin and pancreatin) as well as emerging standards for modern recombinant therapeutics. She discussed measurement challenges associated with different types of standards.

Anne Munk Jespersen and Kirsten Byrialsen of Novo Nordisk A/S presented "A Global Reference Standard Program for Biotech Products." They discussed how their company handles the global

requirement of traceability to all WHO or pharmacopoeial reference standards and associated challenges. They discussed a scenario in which more than one external reference standard is available — such as the USP or the European (PhEur), Japanese (JP), Chinese (CP), and Indian (IP) pharmacopoeia — and the potency values of the in-house working standard (secondary reference standard) differ widely when calibrating against a different external reference standard. They provided an example of traceability through the known difference from an in-house primary reference standard to the relevant external reference standards. Maintaining an in-house primary reference standard in that case secured a more stable baseline for potency values than could be generated from external reference standards.

Jespersen and Byrialsen described two cases in which it might be possible to use an external reference standard to calibrate an in-house working reference standard. One case involved somatropin, for which the International WHO reference standard is defined in both mass (mg) and activity (international units). The other case involved glucagon, for which the one external reference standard is a harmonized USP/PhEur reference standard with one mass value assigned in a shared collaborative study.

To illustrate, Jespersen and Byrialsen discussed shifts in reported potency results that can occur when changing in-house reference standard lots. There may be a slight difference in reportable QC potency results when analyzing the same product lot against the current working reference lot compared with the new working reference lot because of measurement uncertainty in the bioassay. Contributions to this shift can include the inherent variability of analytical methods, the reference standard calibration study design (including data treatment), the qualifications of the participating laboratories, and quality of the reference standard batches (e.g., homogeneity). It is desirable for this shift to be

insignificant relative to other sources of variability. But that is not always the case, and the result can be a shift in the relation between the analytical response and the assigned content or potency of the two working reference-standard lots.

The challenge for a manufacturer is to handle those shifts such that traceability of an internal reference standard is still ensured without introducing bias as a result of uncertainty in reportable QC results. One of the best ways to achieve that goal would be to harmonize WHO and pharmacopoeia reference standards, including those related to mass and content assignment. To achieve that, the assay methods also should be harmonized to ensure that measurements are comparable.

Jane Robinson of the National Institute for Biological Standards and Control presented “Reference Standards to Support the Development of Biosimilars.” She commented that next-generation biopharmaceuticals and biosimilars present challenges for the provision of appropriate reference standards. Such biopharmaceuticals (e.g., biosynthetic structural variants, chemically derivatized natural molecules, MAbs with no natural equivalent, and artificial constructs like receptor-Fc fusion proteins) have no preexisting publicly available standards. Unlike many first-generation therapeutic protein products (which were recombinant versions of natural molecules for which standards existed), next-generation products and biosimilars often get to the market without the existence of applicable WHO standards or traceability of potency to an International Unit.

The session concluded with a panel discussion with questions and answers. Panelists included by Yves Aubin of Health Canada, Anne Munk Jespersen, Tina Morris, and Jane Robinson.

PANEL DISCUSSIONS: PUBLIC STANDARDS AND THEIR ROLE IN GLOBAL HARMONIZATION

Development of product reference standards is a vital part of drug

development. Product reference standards support quality control analysis of products through bioassay and physicochemical techniques. However, for some physicochemical analytical techniques, non-product analytical method performance standards are increasingly being used to support validation of method performance, and they are being incorporated into method system suitability criteria. Provision of a suitable range of analytical method performance standards for use by industry will require a coordinated approach by the WHO and pharmacopoeias.

How are public standards characterized? Most batches of materials used as public standards are obtained by donation from manufacturers and are received with their manufacturers’ characterization data. They are subsequently tested for suitability for purpose and generally reformulated, ampuled, and lyophilized. Lyophilized candidate standards are then compared with original materials for changes in potency and (possibly) physicochemical properties. To test stability and predict any significant change at recommended storage and transport conditions, sample ampules are stored at various temperatures (including over liquid nitrogen) and monitored regularly. Usually, the Arrhenius equation is used to predict any loss of potency. If potency changes are observed, then physicochemical analyses may be used to identify the causes.

Are potency reference standards needed for biosimilars, or can a manufacturer simply have an internal standard if a product is deemed safe and effective and comparable to the innovator product? This is a complex issue. If an in-house product material is used for potency values instead of an international potency reference standard, it may vary from lot to lot within specifications and by region. The IS reduces potency value drift because it is usually extremely stable and can be used in monitoring stability of an in-house potency standard.

DISCLAIMER

The content of this manuscript reflects discussions that occurred during the CMC Strategy Forum. 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.

Reference standards for physicochemical assays are useful for demonstrating system suitability and to ensure that assays are appropriate, robust, sensitive, and capable of detecting relevant differences between products.

How do you ensure suitability and continuity of external reference standards? To ensure the suitability of a reference standard for a particular assay, there must be a sufficient representation of that assay system in the collaborative study. Appropriately large studies permit comparison of different assay systems and ensure that a few anomalous results do not influence greatly the overall outcome of the study. If the standard is used for a single method, then the study is less complex and generally can be smaller. The more assays are intended for the standard, the more challenging it becomes. If the standards are not commutable between assays, then using the IS for some assay systems may not be possible.

What is the difference between an in-house reference standard and a reference licensed product in biosimilar development? They are not the same thing at all. The reference licensed product is the commercially available drug that has been through regulatory agency scrutiny and is approved as being safe and effective. Analytically comparing the biosimilar product to the reference licensed product generates data that may allow for reduced clinical studies. But biosimilar companies are held to the same paradigms as originator companies when creating and using in-house reference standards for product quality control and stability testing. So everything that has been discussed regarding establishment of a

tiered-approach system for in-house primary and secondary (working) reference standards applies to biosimilar products.

What special challenges are presented by biopharmaceutical drugs with respect to establishing and maintaining equivalent official reference standards? Developing formulation and lyophilization processes to produce a highly stable reference standard are some of the biggest challenges for a public standard. Design of a collaborative study is important because many different assays may be required — and both a physicochemical and potency standard are often needed. If a WHO standard is available, then the national standard needs to be calibrated to the WHO standard. Replacing the standard with material that both behaves similarly and looks similar (that is, the manufacturer hasn't made too many changes) or preventing shifts in potency can be challenging.

Do regulators review international standard potency results when executed by public authorities? WHO does review data with some regulators, but those data are not approved specifically by regulatory authorities. If regulators take part in a collaborative study, then they can review the collated results before ratification by the WHO Expert Committee for Biological Standardization.

Should national compendial agencies (e.g., PhEur, JP, and USP), WHO, and The National Institute for Biological Standards and Control collaborate to establish one international standard per product? Should WHO offer the international standard and USP offer a US national standard traceable to the WHO international standard? National compendial agencies and WHO should be encouraged to create and distribute only a single international reference standard to prevent issues with calibrating between a number of materials. If a separate pharmacopoeial standard is created, then it must be carefully calibrated and/or similar for the needs of the

assay to the WHO international standard. Otherwise the results provided by two different standards could be different and cause confusion.

How have multiple analytical methodologies for one product changed the way international standard are calibrated? Would harmonization of compendial and public standards necessitate designation of a "preferred" method? The answer depends on whether a standard yields the same results independent of the assay system used. public standards are intended to be independent of the assay system, so that different cell lines, readouts, and so forth should yield the same result for relative potency. If that is not the case, then manufacturers using different approved assays can get different results for the same standard. That has happened, for example, when the IS material was not identical to the existing company commercial material. Having a standard method prevents this risk but then requires each company to file a new assay. That can be an issue, especially if the unitage, the relative potency, or some other assigned value changes when switching test methods.

It is not easy to harmonize across compendia and regions because regulatory and scientific opinions may differ. The challenge of creating a sufficient quantity of a fully harmonized standard is huge (e.g., with 75,000 vials, it can take up to six years to complete a study). Procuring representative material for a global standard isn't easy, and the product may vary across regions.

FACTORS TO TAKE INTO ACCOUNT

Although many common strategies are used, including many shared elements of best practices, there is no single recommended way to execute assigning a potency value to an in-house primary or secondary reference standard. Every strategy should take into account numerous variables associated with type of therapeutic protein product, its mechanism(s) of action, the nature of

Continued on page 31

Continued from page 22

the bioassay(s) used to assess product quality and stability, and the manner in which potency values will be determined (empirical or relative). Statistical approaches should be used as needed to ensure that the accuracy and reliability of the data set used to establish potency values and bridge between reference standards. Whatever approach is used should be technically appropriate, scientifically sound, justified by supporting data, discussed with your regulatory authority, and included in your product filing.

Agencies for the establishment of international and national reference standards have a long history in providing materials against which industry can calibrate the potency of its versions of those products. Practices have been developed in these agencies to generate the most robust collaborative data and produce the most stable form of reference standards for designated protein products. The emergence of biosimilar products — for which comparison with a reference licensed originator product is an additional type of reference standard study — has opened questions of whether and how a single global reference version of a product could be established by international biological standards agencies.

REFERENCES

- 1 2013 CMC speaker presentations available at <http://cass.org/displaycommon.cfm?an=1&subarticlenbr=855>.
- 2 CMC Forum: Standard Reference Materials for Biopharmaceutical Products. *The Gold Sheet* 40(6) June 2006.
- 3 2012 WCBP speaker presentations available at <http://www.casss.org/displaycommon.cfm?an=1&subarticlenbr=673>.
- 4 2013 WCBP speaker presentations available at <http://www.casss.org/displaycommon.cfm?an=1&subarticlenbr=799>.
- 5 2011 IABS program and slides available at www.iabs.org/index.php/conferences/iabs-conferences/past-iabs-conferences/104-september-20-21-2011-bethesda-maryland-us.
- 6 Mire-Sluis A, et al. Reference Standards for Therapeutic Proteins: Current Regulatory and Scientific Best Practices and Remaining Needs, Part 1. *BioProcess Int.* 12(3) 2014: 26–36.
- 7 ICH Q6B: *Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*. March 1999.
- 8 ICH Q7A: *Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients*, November 2000.
- 9 ISO Guide 30:1992/Amd 1:2008: *Revision of Definitions for Reference Material and Certified Reference Material*. International Organization for Standardization: Geneva, Switzerland, 1992.
- 10 Rieder N, et al. The Roles of Bioactivity Assays in Lot Release and Stability Testing. *BioProcess Int.* 8(6) 2010: 33–42.
- 11 <1034> Analysis of Biological Assays. *The United States Pharmacopeia and The National Formulary* (USP–NF). US Pharmacopeial Convention: Rockville, MD.
- 12 <111> Design and Analysis of Biological Assays. *The United States Pharmacopeia and The National Formulary* (USP–NF). US Pharmacopeial Convention: Rockville, MD.
- 13 5.3. Statistical Analysis of Results of Biological Assays. *European Pharmacopoeia*. European Directorate for the Quality of Medicines and Healthcare (EDQM): Strasbourg, France. 🌐

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