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Biological Assays:
Development, Validation & Maintenance

24-26 September 2014 Barcelona, Spain

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Conference Topics

Come hear the nitty-gritty details of the emerging new bioassay development tools, and importantly what can go wrong. Learn how you can avoid the looming pitfalls and use new approaches to accelerate your product development. Bioassay scientists gather here because they know that the most current topics, trends and problems will be discussed in an open, friendly and dynamic environment. This conference is designed by scientists, like you who must overcome the challenges of developing, validating, transferring and maintaining bioassays under strict, aggressive timelines.

Main Conference Topics Include:

Keynote Address: Gaze into the bioassay crystal ball with one of the industry's most seasoned and knowledgeable practitioners. Hear what she thinks has impacted development practices and what is most likely to do so in the future. Featuring:
Dr. Hélène Gazzano-Santoro of Genentech

Case Studies Include:

- ***Case Study***: Use of DOE Tools During Development and Optimization
- ***Case Study***: PCR Read-out as a Bioassay Readout (Two Case Studies)
- ***Case Study***: Blood Clotting Bioassay Development
- ***Regulator Perspective***: Gene Therapy Vector Activity Assessment
- ***White Paper Review***: Assay Acceptance Criteria Draft Paper
- ***Case Study***: Bioassay as the Key Stability Indicating Assay
- ***Statistical Tools***: Use of Bayesian Statistics to Solve Bioassay Problems
- ***Case Study***: Developing Antibody Drug Conjugate Potency Bioassay
- And much more!

Plus! 2 Pre Conference Workshops

Workshop #1*:Statistical & Practical Approaches to Handling Outliers & Noisy Data*
This session starts with an overview of the regulatory environment, continues with an easy to understand, clear mini-tutorial to give you the vocabulary and knowledge to approach this topic and then follows with several case studies to highlight handling outliers within dose-response curve replicates and the dreaded product OOS

Workshop #2: *In-depth Look at Validation Practices for Bioassays*
With the advent of Quality by Design validations and the continued use of ICH approaches, the somewhat mundane activity of bioassay validation feels like a minefield. This workshop will review validation basics, which haven't really changed and explore how it can be done today. As part of this workshop, case studies will be presented and a real time survey will be taken to help everyone understand the current practices.

Join us Wednesday, September 24th for a Covance Sponsored Networking Reception

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BEBPA's 7th Annual Biological Assay Conference

Pre Conference Workshop #1 24 September 2014~ Morning

Statistical & Practical Approaches to Handling Outliers & Noisy Data

Schedule

9:00-9:10: Opening remarks
Chaired by Dr. Laureen Little, Principal Consultant, **Quality Services**

9:10-11:00: Mini-tutorial on Outliers
An "outlier" can be defined as an observation that is discordant with the pattern exhibited by other observations in the data set. The question raised by the existence of an "outlier" is: should it be assumed to belong to the whole data set and be retained, or should it be assumed to belong to a different data set and be rejected? Statistical outlier tests are often used to answer this question. Four outcomes are possible: (1) If the "outlier" belongs to the data set and it's retained, that's a good decision. (2) If it doesn't belong to the data set and it's rejected, that's also a good decision. (3) If it belongs to the data set and it's rejected, that's not a good decision -- in general, researchers don't worry about this, but regulatory agencies do. (4) If it doesn't belong to the data set and it's retained, that's not a good decision -- in general, researchers worry about this, but regulatory agencies don't. There is no easy answer --usually assumptions and probabilities are involved. Two common statistically-based outlier tests will be presented: Dixon's Q-test and the Studentized T-test. Although researchers are often most interested in the effect of an outlier on the estimated mean of a data set (or the estimated parameters of a fitted model), it is also important to understand that an outlier can also affect the estimated standard deviation of the raw data about the mean (or about the fitted model). This, in turn, can affect the significance (p-values) of subsequent statistical tests on the data.
Dr. Stan Deming, President**, Statistical Designs**

10:30-11:00: Morning Break

11:00-11:30: Statistical Approaches to Outliers in Bioassay Data
Outliers in bioassay data are not uncommon. It may be necessary to estimate relative potencies from data that includes outliers; for example, if the relative potency result is required for batch release or stability testing. It is not ideal to either include the outliers in the analysis or exclude them altogether. If outliers are included in the analysis, they have the potential to compromise parallelism, skew the estimate of relative potency and inflate its uncertainty. Excluding outliers is wasteful of data and will underestimate the uncertainty. Rather than simply including or excluding outliers, it is better to apply more sophisticated statistical approaches, such as robust regression. Robust regression is mentioned very briefly in USP <1032>. This talk will explore methods for analysing bioassay data that includes outliers. It will demonstrate how to make the best use of bioassay data with outliers.
Kelly Fleetwood, Principal Statistician, **Quantics Consulting**

11:30-12:00: QC/QA Techniques for Outliers
The recently revised USP chapters on bioassays (USP <111>, <1032>, <1033>, and <1034>) recommend outlier detection methods that are substantially different from the usual Dixon's method applied to three replicate wells at each sample by dose combination. The suggested methods fit all the data in an assay together, typically after a transformation that stabilizes the variance, using a model that makes minimal assumptions about the shape of the concentration-response curve and includes the design structure of the assay. These and other good outlier practices will be discussed. Modified versions (to protect confidentiality) of real examples will illustrate many of the reasons for and benefits of modern outlier detection methods.
David Lansky, President**, Precession Bioassay**

12:00-12:30: Developing a Product OOS Strategy for Bioassay Results
Rebecca Munns, Quality Leader, **Public Health England**

12:30-2:00: Lunch

Pre Conference Workshop #2: 24 September 2014~ Afternoon

In-depth Look at Validation Practices for Bioassays

2:00-3:00: Mini Tutorial on Validation Basics & Emerging Trends
This session will discuss validation in today's somewhat complicated environment, where both QbD and ICH validation approaches exist. What do we do and how do regulators perceive these approaches? The session will include an interactive, on-the-spot survey which will allow you to learn standard industry practice during this highly interactive session.
Dr. Laureen Little, Principal Consultant**, Quality Services & Dr. C. Jane Robinson**, Principal Scientist, **NIBSC**

3:00-3:30: Validating QPCR endpoints for Bioassays
Bioassays with QPCR endpoints have significant benefits with regards to timelines, precision and robustness of data when compared to the standard endpoints for Biological Assays. However, it is also critical that the results generated from the QPCR analysis of mRNA signals are relevant and comparable to standard endpoint results following cellular processing of these molecules. This presentation will focus on the validation of the QPCR endpoint assay and comparability with the standard endpoint(s) for bioassays, and will include data from 2 case studies.
Paul Byrne, Scientific Manager**, Covance Laboratories, Ltd.**

3:30-4:00: Afternoon Break

4:00-4:30: Validation of CMC Bioassay Methods According to USP 1033, Case Studies
Dr. Gael Debauve, Group Leader**, UCB Pharma S.A.**

4:30-5:00:Development & Qualification of an Enzyme Linked Lectin Assay for Determining Functional Antibody Response Against Influenza Virus Neuraminidase
Dr. Dewal Jani, Director**, Novavax Inc**

5:00-5:30: Validation challenges when test samples are not parallel/equivalent to the reference
Kim Steeds, Scientist, **Public Health England**

5:30: Workshop Adjourns

Come prepared to take part in our first ever real time validation survey. We will be taking an electronic survey covering such nitty-gritty topics as: What do you use for validation samples? Do you include stability indicating studies in your Validation? Do you include Robustness studies?

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BEBPA's 7th Annual Biological Assay Conference

Main Conference, Day 1, 25 September 2014

9:00-9:10: Open on Conference: Welcome and BEBPA Update
Dr. Laureen Little, Principal Consultant, **Quality Services**

Keynote Address

9:10-10:00: Advances in Bioassays: New Technologies & Approaches
Bioassays are the most challenging assays to develop. They are expected to reflect the mechanism of action (MOA) of the biologic drug and at the same to fulfill all criteria of potency assay for lot release and stability. Different antibody formats, an increasing diversity of clinical indications with complex MOAs, and reduced timelines due to increased competition, make assay development more challenging. This presentation will highlight the advances in bioassays over the last decade with the incorporation of new technologies and approaches which have significantly increased speed, sensitivity, efficiency, throughput and consistency. Special focus will be given to the development of bioassays for multi-domain proteins, new assay formats, implementation of automation, use of physicochemical surrogate measure of bioactivity and the use of ready-to-use cryopreserved cells. Regulatory updates and expectations will also be shared.
Dr. Hélène Gazzano-Santoro, Director**, Genentech**

Bioassay Development for Specific Product Types

10:00-10:30: Case Study: Blood Clotting–the Troubles & the Fix
Dr. Jan Amstrap, Senior Research Scientist**, Novo Nordisk A./S.**

10:30-11:00: Morning Break

11:00-11:30: Case Study: Monoclonal Antibody Analyzed with Three Different Bioassays
A case study of a monoclonal antibody for which several bioassays were developed will be shown. The presentation will focus on the bioassays, their principle and their results.
Marie Gottar-Guillier, Principal Scientist, **Novartis Pharma AG**

11:30-12:00: Development & Validation of a Potency Assay for a Peptide Immunotherapeutic
This talk focuses on the development of a bioassay for a multi peptide immunoregulator. The mode of action of the drug includes the activation of T cells which can be demonstrated in primary murine splenocyte cultures. We have developed a method suitable for routine, cGMP compliant, potency testing using ELISpot for the detection of IL-2 release from activated T cells. A procedure for the production, qualification and use of frozen rodent splenocytes, immunised with individual peptide components of the drug, was developed and the assay procedure and IL-2 ELISpot read-out were optimised using Design of Experiment. In addition, a bespoke method for the statistical data analysis and assessment of system suitability was developed.
Dr. Barbara Hebeis, Principle Scientist**, NDA-Analytics**

12:00-12:30: Candidate Ranking of Antibody Drug Conjugates via Cell Killing & Bystander Effect Assays
Effective candidate selection for ADC's requires the assessment of the different linker and payload options in different *in vitro* cell killing assays. This presentation will outline work undertaken to rank a series of Herceptin conjugates with different linkers and payloads using cell killing and bystander effect assays. These assays were developed and optimised and using the EC50 from these assays it was possible to provide a ranking for each different conjugate.
Dr. Joanne Cooper, Principal Scientist**, NDA-Analytics**

12:30-2:00: Lunch

Bioassays for Gene Therapy Products

Session Chaired by Dr. Jane Robinson, Principal Scientist, **NIBSC**

2:00-2:30: Strategies & Challenges in Selecting Bioassays for Quality Control of Cell & Gene Therapy Products
Quality control of Cell and Gene Therapy (CGT) products provides new challenges due to the different nature and sometimes patient-specific properties of the therapeutic product. Bioassays are important components for controlling quality attributes like purity, identity and potency for biologicals and even more for CGT therapeutics. Developing a comprehensive testing strategy which often may consist of several different assays is important. As an example, the role of bioassays in the quality control strategy of CTL019, a cell therapy product using T cells modified to express a chimeric antigen receptor against CD19, is presented. This strategy uses several different bioanalytical assays for both release testing and characterization. Current challenges like validation of assays and developing potency assays for such a complex product are discussed.
Dr. Dirk Huebert, Principle Scientist**, Novartis Pharma AG**

2:30-3:00: Bioassays for the Characterization of Cellular & Gene Therapy Medicinal Products
Dr. Jens Reinhardt, Sr. Scientific Assessor, **Paul-Ehrlich-Institut Germany**

3:00-3:30: Potential Potency Assays in Cell Therapy
Dr. Paul Kemp, Executive Officer, **Intercytex Ltd**

3:30-4:00: The Challenges of Gene Therapy Vector Production & Activity Measurement
Dr. Mary Collins, Professor, **University College London**

4:30-5:00: Afternoon Break

New Statistical Tools to Solve Old Problems

Session Chaired by Dr. Stan Deming, President, **Statistical Designs**

5:30-6:00: Bayesian Statistics: An Introduction
Bruno Boulanger, Chief Scientific Officer, **Arlenda**

6:00-6:30: A Bayesian Framework for Conducting Effective Bridging Between References Under Uncertainty
The efficacy of a specific batch of a biopharmaceutical (e.g. vaccine) is commonly measured by its relative potency (RP). Reference batches are needed to estimate the RP. Bridging studies between references are required, as the shelf life of the primary

reference (the very first reference) is shorter than the commercial life of the vaccine. Regulatory authorities recommend the RP of new batches be always expressed as a function of the primary reference when measured using a new reference. This requires the calculation and application of a correction factor to link newly computed RP to the results that would have been obtained if the primary reference was still available. However, because correction factors are subject to uncertainty, the new reference can drift from the primary reference over successive bridging studies. This induced bias in the RP can lead to the rejection of a manufactured batch when truly acceptable (producer risk), or acceptance of a batch that should be rejected (consumer risk). In this talk, we focus on Bayesian methods to compute the correction factors. One advantage of Bayesian methods is the prior information. In this particular context, previous references contains prior information. Incorporation of this prior information reduces the bias between successive references, compared to other methods.
Jean-Francois Michiels, Statistician, **Arlenda**

6:30: Conference Adjourns

Main Conference, Day 2: 26 September 2014

8:30-8:40: Welcome by Chairperson
Dr. Hans-Joachim Wallny, Technical Project Leader, **Novartis Vaccines**

Keynote Address

8:40-9:10: Bioassays in the 21st Century–Where are We Now?
It has long been the paradigm that bioassays are required to assess the potency of biological products and yet the purpose, development and execution of bioassays often remain a source of deliberation. Purpose, stage of development and the nature of the product drives what sort of bioassay is required – for example, when can a ligand binding assay be used instead of, or in parallel with, a cell based bioassay? With the advances in understanding of structure/function and degradation pathways, one can argue if a bioassay is necessary or informative in routine stability studies. The relationship of the bioassay to the mechanism of action continues to be a constant source of discussion and influences the choice of assay as well as whether more than one assay to assure potency is required. The role of potency standards in assuring the ability to detect assay drift and assist in assay suitability and data reporting is also as important now as was fifty years ago. Scientific advances in cellular manipulation to create 'designer' cell lines, use of frozen 'ready to plate' cell banks and assay automation is resulting in much more relevant and reproducible bioassays.
Dr. Anthony Mire-Sluis, Vice President, **Amgen**

9:10-9:40: Development of a Robust & Easily Executable Bioassay using mRNA Transcription, Assessed with Dual RT-qPCR, as Assay Response
Transcriptional gene regulation network within cells is one of the most explored biological responses to study normal and/or disease biological mechanisms. However, using such responses as a biological read out for drug potency bioassays have been challenging. The challenges have been due, in part, to the invariable need of cellular extraction of "good quality RNA" after cell stimulation. A potency bioassay using real-time quantitative reverse transcription assay (RT-PCR) has been developed by Catalent using the TaqMan® assay chemistry in a duplex format. Using a cell line, the assay was developed to assess neutralization (inhibition) of ligand-induced bioactivity, in terms of induction of an immediate response gene. Two different fluorescent dyes, with the greatest degree of spectral separation, are used, allowing real time monitoring of gene expression of both target and normalizer gene in each individual well of an assay plate. Additionally, the assay uses cell lysate preparation requiring no RNA extraction procedure. The result is a potency bioassay with an excellent precision.
Dr. Michael Sadick, Sr. Manager, **Catalent Pharma Solutions**

9:40-10:10: To Translate or Not to Translate (PCR)
This presentation explores and provides a blueprint for the application of PCR for potency testing of biopharmaceuticals.
Bioassays form a fundamental part of the control of biopharmaceutical products. They can mimic the mechanism by which the product works in a patient and can be used to establish structure-function relationships. However, there is always the tension between a biologically relevant output and the robustness required for a QC assay. Listen to this presentation to learn:

- Why PCR offers improvements over other endpoints for potency assays
- Key considerations when developing a PCR based assay
- Examples of the application of PCR based potency assays

The presentation offered theoretical and practical insights, and educational content throughout with an overall aim of sharing experiences and expertise.
Chaminda Salgado, Head of CMC, **NDA-Analytics**

10:10-10:40: Morning Break

Tracking Your Assay: What to Watch and How

10:40-11:10: Bioassay Assay Acceptance Criteria White Paper
Dr. Jane Robinson, Principal Scientist, **NIBSC**

11:10-11:40: Bioassay Monitoring Data: Use for Statistical Process Control, Qualification of Critical Reagents, Reference Standard Requalification & Dermination of Process Capability Index (CpK)
Typically during QC bioassay performance multiple monitoring data are generated. Examples will be shown for using cell culture monitoring data and bioassay monitoring data for Statistical Process Control (SPC) and for qualification of critical reagents for a bioassay. Monitoring data can also be used for housekeeping activities like Reference Standard requalification. In addition, examples of process capability index (CpK) determination of bioassays will be presented.
Dr. Alex Knorre, Department Head, **BSL Bioservice Scientific Laboratories GmbH & Liming Shi**, Sr. Research Scientist, **Amgen**

11:40-12:10: Novel Data Analysis Methods for Continued Process Verification Using Change-point Analysis
The pharmaceutical industry has been encouraged to implement the concept of "Continued Process Verification" and the requirement to institute analysis of process stability and capability. Most guidance documents recommend the use of appropriate process behaviour

charts such as I-MR control charts. However these tools have some significant drawbacks and often result in false alarms. A novel integrated approach, combining change-point analysis, process capability analysis and a simple Shewhart charts is presented. A highly automated tool for data management, analysis and reporting is presented.
Dr. Jochen Giese, Sr. Statistician, **Novartis Vaccines**

12:10-1:30: Lunch

Practical Statistical Tools for Bioassays

Session Chaired by Dr. Bassam Hallis, General Project Manager**, Public Health England**

1:30-2:00: Incorporation of DOE Methodology in Bioassay Development & Optimization
In this case study, the original potency assay was complicated and highly variable, and lacked the capacity to meet phase II/III process development and manufacture needs. The potency assay is a DELFIA-based assay with 4 major interacting components. With the goal of improving assay performance and robustness, DOE studies were carried out to assess how assay components affected the performance of the assay individually and interactively. Application of the DOE methodology significantly shortened the assay development time. Using subject matter expertise, we further improved assay performance and expanded the design space for assay optimization. Combining these two approaches, we implemented several major changes that significantly improved the assay performance and robustness, resulting in reduced the assay replicates and increase assay throughput. This new QC-friendly potency assay satisfies the upcoming testing needs, and serves as a good example for how DOE can help in assay development and optimization.
Dr. Xiaohui Lu, Scientist, **Biogen Idec**

2:00-2:30: Dealing with Values Less than LOQ (or NRs)
Bioassay data often contains values below the limit of quantitation (LOQ), sometimes termed non-reportables (NRs). NRs can be analysed by setting them to a fixed value, for example half the LOQ. However, this can bias the results, for example reducing the estimate of the slope. A better approach is to use survival analysis methods to treat NRs. NRs are known to be below the LOQ, but no other information is known about them. In terms of the statistical analysis, this is the same situation as is encountered in survival analysis. This talk will explore how survival analysis methods can be adapted to analyse bioassay data with NRs. In practice, NRs are often encountered in assays which also contain outliers. Bioassays containing outliers can be analysed using robust regression methods. This talk will describe how robust regression can be adapted to deal with both outliers and NRs correctly.
Dr. Ann Yellowlees, Director, **Quantics Consulting Ltd**

2:30-3:00: Determination of the Limit of Detection & the Limit of Quantitation During Assay Development in the Biopharmaceutical Industry
The determination of LOD and LOD is increasingly becoming a regulatory concern for calculation of sero-conversion, sero-protection or geometric mean when licensing a vaccine product. *The* usefulness and optimal throughput of an assay may depend on the appropriate determination of the LOD and the LOQ. The experiment design and statistical analysis method used for the determination of LOD and LOQ is dependent on the assay type (e.g., ELISA, Functional or PCR). This presentation describes the design, testing and statistical procedures required to determine the LOD and LOQ during assay development. The procedures to be used to confirm the LOD and the LOQ during assay validation are discussed.
Dr. Eloi Kpamegan, Executive Director, **Novavax, Inc**

3:30-4:00: Afternoon Break

Stability Indicating Assays

4:00-4:30: When Nothing Else Fails: Bioassays as Early Instability Indicators, a Case Study
Bioassays provide a mechanistic model to monitor function and determine relative potency to a reference and are required for the licensing biological products. The testing supplements physicochemical methods as part of a complete panel of analytical characterization of product consistency and stability. Despite the known variability bioassays exhibit, they are validated for robustness and sensitivity to support identification of lot variation, degradation and instability, but they are rarely the earliest indicators of instability. A validated bioassay applied routinely for product lot and formulation stability testing demonstrated potency failures that were not obvious indications of product instability, and were unsupported by other analytic results. Extensive investigation of the method was performed, and evaluation of the in-use reference standard against a characterized replacement standard demonstrated loss of the reference standard's potency. This confirms the requirement for applying robust bioassays to monitor biological product performance.
Peter Wunderli, Associate Director of Biopharmaceutical Services, **PPD, Inc**

4:30-5:00: Proving Your Bioassay is Stability Indicating
Dr. Nicola Crawford, Senior Scientist, **MedImmune**

5:00-5:30: Update of the HCP Conference
Dr. Martin Vanderlaan, Director, **Genentech**

5:30: Conference Adjourns

BEBPA has published 2 white papers that can be viewed HERE.

- **Assay Acceptance Criteria for Multi-well Plate-Based Biological Potency Assays**
- **October 2013: HCP Testing, Some Points to Consider**

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