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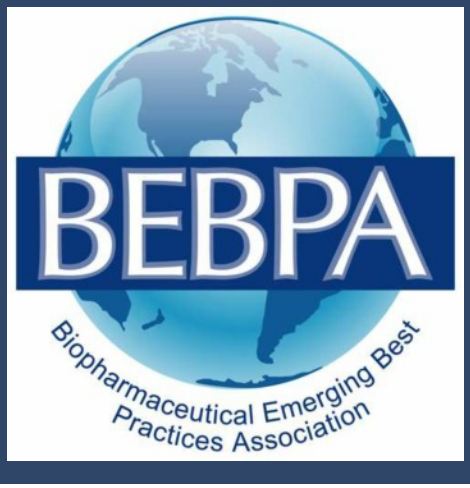

First Annual: Workshop on Host Cell Protein Assays

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Host Cell Protein Assay Workshop

BEBPA is proud to announce its first European conference dedicated to the development of Host Cell Protein Assays. Last year we sponsored a one day workshop, which was so popular, we are now sponsoring this dedicated HCP conference. Right now both the USP and EP are drafting chapters about how to develop these critical methods. This conference will give you a glimpse of where the field isright now and where it is going in the near future. Come hear the thought leaders in the field, and understand the expectations for your HCP assay

Main Topics:

- Screening and Assessing HCP Rare Reagents
- Selecting Appropriate HCP assays for Early vs. Late Stage Development
- Assessment of Various Technologies
- Overcoming Common Analytical Development Hurdles
- Update on USP General Chapter on HCP Assays
- Impact of HCP Immune Responses in Humans

Over 15 Case Studies including:

- **Demonstration of antibody coverage. Alternatives to Western Blot**
- **Characterization of new HCP reagents for Chinese Hamster Ovary Cells by High-Throughput Simple Western Technology**
- **Measurement and Determination of Host Cell Proteins; What Methods to Use and When?**
- **Strategies for the Replacement of the Host Cell Protein Immunoassay with "as good as or better" assay**
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BEBPA's First European HCP Workshop

Host Cell Protein Workshop

Day 1

8:00-8:30: Open of Workshop: Comments by organizer
Dr. Martin Vanderlaan, Director, **Genentech**

Imunnoassay Development & Validation I

8:30-9:00: Evaluation of Immune Reagents of Multi-product HCP Assay for Suitability of Use

Host cell proteins (HCPs) are process-related impurities and critical process parameters in the manufacturing of protein-based pharmaceuticals. HCPs are undesirable in the final drug product as they may act immunogenic and potentially exhibit biological activity. Hence, the clearance of HCPs in the manufacturing process must be controlled. Often platform-based immunoassay approaches for the control of HCP removal in the production process are in routine use. The coverage of HCP of these test methods must be evaluated product specifically with orthogonal methods. Typically two-dimensional difference in gel electrophoresis (2-D DIGE), Western blot techniques and mass spectrometric methods are used for this purpose.

Dr. Micahel Weidmann, Manager, **Roche Diagnostics**

9:00-9:30: Development of a cell line specific ELISA-HCP assay

Host cell proteins (HCPs) are process-related impurities derived from host cells expression system that may be present in trace amounts in a final drug substance. Although generic ELISA kits are commercially available to quantify HCP from different recombinant systems, a specific assay for biologicals is required before registration. Here we describe the development of a cell line specific ELISA-HCP assay for one of UCB's biopharmaceuticals. In order to mimic HCP from the expression cell line, we developed a mock cell line that was transfected with a point-mutated expression vector. Then, an immunization strategy was defined using upstream and downstream process material from a mock run to generate anti-HCP antibodies. The obtained sera were characterized by 2D Western Blot to select antibodies for the development of a cell line specific ELISA-HCP assay. This presentation will present our strategy and the challenges encountered during all steps of development.

Aurelie Delangle, Scientist, **UCB**

9:30-10:00: Host Cell Protein Assay Development: ...From Antibody Characterization to Assay Performance, a Platform Update!

Host cell protein assays are multi-analyte, quantitative systems developed and implemented using a broad range of technologies. They all rely on the demonstrated performance and characteristics of the antibody preparations used. In the end they are expected to meet the performance characteristics of more traditional single analyte quantitative assay systems. Generation of the necessary antibodies, thorough characterization, and development of solid assay designs allow for these methods to achieve acceptable performance. This performance can be extended within a production platform, across multiple products and used to generate significant savings in headcount, and time within the product development process.

Patrick Niven, Associate Director, **Janssen Research &Development**

10:00-10:30 Morning Break

10:30-11:00: Case Study - Choice of Immunization Materials as well as ELISA Standards and Antibody

Different antigen preparations were combined with different antibodies in a sandwich ELISA for the quantitation of HCP in real downstream process samples of a certain drug substance. These combinations demonstrated very different results toward realistic determination of HCP content and dilution linearity characteristic. Consequently a suitable compromise is discussed, balancing these two basic analytical parameters. The influence of total IgG versus affinity purified specific IgG from the same HCP-specific antiserum source on the detection of HCP was evaluated in a sandwich ELISA system. Due to the obvious lower sensitivity of the total IgG-based ELISA, the use of specific IgG as ELISA capture and detector antibody has to be favoured and is almost essential for samples with very low HCP content.

Dr. Kathrin Gunther, Director Marketing International, **BioGenes GmbH**

11:00-11:30: Analytical detection of host cell proteins

Presence of host cell proteins (HCP) derived from production cell lines in biologics poses a potential safety risk for patients. Therefore, control of HCP impurities is critical. During early development phases, commercially available kits are frequently used, because process-specific assays remain to be developed. Therefore, we have compared the suitability of different commercially available ELISA assays. In particular, we have focused on the recovery of process-specific mock HCP and dilutional linearity. At later stages of product development, usually process-specific HCP assays are established. Development of process-specific HCP ELISAs poses several challenges. For instance, it requires generation of new antibodies with broad coverage of HCP. Two dimensional (2D) gel electrophoresis, which is frequently used as an established technique for coverage determination, is limited to the detection of linear epitopes. Here, we introduce the concept of additionally employing a depletion assay to determine conformational coverage. Data on the development and suitability of the depletion assay including a comparison of these methods will be presented.

Dr. Stefanie Fas, Associate Principal Scientist, **MSD The Netherlands**

11:30-12:00: The Automation of a High Throughput Residual Host Cell Protein Assay to Support In-Process Development of Biologics Using Microfluidic Assay System

Quantification of impurities in biological products is challenging process as commonly used ELISA is limited in providing consistency and throughput. In this work, a fully automated HCP assay was developed using new microfluidic system (Gyrolab) to support in-process development of monoclonal antibodies. The newly developed HCP assay significant improved throughput (>5x), dynamic range (>100x), sample consumption (<5x) with excellent consistency compared to ELISA. The Gyrolab assay has been evaluated for analysis of therapeutic antibodies from all purification processes and compared with Tecan-automated ELISA and manual ELISA. As a result, it demonstrated the superiority over plate-based ELISA for in-process HCP clearance monitoring and quantification of final HCP in the drug substance. This microfluidic system also has a great potential to be used for other residual impurities such as protein A and enable accelerated purification development.

Jun Hyuk Heo, Analytical Scientist, **Merck & Co**

Immunoassay Development & Validation II

12:00-1:30: Lunch

1:30-2:00: Characterization of new HCP reagents for Chinese Hamster Ovary Cells by High-Throughput Simple Western Technology

Many biopharmaceutical protein drugs are recombinantly produced in Chinese hamster ovary cells. Therefore monitoring of residual host cell proteins (HCPs) by specific antibodies in various immunoassay formats is mandatory to ensure their removal during purification. Since the performance of such assays is directly dependent on the quality of the antibody reagents, we produced new goat anti-HCP antibodies using an improved immunization protocol. The performance of the new antibodies was studied in 2D gel electrophoresis experiments comparing their HCP coverage to commercial anti-HCP antibodies. Since 2D gel electrophoresis is labor-intensive and limited in reproducibility, the Simple Western Technology was assessed as an automated high throughput screening approach for antibody profiling. In this application proteins are separated based on size or charge by capillary electrophoresis and visualized directly within the capillary by immunodetection. Application of the charge-based separation mode resulted in distinct HCP patterns that enabled site-by-site comparison of the different antibodies.

Dr. Alexandra Krog, Head of Electrophoresis, **Merck Millipore**

2:00-2:30: Strategies for the Replacement of the Host Cell Protein Immunoassay with "as good as or better" assay.

Replacement of the HCP assay reagents and development of a new HCP assay may become necessary due to a low inventory of the current HCP reagents, a major change in the manufacturing process or inadequate HCP assay performance. Regulatory agencies require the new assay to be "as good as or better" than the assay that is being used. Replacing HCP assay reagents is a complicated process that requires considerations of multiple influencing factors, from the choice of the null cell line and blank run process parameters for antigen production to antibody production and purification scheme. In this presentation different factors that can affect the quality of the HCP reagents will be discussed and the strategies for HCP reagent replacement will be proposed.

Dr. Svetlana Bergelson, Director, **Biogen Idec**

2:30-3:00: Measurement and Determination of Host Cell Proteins; What Methods to Use and When?

The measurement of host cell protein (HCP) concentrations during bioprocessing has traditionally relied upon ELISA based technology. Whilst this is a very rapid and useful technology, this gives little information as to which HCPs are present and how these change during a bioprocess. Further, not all HCPs will be detected by such approaches. More recently a number of other technologies including 2D--PAGE based approaches and mass spectrometry based approaches have been used to investigate and determine the host cell proteome and how this changes in various systems. Here the different approaches, the advantages and disadvantages of each and the information that might be gather from these is discussed alongside how such information might be used or be required in the future by the regulatory authorities.

Mark Smales, Professor**, University of Kent**

3:00-3:30: The Devil in the Detail–HCP Assay Platform by Design

A critical task in biologics manufacturing is the control of process-related impurities such as Host cell proteins (HCP) which co-purify with the drug substance and which are known for adverse effects such as immunogenicity. For decades the method of choice are immunoassays based on polyclonal antisera raised against a population of HCPs. Although immunoassays such as ELISAs are well established in many QC applications, especially for HCP determination, there are some inevitable risk factors (antigen, animals etc.) associated with this approach and the fi-

nal performance of the HCP assay strongly depends on the preparation work and materials used at the start. In this presentation we will show success and non-success case studies from more than twenty years experience in the field of HCP assay development. We will highlight critical parameter and quality attributes along the entire assay development process such as:

- Considerations for the choice of a suitable antigen including regulatory and practical requirements
- Optimization of the immunization process by cascade/subtractive approaches
- Qualification of antigen and antisera as reagents for a GMP compliant assay development
- Targeted enhancement of coverage by combining tools from proteomics, protein synthesis and immunization

Dr. Olaf Stamm, Sr. Specialist, **Charles River**

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

4:00-4:30: How Many are there? The Difficulty of Coming Up with a Single Number for Host Cell Protein Coverage
Dr. Oliver Anderka, Fellow, **Novartis**

4:30-5:00: Case Study: Demonstration of antibody coverage. Alternatives to Western Blot

2D-Western Blot is still the most accepted method for antibody coverage assessment. One limitation of this method is the use of denaturing conditions and the resulting conformational changes that make the comparisons of this method with ELISA methods questionable. Therefore case studies from alternative methods, where the recognition of the Antigen occurs under native conditions were evaluated. In one approach, the antigen is fractionated by 2D chromatography using SEC and IEC. The fractions were analyzed subsequently in an ELISA. The chromatographic signal and the ELISA reactivity were compared. In a second approach, the antigen was separated in a bound (recognized) and unbound (unrecognized) population on an immunoaffinity column immobilized with the relevant antibody. Both populations were analyzed alongside an unseparated original antigen sample then visualized and compared in a 2D-SDS-Gel. In both cases the antibody coverage is assessed under native conditions. Relevance for the subsequent ELISA application is discussed

Dr. Norbert Berwanger, Lab Supervisor, **Boehringer Ingelheim Pharma**

5:30: Workshop Adjourns

8:00-8:30: Opening Remarks

Orthogonal Methods for HCPs & Implications for Process Development

8:30-9:00: Characterization of Host Cell Proteins in Biotherapeutics by Mass Spectrometry

Sensitive detection and quantitation of residual host cell proteins (HCPs) during purification process development is critical in the design of robust and well-controlled manufacturing processes that yield high quality biotherapeutics. The enzyme-linked immunosorbent assay (ELISA) is the current standard assay for determining residual HCP levels in the purified biotherapeutic drug substance. Mass spectrometry-based methods are emerging as a routine approach for HCP analysis where residual HCPs can be detected, identified, and quantitated directly due to ever increasing instrument performance. In this study, we have developed proteomic approaches to identify and quantify residual HCPs in biotherapeutics derived from both mammalian and bacterial expression systems in an effort to complement ELISA results. Spiking studies were employed to determine the limits of quantitation and detection for a wide variety of possible HCPs. The proteomic method employs proteolytic digestion, one dimensional chromatographic separation by RP-HPLC, ultrahigh-resolution mass spectrometry, and database searching to definitively identify potential HCPs. A comparison of analytical approaches for HCP detection and quantitation will be discussed.

Dr. Justin Sperry, Senior Principal Scientist**, Pfizer**

9:00-9:30: Mass Spectrometric Detection & Quantification of Host Cell Proteins in Support of Bio-process Development
Dr. Christopher Yu, Senior Scientist, **Genentech**

9:30-10:00: Improved Detection of Host Cell Proteins (HCPs) in a Mammalian Cell-Derived Antibody Drug Using Liquid Chromatography-Mass Spectrometry in Conjunction with an HCP Enrichment Strategy
Dr. Jenny Heidbrink Thompson, Scientist, **MedImmune**

10:00-10:30: Production of HCP by CHO Cells: Insights from Cell Culture

To increase our understanding of HCP from a cell culture perspective, we profiled the production of HCP by three CHO hosts. We also investigated the impact of CHO host and process conditions on the production of a specific HCP species.

Dr. Inn Yuk, Principal Engineer, **Genentech**

10:30-11:00: Survey of HCP data from a biopharmaceutical pipeline: Are process-specific HCP assays superior to a platform assay?
Dr. Frieder Kroner, Post-doctoral Scientist, **Novartis**

BEBPA's First European HCP Workshop

Host Cell Protein Workshop

Day 2

11:00-11:30: Morning Break

11:30-12:00: Specific Assays to Ensure Chaperones Don't Crash the Final Product

During the development of biologicals, high titer producing cell lines are highly desired. For *E. Coli* hosts, endogenous chaperones (eg, DsbA, and DsbC) can be engineered to express over endogeneous levels to help proper folding and assembly of the product protein, which leads to increased product titer. Since the host cells were engineered to overexpress the chaperones, the levels of the chaperones require monitoring to ensure that the chaperones are cleared to acceptable levels. The over-expressed levels of the chaperones may not be detected in the normal HCP assays, and specific assays for the chaperones are needed. Custom reagents can be used to develop specific chaperone assays. The chaperone specific assays can be used as a monitoring tool during process development to help characterize the manufacturing process.

Chris Fong, Sr. Supervisor, **Genentech**

12:00-1:30: Lunch

Quality, Regulatory & Clinical Perspectives

1:30-2:00: Factors Leading to Sample Dilution Dependence

Dr. Heather Boux, Sr. Specialist, **Amgen**

2:00-2:30: Identification of an antibody-bound HCP and the impact to Process Development

Dr. Denise Krawitz, Sr. Manager, **Genentech**

2:30-3:00: A New USP General Chapter: <1132> Residual Host Cell Protein Measurement in Biopharmaceuticals

In 2013, USP formed an Expert Panel to produce a general chapter containing current best practices for host cell protein (HCP) assays and reagent development. Regulators expect low levels of HCPs in biopharmaceuticals and manufacturers use these methods to monitor their clearance during production and in the final drug substance. The chapter proposal is now finalized and will appear online in the *Pharmacopoeial Forum* in the summer of 2014. The chapter will provide expert guidance on (1) HCP immunogen and standard preparation and characterization; (2) immunization; (3) purification of characterization of antibodies; (4) immunoassay development and validation; (5) lifecycle management; and (6) orthogonal tools to supplement the primary immunoassay technology. Due to the complexity of the multi-antigen HCP immunoassays, emphasis is placed on unique challenges such as sample dilution linearity, validation, and reporting of results.

Maura Kibbey, Sr. Scientific Liaison, **USP**

3:00-3:30: Afternoon Break

3:30-4:00: Host Cell Protein Analytics: Seen from the Quality Control Perspective

Only rare published cases exist that show clinical incidents referring to Host Cell Protein (HCP) impurities in Biopharmaceuticals. Despite this fact, it is essential to deplete HCPs during the downstream process to ensure a consistently high level of drug purity and to guarantee the best possible patient safety. HCPs are never absolutely depleted because of variable HCP properties and technical limitations. This talk provides assistance for setup of an individual HCP control strategy by discussing the suitability of different types of HCP assays and the generation of supportive data to fulfil current scientific and regulatory requirements.

Dr. Thomas Waerner, Associate Professor, **Boehringer Ingelheim**

4:00-4:30: Assessing Immunogenicity Risk from Host Cell Proteins in Biotherapeutic Development

Protein therapeutics may elicit unwanted immune responses in some patients, and these may compromise product safety and efficacy. A number of tools and technologies have been developed to assess the likelihood that a protein therapeutic may be immunogenic, and these are typically used during therapeutic lead selection and optimization. Some of these approaches may also be used to assess the risk of immunogenicity from exposure to host cell proteins. This talk will discuss some of the systems that are used for immunogenicity risk assessment and their potential utility in assessing whether or not host cell proteins will be immunogenic in humans.

Dr. Valerie Quarmby, Staff Scientist & Director, **Genentech**

4:30-5:00: CHOPPI: a web tool for the analysis of immunogenicity risk from host cell proteins in CHO-based protein production-

Despite high standards for purity, co-purifying host cell protein (HCP) impurities remain a risk for biological products. Residual HCPs can induce a detrimental immune response potentially compromising a biologic. To enable rapid assessment of the risk posed by HCPs in Chinese Hamster Ovary (CHO)-based protein production, we have developed a web tool called CHOPPI, for CHO Protein Predicted Immunogenicity. CHOPPI integrates information regarding the possible presence of CHO-derived impurities (expression and secretion) with characterizations of their immunogenicity (T cell epitope count and density, and relative conservation with human counterparts). We show that CHOPPI can identify clear differences in immunogenicity risk CHO HCPs, and identify additional "risky" CHO proteins that may co-purify during production. CHOPPI provides a computational complement to existing experimental approaches for HCP risk assessment.

Phoebe de Groot, Business & Science Associate, **EpiVax Europe**

5:00-5:30: Close of Workshop Remarks

Ned Mozier, Sr. Director , **Pfizer**

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