FEATURE ARTICLE

Biological Potency Assays Grow in the Importance
Assays have Both a Long History and a Critical Modern Role

Story by Laureen Little, PhD.

Biological potency has long been considered a critical quality attribute, (CQA), in fact longer than the snappy CQA acronym. The first FDA regulated product, with a quantitative potency assay, surfaced in 1949. Dr. Margaret Pittman, director of the Biologic Control Laboratory (fore-runner of CBER), developed a potency assay for the pertussis vaccine and correlated potency with human efficacy. It quickly became a stan-

Another regulatory problem can occur with bioassays at BLA time.

If a company has not tested pivotal clinical batches with the appropriate bioassay, the FDA will often not accept the clinical data from that trial

The method was a lethal dose 50% (LD50) assay. The LD50 value was estimated without computers using a probit. The assay required 16 or 32 animals per testing group.

Compare to today, in which commercial software providers duke it out for market share, biological read-outs include animals, tissues, cultured cells, frozen ready-to-use cells and statisticians argue nuances of determining similarity between test and reference samples.

However, what has not changed since the 1940’s when Dr. Pittman was doing her ground-breaking work, is the singular lack of guidance on what the regulatory expectations are for the method.

Since the late 1990’s, the potency assay has been touted as the critical quality assay to support comparability studies, development and release of biopharmaceuticals. Now with the advent of BioSimilar products, there is an even stronger emphasis on potency bioassays.

The original pertussis potency assay become both a release assay and a surrogate for human efficacy. This is now considered the holy grail of bioassay, but is not typically achieved outside of the vaccine industry. Even here, it is only commonly accepted for challenge animal potency assays. Newer, binding or neutralization vaccine potency assays still meet strong resistance if trying to claim the ability to predict efficacy.

An interesting exception to this is the Hepatitis B (HB) vaccine field. The commercial vaccines; Engerix-B, Recombivax HB®, and Twinrix®, utilize some form of binding assay either as part of an In-vivo immunogenicity assay (Engerix B®) or a direct quantitative determination of the antigenicity of the Hepatitis B Antigen (HBsAg) in the final drug product (Twinrix® and Infanrix®).

It is the latter method, the direct

(Continued on page 2)
The poster-child for this trend are the numerous monoclonal antibody products.

The binding of a neutralizing monoclonal antibody to the HBsAg which is absorbed to the solid Alhydrogel particle which is of the most interest. Originally the binding assay, was based upon a commercially available kit, Auszyme®, manufactured by Abbott.

The monoclonal antibody which drove the specificity of the Auszyme was specific for the a-determinant of the HBsAg. This is a double-loop structure which projects from the surface of the HBV particle and is known to be the major neutralizing epitope. Antibodies to the a-determinant confer protection in adults against all common subtypes of HBV. The scientific rationale for a binding/epitope assay relies specifically upon this clinical bridge to human efficacy data.

The Abbott kit was discontinued in 2005 and several companies since have developed replacement in-house methods which focus on developing mAbs to the a determinant.

Today, there is an increasing emphasis on demonstrating the linkage of the potency to the proposed mechanism of action (MOA) of the therapeutic. Although few products outside of the vaccine world have achieved the status of predictive efficacy.

Also with the appearance of large, complex biotech products, such as monoclonal antibodies, it has become apparent that many products may have more than one of MOA and that a single assay may not supply sufficient coverage.

The poster-child for this trend are the numerous monoclonal antibody products. Antibody products contain the two distinct functional regions, the constant region (Fc) and the binding region (Fab), if both are involved in the MOA then there must either be a single assay which covers both functions or two methods, one for each distinct structure.

A recent paper authored by multiple industry scientist (Nature Reviews/Drug Discovery, Vol. 10 Feb. 2011, Jiang, XR, et. al.) strives to classify various classes of mAb products and provide a scientific rationale for the required potency assay. This paper describes three classes of products:

1. Ab which binds to a target cell and use the Fc region of the Ab to mediate an effector function, resulting in the death of the target cell.
2. Ab which binds to a target cell, but does not result in its death. (Thus the Fc is not part of the MOA)
3. Ab which binds to a soluble receptor.

The paper includes a table containing 27 commercial Ab products, 5 are class I, 15 class II and 7 class III.

The class I molecules are considered bifunctional and therefore may have inherently more difficult potency assay development. The two most common effector functions for Class I products are the activation of either the Antibody Dependent Cell Mediated Cytotoxicity (ADCC) or the Complement Dependent Cytotoxicity (CDC) systems.

Traditionally CDC assays are easier to develop, and more precise. One regulatory document suggested that the potency assay for rituximab had 97-102% accuracy, with an overall % RSD = 7%. (The largest contribution to this error was found to be well-to-well variability.)

The ADCC assay, has traditionally been a more difficult assay to develop, as depending on which specific Fc receptor is required on the effector cell, it may be a primary cell-based assay. Primary cells are those which cannot be maintained in tissue culture and must be obtained from donors. To add complexity, there is a great deal of receptor heterogeneity among donors.

Initially many sponsors attempted to convince the FDA and other regulators that the ADCC assay should not be part of the quality control (QC) strategy. However, this had limited success for Class 1 molecules. Thus many firms are now pursuing alternative approaches such as developing two binding assays, one for the Fab and another for the Fc portion of the molecule. Also commercial vendors are developing cell lines which express various Fc receptors. This removes the need for primary cells and improves ADCC assay precision.

The advent of bioSimilar mAb products has been driving the interest in mAb potency assays. This is not surprising when one looks at the predicted market numbers for top pharmaceutical products and realizes that six of the top 10 products are antibody products. (See page 3 this issue.)

Because potency assays are often difficult and expensive to develop (especially animal based bioassays) sponsors wait too long to start developing them or drop critical methods too soon. Clinical holds because of potency assays can happen as early as Phase 1, especially if a company has not shared their plans and development progress with the agency.

Another regulatory problem can occur with bioassays at BLA time. If a company has not tested pivotal clinical batches with the appropriate bioassay, the FDA will often not accept the clinical data from that trial—as there is not proof it has the typical potency claimed for the proposed commercial material. This can be deadly as often firms do not find this out until well past the shelf-life of the pivotal lot.

Potency assays vary in their underlying technology, their design and their complexity, however what doesn’t appear to change is their importance in the industry.

Laureen E. Little, PhD is a consultant with over 25 years of experience consulting in potency assays. She can be contacted at: Biotech@ix.netcom.com or 951-659-1957
Does anyone still doubt that biotechnology is an important part of the pharmaceutical industry? Certainly not if they have been looking at recent sales predictions. Recent industry watch-dogs are predicting that 7 of the top 10 pharmaceutical products in terms of sales will be biotech product. Six of the ten are listed below with some available potency information. (The seventh product is an insulin recombinant product which doesn’t have a potency assay.)

<table>
<thead>
<tr>
<th>Rank</th>
<th>Product</th>
<th>Technology</th>
<th>MOA</th>
<th>WW sales ($m)</th>
<th>Available Information about the Potency Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Avastin</td>
<td>Monoclonal antibody Class III</td>
<td>Anti-VEGF</td>
<td>9,232</td>
<td>The potency assay carried out is an anti-proliferation bioassay based on the ability of bevacizumab to inhibit rhVEGF-induced proliferation of Human Umbilical Vein Endothelial Cells (HUVEC). It is performed in microtitre plates and the relative number of viable cells, proportional to inhibition of rhVEGF-induced HUVEC proliferation, is quantified by fluorescence. This assay was chosen as drug substance release test based on its sensitivity (ability to detect significant changes in the activity), robustness, precision (RSD&lt;10%) and accuracy (98-102%).</td>
</tr>
<tr>
<td>2</td>
<td>Humira</td>
<td>Monoclonal antibody Class II</td>
<td>Anti-Tumor Necrosis Factor (anti-TNF)</td>
<td>9,134</td>
<td>Commercially available bioassay is a neutralization ass which binds the activity of the Fab binding against TNF-Alpha. The cell line is usually L929, WeHi or U937 grown in the present of TNF-Alpha. A relative potency assay is established using a reference product versus the test lot by measuring cell-death at various Humira concentration.</td>
</tr>
<tr>
<td>3</td>
<td>Rituxan</td>
<td>Monoclonal antibody Class I</td>
<td>Binds the CD20 on malignant B Cells</td>
<td>7,815</td>
<td>Potency-CDC: The sponsor has validated the complement dependent cytotoxicity assay in a very detailed manner. Some of the parameters that were found to be critical were: the lot and dilution of human complement, and the galactose content on the heavy chain (and such, one of the lot release specs from IDEC is to assay for glycans). Items that were found to have very little effect on the assay include: cell passage number (the cell line used for the assay is the human B-lymphoblastoid cells, XXX cell density, cell suspension storage time, assay incubation time, PCX, BSA, tissue culture plates and the source of human complement. The sponsor in conjunction with IDEC have shown that while the binding of Rituximab to CD-20 is unaffected by the galactose content the complement dependent cytotoxicity assay is affected by the galactose content. These galactose molecules are on the heavy chain of the chimeric molecule. Below is a graph taken from the IDEC submission which shows how the number of Galactose molecule (0-2 moles/mole of heavy chain) will affect the complement dependent cytotoxicity assay giving a result of 80-150% of the maximum depending on the gal content. 97-102% accuracy, with an overall %RSD = 7%. The largest contribution to this error was found to be well-to-well variability.</td>
</tr>
<tr>
<td>4</td>
<td>Enbrel</td>
<td>Recombinant product</td>
<td>TNF receptor (human) fusion protein</td>
<td>6,583</td>
<td>Potency is determined by measuring the ability of etanercept to neutralise the TNFa-mediated growth inhibition of A375 cells. The specific activity of etanercept is 1.7 x 106 units/mg.</td>
</tr>
<tr>
<td>5</td>
<td>Herceptin</td>
<td>Monoclonal antibody Class I</td>
<td>Anti-human epidermal growth factor receptor 2 protein.</td>
<td>5,796</td>
<td>(The following came from an FDA biopotency review available at: <a href="http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm091362.pdf">http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm091362.pdf</a>) The two in vitro biological properties for Herceptin are ADCC and antiproliferation activities. The former ADCC assay is inappropriate because it requires fresh donor cells. An advantage of the latter is that cell surface binding examines both HER2 down-regulation and interruption of mitogenesis. Hence biological potency of Herceptin is ascertained by an anti-proliferative assay using [redacted] which over-express the p185 HER2 protein by about 20-fold compared to normal breast epithelial cells. This assay was able to differentiate several product variants in respect to biological activity and under the stress conditions... and consequently is used as the lot release potency test.</td>
</tr>
<tr>
<td>6</td>
<td>Remicade</td>
<td>Monoclonal antibody Class II</td>
<td>Anti-TNF</td>
<td>5,220</td>
<td></td>
</tr>
</tbody>
</table>

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FEATURE ARTICLE (REPUBLISHED FROM BQ; DECEMBER 2009)

BioAssay Conferences: Technical and Regulatory Info - All Agree that Mechanism of Action is the Key

This article was first published in December 2009, promptly after the meetings occurred. BQ routinely attends conferences and provides our readers with meeting highlights.

Biological potency assays have been the rising star of analytical methods for the last decade. Many improvements in analytical approaches were heralded during this time. Also, regulatory scrutiny increased dramatically. A number of new bioassay conferences have hit the meeting circuit providing a venue for regulatory and technical discussion.

The California Separation Science Society (CASSS), an organization well-known for their regulatory/analytical conference, WCBP, held their first bioassay conference the first week in November, on the NIH campus in Bethesda, MD. (www.casss.org) Al- though speakers were primarily industry representatives, the panels included two regulators each and a large portion of the attendees included FDA employees.

Chana Fuchs, PhD, from CDER’s Office of Biotechnology summarized the role of bioassays in the Quality Control approach stating, “Bioassays test for what the product does rather than what it is.”

A commonly-heard lament in the field is the lack of regulatory guidances on bioassays. The US Pharmacopeia (USP) (www.usp.org) has been actively rewriting the current chapter <111>: design and analysis of bioassays. Bob Singer, from Biometry Associates LLC, chairs the panel revising this chapter [BQ 13 (9)]. He outlined and updated the proposed <111> revision which now includes a suite of inter-related chapters including 1032: validation of bioassays and 1034: analysis of biological assays.

Since biological assays are more resource and time consuming than other analytical methods to develop, spon- sors worry about when to initiate their development. Some large firms insist they initiate their development early in phase 1, while others claim to wait until phase III. Making the waters even mud- dier, there is no written regulatory guid- ance and the industry has been told many things for many different types of products. Dr. Fuchs take on this issue was “For your dose escalation studies to be meaningful you have to have ade- quate evaluation of the potency.” This is in line with a common industry experience of pre-phase II clinical holds for products without adequate bioassays.

Another common question during product development is when do analytical methods have to be validated and what the standards are for methods releasing clinical material. Dr. Kathleen Clouse, CBER replied “Generally we have two levels: lot release “validated” and comparability characterization.”

Earlier in the year, Sept 30 – October 1, another bioassay conference occurred in Rome, Italy. This conference, sponsored by the non-profit association Biopharmaceutical Emerging Best Practices Association (BEBPA pronounced Beh-pah) was the second annual conference. (www.bebpa.org) It delved deeply into technical aspects, including assay design, system suitability, and the use of various statistical approaches for method development.

The buzz phrase at the CASSS conference was “Mechanism of Action” (MOA). Many speakers and attendees taking part in the discussion discussed the link between MOA and po- tency assay. The potency assay should not only reflect the mechanism of action, but if there is more than a single functional structure on the molecule the po- tency assay should reflect both functions.

A classic example are Antibody-Dependent Cellular Cytotoxicity (ADCC) assays developed to monitor the two functional areas of antibody products. The antibodies require functional Fc and CDR regions and requiring an assay which demonstrates functionality in both regions.

A case study, presented by Jens Lohr- mann, Novatis Biolgics illustrated many common issues encountered during ADCC assay development. ADCC assays are unusual in that two cell types are required; an effector cell and a target cell. The target cell, the cell with the therapeutic target, binds to the antibody variable region while the Fc region binds to the effector cell. The bi- functional binding brings together the two cells and triggers the Natural Killer cell to release cytokines such as IFN-γ, and cytotoxic granules containing perforin and granzymes that enter the tar- get cell and promote cell death.

In Dr. Lohrmann’s case study a target cell line with sufficient expression of the mAb target receptor was not available and a human embryonic kidney (HEK) line was used to express the appropriate receptor. The advantages of this approach was the availability of a well-behaved cell-line for use in the assay.

Dr. Stan Deming, Statistical Designs, introduced the use of sequential simplex optimization in the context of bioassays. This approach identifies parameter values giving optimum assay performance through a series of iterative experi- ments. It allows scientists to quickly concentrate their investigation in the region of best performance.

Additional talks focused on the more traditional use of Design of Experiment (DOE) – especially the use of the Plack- ett-Burman designs to quickly perform robustness studies.

The potency bioassay has gone from the poor step-daughter to a rising star. The analytical and statistical ap- proaches used are some of the most sophisticated in the biopharmaceutical analytical arena. Soon the physical/ chemical analytical chemists will be at- tending just to gain some new ideas.
Recent Conferences Put Bioassays in the Spotlight Assessing similarity causes controversy

Biological assays come in many forms, and are used at a variety of stages during development of a biopharmaceutical product. In the past decade scientific and regulatory scrutiny of these assays has escalated. With this escalation has come a rapid increase in the number of conferences, guidelines and recent publications covering this field.

Three recent bioassay conferences, two in the US and one in the EU, provided insights into a field as varied as the drugs it supports. The conferences included two sponsored by non-profit organizations, the United States Pharmacopeia (USP) and Biopharmaceutical Emerging Best Practices Association (BEBPA). The longest running conference in the field, heading for its 16th year, is organized by IBC.

All three conferences covered technical aspects of bioassays, albeit each with its own emphasis. The IBC conference, held May 2010 in San Francisco, focused on case studies, educational workshops and talks. This year several sessions emphasized practical statistical tools available for those tasked with hard core assay development. This included case studies using Design of Experiments (DOE) tools for robustness studies and the overall implementation of Quality by Design for bioassays.

Dr. David Lansky, presented a workshop discussing outlier analyses. He emphasized how to use dose-response curve modeling to locate and eliminate single outlier values within a dose curve. Current industry practice typically uses a simple replication scheme which compares replicates within a single concentration in the curve (e.g. a Dixon analysis).

IBC’s next annual bioassay conference will be held in San Francisco, CA, at Fisherman’s Warf on May 11-13, 2011. More information can be found at www.ibclifesciences.com/events/

BEBPA held its third annual conference in September 2010 in Barcelona, Spain. This conference, which is organized by a scientific committee with vast experience in the field and in putting together bioassay conferences, focuses on practical case studies and evolving industry practices. This year Dr. Michael Sadick, from Aptuit, led a lively evening discussion session on assay monitoring. During this session, it became apparent that current monitoring practices vary widely and the nomenclature in the field is undefined. Proposed terms included system suitability criteria, assay acceptance criteria, and quality criteria.

The group agreed there are three types of “assay” monitoring criteria:

- Those which monitor the biological aspects of the rare reagents, (cells, conjugates, etc.),
- Those tracking analytical characteristics (overall plate precision, reference material characteristics)
- And finally the individual sample criteria (e.g. similarity of the dose-response curves of the test sample versus the reference curve.)

BEBPA’s fourth annual conference will be held in Nice, France on September 28-30, 2011. Each year the conference is held in a different European city and is chosen by meeting attendees by vote during the conference. More information can be found at www.BEBPA.org.

The USP bioassay conference focused primarily on their newly released bioassay chapters. (Available at http://www.usp.org/meetings/workshops/bioassayGuidance.html) These chapters were written by a group of industry volunteers, including statisticians specializing in bioassays.

Originally, the intent of the USP bioassay working group was to provide a rewrite the USP general chapter <111>

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Our Most Popular Course

Development & Validation of Biological Potency & Other Bioassays taught by Laureen E. Little, PhD.
Course Date: November 12-13, 2012
Course Location: The Hilton at Palm Springs, California
About this Course: A highly technical course designed to speed up your development and validation of biological potency assays. Tricks of the trade, regulatory requirements and emerging trends are discussed in practical, no-nonsense terms allowing you to return to your labs with a clear vision of how to design and implement bioassays. Scientific, regulatory and statistical tools are all covered giving you a balanced working knowledge of all aspects of these critical assays.

Check out our other great courses!

Stability Studies to Determine Shelf Life of Biopharmaceuticals & Biologics
Course Date: November 12-13, 2012, taught by Thomas J. Pritchett, PhD
Course Location: The Hilton at Palm Springs, California
About This Course: A comprehensive and up-to-date course on stability testing for quality assurance and control of protein and peptide biopharmaceuticals and biologics and will prepare attendees to plan and execute effective and compliant stability programs. The guidelines of the International Conference on Harmonization (ICH) will receive special attention and coverage.

Method Development & Validation for Assessing Unwanted Immunogenicity during Clinical Trials
Course Date: November 1-2, 2012, Laureen E. Little, PhD.
Course Location: The Hilton at Palm Springs, California
About This Course: Gain technical insight and approaches to anti-drug antibody (ADA) assays at this course. It will help attendees understand how to produce critical rare reagents, format the assays for sensitivity and ruggedness, and finally highlight phase specific validation.

Global GMPs for Pharmaceuticals, Biopharmaceuticals & Biologics
Course Date: November 1-2, 2012, Thomas J. Pritchett, PhD.
Course Location: The Hilton at Palm Springs, California
About This Course: This course gives participants a thorough grounding in the Current Good Manufacturing Practices in force in the United States and Europe. Course starts with a comprehensive introduction to international CGMPs, then covers intermediate level topics such as compliance “hot spots,” deviations, out-of-specification results, risk analysis, and auditing for compliance. The final day covers such advanced topics as compliance during development, effective compliance strategies, current hot topics, predicting regulatory trends, and shortcuts for staying at the forefront of compliance knowledge.

GMP Compliance during Clinical Development of Pharmaceuticals, Biopharmaceuticals & Biologics
Course Date: November 14-15, 2012, Thomas J. Pritchett, PhD.
Course Location: The Hilton at Palm Springs, California
About This Course: The goal of the course is to give attendees the knowledge they need to achieve a Phase-of-Development-Appropriate level of Compliance for each clinical development phase. Participants will gain a thorough grounding in the Current Good Manufacturing Practices in force in the United States and Europe, and understand how to apply those regulations during clinical development of Pharmaceuticals, Biopharmaceuticals, and Biologics. Over-compliance wastes scarce and valuable company resources, while under-compliance puts the trial in danger of a clinical hole and even of product approval delays.

Analytical Comparability Studies for BioSimilars & Innovator Products; Analytical and Regulatory Aspects
Course Date: October 29-31, 2012, Laureen E. Little and Thomas J. Pritchett, PhD.
Course Location: The Hilton at Palm Springs, California
About This Course: Comparability protocols are a fact of life for BioSimilar and Innovator products alike. This course tackles the nitty-gritty about putting together a comparability protocol. The analytical requirements, statistical aspects and regulatory requirements will be addressed. Topics include: comparing to international references, content of submission, appropriate level of analytical validation for one time methods, target equivalency values and appropriate numbers of types of lots will be covered. This course is appropriate for those seeking approval of a BioSimilar, comparing Phase I/II/III materials, or planning significant manufacturing changes for an approved product.
### Category: Adverse Events, Complaints, and FDA Notification

<table>
<thead>
<tr>
<th>Cause for Recently Issued 483 Notice of Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Q) No BPDR was filed for vaccine lot which was OOS for potency during stability testing</td>
</tr>
<tr>
<td>(Q) Biologic Product Deviation Report not filed for vaccine deviation involving failure to meet potency specification at [redacted] month stability time point</td>
</tr>
<tr>
<td>(Q) Inadequate investigation into complaint of low potency</td>
</tr>
</tbody>
</table>

### Category: Analytical Methods, Sampling, In-process Controls

<table>
<thead>
<tr>
<th>Cause for Recently Issued 483 Notice of Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>(L) OOS results for potency were invalidated even though lab investigations state that no clearly-assignable laboratory error were found</td>
</tr>
<tr>
<td>• Moreover, retesting was performed and lots were released on in-specification retest data</td>
</tr>
<tr>
<td>• In addition, for one lot, an OOS result was obtained upon the first retest, but the lot was released based upon 6 subsequent retests</td>
</tr>
<tr>
<td>⇒ This retest OOS was also invalidated without justification</td>
</tr>
<tr>
<td>⇒ And, only the in-specification results were reported</td>
</tr>
<tr>
<td>• Additionally, no manufacturing failure investigations were conducted</td>
</tr>
<tr>
<td>(L) Initial potency results invalidated without laboratory investigation or justification</td>
</tr>
<tr>
<td>(L) No investigation into conflicting assay results between release potency results and:</td>
</tr>
<tr>
<td>• the results from a second subsequent test on a retain sample, performed under:</td>
</tr>
<tr>
<td>⇒ special request from the production department</td>
</tr>
<tr>
<td>• In addition, raw data from the second test is not available</td>
</tr>
<tr>
<td>(L) Potency SOP allows averaging of in- and out-of-specification results</td>
</tr>
<tr>
<td>(L) Control Procedure (CP) for performing plaque assays to measure potency are deficient:</td>
</tr>
<tr>
<td>• There is inadequate monitoring of [redacted] prior to inoculation with virus</td>
</tr>
<tr>
<td>• An insufficient number of plates are examined to provide a thorough overview of: the cell density of all plates to be used in the assay</td>
</tr>
<tr>
<td>• There is no indication in the CP of what proportion of plates should be examined, or where in the sequence of plating these should be selected, for example:</td>
</tr>
<tr>
<td>⇒ at the beginning, middle or end of the plating procedure</td>
</tr>
<tr>
<td>• Extensive cell sheet destruction due to re-feeding or plate manipulation was evident:</td>
</tr>
<tr>
<td>⇒ on multiple plates, present in the laboratory, that had been prepped and were waiting for cell counting, and:</td>
</tr>
<tr>
<td>• The procedure to re-feed the infected cell monolayer, after infection, with medium did not specify methods to reduce cell sheet disruption caused by the force of:</td>
</tr>
<tr>
<td>⇒ media addition and other factors</td>
</tr>
<tr>
<td>• In addition: the estimation procedure for voiding plaque assays is not adequate:</td>
</tr>
<tr>
<td>⇒ no distinction is made between excessive plaques at that dilution and poorly manipulated plates, which should not be routinely discarded without follow-up</td>
</tr>
<tr>
<td>• In addition, CP does not provide:</td>
</tr>
<tr>
<td>⇒ criteria to evaluate whether a stained plate is invalid</td>
</tr>
<tr>
<td>⇒ stipulation for re-training of technicians when needed</td>
</tr>
<tr>
<td>(L) The in-house potency standards are not fully characterized</td>
</tr>
<tr>
<td>(L) Potency testing not designed so as to indicate potency of the product in an adequate manner</td>
</tr>
</tbody>
</table>
## cGMPs at a Glance...continued

<table>
<thead>
<tr>
<th>Category</th>
<th>Cause for Recently Issued 483 Notice of Deficiency</th>
</tr>
</thead>
</table>
| **Analytical Methods, Sampling, In-process Controls (continued)** | (L) A high number of animals used in potency testing were found to have lymphosarcoma, and: The firm’s veterinarian indicated that this can affect test results, but Corrective action has been proposed but not implemented  
(L) Labeling of reference vaccine is inconsistent:  
- Lot numbers on labels of vials used for potency testing do not correspond to:  
  ⇒ the lot number for reference standard vaccine listed in the SOP and  
  ⇒ the lot number for reference standard recorded in the Dilution Record  
(L) No written procedure governing use of vaccine lot as potency reference standard including  
- Procedures for requesting, labeling, and transfer of vials to become standards  
- Validation/qualification of lot as reference standard |
| **Investigations, Tracking, Trending, and Corrective and Preventive Actions (CAPA)** | (Q) Deficient handling of lot which had 9 month stability OOS result for potency and was recalled:  
- Failure investigation was incomplete  
- Investigation did not use reserve sample stored under same conditions as sample which had the OOS result  
- Investigation did not reveal that the recalled lot differed in any way from similar lots which were produced and remained in distribution, and:  
  ⇒ These other lots, produced under the same conditions, were not placed on stability and monitored to expiration  
(Q) Inadequate investigation into the death of more than 10% of guinea pigs in the colony used for potency testing:  
- Most of the animals were merely noted as “found dead,” with no investigation into, or documentation of, the cause of death  
- An investigation with QA oversight was not conducted  
- Proposed CAPA were not implemented  
(Q) Investigation into deaths of animals during safety and potency testing is incomplete  
- No manufacturing investigation was conducted  
- Investigation Report did not document the incidence rate of deaths of:  
  ⇒ animals injected with the same product lot  
- This lot was released and there were adverse events involving:  
  ⇒ shaking, hypotension, tachycardia, and extreme thrombocytopenia  
(Q) Bulk Drug Substance lots which were high for potency were retested and averaged into compliance without an investigation  
(Q) Investigation into potency failure during stability testing did not include other bulk and final lots  
(Q) Responsibilities/procedures applicable to the quality control unit not fully followed, specifically:  
- Guinea pigs are used for potency testing, and Deviation Investigation Procedure was not followed in that:  
  ⇒ A Notice of Event (NOE) was not initiated for an incidence of Lymphosarcoma identified in the laboratory guinea pig colony, and  
- An investigation with QA oversight was not conducted for the event |
| **Specifications and Limits** | (Q) Vaccine lots which exceeded potency specifications were released by QA  
(Q) Several sub-potent lots were “blended off” with lots with potency at or above target values, and:  
- There is no formal documentation or justification for this blend-off process  
- This was performed at the instruction of upper management and was accepted by Quality Assurance |
| **Stability and Expiration Dating** | (L) No actions were taken when stability data showed that potency data trended downward |
**cGMPs at a Glance...continued**

<table>
<thead>
<tr>
<th>Category</th>
<th>Cause for Recently Issued 483 Notice of Deficiency</th>
</tr>
</thead>
</table>
| Training and Personnel Issues    | Training of staff to perform plaque assays to measure Varicella potency is deficient. Specifically, training of staff is inadequate to assess:  
• cell monolayer damage due to viral infection versus  
• damage due to poor manipulation of the plates  
• In addition, the Control Procedure does not provide guidelines for monitoring techniques:  
⇒ if re-training of technicians in cell culture re-feeding procedures is required  
⇒ In addition, laboratory staff were unable to adequately distinguish between “clearings” in the stained monolayers that were due to:  
⇒ large numbers of plaques, and:  
⇒ those that were cell sheet disruptions due to poor re-feeding or plate manipulation technique |

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**Biological Potency for Comparability**


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In the Literature Continued

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Cellular Therapy Potency Assays

Comparison of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process, June 2005


Chapter <111> Design and Analysis of Biological Assays. US Pharmacopeia 28, United States Pharmacopeia Convention, Inc., Rockville, MD.


Characterization of Cell/Scaffold Products, presentation at the Workshop on In Vitro Analysis of Cell/Scaffold Products, December 6, 2007, by Kimberly Benton, PhD, Deputy Director, Division of Cellular & Gene Therapies, Office of Cellular, Tissue, & Gene Therapies, CBER. FDA


International Conference on Harmonisation: Guidance for Industry: Q5E

Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process, June 2005

Summary Basis for Regulatory Action, PROVENGE (sipuleucel-T), Thomas Finn, PhD, Chair of the FDA/CBER Review Committee, May 1, 2010

Prochymal, The dynamics of a new age in medicine, presentation at the February 9, 2006 meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee by Alla Danilkovitch, PhD, Senior Scientist, Prochymal, Osiris Therapeutics Inc. Slides and transcript available at: http://www.fda.gov/ohrms/dockets/ac/accber06.html#CellularTissueGeneTherapies


Final Questions for Committee Discussion of BLA 125400, Cellular, Tissue and Gene Therapies Advisory Committee Meeting, November 17, 2011, available at http://www.fda.gov/AdvisoryCommittees/
In the Literature Continued

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Comparing Potency Assays


Data Analysis and Statistics


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Gene and Nucleic Acid Therapy Potency


Physicochemical methods for Potency

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rDNA Protein Therapeutic Potency


Reassessing, Reducing, Replacing, Refining Potency Methods
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Epub 2006 Jan 20. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17012901

Reference Materials & Standards


Calibration of the Ph. Eur. Biological Reference Preparation (BRP) for tetra-

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Robustness Testing of Potency Methods

Stability Determination using Potency


Toxin Potency


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Vaccine Potency


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Validation of Potency Methods
A practical approach for the validation of sterility, endotoxin and potency testing of bone marrow mononucleated cells used in cardiac regeneration in compliance with good manufacturing practice. Soncin S, et al. J Transl Med. 2009 Sep 8;7:78. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/19737416


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