BioQuality™

Bioassay Special Edition 2012

Bioassay Special Edition 2012

ISSN: 1090-2759

BioQuality Goes Weekly!

We are now sending you weekly BioQuality update editions three times each month, in addition to the monthly edition you are now receiving. There will be no additional charge for this enhanced service. We just want to be sure you get the knowledge you need in a timely fashion.

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 (forerunner of CBER), developed a potency assay for the pertussis vaccine and correlated potency with human efficacy. It quickly became a standard release assay.

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% (LD_{50}) assay. The LD_{50} 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®).

Biological potency has long been considered a critical quality attribute, (CQA), in fact longer than the snappy CQA acronym.

It is the latter method, the direct

(Continued on page 2)

BioQuality is published twelve times a year , PO Box 7087, Citrus Heights, CA 95621. Annual subscription is 695.00.

Biological Potency Assay Continued...

(Continued from page 1)

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 poster-child for this trend are the numerous monoclonal antibody products.

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 many 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.)

Rank	Product	Technology	MOA	WW sales ($m)	Available Information about the Potency Assay
1	Avastin	Monoclonal antibody Class III	Anti-VEGF	9,232	The potency assay carried out is an anti-proliferation bioassay based upon the ability of bevacizumab to inhibit rhVEGF-induced proliferation of Human Umbilical Vein Endothelial Cells (HUVEC). It is performed in micro-titre 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<10%) and accuracy (98-102%).
2	Humira	Monoclonal antibody Class II	Anti-Tumor Necrosis Factor (anti-TNF)	9,134	Commercially available bioassay is a neutralization ass which binds the activity of the Fab binding agains 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.
3	Rituxan	Monoclonal antibody Class I	Binds the CD20 on malignant B Cells	7,815	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 wer: the lot and dilution of human complement, and the galactose content on the heavy chain (and s such, one of the lot release specs from IDEC is to assay for glycan). 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 supsension 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 cytoxtoxicity assay is affected by the galactose content. Thes 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.
4	Enbrel	Recombinant product	TNF receptor (human) fusion protein	6,583	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.
6	Herceptin	Monoclonal antibody Class I	Anti-human epidermal growth factor receptor 2 protein.	5,796	(The following came from an FDA biopotency review available at: http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm091362.pdf) The two in vitro biological properties for Herceptin are ADCC and antiproliferation activities. The former ADCC assay is inappropriate because it requires fres 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.
9	Remicade	Monoclonal antibody Class II	Anti-TNF	5,220	

Bioassay Special Edition 2012

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) Although speakers were primarily industry representatives, the panels included two regulators each and a large portion of the attendees included FDA employees.

"Bioassays test for what the product does rather than what it is."
Chana Fuchs, PhD, CDER

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 Pharmacoepia (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, sponsors 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 muddier, there is no written regulatory guidance 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 adequate 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."

"Generally we have two levels: lot release "validated" and comparability characterization."
Kathleen Clouse, CBER

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 potency 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 potency 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 Lohrmann, 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 portion while the Fc region binds to the effector cell. The bifunctional 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 target 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 experiments. 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 Plackett-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 approaches used are some of the most sophisticated in the biopharmaceutical analytical arena. Soon the physical/chemical analytical chemists will be attending just to gain some new ideas.

FEATURE ARTICLE (REPUBLISHED FROM BQ; OCTOBER 2010)

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 Pharmacoepia (USP) and Biopharmaceutical Emerging Best Practices Association (BEBPA). The longest running conference in the field, heading for its 16th year, is organized by IBC.

Three recent bioassay conferences, two in the US and one in the EU, provided insights into a field as varied as the drugs they support

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/topics.xml

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.)

During this session, it became apparent that not only do the monitoring practices vary widely, the nomenclature in the field is currently undefined

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> *Design and Analysis of Biological Assays.* This initial draft rewrite was published in *Pharmacopeial Forum 34(3)* [March April, 2008]. The statistical expert committee and the USP provided numerous presentations at all the bioassay conferences, and made the chapter available free of charge on the web. This allowed a larger audience to participate during the comment period.

The chapter contained recommendations to implement a new approach to determining similarity of test versus reference sample dose-response curves, often referred to as equivalence testing. It also recommended against the use of weighting in specific aspects of the data analysis.

These recommendations were greeted with some concern by the bioassay community at large, especially as the chapter contained a strongly-worded caution against using a difference approach to assessing similarity.

Difference testing is currently recommended in the European Pharmacoepia (EP) and is thus part of many monograph methods. Therefore there would be legal problems with changing the approach to determining similarity in Europe. Three digit chapters in the USP also have a legal status in the US—thus a strongly worded caution against using difference testing in a three digit chapter would have forced a lack of harmony in many bioassays.

The newest rewrites includes putting all of the more controversial material in 4-digit chapters, which means they are general guidance chapters and no longer have legal status. The original General Chapter <111> was put it into a suite of the following 3 chapters:

- <1032>*Design and Development of Bioassays*
- <1033>*Biological Assay Validation*
- <1034>*Analysis of Biological Assays*

Slides from the most recent conference are available at: http://www.usp.org/meetings/workshops/2010Bioassay.html

The next bioassay conference, is scheduled to be part of a larger USP analytical conference, to be held in Seattle, WA in October 2011. See www.usp.org for more information.

BioQuality Presents: FasTrain Destination Courses

Register at: www.fastraincourses.com
Courses start at less than $690 per day

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.

Note for This Month's 483s:

The letter(s) in brackets preceding each observation represent the system category of the deficiency. Q = Quality System; FE = Facilities and Equipment System; M = Materials System; Pr = Production System; PL = Packaging and Labeling System; L = Laboratory Controls System

Category	Cause for Recently Issued 483 Notice of Deficiency
Adverse Events, Complaints, and FDA Notification	(Q) No BPDR was filed for vaccine lot which was OOS for potency during stability testing (Q) Biologic Product Deviation Report not filed for vaccine deviation involving failure to meet potency specification at [redacted] month stability time point (Q) Inadequate investigation into complaint of low potency
Analytical Methods, Sampling, In-process Controls	(L) OOS results for potency were invalidated even though lab investigations state that no clearly-assignable laboratory error were found • Moreover, retesting was performed and lots were released on in-specification retest data • In addition, for one lot, an OOS result was obtained upon the first retest, but the lot was released based upon 6 subsequent retests ⇒ This retest OOS was also invalidated without justification ⇒ And, only the in-specification results were reported • Additionally, no manufacturing failure investigations were conducted (L) Initial potency results invalidated without laboratory investigation or justification (L) No investigation into conflicting assay results between release potency results and: • the results from a second subsequent test on a retain sample, performed under: ⇒ special request from the production department • In addition, raw data from the second test is not available (L) Potency SOP allows averaging of in- and out-of-specification results (L) Control Procedure (CP) for performing plaque assays to measure potency are deficient: • There is inadequate monitoring of [redacted] prior to inoculation with virus • 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 • 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: ⇒ at the beginning, middle or end of the plating procedure • Extensive cell sheet destruction due to re-feeding or plate manipulation was evident: ⇒ on multiple plates, present in the laboratory, that had been prepped and were waiting for cell counting, and: • 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: ⇒ media addition and other factors • In addition: the estimation procedure for voiding plaque assays is not adequate: ⇒ no distinction is made between excessive plaques at that dilution and poorly ⇒ manipulated plates, which should not be routinely discarded without follow-up • In addition, CP does not provide: ⇒ criteria to evaluate whether a stained plate is invalid ⇒ stipulation for re-training of technicians when needed (L) The in-house potency standards are not fully characterized (L) Potency testing not designed so as to indicate potency of the product in an adequate manner

cGMPs at a Glance...continued

Category	Cause for Recently Issued 483 Notice of Deficiency
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

Category	Cause for Recently Issued 483 Notice of Deficiency
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

Biological Potency Assays: The Scientific Literature

date of most recent search (searched papers in the last 10 years): April 21-22, 2012

Categories

General Reviews

Quality control and analytical techniques for biopharmaceuticals. Robinson CJ, Jones C. Bioanalysis. 2011 Jan;3(1):81-95. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21175369

Evaluation of standard and state of the art analytical technology-bioassays. Indelicato SR, et al. Dev Biol (Basel). 2005;122:103-14.To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/16375255

Measurement of cytokines by bioassays: theory and application. Meager A. Methods. 2006 Apr;38(4):237-52. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/16481200

Increased consistency and efficiency in routine potency testing by bioassay with direct use of cryopreserved (ready-to-plate) cells. TerWee JA, et al. J Immunol Methods. 2011 Jul 29;370(1-2):65-74. Epub 2011 Jun 12. 21664360

Antibody and Antiserum and Immune Globulin Potency

Immunopurification and mass spectrometric quantification of the active form of a chimeric therapeutic antibody in human serum. Dubois M, et al. Anal Chem. 2008 Mar 1;80(5):1737-45. Epub 2008 Jan 29. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/18225864

A new in-vitro agglutination technique for potency estimation of antisnake venom serum (ASVS). Chander R, et al. Toxicon. 2006 Dec 15;48 (8):1011-7. Epub 2006 Aug 17. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/16982078

Preclinical manufacture of anti-HER2 liposome-inserting, scFv-PEG-lipid conjugate. 2. Conjugate micelle identity, purity, stability, and potency analysis. Nellis DF, et al. Biotechnol Prog. 2005 Jan-Feb;21(1):221-32. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15903261

A simple and rapid Hepatitis A Virus (HAV) titration assay based on antibiotic resistance of infected cells: evaluation of the HAV neutralization potency of human immune globulin preparations. Konduru K, et al. Virol J. 2008 Dec 18;5:155. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/19094229

Standardization and validation of Vero cell assay for potency estimation of diphtheria antitoxin serum. Kumar S, et al. Biologicals. 2009 Oct;37 (5):297-305. Epub 2009 Jun 18. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/19540135

Collaborative study for the validation of alternative in vitro potency assays for human tetanus immunoglobulin. Gross S, et al. Pharmeur Bio Sci Notes. 2009 Oct;2009(1):11-25. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20144449

Biological Potency for Comparability

Bioanalytical considerations in the comparability assessment of biotherapeutics. Lee JW, et al. Bioanalysis. 2011 Mar;3(6):613-22. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21417731

An assessment of biological potency and molecular characteristics of different innovator and noninnovator interferon-beta products. Meager A, et al. J Interferon Cytokine Res. 2011 Apr;31(4):383-92. Epub 2010 Dec 7. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21138379

Measuring the potency labelling of onabotulinumtoxinA (Botox(®)) and incobotulinumtoxinA (Xeomin (®)) in an LD50 assay. Dressler D, et al. J Neural Transm. 2012 Jan;119(1):13-5. Epub 2011 Oct 5. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21971766

Bioactivity determination of native and variant forms of therapeutic interferons. Larocque L, et al. J Biomed Biotechnol. 2011;2011:174615. Epub 2011 Mar 3. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21403871

A multi-centre collaborative study on the potency estimation of ReFacto. Hubbard AR, et al. Thromb Haemost. 2003 Dec;90(6):1088-93. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/14652641

(Continued on page 11)

In the Literature Continued

(Continued from page 10)

Cellular Therapy Potency Assays

Guidelines for the development and validation of new potency assays for the evaluation of umbilical cord blood. Spellman S, et al. Cytotherapy. 2011 Aug;13(7):848-55. Epub 2011 Mar 30. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21449685

Potency analysis of cellular therapies: the emerging role of molecular assays. Stroncek DF, et al. J Transl Med. 2007 May 30;5:24. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17537259

Validation of the COSTIM bioassay for dendritic cell potency. Shankar G, et al. J Pharm Biomed Anal. 2004 Oct 29;36(2):285-94. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15496321

Development of a potency assay for human dendritic cells: IL-12p70 production. Butterfield LH, et al. J Immunother. 2008 Jan;31(1):89-100. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/18157016

Guidance for Industry, Potency Tests for Cellular and Gene Therapy Products, U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research January 2011

Guidance for Reviewers: Instructions and Template for Chemistry, Manufacturing, and Control (CMC) Reviewers of Human Somatic Cell Therapy Investigational New Drug Applications (INDs), U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research, April 2008

International Conference on Harmonisation: Guidance for Industry: Q5E Comparability of Biotechnological/ Biological Products Subject to Changes in Their Manufacturing Process, June 2005

Potency Measurements for Cellular and Gene Therapy Products, Cellular, Tissue and Gene Therapies Advisory Committee (CTGTAC) Meeting. Gaithersburg Hilton, February 9, 2006. Slides and transcript available at: http://www.fda.gov/ohrms/dockets/ac/cber06.html#CellularTissueGeneTherapies

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

Cellular Therapy Products, at 2006 Advisory Committee meeting on stability testing (presenter not listed). Available at http://www.fda.gov/downloads/BiologicsBloodVaccines/InternationalActivities/UCM273197.pdf

Guidance for FDA Reviewers and Sponsors, Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Application (INDs), U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research, 2008

Characterization of Cell/Scaffold Products, presentation at the Workshop on In VitroAnalysis 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

Potency Testing for an Autologous Cellular Immunotherapy, presentation at the February 9, 2006 meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee by Nicole Provost, PhD, VP Product Development, Dendreon Corporation, Slides and transcript available at: http://www.fda.gov/ohrms/dockets/accber06.html#CellularTissueGeneTherapies

Summary Basis for Regulatory Action, PROVENVGE (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/accber06.html#CellularTissueGeneTherapies

Potency Assays for Adenovector-Based Therapies, presentation at the February 9, 2006 meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee by Brian T. Butman, Ph.D., Senior Vice President Vector Operations, GenVec Inc., Transcript available at: http://www.fda.gov/ohrms/dockets/ac/06/transcripts/2006-4205T1.pdf

Potency Assays for Recombinant Viral Vaccines for Cancer Therapy, presentation at the February 9, 2006 meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee by Kelledy manson, M.T., M.S., Senior Director Bioanalytical Development, Therion Biologics Corporation, Transcript available at: http://www.fda.gov/ohrms/dockets/ac/06/transcripts/2006-4205T1.pdf

February 9, 2006 meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee, Transcript available at: http://www.fda.gov/ohrms/dockets/ac/06/transcripts/2006-4205T1.pdf

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/

(Continued on page 12)

In the Literature Continued

(Continued from page 11)
CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/CellularTissueandGeneTherapiesAdvisoryCommittee/ucm280225.htm

Sponsor-supplied Briefing Document for Cellular, Tissue and Gene Therapies Advisory Committee Meeting, November 17, 2011, available at 24. FDA-supplied Briefing Document for Cellular, Tissue and Gene Therapies Advisory Committee Meeting, November 17, 2011, available at http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/CellularTissueandGeneTherapiesAdvisoryCommittee/ucm279851.htm

FDA Briefing Document for Cellular, Tissue and Gene Therapies Advisory Committee Meeting, November 17, 2011, available at http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/CellularTissueandGeneTherapiesAdvisoryCommittee/ucm279851.htm

Presentation by Terrig Thomas, Ph.D., Division of Cellular and Gene Therapies, Office of Cellular, Tissue and Gene Therapies, CBER FDA, at the October 9, 2009 meeting of the Cellular, Tissue and Gene Therapy Advisory Committee, available at www.fda.gov/downloads/.../UCM226778.ppt

FDA Briefing Document for Cellular, Tissue and Gene Therapies Advisory Committee Meeting, September 22, 2011, available at http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/CellularTissueandGeneTherapiesAdvisoryCommittee/UCM272535.pdf

Comparing Potency Assays

Botulinum type A toxin neutralisation by specific IgG and its fragments: a comparison of mouse systemic toxicity and local flaccid paralysis assays. Jones RG,et al. Toxicon. 2006 Sep 1;48 (3):246-54. Epub 2006 Jun 14. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/16870221

Quality assurance of C. perfringens epsilon toxoid vaccines--ELISA versus mouse neutralisation test. Rosskopf-Streicher U, et al. ALTEX. 2004;21 Suppl 3:65-9. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15057410

Comparison of the primary rat spinal cord cell (RSC) assay and the mouse bioassay for botulinum neurotoxin type A potency determination. Pellett S, et al. J Pharmacol Toxicol Methods. 2010 May-Jun;61(3):304-10. Epub 2010 Jan 25. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20100585

Data Analysis and Statistics

Equivalence testing for parallelism in the four-parameter logistic model. Jonkman JN, Sidik K. J Biopharm Stat. 2009 Sep;19(5):818-37. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20183446

Measuring parallelism, linearity, and relative potency in bioassay and immunoassay data. Gottschalk PG, Dunn JR. J Biopharm Stat. 2005;15(3):437-63. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15920890

Development of parallel line analysis criteria for recombinant adenovirus potency assay and definition of a unit of potency. Ogawa Y, et al. PDA J Pharm Sci Technol. 2007 May-Jun;61 (3):183-93. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17722485

Statistical evaluation of numbers of animals to be used in vaccine potency testing: a practical approach. Akkermans AM, Hendriksen CF. Dev Biol Stand. 1999;101:255-60. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/10566799

ECVAM's contributions to the implementation of the Three Rs in the production and quality control of biologicals. Halder M, et al. Altern Lab Anim. 2002 Jan-Feb;30(1):93-108. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/11827574

Gene and Nucleic Acid Therapy Potency

Optimization of transfection conditions and analysis of siRNA potency using real-time PCR. Cheng A, et al. Methods Mol Biol. 2011;764:199-213. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21748642

A potency assay for a replication incompetent adenovirus type 5 carrying a human fgf-4 gene. Fawaz FS, et al. Anal Biochem. 2005 Jul 1;342(1):34-44. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15958178

A receptor-binding-based bioassay to determine the potency of a plasmid biopharmaceutical encoding VEGF-C. Waerner T, et al. Anal Bioanal Chem. 2007 Dec;389(7-8):2109-13. Epub 2007 Oct 24. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17957358

Determination of silencing potency of synthetic and RNase III-generated siRNA using a secreted luciferase assay. Morlighem JE, et al. Biotechniques. 2007 May;42(5):599-600, 602, 604-6. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17515198

Physicochemical methods for Potency

(Continued on page 13)

In the Literature Continued

(Continued from page 12)

An alternative to animal testing in the quality control of erythropoietin. Zimmermann H, et al. Pharmeur Bio Sci Notes. 2011 Jun;2011(1):66-80. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21619857

Physicochemical methods for predicting the biological potency of recombinant follicle stimulating hormone: an international collaborative study of isoelectric focusing and capillary zone electrophoresis. Storring PL, et al. Biologicals. 2002 Sep;30(3):217-34. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12217346

A pilot study on potency determination of human follicle-stimulating hormone: a comparison between reversed-phase high-performance liquid chromatography method and the in vivo bioassay. Almeida BE, et al. J Pharm Biomed Anal. 2011 Mar 25;54 (4):681-6. Epub 2010 Oct 30. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21093191

Correlation of liquid chromatographic and biological assay for potency assessment of filgrastim and related impurities. Skrlin A, et al. J Pharm Biomed Anal. 2010 Nov 2;53(3):262-8. Epub 2010 Feb 12. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20478679

Correlations between in vitro potency of polyethylene glycol-protein conjugates and their chromatographic behavior. Caserman S, et al. Anal Biochem. 2009 Jun 1;389(1):27-31. Epub 2009 Mar 21. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/19306838

rDNA Protein Therapeutic Potency

Development of an in vitro potency assay for therapeutic TGFbeta antagonists: the A549 cell bioassay. Rapoza ML, et al. J Immunol Methods. 2006 Oct 20;316(1-2):18-26. Epub 2006 Aug 18. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17010369

Development of an in vitro potency bioassay for therapeutic IL-13 antagonists: the A-549 cell bioassay. Miller R, et al. J Immunol Methods. 2008 May 20;334(1-2):134-41. Epub 2008 Mar 7. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/18355834

Measurement of sialic acid content is insufficient to assess bioactivity of recombinant human erythropoietin. Yanagihara S, et al. Biol Pharm Bull. 2010;33(9):1596-9. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20823580

A quantitative method for measuring the antitumor potency of recombinant human endostatin in vivo. Xu YF, et al. Eur J Pharmacol. 2007 Jun 14;564(1-3):1-6. Epub 2007 Feb 15. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17346697

Detection and consequences of recombinant protein isoforms: implications for biological potency. Federici MM, et al. Dev Biol (Basel). 2003;113:53-7; discussion 113-4. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/14620852

Development of a STAT5 phosphorylation assay as a rapid bioassay to assess interleukin-7 potency. Zumpe C, et al. Curr Pharm Biotechnol. 2011 Oct;12(10):1580-8. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21542800

Potency determination of recombinant IFN-alpha based on phosphorylated STAT1 using flow cytometry. de Oliveira ER, et al. J Immunol Methods. 2012 Jan 31;375(1-2):271-5. Epub 2011 Nov 12. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/22115721

Reassessing, Reducing , Replacing, Refining Potency Methods

Three Rs potential in the development and quality control of immunobiologicals. Halder M. ALTEX. 2001;18 Suppl 1:13-47. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/11854853

Three Rs potential in the development and quality control of pharmaceuticals. Hartung T. ALTEX. 2001;18 Suppl 1:3-13.

Evaluation of two serological methods for potency testing of whole cell pertussis vaccines. von Hunolstein C, et al. Pharmeuropa Bio. 2008 Dec;2008 (1):7-18. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/19220977

Replacement, reduction and refinement alternatives to animal use in vaccine potency measurement. Hendriksen CF. Expert Rev Vaccines. 2009 Mar;8(3):313-22. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/19249973

Towards eliminating the use of animals for regulatory required vaccine quality control. Hendriksen CF. ALTEX. 2006;23(3):187-90. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17086348

The consistency approach for quality control of vaccines - a strategy to improve quality control and implement 3Rs. De Mattia F, et al. Biologicals. 2011 Jan;39(1):59-65. Epub 2011 Jan 31. To see the abstract and a link to obtain this

(Continued on page 14)

In the Literature Continued

(Continued from page 13)
paper: http://www.ncbi.nlm.nih.gov/pubmed/21277791

A short history of the use of animals in vaccine development and quality control. Hendriksen CF. Dev Biol Stand. 1996;86:3-10. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/8785959

Quality assurance of C. perfringens epsilon toxoid vaccines--ELISA versus mouse neutralisation test. Rosskopf-Streicher U, et al. ALTEX. 2004;21 Suppl 3:65-9. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15057410

Refinement, reduction, and replacement of animal use for regulatory testing: current best scientific practices for the evaluation of safety and potency of biologicals. Hendriksen CF. ILAR J. 2002;43 Suppl:S43-8. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12388851

Reduction of animal use in human vaccine quality control: opportunities and problems. Metz B, et al. Vaccine. 2002 Jun 7;20(19-20):2411-30. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12057596

Consistency testing of diphtheria and tetanus to replace potency testing for lot release. Kreeftenberg JG. Dev Biol (Basel). 2002;111:291-8. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12678252

Do we need a "Chair of alternative methods", and where? Wendel A. ALTEX. 2002;19(2):64-8. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12098011

Adoption of three Rs alternatives for regulatory testing of vaccines in the developing world: possibilities and barriers. Di Fabior JL, et al. Dev Biol (Basel). 2002;111:195-8. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12678241

ECVAM's role in the implementation of the Three Rs concept in the field of biologicals. Hendriksen C, et al. Altern Lab Anim. 2002 Dec;30 Suppl 2:41-6. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12513650

Regulatory processes and three Rs alternatives. Milstien J. Dev Biol (Basel). 2002;111:15-9. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12678220

The consistency approach for the quality control of vaccines. Hendriksen C, et al. Biologicals. 2008 Jan;36 (1):73-7. Epub 2007 Sep 24. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17892948

Collaborative study on a Guinea pig serological method for the assay of acellular pertussis vaccines. Winsnes R, et al. Pharmeur Bio Sci Notes. 2009 Oct;2009(1):27-40. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20144450

Application of the Three Rs to challenge assays used in vaccine testing: tenth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Jennings M, et al. Biologicals. 2010 Nov;38(6):684-95. Epub 2010 Aug 17. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20724180

Tuberculin purified protein derivative (PPD) immunoassay as an in vitro alternative assay for identity and confirmation of potency. Ho MM, et al. Hum Vaccin. 2006 Jan-Feb;2(1):29-33. 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

Establishment of a biological reference preparation for hepatitis A vaccine (inactivated, non-adsorbed). Stalder J, et al. Pharmeur Bio Sci Notes. 2010 Apr;2010(1):15-29. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20223187

Biological standardization of human interferon beta: establishment of a replacement world health organization international biological standard for human glycosylated interferon beta. Meager A, Das RG. J Immunol Methods. 2005 Nov 30;306(1-2):1-15. Epub 2005 Sep 26. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/16226271

Calibration of European pharmacopoeia biological reference preparation for diphtheria vaccine (adsorbed) batch 4. Stickings P, et al. Pharmeur Bio Sci Notes. 2009 Oct;2009(1):1-9. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20144448

Calibration of human coagulation factor VII concentrate Ph. Eur. BRP batch 2. Dodt J, et al. Pharmeur Bio Sci Notes. 2010 Apr;2010(1):31-8. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20223188

Calibration of human coagulation factor VIII concentrate Ph. Eur. BRP Batch 4 for use in potency assays. Raut S, et al. Pharmeur Bio Sci Notes. 2010 Oct;2010(2):1-29. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21144486

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

(Continued on page 15)

In the Literature Continued

(Continued from page 14)

nus vaccine (adsorbed) batch 3. Tierney R, et al. Pharmeur Bio Sci Notes. 2011 Jun;2011(1):1-26. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21619853

Calibration of the Ph. Eur. BRP Batch 3/Mega 2 (US/FDA) standard for human coagulation factor VIII concentrate for use in the potency assay. Kirschbaum N, et al. Pharmeuropa Spec Issue Biol. 2002 Jun;2002(1):31-64. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12448031

Calibration of the second International Standard for hepatitis B immunoglobulin in an international collaborative study. Ferguson M, et al. Vox Sang. 2010 Jul 1;99(1):77-84. Epub 2010 Feb 25. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20202182

Establishment of the European Pharmacopoeia Biological Reference Preparation (Ph. Eur. BRP) for hepatitis A vaccine type B (Aventis Pasteur) batch 2. Buchheit KH, Daas A. Pharmeuropa Spec Issue Biol. 2002 Jun;2002(1):95-108. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12448033

Establishment of European Pharmacopoeia (Ph. Eur.) Biological Reference Preparations (BRP) batch 2 for rDNA hepatitis B vaccine (method A and B). Dobbelaer R, et al. Pharmeuropa Bio. 2004 Jan;2003(2):77-90. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/14960264

Establishment of the human coagulation factor VII concentrate European Pharmacopoeia biological reference preparation batch 1. Dodt J, et al. Pharmeuropa Bio. 2006 Nov;2006(1):15-22. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17270128

WHO Expert Committee on biological standardization. World Health Organization. World Health Organ Tech Rep Ser. 2011;(962):1-206. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/22397172

Collaborative study for establishment of a global standard for the potency assay of human anti-D immunoglobulin. Thorpe SJ, et al. Pharmeuropa Bio. 2004 Jan;2003(2):9-26. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/14960260

Collaborative study for the establishment of a European Phamacopoeia Biological reference preparation for Bordetella pertussis mouse antiserum for serological potency testing of acellular pertussis vaccines. Poirier B, et al. Biologicals. 2003 Mar;31(1):25-38. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12623057

Collaborative study for the establishment of the Ph. Eur. BRP for oral poliomyelitis vaccine (OPV) Batch 3 for use in the potency assay. Buchheit KH, et al. Pharmeuropa Spec Issue Biol. 2002 Jun;2002(1):67-91. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12448032

Robustness Testing of Potency Methods

Robustness testing of live attenuated rubella vaccine potency assay using fractional factorial design of experiments. Kutle L, et al. Vaccine. 2010 Jul 26;28(33):5497-502. Epub 2010 May 14. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20472023

Stability Determination using Potency

Stability of recombinant human thyrotropin potency based on bioassay in FRTL-5 cells. Lin R, et al. Thyroid. 2010 Oct;20(10) :1139-43. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20615135

Stability and potency of the Plasmodium falciparum MSP1-19/AMA-1(III) chimeric vaccine candidate with Montanide ISA720 adjuvant. Xue X, et al. Vaccine. 2010 Apr 19;28 (18):3152-8. Epub 2010 Mar 1. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20197139

Thermal stability of the WHO international standard of interferon alpha 2b (IFN-alpha 2b): application of new reporter gene assay for IFN-alpha 2b potency determinations. Caserman S, et al. J Immunol Methods. 2007 Jan 30;319(1-2):6-12. Epub 2006 Dec 12. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17196611

Toxin Potency

Potency evaluation of a formulated drug product containing 150-kd botulinum neurotoxin type A. Hunt T, Clarke K. Clin Neuropharmacol. 2009 Jan-Feb;32(1):28-31. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/18978494

Experimental conditions substantially influence botulinum toxin potency testing. Mander G, et al. Clin Neuropharmacol. 2009 Jul-Aug;32 (4):234; author reply 235. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/19644233

A functional dual-coated (FDC) microtiter plate method to replace the botulinum toxin LD(50) test. Liu YY, et al. Anal Biochem. 2012 Mar 6;425 (1):28-35. [Epub ahead of print] To see the abstract and a link to obtain this

(Continued on page 16)

In the Literature Continued

(Continued from page 15)

paper: http://www.ncbi.nlm.nih.gov/pubmed/22406430

Vaccine Potency

Evaluation of allergen vaccine potency. Esch RE. Curr Allergy Asthma Rep. 2006 Sep;6(5):402-6. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/16899202

Development of a rapid biological assay for determination of potency of Newcastle disease vaccine (strain I-2). Wambura PN, et al. Trop Anim Health Prod. 2006;38(6):463-6. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17243473

Development of an edema factor-mediated cAMP-induction bioassay for detecting antibody-mediated neutralization of anthrax protective antigen. Zmuda JF, et al. J Immunol Methods. 2005 Mar;298(1-2):47-60. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15847796

Development of an in vitro potency assay for anti-anthrax lethal toxin neutralizing antibodies. Whiting G, et al. Toxins (Basel). 2012 Jan;4(1):28-41. Epub 2012 Jan 19. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/22347621

Potency assay design for adjuvanted recombinant proteins as malaria vaccines. Giersing BK,et al. Vaccine. 2006 May 15;24(20):4264-70. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/16767804

Potency assays for therapeutic live whole cell cancer vaccines. Petricciani J, et al. Biologicals. 2007 Apr;35 (2):107-13. Epub 2006 Aug 1. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/16882459

Potency estimation of measles, mumps and rubella trivalent vaccines with quantitative PCR infectivity assay. Schalk JA, et al. Biologicals. 2005 Jun;33(2):71-9. Epub 2005 Apr 7. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15939284

Potency testing for the experimental Na-GST-1 hookworm vaccine. Jariwala AR, et al. Expert Rev Vaccines. 2010 Oct;9(10):1219-30. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20923271

Tail scarification with Vaccinia virus Lister as a model for evaluation of smallpox vaccine potency in mice. Melamed S, et al. Vaccine. 2007 Nov 7;25(45):7743-53. Epub 2007 Sep 29. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17928110

A TaqMan reverse transcription polymerase chain reaction (RT-PCR) in vitro potency assay for plasmid-based vaccine products. Mahajan R, et al. Mol Biotechnol. 2008 Sep;40 (1):47-57. Epub 2008 Mar 26. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/18365771

WHO Working Group meeting on standardization of acellular pertussis vaccines: Potency assay Beijing, China, 7-9 November 2007. Knezevic I, et al. Vaccine. 2008 Jul 29;26(32):3960-8. Epub 2008 May 15. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/18514369

An alternative method for preparation of pandemic influenza strain-specific antibody for vaccine potency determination. Schmeisser F, et al. Vaccine. 2010 Mar 11;28 (12):2442-9. Epub 2010 Jan 12. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20074687

Assessing vaccine potency using TCRmimic antibodies. Neethling FA, et al. Vaccine. 2008 Jun 13;26 (25):3092-102. Epub 2008 Feb 25. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/18353510

Attempt to curtail the observation period of mice in the tetanus vaccine potency tests. Fukuda T, et al. Jpn J Infect Dis. 2004 Dec;57(6):257-9. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15623950

Correlation between mouse potency and in vitro relative potency for human papillomavirus Type 16 virus-like particles and Gardasil vaccine samples. Shank-Retzlaff M, et al. Hum Vaccin. 2005 Sep-Oct;1(5):191-7. Epub 2005 Sep 20. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17012876

Determination of H5N1 vaccine potency using reference antisera from heterologous strains of influenza. Vodeiko GM, Weir JP. Influenza Other Respi Viruses. 2012 May;6(3):176-87. doi: 10.1111/j.1750-2659.2011.00285.x. Epub 2011 Sep 8. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21902817

Development and application of a flow cytometric potency assay for DNA vaccines. Badger CV, et al. Vaccine. 2011 Sep 9;29(39):6728-35. Epub 2011 Jan 8. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21219978

(Continued on page 17)

In the Literature Continued

(Continued from page 16)

Development and application of a quantitative RT-PCR potency assay for a pentavalent rotavirus vaccine (RotaTeq). Ranheim T, et al. J Virol Methods. 2006 Feb;131(2):193-201. Epub 2005 Oct 7. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/16214228

Development and validation of an egg-based potency assay for a trivalent live attenuated influenza vaccine. Yeolekar LR, Dhere RM. Biologicals. 2012 Mar;40(2):146-50. Epub 2012 Jan 23. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/22269606

Development of an in vitro-based potency assay for anthrax vaccine. Little SF, et al. Vaccine. 2004 Jul 29;22 (21-22):2843-52. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15246620

Feasibility of the use of ELISA in an immunogenicity-based potency test of anthrax vaccines. Jiménez-Alberto A, et al. Biologicals. 2011 Jul;39(4):236-41. Epub 2011 Jun 12. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21664832

Immunocapture enzyme-linked immunosorbent assay for assessment of in vitro potency of recombinant hepatitis B vaccines. Shanmugham R, et al. Clin Vaccine Immunol. 2010 Aug;17(8):1252-60. Epub 2010 Jun 30. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20592114

Improved ELISA test for determination of potency of Inactivated Poliovirus Vaccine (IPV). Rezapkin G, et al. Biologicals. 2005 Mar;33(1):17-27. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15713553

New generation of cell culture assay for smallpox vaccine potency. Leparc-Goffart I, et al. J Clin Microbiol. 2003 Aug;41(8):3687-9. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12904376

Potency assays for novel T-cell-inducing vaccines against malaria. Reyes-Sandoval A, et al. Curr Opin Mol Ther. 2009 Feb;11(1):72-80. To see the abstract and a link to obtain this paper: www.ncbi.nlm.nih.gov/pubmed/19169962

Potency evaluation of rabies vaccine for human use: the impact of the reduction in the number of animals per dilution. de Moura WC, et al. J Virol Methods. 2009 Jun;158(1-2):84-92. Epub 2009 Jan 30. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/19428574

Rapid and accurate determination of the potency of varicella vaccine by quantitative polymerase chain reaction. Russell MS, et al. Vaccine. 2011 Nov 3;29(47):8490-5. Epub 2011 Sep 20. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21939719

Toxicity and potency evaluation of pertussis vaccines. Corbel MJ, Xing DK. Expert Rev Vaccines. 2004 Feb;3 (1):89-101. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/14761246

Using QPCR to assign infectious potencies to adenovirus based vaccines and vectors for gene therapy: toward a universal method for the facile quantitation of virus and vector potency. Wang F, et al. Vaccine. 2005 Aug 22;23(36):4500-8. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/16002190

Validation of a new ELISA method for in vitro potency assay of hepatitis B-containing vaccines. Giffroy D, et al. Pharmeuropa Bio. 2006 Nov;2006(1):7-14. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17270127

Would an in vitro ELISA test be a suitable alternative potency method to the in vivo immunogenicity assay commonly used in the context of international Hepatitis A vaccines batch release? Poirier B, et al. Vaccine. 2010 Feb 17;28(7):1796-802. Epub 2009 Dec 16. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20018270

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

Collaborative study for the validation of serological methods for potency testing of diphtheria toxoid vaccines-part 1. Winsnes R, et al. Pharmeuropa Bio. 2004 Jan;2003 (2):35-68. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/14960262

Validation of in vitro potency assays for tetanus immunoglobulin. Gross S, et al. Pharmeuropa Bio. 2006 Nov;2006(1):1-6. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17270126

(Continued on page 18)

In the Literature Continued

(Continued from page 17)

Collaborative study for the validation of serological methods for potency testing of diphtheria toxoid vaccine (part 2). Winsnes R, et al. Pharmeuropa Bio. 2006 Nov;2006(1):73-88. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/17270133

Serological methods for potency testing of tetanus toxoid vaccines for human use. Hendriksen C, Winsnes R. Dev Biol (Basel). 2002;111:131-40. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/12678232

Collaborative study for the validation of alternative in vitro potency assays for human tetanus immunoglobulins. Gross S, et al. Biologicals. 2010 Jul;38(4):501-10. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20399681

Validation of the normocythemic mice bioassay for the potency evaluation of recombinant human erythropoietin in pharmaceutical formulations. Barth T, et al. J AOAC Int. 2008 Mar-Apr;91(2):285-91. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/18476339

Validation study to evaluate the reproducibility of a candidate in vitro potency assay of newcastle disease vaccines and to establish the suitability of a candidate biological reference preparation. Claassen I, et al. Pharmeuropa Bio. 2004 Dec;2004(1):1-15. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15659281

Collaborative study for the validation of alternative in vitro potency assays for human tetanus immunoglobulins. Gross S, et al. Biologicals. 2010 Jul;38(4):501-10. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/20399681

Collaborative study for the validation of serological methods for potency testing of diphtheria toxoid vaccines - extended study: correlation of serology with in vivo toxin neutralisation. Sesardic D, et al. Pharmeuropa Bio. 2004 Jan;2003(2):69-76. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/14960263

Validation of an anti-PA-ELISA for the potency testing of anthrax vaccine in mice. Pombo M, et al. Biologicals. 2004 Sep;32(3):157-63. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/15536047

Validation of an in-vitro method for Hepatitis B vaccine potency assay: specification setting. Karimzadeh H, et al. Panminerva Med. 2010 Sep;52(3):177-82. To see the abstract and a link to obtain this paper: http://www.ncbi.nlm.nih.gov/pubmed/21045773

Enjoy the easy way to keep up! Subscribe to the knowledge source dedicated to your needs...

BioQuality, The Quality Resource

Don't Miss the Next Issue of BioQuality - Fill out this Order Form Today!

Fax: 916-729-0134 Phone: 916-729-0109 E-mail: bq_editor@surewest.net

or Mail: PO Box 7087, Citrus Heights, CA 95621

_____ _____ _____
Name *Title/Position/Dept* *Company Name*

_____ _____ _____
Street or PO Box (Billing Address) *Voice Phone* *Fax Phone*

_____ _____
City *State* *Zip Code* *e-mail*

Subscription Information

☐ Single Subscription $695.00
(No reproduction allowed)

☐ Unlimited Site License
(please inquire)

Delivery Method

☐ Mail

☐ Email

Payment Information

☐ Credit Card
(Billed by BioQuality)

☐ Bill me later

☐ Payment Enclosed
(Payable to BioQuality)

Charge to: ☐ AMEX ☐ MC ☐ VISA

Account Number _____ Expiration Date _____

Name, exactly as it appears on card _____

Signature _____ Date _____