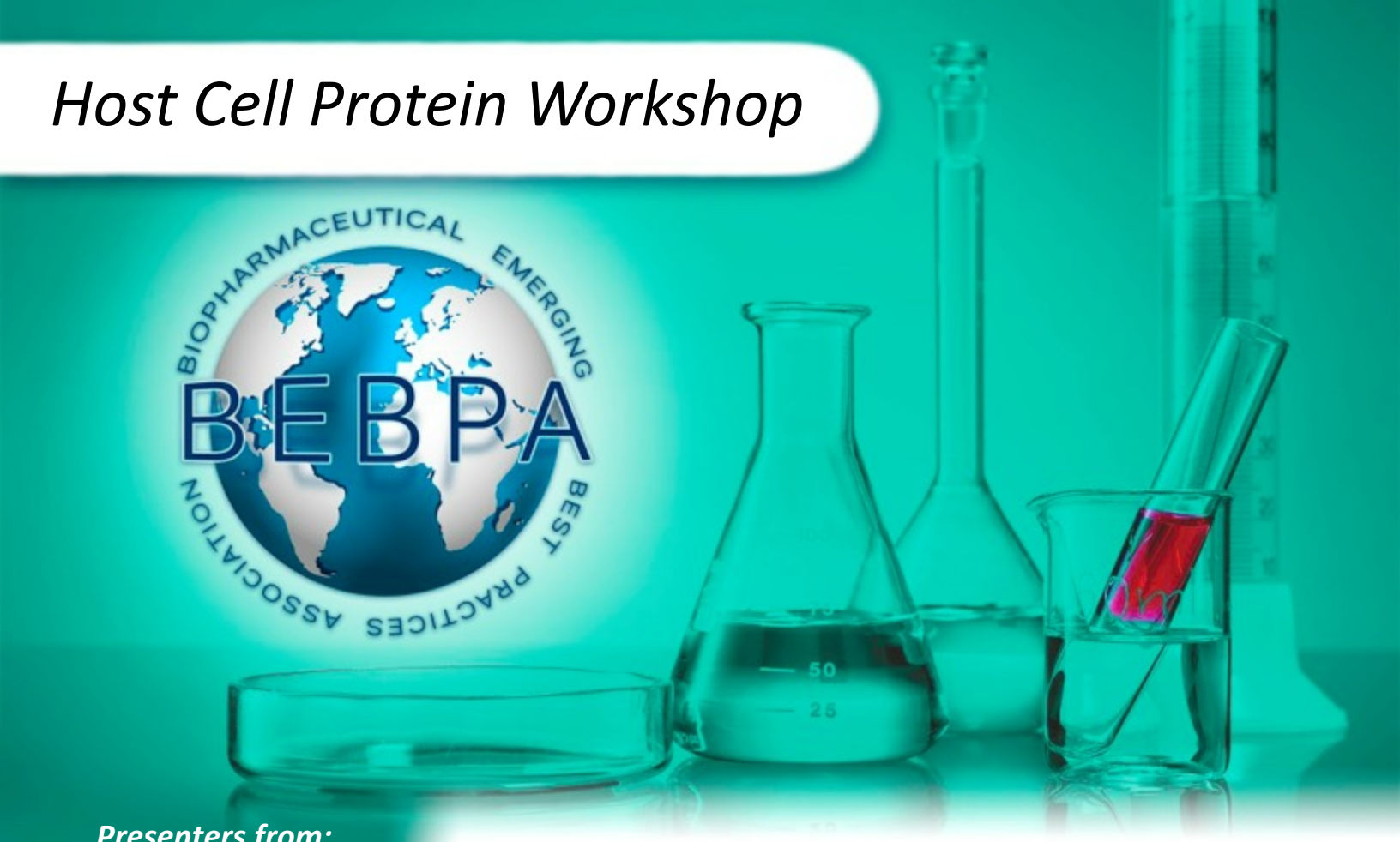
Host Cell Protein Workshop



Presenters from:

Amgen
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Bioprocessing Tech. Instit.
Caprion Proteomics
Charles River Labs
Cygnus Technologies
EMD Millipore
Genentech
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Jansen R & D
Mabworks Biotech Co.
Merck
Novo Nordisk
Pfizer
Quality Assistance
Roche Penzberg
Sanofi
University of Delaware
University of Nebraska
Waters Corporation

What's in your product? Come hear the latest advances in the field of biopharmaceutical HCP Testing

- **Rare Reagents:** Multiple Case Studies on how to develop your rare reagents, including selection of null cell lines, developing immunization strategies and assessing the specificity of your antibodies, characterization of antibodies and standards by 2-D Gels, western blots, and immunofractionation
- **Platform Specific CHOP Immunoassays:** Strategy and Points to Consider for the Successful Development
- **Risk Assessment Framework**: Presentation of a systematic risk assessment of HCP impurties based on such factors as dose, dose frequency, route of exposure, and patient population.
- **LC/MS** is quickly becoming the orthogonal method for detecting HCPs, come hear case studies about getting this powerful technique working in your lab, talks include updates on the CHO genome, proteolytic techniques and the use of SWATH data acquisition.
- **HCP enrichment** as a way to improving sensitivity of the LC-MS methods. Details are shared about all the critical details.
- **Immungenicitiy of HCP** , either bound or unbound to the product is a key safety concern. Several case studies explore the impact of "stealth" contaminants and how to assess their potential for causing clinical problems

For more information: www.bebpa.org **HCP Workshop, May 14-15, 2015**

HCP Workshop Day 1, May 14, 2015

8:30: Keynote Address: Current State of HCP Monitoring Technologies and Significance
Dr. Martin Vanderlaan, Genentech

9:00: Survey of HCP Testing
Dr. Laureen Little, Quality Assistance

Session 1: Immunoassays for HCP Monitoring

Session Chair:
Feny Gunawan, Sr. Research Sceintist, **Genentech, Inc.**

9:30: CHO Anti-HCP Antibodies OK for CHO HCP Samples, Right? Depends…

The measurement of HCPs during bioprocess development is highly dependent on the antigen and antibody reagents used in HCP assays. This talk presents a case study that describes the evaluation of several sets of HCP reagents derived from one host cell line which span different immunizing species, rabbit and goat, and different antigens, cell culture fluid and cell pellet lysate. The presentation will describe the experimental strategy used to characterize and select the final reagents used for in method development. This case study will discuss how multiple orthogonal techniques including 2D-DIGE, immunoassays and mass spectrometry were used to determine that the selected HCP reagents were appropriate for use.
Dr. Carl Co, Scientist, **Biogen Idec**

10:00: Avoiding common mistakes in developing HCP ELISAs
Ken Hoffman, President, **Cygnus Technologies**

10:30-11:00 Morning Break

11:00: Platform Specific CHOP Immunoassays: Strategy and Points to Consider for the Successful Development

Development and manufacturing of active biopharmaceutical ingredients are extremely cost-intensive and time-consuming. To standardize and focus the operational activities the pharmaceutical industry developed platform approaches for upstream and downstream processes. Therefore a standardized and harmonized set of process materials and process steps is used for defined classes of molecules. This platform approach is also implemented for analytical procedures, which monitor the critical quality attributes (CQAs). One of the most important CQA for cell culture dependent processes is the residual content of host cell protein. The strategy and points to consider for the generation of an immunoassay to determine the content of Chinese Hamster Ovary Cell Protein (CHOP) will be outlined and discussed. This comprises the selection of the null cell line, the preparation and characterization of the immunogen/assay standard, the immunization strategy and the evaluation of the resulting anti CHOP polyclonals.
Dr. Frank Wedekind, Manager, **Roche Diagnostics**

11:30: Antibody Qualification, Risk Assessment, and Mitigation Strategy for HCP Immunoassay Development

Residual host cell proteins (HCP) are of immunogenicity concern for biotherapeutics. Recently, the USP released a chapter for residual HCP measurement in biopharmaceuticals, including guidance for testing the residual amount of these proteins in biotherapeutic products. It is a regulatory requirement to demonstrate clearance of residual HCP following downstream processing of biological therapeutics. Greater than 1000 non-product proteins are routinely detected in the cell culture supernatant of mock CHO cells, and assessing coverage of the polyclonal antibodies for a complete match is challenging. The industry gold standard approach is to utilize 2D-silver and Western blotting to assess the coverage of polyclonal antibody reagent used in ELISA. The 2D silver/western blot method is time consuming, tedious, and prone to reproducibility error. Analysis can vary widely, resulting in significantly different coverage assessment values that in turn can cause concern regarding the applicability of the ELISA method. To make the 2D silver/ western blot more reliable, a systematic approach has been developed to assess the analytical variability and more accurately report the results. Additional techniques for characterizing the removal of process-specific HCPs are also being developed, including 2D-DIGE and 2D LC-MS. These emerging techniques have been investigated to provide a thorough assessment of the HCP polyclonal reagent coverage and HCP process clearance to improve the understanding of the HCP impurity profile.
Dr. Fengqiang Wang, Principal Scientist, **Merck**

12:00: Development of Immunoassays for Monitoring Clearance of HCP: Antibody Selection and Characterisation Strategy

Initially, immunoassays were developed to monitor specific components in low concentrations in highly complex matrices such as human sera. The immunoassay technology was applied also in the biopharmaceutical industri as a tool for designing and documenting purification processes with aim of providing more pure and less immunogenic products. Application of the ELISA format for monitoring HCP i biophar-

maceuticals involves a number of choices, including: source of immunogen, procedure and species for immunisations, selection of antisera, purification of antibodies, preparation and storage of standard. We have developed multiple assays for process development and quality control for biopharmaceutical products derived from E. coli, S. cerevisae, BHK or CHO cells. The assays are process specific sandwich ELISAs based on in-house produced polyclonal antibodies developed using a strategy of minimising risk of deselection of antibodies during antibody purification. Examples will be given of characterisation of the multispecificity of the employed antibodies, and the performance of the resulting ELISAs.

Dr. Lise Rønnedal, Novo Nordisk

12:30-1:30: Lunch

Session 2: Immunoassays for HCP Monitoring, Continued

Session Chaired by: Chris Fong, Genentech

1:30: Host Cell Protein Assay Development: ..... A CHO HCP Platform Update!

Patrick Niven, Manager, **Janesen R & D**

2:00: Stealth Contaminants in Therapeutic Antibody Purification

Recent experimental work has documented that traditional host protein ELISAs fail to detect histone cell proteins (HCP) released by dead cells into cell culture harvests. This causes HCP levels to be significantly underestimated, not only at harvest but through the course of downstream purification. Besides the obvious problem of inaccurate contaminant measurement, this is a significant problem for fractionation. Histones and other chromatin-associated species have been shown to depress capacity, fractionation performance, and antibody recovery from all chromatography methods. This presentation will show how histones interfere with protein A affinity chromatography, and reveal practical methods for accurate measurement of histone content in cell culture harvests, chromatography fractions, and final product.

Dr. Hui Theng, Scientist, **BioProcess Technology Instituite**

2:30: Effect of antibody interaction on host cell protein co-purification

Identification and unbiased quantification of individual host cell proteins (HCPs) by mass spectrometry can facilitate assessment of potential HCP-related safety risks to patients as well as forming the basis for a mechanistic understanding of HCP-mAb interactions with a view to eventual mitigation of these interactions. We have undertaken a systematic study to investigate how specific HCPs are retained or cleared at each step of a platform mAb purification process. The bulk of the HCP content remaining after Proteins A affinity chromatography consists of some of the most abundant HCPs present in cell culture fluid, suggesting a roughly similar clearance factor for different HCPs at this step. HCPs remaining after the Protein A step exist bound to mAb, and a challenge for subsequent purification steps is to separate these complexes from free mAb. We will illustrate, with example, insights gained from these studies and how they may lead to refinements of mAb process development strategies.

Qingchun Zhang, Sr. Scientist, **Amgen**

3:00-3:30: Afternoon Break

3:30: A New Perspective on Immunization Strategies – Cascade and Chicken Immunization

Immunoassays for host cell protein (HCP) quantitation require high quality polyclonal antisera which is generated by immunization of common animal models such as rabbits, goats and sheep. The challenge in HCP antisera development is the large number of proteins contained in the antisera and their heterogeneity. The antigens can differ widely with respect to size, physic-chemical properties and immunogenic profile. Typically there is a fast and strong immune response against the very immunogenic proteins, whereas there are much less antibodies developed against the weaker antigens resulting in incomplete coverage of the assay. There are multiple ways to address this challenge and the following presentation is focused on two strategies which have been studied over the last year a) cascade immunization and b) chicken immunization. This presentation will first examine the results from a systematic year long comparison study between a conventional immunization strategy and a cascade approach performed with CHO antigen in rabbits. The same CHO antigen was also used in pilot study for the immunization of chickens. The goal was to verify the expectation that an antigen from a mammalian cell line would be much more immunogenic in a non-mammalian animal resulting in improved coverage. Also examined was if the immunization of chickens and the production of IgY would be associated with other advantages such as an increased yield, ease of purification and animal welfare considerations. The results of both case

studies show interesting alternatives to overcome the limitations of the traditional immunization strategies and animal models.
Dr. Olaf Stamm, Sr. Specialist**, Charles River Labs**

4:00: New Approaches to Characterizing HCPs and Recent Case Studies

Host cell proteins (HCPs) are critical quality attributes in the manufacturing of biopharmaceuticals. Hence, the clearance of HCPs in the manufacturing process must be controlled. HCPs are generally measured using an immune-based assay with HCP antigens as standard and polyclonal antibodies specific for the HCP antigens. Comparative characterization of the standard antigen with HCPs of manufacturing process is performed for assessment of suitability of the HCP assay. Moreover, HCP specific antibodies have to be evaluated to cover a broad spectrum of HCPs present in the product. Orthogonal analytical methods (e.g. Western blot, two dimensional difference in gel electrophoresis, mass spectrometry based methods) are used for this purpose. Quantitative HCP detection may be compromised by missing weak- or non-immunogenic HCPs. To close this potential gap, orthogonal methods are performed for extended in depth characterization. Case studies on evaluation of critical reagents as well as characterization of process HCPs will be given.
Dr. Michael Wiedmann, Manager, **Roche Penzberg**

4:30: Host Cell Protein Characterization for Bioprocess and Product Improvement

Host cell proteins (HCPs) are biomanufacturing process-related impurities which must be closely monitored to ensure process consistency and product quality. ELISA is the most common method for HCP quantification. Validation of HCP ELISA with orthogonal methods is essential to ensure that in-house developed ELISA assays are able to detect the majority of HCP impurities. We have implemented advanced analytical technologies (such as 2D western and LCMSMS) for HCP quantification and characterization.
In this talk, I will present results on:

1. Evaluation the antibody coverage of commercial ELISA kits by 2D western and LCMSMS
2. Identification of high risk HCP to product quality by LCMSMS
3. New downstream processes in reducing high risk HCP

Dr. Rong-Rong Zhu, Sr. Scientist, **EMD Millipore**

5:00: Survey: Use of Mass Spectrometry in HCP Detection
Dr. Laureen Little, Principal Consultant, **Quality Assistance**

5:30: Workshop Adjourns

HCP Workshop Day 2

8:55: Opening Remarks
Dr. Laureen Little, Principal Consultant, **Quality Services**

Session 3: Application of Mass Spectrometry to HCP

Session Chair: Chris Yu, Genentech

9:00: A risk assessment framework for evaluating the impact of host cell protein(s) in biotechnology-derived products

Biotechnology-derived drugs, produced using engineered bacterial or mammalian cells, have been manufactured for over 30 years. These host cells contain a repertoire of proteins essential for their own function and survival, some which may copurify with the therapeutic protein and ultimately become a part of the final drug substance. The thorough characterization of biotherapeutics includes the measurement of host cell protein (HCP) levels. A focus of the manufacturing process is the production of material with appropriate purity; a risk assessment framework that considers a number of important factors can help to inform decision-making about appropriate process development strategies designed to manage the levels of HCPs.
Dr. Christina L. Zuch de Zafra, Sr. Scientist, **Genentech, Inc.**

9:30: An Update from the CHO Genome Community

The CHO K1 cell line is an ancestor to many production cell lines and the ATCC version was recently sequenced. In addition, there have been efforts in the community to sequence K1 variants, other CHO cell lines, and there are two versions of the Chinese hamster genome that have been published. The organization and continual updating of genomic information, including assemblies, annotations, data from RNASeq, microRNA, proteomics, and metabolomics studies, benefits from a community-wide effort to disseminate and share publicly-available information as broadly as possible but also comes with a number of challenges. We will discuss aspects of the international community's efforts at developing an infrastructure to support, host, disseminate, and update genome-scale data related to CHO cell lines. This includes sharing of both the Genbank and the RefSeq ver-

sions of the genome, the availability of a BLAST server, and GMOD-based genome browser for CHO
Dr. Kelvin Lee, Professor, **University of Delaware**

10:00: Identification and Quantification of Host Cell Proteins in Biotherapeutics by Ion-Mobility Mass Spectrometry
Dr. Weibin Chen, Sr. Manager, **Waters Corporation**

10:30-11:00 Morning Break

11:00: Assessment of Residual Host Cell Proteins Using Mass Spectrometry
Mass spectrometry-based methods are emerging as a routine approach for host cell protein (HCP) analysis where residual HCPs can be detected, identified, and quantitated directly due to ever increasing instrument performance. 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. In this study, we have applied this state-of-the-art approach for analyzing residual host cell proteins in mammalian expressed biotherapeutics. In particular, focus areas include downstream process clearance studies and those HCPs that can affect final product attributes.
Dr. Justin Sperry, Sr. Principal Scientist, **Pfizer**

11:30: Host Cell Protein Enrichment For Efficient Identification
Identification of host cell proteins (HCPs) in biopharmaceuticals drug is hampered by the presence of overwhelming amount of the products. Product removal via affinity chromatography carries the inherent risk of losing certain product-associated HCPs. In this case study, HCPs from drug substances were enriched using ProteoMiner™ beads, a bead-based library of combinatorial peptide ligands, and resulted in significant improvements in HCP characterization by 2D-gels and HCP identification by LC-MS (>10-fold increases). Anti-HCP affinity columns were used as an alternative approach for HCP enrichment. Following LC-MS, overlapping yet different HCPs were identified from affinity enriched samples. ProteoMiner™ beads have advantages in preserving the relative abundance information after enrichment, and can be easily incorporated into different HCP characterization workflows. Applying these enrichment technologies will greatly improve MS-based HCP identification, and provide much needed HCP information for purification process development and risk assessments.
Dr. Xiaohui Lu, Sr. Scientist, **Biogen Idec**

12:00: Identification and Quantification of HCPs by LC-MS/MS and LC-MRM
Mass spectrometry can be used to obtain a nearly comprehensive assessment of host cell proteins without the use of antibodies or any prior knowledge of the protein contents. A method will be described that includes an initial phase of protein digestion with trypsin, followed by peptide fractionation by two orthogonal methods to ensure good coverage. Each fraction is analyzed by LC-MS/MS to identify peptides and their parent proteins and to determine relative quantification in each sample. This approach can be used directly to compare purity of different batches, monitor batch uniformity or, for example, to compare product at different production sites. The assay can be further refined by developing a targeted multiple reaction monitoring (MRM) assay to quantify hundreds of peptides/proteins of interest with the aid of isotopically labeled reference peptides. In addition to higher sensitivity and accuracy of the MRM assay, actual protein concentration can be determined. A case study will be used to demonstrate the approach.
Dr. Daniel Chelsky, Chief Scientific Officer, **Caprion Proteomics**

12:30-1:30: Lunch

Session 4: Application of Mass Spectrometry to HCP

Session Chair: Carl Co, Scientist, **Biogen Idec**

1:30: Mass Spectrometric Approaches to HCP Identification- A State of the Technology
Dr. Matthew Schenauer, Gilead

2:00: A Flexible, Robust LC-MS/MS Workflow for Identification and Quantification of HCP Impurities
Donald Walker, Jr., Genentech

2:30: Using LC-MS/MS SWATH Method to Demonstrate the Protein Coverage of Anti-HCP Antibodies and Identify Specific HCP
Enzyme-linked immunosorbent assays (ELISA) are typically used to measure total HCP concentrations. Unfortunately, Anti-HCP antibodies used in the ELISA often failed to recognize all HCP proteins. Some low immunogenic or low

abundant HCPs are hard to be measured by traditional ELISA assay. This can be complemented by LC-MS/MS method. Sequential window acquisition of all theoretical spectra (SWATH) is a LC-MS/MS data independent acquisition method, which benefit of reproducibility and comprehensiveness. We are trying to apply SWATH approach for verifying the coverage of anti-HCP antibody and identifying specific HCP in the final product. HPLC and MS parameters were optimized to identify more than 1000 HCPs in the 1-D LC-MS/MS analysis. Off-line high pH-RPLC coupled with online low pH-RPLC-MS/MS was used to obtain high coverage of the HCP proteome and generate the ion library. These works are on-going. Affinity purification products of anti-HCP antibody and negative control will be analyzed in the SWATH situation. The study results will be reported at the meeting.

Dr. Boyan Zhang, Vice President, **Mabworks Biotech Co**

3:00-3:30: Afternoon Break

3:30: HCP Identification and Quantitation in Biological Products via LC-MS/MS

Dr. Martha Stapels, Sr. Scientist, **Sanofi**

4:00: Identification of an Immunogenic HCP Impurity

Dr. Kevin van Cott, Assoc. Professor, **University of Nebraska**

4:30: Meeting Summary and Looking Forward

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

5:00: Workshop Adjourns

Comments from last year's workshop:

It was very useful to see how different companies look at the HCP problems and issues from different perspective. Knowledge sharing was amazing.
Kasia M., UCB Pharma

Excellent idea to concentrate on one topic which becomes more and more challenging. Nice selection of speakers & topics
Felix D., Sandoz

It was nice to hear the common issues/problems and resolutions from others
Tiffanie H., Coherus Bioscience

This workshop has been both a great review of immunoassay development and a valuable insight into characterization of HCPs and impact in development/validation
Sarah V., MedImmune

Finally a relevant conference focused on host cell proteins that brings everyone up to date on all the best approaches
Georgeen G., Abbvie

Excellent talks over a wide range of topics, but orchestrated so that the sessions flowed.
Rick C, GSK

Open dialogues, very honest, totally worthwhile~
Harold T., Merz Pharmaceuticals

Very high quality of information in a very "familiar" atmosphere. One of the most useful workshops I ever visited. Asked many of my burning questions or give new ideas where to continue. Highly actual state of the art
Anke F., Glycotope

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