# *Annual Bioassay Conference*


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The only constant about bioassays is that they are always changing. Regulatory requirements change, technologies improve and their uses during product development expand. It is critical for your company to know the latest; attend and find it all out.

**2 Workshops:**

- The Course Awakens: Moving Bioassays from Development to Phase-Appropriate Validation
- Setting Intervals for Bioassay Similarity (Parallelism)

**<u>Main Conference Topics Include:</u>**

- Stability indicating properties of bioassays by Juan Arciniega, FDA regulator
- How to apply new technologies and approaches in Bioassays
- Case Studies on biological events, Adalimumab and more
- An interactive survey on the use of DOE during method development
- Poster sessions
- Approaches to assay development and much more…

**For more information: www.bebpa.org**

**Annual Bioassay Conference**
**September 28-30, 2016**

# Bioassay Workshops September 28, 2016

## Workshop 1

**The Course Awakens: Moving Bioassays from Development to Phase-Appropriate Validation.**

Michael Merges and Michael Sadick, back by popular demand, present the Mike[2] Workshop on tackling phase appropriate bioassay validations. The different requirements and expectations between an early phase (IND/Phase I/II) validation and a late phase (Phase III/NDA/BLA/Phase IV) validation, from assay number to range of sample numbers used, will be highlighted. The phase appropriate 'pre-validation' critical parameters needing definition prior to finalization of the validation protocol will be discussed as will the relationship to the ICH Q2R validation guidelines. Focus will also be paid to evaluating validation results. Mike and Mike will have invited guest speakers to present case studies throughout the workshop highlighting and reinforcing the topics presented. The presentation atmosphere of the many topics is designed to encourage active audience participation.

Topics Include:

- Review the bioassay parameters addressed in early vs. late phase development?
- How many assays and how many samples do I have to run?
- What are acceptable criteria for each phase?
- Are ICH Q2 and USP <1033> the same things and which do I follow?

Course Instructors:
**Mike Merges**, **Catalent Pharma Solutions** &
**Dr. Mike Sadick**, **Catalent Pharma Solutions**

**Workshop Schedule:**

**9:00-9:05 Open of Workshop**

**9:05-10:00 Workshop in session**

**10:00-10:30 Morning Break**

**10:30-12:00 Workshop in session**

**12:00-1:30 Lunch**

**1:30-3:00 Workshop in session**

**3:00-3:30 Afternoon break**

**3:30-5:00 Workshop in session**

**5:00 Workshop Concludes**

## Workshop 2

**Setting Intervals for Bioassay Similarity (Parallelism)**

The development of Equivalence Margins and Equivalence Test Systems is a huge challenge with many side effects. The workshop covers how to develop a system of test margins and how to qualify this system of tests for the correct discrimination acceptable and unacceptable assay runs. The challenges and risks of the equivalence test approach will be discussed. Meaningful limits link the test system to the purpose of the bioassay.

- Introduction
- The USP Equivalence Testing Approach (USP <1032>)
- Basic Concepts
- System Suitability Testing
- Similarity Testing
- Developing Margins with the Tolerance Interval Method Diving into the Models Challenges of the approach: How to avoid bias Equivalence Tests beyond the Guidance Control limits vs. prediction (tolerance) intervals Emphasis on the purpose of a bioassay Specifications, and why they're different Ruggedness testing for finding meaningful limits Developing Equivalence Margins based on Historical Data
- Selecting the Data
- Adding challenge data
- Developing Test Margins
- Test Selection Strategies
- Challenging the Developed Test System

Course Instructors:
**Dr. Stan Deming**, President, **Statistical Designs** &
**Dr. Ralf Stegmann**, President, **Stegmann Systems**

Join us after the workshops for a Covance hosted Networking Reception

COVANCE

**9:00:** Opening Remarks by Session Chair
**Laureen Little,** Principal, **Quality Services**

## Session 1: Regulatory Updates

**9:10** Stability-Indicating Properties of Potency Tests of Vaccines: The Anthrax Case

Potency tests aim to ensure, on the basis of the best available scientific information, that vaccines show the expected efficacy. Because stability plays a critical role in vaccine effectiveness, potency is included in all vaccine stability-testing programs. Since all lots entering the National Strategic Stockpile must remain potent throughout protracted storage periods, anthrax vaccines must have reliably long shelf-lives.

Novel anthrax vaccines containing recombinant Protective Antigen (rPA) as the only antigen face a stability issue: purified rPA is particularly susceptible to degradation through non-enzymatic modifications such as deamidation and aggregation.

Spontaneous deamidation of rPA, which occurs at relatively mild temperature, adversely affects vaccine immunogenicity in mice. In addition, rPA forms aggregates in solution after exposure to temperatures ≥40 ºC, losing not only its ability to form lethal toxin (LeTx), but also its immunogenicity. A macrophage lysis assay (MLA) has been proposed to evaluate rPA quality prior to formulation into a final vaccine bulk containing an adjuvant; MLA quantifies the ability of rPA to form LeTx in vitro when mixed with Lethal Factor. We have previously studied ELISA and a LeTx-neutralization assay (TNA), as part of an immunogenicity test in mice to measure anthrax vaccine potency, in terms of their stability-indicating properties. This animal-based potency test is required for the release of drug product, because the interactions between adjuvant and rPA may have an impact on the antigenic structure. However, efforts should be made to reduce, replace and refine use of animals in testing. Thus, we decided to evaluate the MLA as a potential replacement of immunogenicity. We studied the effect of exposure of rPA in solution to temperatures outside refrigeration, including two above its melting point (50 and 75 °C), on the formation of aggregates, and subsequently on the relation of this aggregation to the ability of the protein to form active LeTx by MLA, and on its ability to elicit neutralizing antibody response in mice when combined with adjuvant, by ELISA and TNA. rPA treated at 50 °C for as little as 30 min formed aggregates. While MLA showed that rPA lost about 50% of toxin-forming activity when treated at 50 °C for 1 h before formulation into a final vaccine lot, TNA data showed that antibodies elicited by similarly treated rPA formulated into a vaccine were reduced by over a hundred fold, compared to those elicited by untreated antigen. ELISA data, in contrast to TNA data, indicated only a relatively minor reduction in antibody titer elicited by heat-treated rPA. These findings suggest that although the MLA may not be used to replace immunogen**ic**ity as a potency test for anthrax vaccines, and appears somewhat limited in revealing temperature-driven rPA structural changes, it is still an important in-process test to monitor the immunogenic quality of rPA during the manufacturing of new anthrax vaccines. Our results also confirm previous findings that showed the superiority of the TNA over ELISA for use in the immunogenicity test, in terms of its sensitivity to temperature-mediated changes on rPA.

**Juan Arciniega, FDA**

## Session 2: Method Optimization

**Session Chair: Laureen Little, Quality Services**

**10:10** Bioassays: Applying New Technologies & Approaches

This presentation will highlight some of the advances in bioassays such as the incorporation of new technologies and approaches which have significantly increased speed, sensitivity, efficiency, throughput and consistency of potency assays and the bridging experiments or validation performed to support the implementation of these enhancements. Case studies will focus on the development of bioassays for multi-domain proteins, implementation of automation in QC, and implementing use of ready-to-use cryopreserved cells for a legacy method.

**Pin Yee Wong, Genentech**

***10:40-11:10 Break***

**11:10** Reduction of ELISA Imprecision

Proteins in complex mixtures are quantified using immunoassays such as ELISAs. Typical ELISA protocols are carried out in 96-well microtiter plates and involve multiple steps, wash separations, and the use of multichannel liquid handling equipment such as pipets and washers. At the end, spectrophotometers that acquire assay signals read signals in a row- or column-wise pattern. Many publications report two major sources of bias in ELISAs: 1. Bias from extensive serial dilutions to reach the sensitive assay range and 2. Positional effects with lower signal along the edges of the plate. We addressed dilution bias in a relative potency ELISA by incorporating a fluorescent dye into the first step of the sample and reference preparations. Prior to sample incubation on the assay plate,

the dye signal was measured and used later to correct final assay results by the measured dilution bias. We mitigated positional effects by a block-randomization scheme. Curve points of samples and reference were distributed in a pattern designed to minimize the impact of positional bias on the measured result. Using dilution correction and block-randomization, we were able to reduce ELISA variability by 50% and also improve accuracy.
**Thorsten Verch, Merck**

**11:40 Outlier Detection for Bioassays**

Fitting dose-response models to bioassay data is complex because of: non-linear relationships between log dose and response; non-constant variance; complexities such as blocks, split-unit, or strip-unit designs; pseudo-replicates; missing data; and unusual observations. Good analysis methods for non-linear models usually include: transformation of the response, weights, or mixed models. Both weights and variance components (in mixed models) are particularly difficult to estimate well from small data sets in the presence of unusual observations. Hence, procedures for detecting, removing, and tracking outliers are particularly important. Many bioassays include grouped preparation of samples (which can be created by serial dilution, use of a multichannel pipette, or shared preliminary dilutions); in these assays it is important to check for both group and observation outliers. Because cells (or animals) used in bioassays are inherently sensitive to various departures from normal procedures, multiple unusual observations in a single assay are common. While there are good methods for multilevel outlier detection (using mixed models) and good methods for detecting multiple outliers in a data set (i.e.; Rosner's method), methods for simultaneously detecting at multiple levels and detecting multiple outliers are not broadly available. This talk will focus on some steps to address this problem.
**David Lansky, Precision Bioassay**

**12:10 Early vs. Late Biological Events: A Case Study**

In vitro assays used in QC are aimed to translate complex biological in vivo mechanisms of action into quantitative reproducible results. Currently, reporter assays, based on engineered cell lines and offering the advantage to be robust and extremely fast, are more and more preferred to classical cell-based assays. Based on a case-study, we compare here results obtained from three assays covering early binding: by cell-free assay , middle intracellular signaling: by reporter gene assay and late proliferation: by classical cell- based assay biological events.
**Cecile Avenal, Roche Basel**

**12:40-2:00 Lunch**

## Session 3: More Than One Bioassay? What to do!

**Session Chair: Bassam Hallis, Public Health England**

**2:00 Changing Potency Assays- How Difficult Can It Be?**

Changing potency assays during clinical phase 2 studies requires various considerations. This presentation of a case study is about how a carefully worked-out strategy for changing a potency assay with minimum testing was executed. Results from assay validation and statistical equivalence testing will be discussed. Additionally, the handling of ongoing stability programmes as well as timelines and the strategy for registration will be presented.
**Karen Dixen, Novo Nordisk A/S**

**2:30 Justification of the Use of Surrogate Potency Assays for Lot Release and stability Testing During Clinical Development of Nanobody®-Based Therapies**

Nanobodies® are a class of therapeutic proteins based on single-domain antibody fragments that contain the unique structural and functional properties of naturally-occurring heavy chain only antibodies. During clinical development, a potency assay is required to support lot release and stability studies and is used for biological characterization of a compound. Ideally, the potency assay is reflective of the mechanism of action (MoA) of the biological product. Cell-based assays typically have the potential to reflect different aspects of the MoA, although these often show high variability and poor assay performance and mostly require long assay times. Well-controlled assay conditions are often more easily obtained with a simple, surrogate assay. However, as such surrogate assays typically mimic a single aspect of the MoA (e.g. binding), justification of the surrogate assay is generally required for its use in lot release and stability studies.Through selected case studies, we will discuss our generic justification approach in which the assay performance and stability indicating properties of a surrogate assay and a bio-assay are compared. In order to justify the use of a surrogate assay, the results obtained with this assay should correlate with the results of the bio-assay and the physicochemical characterization of the product-related variants. Therefore, in a first stage during development, mock variants and affinity variants are used. Furthermore, during Phase II clinical development, samples which are exposed to

several stress conditions, such as increased temperature, vibration stress, photo stress, etc., are used to demonstrate and compare the stability indicating capacity of both assays. The overall justification strategy consists first of a general assessment if both assays are able to identify similar changes in potency of the stressed test samples. Therefore, equivalence is assessed by checking whether the confidence interval of the ratio of the potency of a test sample to the control sample, per assay, falls within preset equivalence limits or not. Secondly, statistical equivalence is evaluated of both assays for all stress samples tested. This equivalence test also requires pre-defined equivalence limits which are based on the variability of a representative historical data set.

**Lien Dejager, Ablynx**

**3:00 Fc-Receptors: Challenging Interaction Partners in Analytics**

Receptors that bind the Fc-portion of IgG type antibodies are critical mediators of effector functions and can also critically influence the serum half-life of therapeutic antibodies. Such mediators like the Fc-gamma receptors (FcγR's) and the neonatal Fc-receptor (FcRn) are frequently used in bioanalytics during assessments of therapeutic antibodies by Biacore interaction measurements, cell-based binding measurements and cell-based assays on effector functions. Analysts are confronted with a very heterogeneous receptor population, containing a large diversity in terms of posttranslational modifications, e.g., glycan structures and cells expressing both activating and inactivating receptors in different amounts. Receptor glycan structures are known to depend on the cell line used during production and also affect the interaction with its binding partners. Similarly, the very heterogeneous antibody samples pose challenges on the interpretation of the resulting data and supporting analytical methods. Results from cell-based binding experiments, cell-based effector function assessment and biophysical binding data can only be integrated with a deep knowledge of the respective systems. The basic background on Fc-receptors, their complexities in terms of assay design and possible pros and cons of certain assay setups will be
discussed

**Florian Cymer, Roche Basel**

**3:30-4:00 Break**

***Don't forget to fill out the survey sheets online to receive your password for access to the meeting slides***

## Session 4: Vaccine Potency Assay Development

**Session Chair: Jane Robinson, Consultant**

**4:00 In Vitro Methods to Overcome In Vivo Limitations**

Quality by Design principles together with the typical request for vaccine productivity increase during lifecycle are the main drivers for introducing new in vitro assays in replacement of in vivo testing into vaccine characterization and lot-release testing. In general, the in vitro test has the following advantages: i) a much lower variability as compared to the in vivo method; ii) cost-effectiveness with reduction of time for the test for lot release iii) considerable reduction in the use of animals, in line with the 3R principle. In addition, in vitro tests are generally more sensitive to detect minor o subtle product changes. However, challenges related to the needs of correlation between in vitro and in vivo testing together with expectations from regulatory agencies have to be considered for the replacement of in vivo testing. Here, examples of the development and the path forward for implementation of in vitro tests for measuring safety and potency attributes for vaccines in different stages of development and commercialization will be discussed.

**Marua Prevato, GSK Vaccines S.r.l.**

**4:30 Potency Assay for Polio Vaccines**

Oral live attenuated poliovirus vaccine (OPV) and inactivated poliovirus vaccine (IPV) play an instrumental role in the Global Poliovirus Eradication Initiative (GPEI). The potency of OPV can be assessed by an in vitro assay which measures the infectivity of monovalent polio vaccine bulk harvests using a 50 % end point technique in microtitre system. This assay is used to determine dilutions and validate titres of samples tested for neurovirulence. The potency of IPV can be assessed by an in vitro assay which is based upon the assessment of the quantity of the D-Antigen (D-Ag) units in an IPV. The D-Ag unit is used as a measure of potency as it is largely expressed on native infectious virions and is the protective immunogen. The indirect ELISA is the most commonly used in vitro test. However the potency of IPV is primarily assessed by an in vivo assay developed in rats. This assay is based on the assessment of the neutralising antibody titer within the sera of rats. With the development of transgenic mice expressing the human poliovirus receptor, immunisation-challenge tests have been developed to assess the potency of IPVs.

**Thomas Wilton, NIBSC**

**5:00 What Makes Vaccines Special? A Bio-assayist Perspecitve!**

Successful development of vaccines requires a toolbox of assays to characterise both the product and the patient's reaction to that product. This can present the bio-assayist with "interesting" challenges. Assays for potency or immunogenicity should measure immune responses that correlate with protection against disease and whilst correlates of protection for existing vaccines can be well defined, this is not always the case for vaccines currently in development. Developing assays for new vaccines may require reagents or controls that are hazardous, poorly defined or not readily available. This talk will attempt to illustrate some of the challenges faced and use examples of approaches used to overcome them.

**Sue Charlton, Public Health England**

**5:30 Poster Presentations**

**6:00 Conference Adjourns**

## Bioassay Conference Main Day 2

## Session 5: Bioassays for Biosimilar Products

**Session Chair: Stan Deming, Statistical Designs**

**9:10 The Role of Biological Assays in the Determination of Biosimilarity**

Biological assays play a critical role in the determination of biosimilarity by providing a sensitive method for comparison of potency between the biosimilar and the reference product. Using IgG1 as an example, the potential for various quality attributes, such as structural alterations, glycosylation, deamidation, etc., to impact potency in various ways will be discussed. Biological assays include animal and cell based assays, as well as binding assays, however, animal assays are falling out of use and will not be included in this discussion. Generally, in assessing potency, it is appropriate to apply a range of orthogonal methods in order to ensure that, as far as possible, all subtle differences between biosimilar and reference product are detected. The complexity of this challenge increases with molecules possessing multifunctional properties, such as immunoglobulins. Issues that will be considered include the ability of the assay to detect a meaningful difference and the drivers for this will be discussed. These drivers and their importance will, inter alia, differ depending on the assay platform, the impact of potentially different receptor isotypes within clinical populations and the relevance of the biological attribute to the intended therapeutic effect. These considerations will be illustrated using the potential role of Fc-gamma IIIa binding in respect of a monoclonal anti-TNF alpha. Also, in relation to biosimilarity, immunogenicity assays while not directed to measuring potency have a clear role to play and a brief discussion on this will be included. It could be argued that, in relation to the totality of data used to support biosimilarity, the importance of biological assays transcends that of both structural and clinical data. Regulatory agencies, and notably the FDA, are applying ranking and statistical constraints to best ensure adequate equivalence based on quality attributes and, in this respect, potency assays are always included amongst the highest ranked quality attributes that impact biosimilarity.

**Cecil Nick, Parexel**

**9:40 Platform Bioassay Development for Biosimilar Drug Targets**

One of the key tests in the assessment of Biosimilarity is relative potency. Typically cell based Bioasaays used to determine potency are variable and time consuming. Sensitive bioassays in simple kit form (DiscoverX), targeted at Biosimilar molecules, are currently being developed and assessed. In this way, biosimilar molecule testing can produce relative potency results within reduced time frames e.g. <48hrs. The development of a bioassay kit to measure biosimilar molecules of anti-vascular endothelial growth factor (anti-VEGF) will be presented. VEGF signalling is well established as an inducer of cell proliferation and promotes cell migration (1). Uncontrolled regulation has been implicated in the development of disease states such as polycystic ovaries and ovarian cancer in which abnormal angiogenesis occurs (2). Anti-VEGF molecules have been successfully released onto the market e.g. Avastin (Bevacizumab) and Eylea (Aflibercept) for conditions including metastatic colorectal cancer, metastatic kidney cancer and wet age related macular degeneration. Biosimilars of these drugs are now being tested in preparation for release after current patents end. 1) The FASEB Journal (1999), 13, 9-22 2) Mol Med Rep. 2016 Apr 25. doi:10.3892/mmr.2016.5173.

**Paula Urquhart, Covance**

**10:10 Statistical Aspects of Biosimilar Bioassay Equivalence Testing**

The demonstration of biosimilarity between a candidate biosimilar product and the innovator product involves comparing the two products for a range of properties. The aim is to show that the (putative) biosimilar is equivalent to the innovator for each property. For any given property assay,

demonstrating biosimilarity must therefore be based on demonstrating that a confidence interval for the reportable value, e.g. the relative potency lies entirely within a pre-specified interval – for example 80% – 125%.For relative potency (RP), defined as the ratio of the doses required to achieve the same response, this is only unique (and therefore a useful measure) when the dose-response curves of the two products are parallel – otherwise the ratio varies with the response level. If the curves are not parallel, it is not possible to capture the difference in potency between two products in a single number. This is not necessarily the end of the road for the candidate biosimilar: it may be possible to capture the differences using pair or triplet of numbers, all of which could potentially be used to demonstrate equivalence.The aim of this talk is to explore methods of handling the statistical analysis of reportable values which are relative to a reference standard, where the sample does not behave as a dilution of the reference standard.

**Francis Bursa, Quantics Biostatistics**

**10:40-11:10 Break**

## Session 6: The Care & Feeding of Reference Material

**Session Chair: Hans Joachim Wallny, Novartis**

**11:10 Monitoring the Bioactivity of Reference Standards**

A reference standard is used in biological assays to compare test samples with, such that the bioactivity of the test samples can be determined. For the reference standards in use, it is required to verify and maintain the level of bioactivity over time. This presentation will discuss possible strategies for qualifying and monitoring reference standards both in cases where an International Reference Standards is present and in cases where no International Reference Standard is present.

**Erik Talens, Merck**

**11:40 Experience with Retesting Reference Material**

A bioassay determines potency relative to a reference; thus, evaluating potency changes of the reference substance itself over time requires modifications. Several concepts for retesting reference material have been used; each comes with its own advantages and shortcomings. Reference retesting may be performed, e.g., annually, and may be based on absolute values like bioassay EC50. But, although the EC50 is related to potency, it can also be influenced by other factors that may be difficult to identify and to control. An alternative approach is to evaluate reference potency based on the readout relative potency. This eliminates the influence of extraneous factors; on the other hand, testing against another standard requires the assumption that this other standard would not degrade at the same rate and to the same extent as the reference substance to be tested. The presentation will evaluate selected reference retesting concepts together with measures to handle the respective shortcomings.

**Frank Straube, Novartis**

## Session 7: Use of DOE as a Development Tool

**Session Chair: Bassam Hallis, Public Health England**

**12:10 Optimization & Robustness Study of a Potency Assay Using DOE**

Potency determination is an important part of the quality assessment/control. According to ICH Guideline Q6B, potency must be evaluated by using well-characterized and validated bioactivity assays. A bioassay is often validated at phase I/II, but during the biologic's life cycle, the assay often needs optimization and refinement, to fulfil both internal and external requirements.

Here the optimization of a bioassay used at Novo Nordisk A/S for potency determination of a biopharmaceutical protein will be shown. By implementing Design of Experiments DoE in the optimization process, nine assay factors and four factor interactions based on time consumption and complexity of buffers were evaluated. By using DoE the factors were simultaneously evaluated in an efficient and effective manner. The conclusions drawn from statistical analysis of the results obtained from the DoE provided improved assay conditions and settings that were successfully evaluated in a proof of concept assay. Furthermore, how to implement the optimizations will be discussed with regard to validation status of the assay.

**Jan Amstrup, Novo Nordisk**

**12:40-2:00 Lunch**

**2:00 Interactive Survey Topic: Use of DOE during Method Development**

Abstract Coming Soon

**Laureen Little, Quality Services & Jane Robinson, Consultant**

## Session 8: Product Specific Potency Assay Development

**Session Chair: Hans Joachim Wallny**

**3:00 Development & Validation of Potency Assay for a TNFα Blocking Monoclonal Antibody: Adalimumab as a Case Study**

Anti-tumor necrosis factor (anti-TNF) drugs are a class of drugs that are used worldwide to treat inflammatory conditions. This presentation will give an overview on developing a reliable method for in vitro biological activity measurement of Adalimumab. During inflammatory diseases TNFα binds to its receptor on cell surface and induces cell death. The Mode of Action (MoA) of the drug is reducing the inflammation by binding to TNFα and preventing it from binding to its receptor. We have developed a proliferation-based bioassay method for Adalimumab and a proper method for quantitative measurement of living cells. TNFα blocking ability of the drug was compared with a suitable reference material. Method validation was performed according to ICH guidelines and system suitability parameters were defined. Also, a proper statistical data analysis method was set for the experiment. Here we discuss the assay development steps and the changes which were applied during the development process in order to optimize the method.

**Maryam Varposhti,** CinnaGen Medical Biotechnology Research Center, Alborz University of medical sciences, Karaj, Iran

**3:30 The Testing Strategy to Determine the Pharmacological Activity of Gene Therapy Drug Product (rAd-IFN) to Treat Intravescial Non-Muscle Invasive Bladder Cancer**

rAd-IFN is a recombinant adenoviral gene therapy vector encoding IFNα2b gene for the treatment of refractory non-muscle invasive bladder cancer. The vector transduces the bladder wall cells where IFNα2b gene is expressed leading to death of cancer cells.The advanced testing strategy to determine the pharmacological activity of rAd-IFN drug product involves three key assays:

1. Infectious titer of the virus, quantitative assay.
2. Expression of the transgene (IFNa2b), semiquantitative assay.
3. Potency (IFNa2b mediated cell killing), quantitative assay.

The infectivity and transgene expression assays have been performed for batch release and stability monitoring of activity during Phase 2 and will remain unchanged in principle for Phase 3 and commercial use.

In the infectivity assay, the cells supporting adenovirus replication are infected with three concentrations of adenovirus and left to produce the virus for two days. The percentage of infected cells is then determined with a flow cytometer utilizing a fluorescently conjugated antibody against an adenoviral structural protein. Samples are analyzed in parallel with a reference standard and infectivity is given as Relative Infectious Units / ml.

In expression assay, the IFNα expression capability of the virus preparation is determined by infecting IFN insensitive cells with the rAd-IFN virus and the concentration of produced IFNα is measured with a commercial IFNα ELISA (enzyme-linked immunosorbent assay) from cell culture supernatants. For Phase 3, a new potency assay is developed and added to release and stability testing in order to provide evidence that batches of rAd-IFN are able to produce active IFNa2b which has a relevant pharmacological effect. In this assay, cells are transduced using multiple dilutions of reference standard and test samples leading to expression of IFNα2b and subsequent cell death. Cell killing efficiency is determined using colorimetric method measuring dehydrogenase activity of the living cells. Relative potency of test sample is determined against reference standard response curve after testing parallelism by equivalence test. All activity assays will be fully validated according to ICH Q2 (R1) prior to release testing of Phase 3 clinical study material (Accuracy, Precision, Specificity, Linearity and Range, System Suitability and Robustness). The three validated assays will provide enhanced quantitative measure of biologic function of the rAd-IFN vector and thus demonstrate the quality and efficacy of drug product batches.

**Minna Hassinen, FKD Therapies Oy**

**3:30-4:00 Break**

## Session 9: Approaches to Assay Validation

**Session Chair: Laureen Little, Quality Services**

**4:00 Bioluminescent Bioassays Power Combination Immunotherapy Targeting Two Immune Checkpoint Receptors**

Therapeutic antibodies designed to target immune checkpoint receptors function by modulating a patient's own immune system and are promising strategies to treat cancer. Clinical results showed co-engagement of multiple immune receptors, such as immune inhibitory receptors PD-1 and CTLA4 or PD-1 and TIGIT in combination immunotherapy, elicit much better therapeutic outcomes compared with targeting a single immune receptor. Current methods used to measure the potency of these therapeutic drugs rely on binding assays or primary cell-based assays which are unable to

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provide a mechanism of action-based measure of drug potency with the precision and accuracy required for use in controlled drug development environment. In this talk, we will describe the development of several bioluminescent bioassays that can quantitatively measure the biological activity of antibodies targeting immune checkpoint receptors (PD-1, TIGIT, CTLA4 and others) individually and in combination. For this, we engineered stable cell lines to serve as T effector cells and artificial antigen presenting cells (aAPCs), which are further developed into Thaw-and-Use format to provide convenience and minimize day-to-day assay variation. The T effector cells stably express the immune checkpoint receptors of interest with one or two luciferase reporters responding to signals from TCR or the immune receptors stimulation. The aAPCs are engineered by co-expressing corresponding immune checkpoint receptor ligand and TCR activator which can activate T effector cells upon direct interaction. When the T effector cell is co-cultured with its aAPC cell, the immune receptor/ligand interactions modulate T effector cell activation and luciferase activity, which are blocked or further activated by immune checkpoint antibodies. Furthermore, the combination bioassay is able to provide a quantitative measure of the synergetic effeAct of two immune checkpoint antibodies on T cell activation. These bioluminescent bioassays reflect the mechanism of action for each antibody drug candidate, and exhibit assay specificity, precision, accuracy, linearity and robustness required for drug potency and stability determination. They will empower current and future immunotherapy drug development.

**Jey Cheng, Promega R&D**

**4:30 Case Study: Validation of Cell-based Neutralization Assays**

A cell-based toxin neutralisation assay has been validated to assess the ability of antibodies in sera to neutralise Bacillus anthracis lethal toxin. The assay has been validated for multiple species to test preclinical and clinical samples and as a component for batch release potency tests for anthrax vaccines.

**Pam Proud, Public Health England**

**5:00: Analytical Method Transfer Validation for a Bioassay to a Contract Lab (Including Statistical Tools for Calculation of Sample Size, Critical Difference, Data Evaluation)- Case Study**

When transferring an assay to an external contract lab, the client needs to provide the "know-how" on the in-house assay and has to plan the set-up of the analytical method transfer validation, including relevant acceptance criteria. This includes the calculation of the sample size necessary to detect significant differences between the labs by using statistical tools, selection of suitable samples to be used in the scope of the transfer validation, the calculation of the critical and/or relevant difference as an acceptance criterion for the comparability based on actual process capability data as well as the evaluation of the results generated by the transferring and the receiving lab during parallel testing by using statistical tools, which are not always available at a contract lab. This will be discussed based on the example of a bioassay, which was transferred to a Japanese contract lab

**Iris Unterrieder, Baxalta**

**5:30 Conference Adjourns**

Come and be part of BEBPA's first US Bioassay Conference. This conference has been the must attend Bioassay event for nearly 10 years. Finally, due to attendee requests, we will be hosting a US Conference. Meeting is scheduled for March 8-10, 2016 in San Francisco, CA.

Please come and be a part of this inaugural conference. Be a speaker! Help steer the focus and topics of this conference. Our scientific committee has suggested the following topics:

- Case studies for implementation of USP <1033> style validations
- Bridging studies in the commercial environment and during development
- Assessing comparability of a BioSimilar product utilizing biological assays
- New developments in commercially available technology and their use in bioassays
- Coping with manufacturer-imposed changes in externally sourced reagents for the bioassay
- Use of kits with a research use only designation as part of your biological assay
- Bioassays for coformulated products
- Care and maintenance of the bioassay reference material
- Use of DoE during the development of bioassays
- Modernizing an assay, what is required to establish equivalence
- Comparability of assay performance following assay transfer

We would love to hear case studies on these topics. Have a different idea? Please suggest it. Our best stories often are those we don't expect. If you would like to present, submit an abstract by Oct. 24th HERE

## Poster Presentations:

**Case Study: defining equivalence criteria for a parallelism experiment**
**Anne Benoit, GSK Vaccines, Rixensart, Belgium**

**Development of generic goal posts for equivalence testing of potency assay methods**
**Thorsten Pflanzner, AbbVie**

**Bioassay Trending and Invalid Metrics: The Key to a Successful Bioassay and Lifecycle Management**
**Dyan Sheehan, Janssen Biologics**

**Ready-To-Use Cryopreserved Cells in a GMP Bioassay for an ADC**
**Dr. Stephanie Katzenbach, AbbVie**

**Development of a Cell Surface ELISA for the Detection of Expression of an Immune Modulator Delivered by an Oncolytic Virus**
**Dr. Kate Getliffe, PsiOxus Therapeutics**

**Fc Effector Bioassays for Rapid and Quantitative Measurement of ADCC and ADCP Mechanisms of Action**
**Dr. Vanessa Ott, Promega**

**Hitting the Gas: Quantitative Cell-Based Bioassays to Advance Immunotherapy Programs Targeting Co-Stimulatory Immune Checkpoint Receptors**
**Dr. Richard Somberg, Promega**

**Improved T Cell Activation Bioassays to Advance the Development of Bispecific Antibodies and Engineered T Cell Immunotherapies**
**Dr. Eric Muhr, Promega**

**Qualification of a potency assay for characterization of Golimumab Fab functional activity**
**Sebastian Königsberger, Eurofins BioPharma Product Testing Munich GmbH**

**Qualification of a SPR binding for characterization of Golimumab Fc binding to FcgRI (CD64)**
**Ulrike GraabEurofins BioPharma Product Testing Munich GmbH**

**Demonstrating Fingerprint-like Similarity**
**Dr. Debbie Allan, Sartorius-Stedim BioOutsource**

**Development & Evaluation of A Novel Bioassay for Denosumab Activity**
**Abhishek Saharia, DiscoverX**

**Ready-to-Use Potency Assays For Bevacizumab, Aflibercept and Ranibizumab**
**Abhishek Saharia, DiscoverX**

**Novel, Improved Cell-based Assays to Enable Immunotherapy Drug Development for Checkpoint Receptors**
**Abhishek Saharia, DiscoverX**

**Assay of Inactivated Poliomyelitis Vaccine: Comparison of *In vitro* vs. *In vivo* Assays for Potency Determination**
**Dr. Sten Erik Jensen, Statens Serum Institut**

**Quantification of Bevacizumab Activity and Anti-Bevacizumab Neutralizing Antibodies in a Cohort of Patients with Glioblastoma**
**Christophe Lallemand, Florian Deisenhammer, & Michael G. Tovey, Euro Diagnostica Biomonitor**

**iLite™ Reporter Gene Assay for Quantification of the Activity of Anti-IL-23 and Anti-IL-23 Neutralizing Antibodies**
**Christophe Lallemand and Michael G. Tovey, Euro Diagnostica Biomonitor**

4-Parameter Logistic Model: The Slope S and the Dynamic Dose Range Factor B
**Dr. Tina Felber, Dr. Andreas Fromkorth, Dr. Matthias Schmitt, Dr. Ralf Stegmann, Stegmann Systems**

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