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The Organizing Committee gratefully acknowledges the Symposium Program Partners for their generous support of WCBP 2011

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Acknowledgements

Symposium Co-Chairs
Robin Levis, CBER, FDA
Mark Schenerman, MedImmune
Marjorie Shapiro, CDER, FDA

Workshop Committee Co-Chairs
Roman Drews, CBER, FDA
Kathleen Francissen, Genentech, a Member of the Roche Group
Peter Johnson, 3M Drug Delivery Systems
Susan Kirshner, CDER, FDA

Permanent Committee
Robert Cunico, Bay Bioanalytical Laboratory, Inc.
John Dougherty, Eli Lilly and Company
John Frenz, GlobelImmune, Inc.
Christopher Joneckis, CBER, FDA

Michael Kunitani, Marin Analytical Consulting
Thomas Layloff, Supply Chain Management System (Chair)
Robert Sitrin, Merck Manufacturing Division

Special thanks to all the Workshop Session Co-Leaders

Program Committee
Sid Advant, ImClone Systems Corporation
Robert Baffi, BioMarin Pharmaceutical, Inc.
Laura Bass, Pfizer, Inc.
Elizabeth Fowler, Xcellerex, Inc.
John Hennessey, NovaDigm Therapeutics, Inc.
Michelle Frazier-Jessen, Micromet, Inc.
Chulani Karunatilake, Amgen, Inc.
Joseph Kutza, MedImmune
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Lotte K. McNamara, LKM CMC Consulting
Rohin Mhatre, Biogen Idec Inc.

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Katherine Owen, Merck & Co., Inc.
Stefanie Plushkell, Pfizer, Inc.
Nadine Ritter, Biologics Consulting Group, Inc.
Reb Russell, Bristol-Myers Squibb Company
Timothy Schofield, GlaxoSmithKline
Sally Seaver, Seaver Associates LLC
Arne Staby, Novo Nordisk A/S

Collaborating Organizations
American Association of Pharmaceutical Scientists (AAPS)
American Chemical Society, Division of Analytical Chemistry (ACS, DAC)
Association of Biomolecular Resource Facilities (ABRF)
Central New England Chromatography Council (CNECC)

Fédération de l’Industrie Pharmaceutique (FIP)
International Association for Biologicals (IABs)
National Institute for Biological Standards and Control (NIBSC)
PDA, West Coast Chapter

Audio-Visual: Michael Johnstone, MJ Film/Video Productions

CASSS Staff:
Karen A. Bertani, CMP, Symposium Manager
Stephanie L. Flores, CAE, Executive Director
Ann Hein, CMP, Manager of Meetings and Events
Renee Olson, Manager of Meetings and Events
General Information

Name Badges
Please wear your name badge in order to gain admittance to the meetings, poster sessions, exhibit hall and social functions. A name badge with a red circle represents a one-day registration, which does not include the cost of the Welcome Reception for the 15th Anniversary Celebration of WCBP at Almas Hall. Tickets to the Welcome Reception at Almas Hall may be purchased at the registration desk at a cost of $100.00.

Business Center
The Renaissance Mayflower Hotel has a Business Center that is located on the Mezzanine Level. The Business Center is open 24 hours a day, seven days a week and is accessible with your room key. The telephone number for the Business Center is 202.776.9177. The FAX number is 202.776.9182.

Photographic Equipment
The use of cameras is not permitted during the lecture program, workshops or poster sessions. Cameras are permitted on the exhibit floor. However, permission from the vendors involved must be obtained before photographs can be taken.

Poster Sessions
All posters will be set up the entire three days of the Symposium in the East / State Rooms on the Promenade Level. Posters in the P-100-M series will be presented on Monday from 15:00 – 16:00. Posters in the P-200-T series will be presented on 15:05 – 16:05. Poster abstracts can be found beginning on page 88 in this book.

Registration
Registration will be set up in the Cabinet Room on the Promenade Level from Monday, January 10 through Wednesday, January 12.

Social Program
Welcome Reception  Almas Hall  Monday, January 10, 19:15 – 22:30
NOTE: Transportation will be provided.
Exhibitor Reception  East and State Rooms, First Floor  Tuesday, January 11, 17:20 – 19:00
Hosted Luncheon  East and State Rooms, First Floor  Wednesday, January 12, 12:15 – 13:45

If you have special dietary needs, please notify the Symposium Manager Monday morning so that an alternate menu may be prepared. Also, please note that tickets to the Welcome Reception at Almas Hall for non-registered guests or one-day registrants may be purchased at the Symposium registration desk for $100.00.

Technical Seminars
Three technical seminars will be held on Monday, January 10 from 12:30 – 13:30. Two technical seminars will be held on Tuesday, January 11 from 12:15 – 13:15. Please refer to the program summary for more details. Technical Seminar abstracts may be found beginning on page 78.
MONDAY, JANUARY 10, 2011

07:15 – 17:30 Registration in the Cabinet Room

07:15 – 08:15 Continental Breakfast in the East / State Rooms

08:15 – 08:30 Welcome and Introductory Comments
Wassim Nashabeh, Genentech, a Member of the Roche Group
Mark Schenerman, MedImmune

08:30 – 10:30 Regulation of Biopharmaceutical Products: Government Perspectives
Roundtable Discussion in the Grand Ballroom
Session Chair: Mark Schenerman, MedImmune
Facilitator: Kenneth Seamon, University of Cambridge

Panel Members:
Karen Midthun, CBER, FDA, Rockville, MD USA
Peter Richardson, European Medicines Agency, London, United Kingdom
Anthony Ridgway, Health Canada, Ottawa, Canada
Janet Woodcock, CDER, FDA, Bethesda, MD USA

10:30 – 11:00 AM Break – Visit the Exhibits and Posters in the East / State Rooms

11:00 – 11:20 Assay Modernization – Good for Industry, Regulators and Public Health
Rajesh Gupta, OCBQ, CBER, FDA, Rockville, MD USA

11:20 – 11:40 Life Cycle Management for Vaccine Assays
Robert Sitrin, Merck & Co, Inc., West Point, PA USA

11:40 – 12:00 New Analytical Methods for Heparin
Edward Chess, Baxter Healthcare Corporation, Round Lake, IL USA

12:00 – 12:15 Panel Discussion – Questions and Answers
Monday, January 10 continued

12:15 – 13:45  **Lunch Break** - Participants on their own

<table>
<thead>
<tr>
<th>12:30 – 13:30</th>
<th><strong>Technical Seminars</strong></th>
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| **Analytical Characterization of Monoclonal Antibodies: Rapid Glycan Profiling Using the Microfluidic Based mAb-Glyco Chip-LC/MS Solution & Analysis of Biotherapeutics Using the Infinity 1260 Bio-inert LC**<br>Sponsored by Agilent Technologies | **Chinese Room**
| **NOTE:** Lunch is provided for first 100 attendees. |
| **The iCE280, An Excellent Tool for Fast and Efficient Process Development, Product Control and Product Comparability**<br>Sponsored by Convergent Bioscience | **Colonial Room**
| **NOTE:** Lunch is provided for first 100 attendees. |
| **From Discovery to the Routine – Mass Spectrometric Workflows for the Characterization of Protein-Based Biopharmaceuticals**<br>Sponsored by Bruker Daltonics | **Senate Room**
| **NOTE:** Lunch is provided for first 100 attendees. |

### Linking Quality Attributes to Safety and Efficacy

**Parallel Session** in the Grand Ballroom

**Session Chairs:** Chulani Karunatilake, *Amgen, Inc.* and Reb Russell, *Bristol-Myers Squibb Company*

<table>
<thead>
<tr>
<th>13:45 – 14:05</th>
<th><strong>Using Clinical Study Samples for Quality Attribute Assessments</strong>&lt;br&gt;Greg Flynn, <em>Amgen, Inc.</em>, Thousand Oaks, CA USA</th>
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<tr>
<td>14:05 – 14:25</td>
<td><strong>Protein Analytical Approaches: In-vivo / Ex-vivo Biologics Characterization</strong>&lt;br&gt;Ashok Dongre, <em>Bristol-Myers Squibb Company</em>, Princeton, NJ USA</td>
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<td>14:25 – 14:45</td>
<td><strong>Monoclonal Antibody Charge Variants: Physicochemical Characteristics and Impact on Pharmacokinetics</strong>&lt;br&gt;Paul Motchnik, <em>Genentech, a Member of the Roche Group</em>, South San Francisco, CA USA</td>
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<td>14:45 – 15:00</td>
<td><strong>Questions and Answers</strong></td>
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### Small Company Key Issues – Pay Now or Pay Later: Early Product Development Strategies

**Parallel Session** in the Colonial Ballroom

**Session Chairs:** Chana Fuchs, *CDER, FDA* and Nadine Ritter, *Biologics Consulting Group, Inc.*

| 13:45 – 14:05  | **FDA Perspective: Common Mistakes Made During Biotechnology Product Development**<br>Ruth Cordoba-Rodriguez, *CDER, FDA, Silver Spring, MD USA** |
Monday, January 10 continued

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<th>Time</th>
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<tr>
<td>14:05 – 14:25</td>
<td>Manufacturing Challenges Faced By Biotech Start-Up Companies During Drug Development</td>
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<td>Robert Baffi, <em>BioMarin Pharmaceutical Inc.</em>, Novato, CA USA</td>
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<td>14:25 – 14:45</td>
<td>Biologics Development in a Small Company: Same Game, Different Playbook</td>
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<td>John Hennessey, <em>NovaDigm Therapeutics, Inc.</em>, Lower Gwynedd, PA USA</td>
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<tr>
<td>14:45 – 15:00</td>
<td>Questions and Answers</td>
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<td>15:00 – 16:00</td>
<td><strong>Poster Session One</strong> in the East / State Rooms</td>
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<th>Time</th>
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<td>16:00 – 17:15</td>
<td>Small Company Key Issues: Early Product Development Strategies</td>
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<td>Modernizing Analytical Methods for Legacy Products</td>
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<td>Linking Product Quality Attributes to Safety and Efficacy</td>
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<td>Equipment Cleaning Validation</td>
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<td>Alfredo Canhoto, <em>Genzyme Corporation</em>; Bo Chi, <em>CDER, FDA</em>; John O’Connor, <em>Genentech, a Member of the Roche Group</em>; Destry Sillivan, <em>CBER, FDA</em></td>
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ICH Q10 – Pharmaceutical Quality Systems (PQS): A Key Element in the Implementation of Q8, 9 and 10

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<td>17:30 – 17:55</td>
<td>Continual Improvement Under a Q8, 9 and 10 Framework</td>
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<td>Rick Friedman, <em>CDER, FDA, Silver Spring, MD USA</em></td>
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<td>17:55 – 18:20</td>
<td>PQS – Advantages for a Global Operation in the Implementation of Q8, 9 and 10</td>
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<td>Joseph Famulare, *Genentech, a Member of the Roche Group, Washington, DC USA</td>
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<tr>
<td>18:20 – 18:30</td>
<td>Questions and Answers</td>
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<tr>
<td>19:15 – 22:30</td>
<td><strong>Welcome Reception</strong> at Almas Hall – 15th Anniversary Celebration</td>
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<td>Transportation will be provided</td>
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Transportation will be provided
TUESDAY, JANUARY 11, 2011

07:00 – 17:00 Registration in the Cabinet Room

07:00 – 08:45 Continental Breakfast in the East / State Rooms and Cabinet Room

**Staying One Step Ahead of the Bad Guys: The Science of Drug Counterfeiting**

*Sunrise Plenary Session* in the Grand Ballroom

*Session Chair:* Brian K. Nunnally, Pfizer, Inc.

07:30 – 07:55 Counterfeit Issues Relating to Biologics and Devices
Ray Strucker, *Office of Criminal Investigations, ORA, FDA, Rockville, MD USA*

07:55 – 08:20 Fake Botulinum Toxin Products in Clinical Use – A Growing Market with Issues
Andy Pickett, *Ipsen Biopharm Limited, Wrexham, United Kingdom*

08:20 – 08:30 Questions and Answers

08:40 – 08:45 Announcements by Mark Schenerman, MedImmune

**Innovations in Quality by Design for Biological and Biotechnology Products**

*Parallel Session* in the Grand Ballroom

*Session Chairs:* Timothy Schofield, GlaxoSmithKline and Arne Staby, Novo Nordisk A/S

08:45 – 09:05 Process Simulation, Parameter Uncertainty and Risk in QbD and ICH Q8 Design Space
John Peterson, GlaxoSmithKline, King of Prussia, PA USA

09:05 – 09:25 Development of a Modeling Predictive and Visualization Tool for Tracking Quality Attributes Across Upstream, Downstream and Stability Testing
José Casas-Finet, MedImmune, Gaithersburg, MD USA

09:25 – 09:45 Mechanistic Modeling – The Ultimate QbD Tool for Process Understanding
Marcus Degerman, Novo Nordisk A/S, Gentofte, Denmark

09:45 – 10:00 Questions and Answers

**Small Company Key Issues – 21st Century Principles for Early Product Development**

*Parallel Session* in the Grand Ballroom

*Session Chairs:* Michelle Frazier-Jessen, Micromet, Inc. and Joseph Kutza, MedImmune

08:45 – 09:05 Prioritizing and Managing Key CMC Elements
Laurie Graham, CDER, FDA, Bethesda, MD USA

09:05 – 09:25 Applying Big Principles for Small Companies
John Frenz, GlobalImmune, Inc., Louisville, CO USA
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<tr>
<td>09:25 – 09:45</td>
<td>You’re Virtually There – Virtual Product Development</td>
<td>Joseph Tyler, Proteon Therapeutics, Inc., Waltham, MA USA</td>
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<td>09:45 – 10:00</td>
<td>Questions and Answers</td>
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<td>10:00 – 10:30</td>
<td>AM Break – Visit the Exhibits and Posters in the East / State Rooms</td>
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<td>10:30 – 11:45</td>
<td>Workshop Session 2</td>
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<td></td>
<td>Small Company Key Issues – It’s a Risky Business…Or Is It? Managing Risk Associated with Early Product Development</td>
<td>Chinese Room</td>
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<td>John Frenz, GlobalImmune, Inc.; Michael Havert, CBER, FDA; Kathy Lee, CDER, FDA; Jeffrey Staeker, PPD, Inc.</td>
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<td>TUTORIAL: Assessment and Control of Fc Effector Functions of Therapeutic Antibodies</td>
<td>Colonial Room</td>
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<td>Xu-Rong Jiang, MedImmune; Kimberly May, Merck &amp; Co., Inc.; Marjorie Shapiro, CDER, FDA</td>
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<td>Practical and Innovative Solutions for Applying QbD Principles to Complex Biologics</td>
<td>Grand Ballroom</td>
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<td>Ilse Blumentals, GlaxoSmithKline; Michele Dougherty, CDER, FDA; Christopher Joneckis, CBER, FDA; Stefanie Pluschkell, Pfizer, Inc.</td>
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<td>Characterization of Higher Order Structure in Protein and Monoclonal Antibody Therapeutics</td>
<td>Senate Room</td>
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<td>Michail Alterman, CBER, FDA; Sarah Kennett, CDER, FDA; Andrew Kosky, Genentech, a Member of the Roche Group; Alla Polozova, MedImmune</td>
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<tr>
<td>11:45 – 13:30</td>
<td>Lunch Break - Participants on their own</td>
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<tr>
<td>12:15 – 13:15</td>
<td>Technical Seminars</td>
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<td>Deploying High Resolution Protein Analytics Across Biopharma Organizations: New Technologies, Informatics and Compliance (Oh My!)</td>
<td>Chinese Room</td>
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<td>Sponsored by Waters Corporation</td>
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<td>NOTE: Lunch is provided for the first 100 attendees.</td>
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<td>High Throughput / High Resolution Glycosylation Pattern Analysis of Monoclonal Antibodies by Capillary Electrophoresis</td>
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<td>Sponsored by Beckman Coulter, Inc.</td>
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Tuesday, January 11 continued

Adventitious Agent Testing – The Evolving Reality
Plenary Session in the Grand Ballroom
Session Chairs: Robin Levis, CBER, FDA and Sally Seaver, Seaver Associates LLC

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<tr>
<td>13:30 – 13:50</td>
<td>Use of New Technologies to Detect Adventitious Agents in Biological Products</td>
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<td>Philip Krause, CBER, FDA, Rockville, MD USA</td>
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<td>13:50 – 14:10</td>
<td>Commercial Vaccines and New Technologies: Impact and Learning’s</td>
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<td>Anne-Françoise Macq, GlaxoSmithKline Biologicals, Wavre, Belgium</td>
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<td>14:10 – 14:30</td>
<td>Virus Detection and Identification: A General Process Overview</td>
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<td>Mark Plavsic, Genzyme Corporation, Framingham, MA USA</td>
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<td>14:30 – 14:50</td>
<td>The Importance of Classical Virology Training for Quality and Regulatory Staff in the Biopharmaceutical Industry</td>
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<td>Barbara Potts, Biologics Consulting Group, Inc., San Francisco, CA USA</td>
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<tr>
<td>14:50 – 15:05</td>
<td>Questions and Answers</td>
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<td>15:05 – 16:05</td>
<td>Poster Session Two in the East / State Rooms</td>
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Analytical Challenges Associated with New Molecular Modalities and Combination Products
Plenary Session in the Grand Ballroom
Session Chairs: Laura Bass, Pfizer, Inc. and Marjorie Shapiro, CDER, FDA

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<tr>
<td>16:05 – 16:25</td>
<td>Analytical Challenges for Multiple Glycosylated Proteins</td>
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<td>Hansjoerg Toll, Sandoz GmbH, Kundl, Austria</td>
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<tr>
<td>16:25 – 16:45</td>
<td>Analytical Challenges in the Analysis of Peptide Bioconjugates</td>
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<td>Jeffrey Schneiderheinze, Pfizer Biotherapeutics, St. Louis, MO USA</td>
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<tr>
<td>16:45 – 17:05</td>
<td>Analytical and Development Challenges with Recombinant Antibody Mixtures for the Treatment of Infectious Disease and Cancer</td>
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<td>Torben Frandsen, Symphogen A/S, Lyngby, Denmark</td>
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<tr>
<td>17:05 – 17:20</td>
<td>Questions and Answers</td>
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<td>17:20 – 19:00</td>
<td>Exhibitor and Poster Reception in the East / State Rooms</td>
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WEDNESDAY, JANUARY 12, 2011

07:30 – 17:00  Registration in the Senate Room

07:30 – 08:30  Continental Breakfast in the East / State Rooms

08:30 – 08:40  Announcements by Mark Schenerman, MedImmune

08:40 – 09:00  Acknowledgments
Thomas Layloff, Supply Chain Management System

Blood and Gene/Cell Therapy Complex Biological Products: How Well Can They Be Characterized?
Plenary Session in the Grand Ballroom
Session Chairs: Steven Bauer, CBER, FDA, Roman Drews, CBER, FDA and Timothy Hayes, ProMetic BioTherapeutics Inc.

09:00 – 09:20  rFVIII and rVWF: Strategies to Meet the Challenge of Structural and Functional Characterization of the Most Complex Therapeutic Proteins
Peter Turecek, Baxter BioScience Division, Vienna, Austria

09:20 – 09:40  Critical Parameters for the Characterization of Human Embryonic Cell Based Therapies
Jane Lebkowski, Geron Corporation, Menlo Park, CA USA

09:40 – 10:00  Towards Better Characterization of Recombinant AAV Gene Transfer Vectors: Case Studies Illustrating Challenges with Dose Determining Vector Concentration Methods
J. Fraser Wright, University of Pennsylvania School of Medicine, Philadelphia, PA USA

10:00 – 10:20  Complex Biological Products – The Challenge of Plasma Protein Product Characterization
Stefan Schulte, CSL Behring GmbH, Marburg, Germany

10:20 – 10:35  Panel Discussion – Questions and Answers

10:35 – 11:00  AM Break – Visit the Exhibits and Posters in the East / State Rooms
Wednesday, January 12, 2011 continued

11:00 – 12:15  Workshop Session 3

**Analytical Challenges Associated with New Molecular Modalities**  
Chinese Room  

**How to Get Your Comparability On – Understanding Common Challenges During Comparability Studies**  
Colonial Room  

**Characterizing Complex Biological Products**  
Grand Ballroom  

**Adventitious Agents – Mitigating Risk**  
Senate Room  

12:15 – 13:45  Hosted Lunch in the East / State Rooms

13:45 – 15:00  Workshop Session 4

**Devices: Pre-Filled Syringes**  
Chinese Room  

**Biopharmaceutical Reference Standard Establishment and Life-Cycle Management**  
Colonial Room  
Anu Bansal, *Genentech, a Member of the Roche Group*; Sarah Demmon, *Eli Lilly and Company*; James Kenney, *CBER, FDA*; Carla Lankford, *CDER, FDA*

**Biosimilars – Regulatory Considerations**  
Grand Ballroom  

**Start the Madness: The Role of Methods Monitoring in cGMP Compliant Biotech Manufacturing**  
Senate Room  

15:00 – 15:30  PM Break in the Promenade Foyer
Q11

Plenary Session in the Grand Ballroom
Session Chairs: John Dougherty, *Eli Lilly and Company* and Wassim Nashabeh, *Genentech, a Member of the Roche Group*

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<td>ICH Q11 - An Overview of the Current Draft Guideline</td>
<td>Brian Withers, <em>Abbott Laboratories, Kent, United Kingdom</em></td>
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<td>16:10 – 16:30</td>
<td>Process Validation for Biotech: Concepts Under Consideration for ICH Q11</td>
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Plenary Session Abstracts

Regulation of Biopharmaceutical Products: Government Perspectives
WCBP: 15 Years Later – How Are We Doing and How Can We Tackle the Next Fifteen Years?
Roundtable Discussion

Session Chair: Mark Schenerman, MedImmune
Facilitator: Kenneth Seamon, University of Cambridge

Panel Members:
Karen Midthun, CBER, FDA, Rockville, MD USA
Peter Richardson, European Medicines Agency, London, United Kingdom
Anthony Ridgway, Health Canada, Ottawa, Canada
Janet Woodcock, CDER, FDA, Bethesda, MD USA

It has been fifteen years since the first WCBP conference. The application of technological advances in manufacturing and analytics along with the increasing genomic knowledge has helped foster a large and significant increase in the investment for biotherapeutics. This investment has occurred along with a number of initiatives aimed at leveraging current science and technology to facilitate development and commercialization of such products. This session will examine some of the areas that impact global development and commercialization of biological products with a particular emphasis on how to take advantage of cross border successes and learnings.

This session will focus on four different areas of drug development commercialization:

1. Streamlining the drug approval process
2. Facilitating global drug development and commercialization
3. Sharing knowledge and experience in advanced technologies
4. Regulatory and scientific considerations for biosimilars

1) Streamlining Drug Approval Process
   a) How have “risk-based” reviews affected approvals?
   b) How far can advanced analytical technologies take us and what areas need further investment? What will be the benefit of these investments?
   c) Has current application of ICH Q8-Q10 principles (also known as QbD) helped to communicate to regulators greater understanding and control of the product and process? Has the QbD approach helped to expedite product reviews and inspections?
   d) There has been some success in regulatory acceptance of biomarkers through consortia and other industry/regulatory authority initiatives. Is this a model that can be extrapolated to other issues related to the manufacturing and characterization of biopharmaceuticals?
2) Globalization of Manufacturing
   a) How can the regulatory compliance challenges related to increased number of manufacturing, testing, and clinical research sites around the world be met?
   b) How will each authority address resource constraints over the next few years and what role can harmonization and mutual recognition play?
   c) Can additional areas of specific advances in unique regions be leveraged more efficiently to facilitate other regions?
   d) What is the status of global acceptance of a particular region’s compendial methods?

3) Personalized Medicine and Advanced Technologies
   a) What are the challenges in co-developing a diagnostic with a therapeutic product?
   b) How have the different regions handled release and stability testing for personalized gene and cell therapy products which have limited manufacturing history and shelf life?
   c) What are the opportunities to facilitate a more global approach for these technologies and approaches?

4) Biosimilars
   a) How much biochemical and clinical information is needed?
   b) What attributes need better methods to assess/monitor them?
   c) What advice would regions with approval pathways and a history of approval for biosimilars give to the other regions?

NOTES:
A fundamental premise for the WCBP conferences has been the application of modern analytical tools to characterize biotech products to better understand production processes and to facilitate process changes. However, those biotech products which had been developed and licensed many years ago, often have dated and widely variable assays associated with them. In many cases, the original potency assays were animal based and susceptible to wide variation. Often the instrumentation used in the original license application is obsolete and no longer supported by the vendor. Furthermore, as older processes are themselves being modernized or revalidated (perhaps because they are being moved to new facilities), appropriate analytical tools with higher levels of precision are expected.

However, introducing new technology brings with it many challenges – bridging, understanding offsets, reacting to new data, and resetting specifications. In this session, several case studies will be presented with issues and surprises when new assays are applied to replace older assay methodologies.

NOTES:
Recent technological developments in the analytical sciences have generated powerful tools for characterization of biological products. New methods based on these advances are being used in characterization and testing of many new products. Legacy products have lagged behind in the implementation of modern methods due to scientific, regulatory and business challenges. Application of modern analytical methods for the evaluation of legacy products will increase our knowledge and lead to improvements in the manufacturing and characterization of these products. This would provide opportunities for better controls in achieving consistency in manufacture, potentials for product improvements and risk mitigation strategies for any potential risks due to new information obtained by using new technologies. In this presentation, CBER’s perspective about introducing modern methods to legacy products will be discussed. Examples from implementation of rapid microbiological methods for sterility testing of biological products and use of mass spectrometry in development of influenza potency reagents will be presented.
Life Cycle Management for Vaccine Assays

Robert Sitrin

Merck & Co., Inc., West Point PA USA

Life cycle management for biotech products has historically focused on process changes based on biochemical comparability facilitated by the application of modern analytical tools to better understand production processes and products. However, those biotech products, particularly vaccines, which had been developed and licensed many years ago, often have dated and widely variable assays associated with them. In many cases, the original potency assays were animal based and susceptible to wide variation. Often the instrumentation used in the original license application is obsolete and no longer supported by the vendor - forcing development of a more modern assay. Furthermore, as older processes are themselves being modernized or revalidated (perhaps because they are being moved to new facilities), appropriate analytical tools with higher levels of precision are expected. Introducing new technology brings with it many challenges – bridging, understanding offsets, reacting to new data, and resetting specifications. This presentation will outline our systematic approach to modernization of our vaccine release tests and present several case studies where major changes were developed and implemented.

NOTES:
New Analytical Methods for Heparin

Edward K. Chess

*Baxter Healthcare Corporation, Round Lake, IL USA*

Heparin was discovered in 1910, first used as an anti-coagulant in 1935, and in 1950 its monograph was introduced in USP 14. In early 2008 a contaminant was discovered in crude heparin produced in China that caused adverse events in some patients in the United States and Europe receiving the therapeutic heparin. The isolation and structure determination of this contaminant, oversulfated chondroitin sulfate (OSCS) was accomplished by an FDA-led group of academic, government, and industrial laboratories. Levels of OSCS in excess of 20% by weight in heparin sodium were not detected by the USP or other pharmacopeial monograph methods. The methods used to evaluate purity and composition of heparin underwent considerable scrutiny during and after the contamination event, and this re-evaluation has led to the development of analytical methods more capable of detecting impurities and providing increased assurance against potential contamination in the future. These orthogonal techniques, such as nuclear magnetic resonance spectrometry, mass spectrometry, capillary electrophoresis, carbohydrate analysis, and strong anion exchange chromatography, have also been employed to characterize related substances produced during heparin manufacture.

NOTES:
Linking Quality Attributes to Safety and Efficacy

Identification of molecule critical quality attributes is fundamental to developing a comprehensive product knowledge, and hence central to the QbD paradigm. CQAs are defined based on a risk assessment exercise that attempts to quantify and rank the safety and efficacy impact of various product variants and degradents present in recombinant protein drugs. Often, these risk assessments are limited by the availability of relevant safety and efficacy data in the targeted patient population. This session will present several in vivo and in vitro approaches to linking specific quality attributes to patient safety and product efficacy outcomes.

NOTES:
Using Clinical Study Samples for Quality Attribute Assessments

Gregory C. Flynn; Andy M. Goetze; Y. Diana Liu; Matt R. Schenauer

Amgen, Inc., Thousand Oaks, CA USA

Abstract: Recombinant therapeutic proteins contain a variety of chemical and physical modifications, whose impact on safety or efficacy may be unknown. Many of the chemical conversions also occur in vivo, and knowledge of these reactions can be applied to assess the attribute’s impact on the safety, potency and/or clearance of the drug. This talk will describe attribute studies conducted using clinical samples and describe the types of information that can be gleaned. Two case studies, N-terminal pyroglutamate and glycation of therapeutic antibodies, will be presented.

NOTES:
Protein Analytical Approaches: *In-vivo / Ex-vivo* Biologics Characterization

Ashok Dongre

*Bristol-Myers Squibb Company, Princeton, NJ USA*

Abstract was not available at time of printing.

NOTES:
Charge variants of monoclonal antibodies are modified forms of the desired product that can be resolved by ion exchange chromatography. Subtle differences in the amount and distribution of charge variants are often observed pre- and post-process change and pose a challenge to demonstrating product comparability. Acidic, basic, and main peak fractions of an IgG1 monoclonal antibody were isolated and characterized for modifications and activity prior to being administered to rats. Acidic variants were composed of deamidated, glycated, sialylated, reduced and cross-linked forms. Basic variants were composed of aggregated, C-terminal lysine and N-terminal valine-histidine-serine forms. Pharmacokinetic results showed that after intravenous or subcutaneous administration to rats, area under the curve were not statistically different for acidic, basic and main peak fractions. These results demonstrate that although charge variants are chemically different from the main peak, these differences are not sufficient to result in changes in pharmacokinetic properties in rats following intravenous or subcutaneous administration.

NOTES:
Small Company Key Issues – Pay Now or Pay Later: Early Product Development Strategies

Although it is a common industry goal to bring innovative biologic products to the clinic, fledgling groups – either independent start ups or within traditional big pharma - are typically limited in terms of resources and/or experience to accomplish this. Unfortunately, many decisions made early in the drug development process can have lasting consequences. For example, lack of careful product characterization or formulation studies early in development can lead to a delay in later stage clinical programs or substantial delays in successful product commercialization. Inadequate early stage planning could result in an inability to advance programs. This session (and associated workshop) will explore the key elements of early development strategies (focusing on pre-IND to end-of-phase 2) and high priority CMC studies as well as potential pitfalls.

Outcomes:

- Analyze/identify strategy gaps in early phase development programs
- Prioritization of key IND enabling and development activities and program advancement
- Understand requirements from regulatory authorities and/or partners

NOTES:
FDA Perspective: Common Mistakes Made During Biotechnology Product Development

Ruth Cordoba-Rodriguez

CDER, FDA, Silver Spring, MD USA

It is common understanding that manufacturers develop drug products using a quality target product profile (QTPP). This term is usually thought as ‘beginning with the goal in mind’ and it is expanded in FDA Guidance. Although the idea of QTPP is to enable the manufacturer to focus product development with the to-be marketed product in mind, there is not much guidance on how to achieve this goal through development. Companies large and small have experienced failures during product development that lead to delays in advancement of clinical phase, stalling of a manufacturing program development and even unmarketable drug products. This presentation will address common mistakes made during biotechnology product development that can critically impact licensure and marketability of the drug product as well as possible early development strategies that can facilitate an integrated lifecycle approach.

NOTES:
Manufacturing Challenges Faced By Biotech Start-Up Companies During Drug Development

Robert Baffi

BioMarin Pharmaceutical Inc., Novato, CA USA

This presentation will focus on the scientific, regulatory and business challenges faced by startup companies during drug development. Emphasis will be placed on the decision to manufacture in-house or to outsource this critical path activity. BioMarin Pharmaceutical examples will be presented that were successfully implemented to bring the first of four products to worldwide approval within six years after founding of the company.

NOTES:
Biologics Development in a Small Company: Same Game, Different Playbook

John P. Hennessey, Jr.

*NovaDigm Therapeutics, Inc., Lower Gwynedd, PA USA*

Development of biological products to benefit human health comes with the same set of rules for all participating companies. Small companies don’t get a stroke-a-hole, a ten-yard-head-start or a close-is-good-enough when it comes to meeting the regulatory standards for testing our products in human subjects or getting our products licensed for commercial use. This presentation will provide one perspective of how a company with ten employees can meet the same standards as much larger companies.

**NOTES:**
Continual Improvement Under a Q8, 9 and 10 Framework

Rick Friedman

CDER, FDA, Silver Spring, MD USA

Abstract was not available at time of printing.

NOTES:
PQS – Advantages for a Global Operation in the Implementation of Q8, 9 and 10

Joseph Famulare

*Genentech, a Member of the Roche Group, Washington, DC USA*

Abstract was not available at time of printing.

**NOTES:**
Counterfeit Issues Relating to Biologics and Devices

Ray Strucker

Office of Criminal Investigations, ORA, FDA, Rockville, MD USA

Abstract was not available at time of printing.

NOTES:
Fake Botulinum Toxin Products in Clinical Use – A Growing Market with Issues

Andy Pickett

*Ipsen Biopharm Limited, Wrexham, United Kingdom*

Abstract was not available at time of printing.

NOTES:
Innovations in Quality by Design for Biological and Biotechnology Products

Quality by design for biotechnology products has evolved in recent years with the launch of the FDA pilot program, and publication of the A-Mab case study. These efforts highlight a number of novel approaches and tools for exploring and modeling biotechnology processes. While industry has readily incorporated some basic tools related to risk assessment, development of design space, and control strategy, these novel approaches offer new opportunities for more refined understanding of biotechnology processes and products.

Key among these is mechanistic modeling. Models are constructed for process optimization which leads to optimized recipes or an optimal process lay-out. Indeed, the differential equations representing one or several unit operations in a production process inherently represent the input-output dynamics, which is precisely the type of information needed to pinpoint the causes of excessive variation of product quality or to select suitable actuators to counteract undesired variations in product quality. To this end, the model can be supplemented by a modeling toolbox including uncertainty and sensitivity analysis to assess the statistical quality (read as reliability) of the simulated scenarios.

Regulators have asked, and industry has responded to the need for an assessment of the uncertainty inherent in the estimation of design space. Recognizing that the approach of overlapping contour regions described in ICH Q8 describes a boundary which predicts quality with limited assurance, several approaches have been described in the literature and in workshops. A method utilizing Bayesian reliability has been illustrated in the A-Mab case study. That approach utilizes the information from multifactor experiments, to calculate the probabilities of material meeting its quality requirements.

Further work is required to address the predictability of small scale experiments on large scale performance. Modeling is limited in its ability to forecast acceptable outcomes. Opportunities may arise during continuous process verification to confirm or update forecasts made at small scale. Standard statistical methods can be used to reliably assess conformance to small scale prediction, with fewer false positive signals.

This session will illustrate innovations in these three areas of process development and manufacture.

NOTES:
Process Simulation, Parameter Uncertainty and Risk in QbD and ICH Q8 Design Space

John J. Peterson

GlaxoSmithKline, King of Prussia, PA USA

A fundamental aspect of quality improvement is to reduce variation about a desired target. Classical design of experiments (DoE) can help one to get their process mean closer to a target, which reduces the systematic variation (i.e. bias) from a target. Reduction in process noise can help to further reduce random variation about a target. There are common statistical methods to do this, but these methods are primarily focused on only one quality response at a time. However, many processes can have multiple quality responses that may be correlated with each other. Conceptually, it is often best to think of an industrial process as a stochastic process producing a distribution of possible responses. This distribution will be a function of process inputs and controllable process factors (e.g. temperature, pressure, etc.). By modeling the process distribution as a function of inputs and controllable factors, one can optimize a process with regard to meeting (multiple) quality specifications, and at the same time, determine the reliability of meeting those specifications. This talk will discuss these concepts and show some examples. Key points include:

- Understanding how the predictive distribution of process responses depends upon process inputs, controllable process factors, and estimated process parameters.
- How Monte Carlo simulation can be used to compute a predictive distribution, which can then be used to compute probabilities of meeting multiple quality specifications.
- How such probabilities can be used to construct an ICH Q8 design space.

NOTES:
Development of a Modeling Predictive and Visualization Tool for Tracking Quality Attributes across Upstream, Downstream and Stability Testing

1Jose Casas-Finet; 1Guillermo Miro-Quesada; 2Husheng Yang

1MedImmune, Gaithersburg, MD USA; 2AstraZeneca, Newark, DE USA

A predictive model was developed to quantify the levels of an impurity throughout the complete manufacturing process, including upstream, downstream and product storage. The model uses a parallel coordinates visualization approach to display in an interactive fashion the impact of various input variables such as culture duration, temperature and pH to column loading and intermediate holding times, on output parameters including impurity levels and process productivity.

The model was used to estimate acceptable limits of product related impurities on the basis of impact on structure-function relationship determined from an Arrhenius model of accelerated stability samples.

NOTES:
Mechanistic Modeling - The Ultimate Qbd Tool For Process Understanding

Marcus Degerman; Lars Sejergaard; Janus Krarup; Ernst Broberg Hansen; Arne Staby

Novo Nordisk A/S, Gentofte, Denmark

Implementing QbD philosophies for process development and process understanding, the use of statistical methods and Design of Experiments has become the method of choice in many biopharmaceutical companies. In this presentation we will show how mathematical models based on mechanistic principles can be used to get an even better understanding of unit operations and to improve the experimental design when performing process challenge. Using simulation for process challenge a full factorial set up may be performed without considering the number of physical experiments. Based on the results of the factorial simulation study or Monte Carlo simulations the design space may be defined and a set of relevant conditions for experimental verification selected. This type of model reduced design of experiments has the huge advantage that experimental conditions may be selected based on CQAs instead of only the input variables.

Case studies showing the complete set up of models to a summary of how the work may be reported to regulatory agencies will be discussed.

NOTES:
Small Company Key Issues - 21st Century Principles for Early Product Development

Early product development groups are faced with many unique challenges due to limited resources and aggressive timelines. This makes implementation of new initiatives such as Quality by Design and Quality Risk Management even more challenging. This session will focus on new initiative and guidances and their applicability to early development programs.

Outcomes:

- New guidances and their applicability to early phase development programs
- The use of a Target Product Profile (TPP) in driving early development strategies
- Determine what value-added principles of QbD and QRM might be realistically applied to an early development program and/or in a small company setting with limited resources
- Understand simple risk management techniques that can apply to early phase programs

NOTES:
Prioritizing and Managing Key CMC Elements

Laurie Graham

CDER, FDA, Bethesda, MD USA

While there has been a recent focus on the use of 21st Century pharmaceutical principles with biotechnology products, the degree to which these principles can be applied may vary depending upon a number of factors. For example, the use of these principles poses a unique set of challenges for sponsors with limited experience/resources. In this situation, more restricted implementation necessitates the identification and prioritization of specific aspects of 21st Century pharmaceutical principles which can be realistically applied. Ms. Graham will give a regulatory perspective on this issue, present some case studies, and describe some of the key elements of new initiatives and guidance, such as ICH Q8(R2), Q9, and Q10, which can be implemented during early product development, even by small companies.

NOTES:
Applying Big Principles for Small Companies

John Frenz

*GlobeImmune, Inc., Louisville, CO USA*

Abstract was not available at time of printing.

**NOTES:**
You’re Virtually There – Virtual Product Development

Joseph Tyler

Proteon Therapeutics, Inc., Waltham, MA USA

Abstract was not available at time of printing.

NOTES:
Adventitious Agent Testing—The Evolving Reality

As long as therapeutics and vaccines are produced in biological systems, there is a risk that a particular lot of product can be contaminated by a known or to-be-discovered virus/adventitious agent that can be propagated by that system. Manufacturers control for this risk by testing the cell banks, animal derived raw materials and the harvest from each culture lot for adventitious agents. However, recent incidents in the manufacture of commercial material have highlighted some issues with this testing: (1) “outside” labs can easily detect viral sequences in vialled product using sensitive techniques such as PCR, (2) the adventitious agent may enter the system through a non-animal derived raw material and (3) the ability to detect or confirm adventitious agents in raw materials may be compromised by antibodies to adventitious agents in the raw material or in the culture media of the cell lines used in the test. Although the first issue does not mean that the vialled product contains “active” (replicating) adventitious agent or that agent is capable of infecting humans, it does affect the public’s perception of the product’s safety. This session will discuss current issues with assessing and handling the (potential) contaminations by adventitious agents.

NOTES:
Use of New Technologies to Detect Adventitious Agents in Biological Products

Philip Krause

CBER, FDA, Rockville, MD USA

The availability of increasingly powerful genomic techniques that can detect potential adventitious agents presents several key challenges. As these technologies improve and become even more widely available, manufacturers and regulators will increasingly need to be prepared to consider the implications of this type of data. The challenges include 1) optimization of techniques for use in a regulatory setting, 2) appropriate standardization and validation of the techniques, and 3) development of approaches to follow up on results obtained using these techniques. The recent detection of porcine circovirus nucleic acids and virus in rotavirus vaccines will be used as a case study to illustrate these scientific and regulatory challenges and potential solutions.

NOTES:
Commercial Vaccines and New Technologies: Impact and Learning’s

Anne-Françoise Macq

GlaxoSmithKline Biologicals, Wavre, Belgium

The technologies for detection and discovery of new adventitious agents continue to evolve at a very rapid pace. GlaxoSmithKline has been made aware of research data using advanced analytical methods suggesting the presence of Porcine circovirus type 1 DNA (PCV-1) in Rotarix™. Circoviruses such as PCV1 is an ubiquitous porcine virus and have not been associated with illnesses in humans.

Rotarix™ vaccine protects young children against mild and severe gastroenteritis that is caused by certain strains of rotavirus. Considering that any potential impact on the safety of the vaccine linked to the presence of PCV-1 material has been extensively studied in infants through Rotarix™ clinical development, GSK has reviewed all the clinical and post-marketing safety experience and the benefit/risk profile of Rotarix™ was concluded to remain unchanged and positive.

This presentation will focus on the GSK investigations with respect to PCV-1 DNA presence in Rotarix™. Specific PCR PCV-1 testing and infectivity testing have been developed by the company as tools to assess the starting materials, the vaccine intermediates and the final product. The technological development launched by the company will be presented as well as the impact in terms of quality control testing and regulatory actions. The preventive actions that have been taken by the Company in order to guarantee continuous quality and safety of GSK vaccines will be discussed.

NOTES:
Virus Detection and Identification: A General Process Overview

Mark Plavsic

*Genzyme Corporation, Framingham, MA USA*

Abstract was not available at time of printing.

NOTES:
Detecting, verifying and identifying viral contamination is not easy. The biotech industry has been "fooled" by the data claiming viral contamination as well as exonerating viral contamination, mainly due to a lack of scientists with broad virology training that encompasses pathogenesis, viral transmission, detection and expectations from virology studies. Viral genome or mRNA expression does not equal viral replication. Lack of detection of viral replication does not mean there is no infectious virus present. The importance of using the full spectrum of virology methods and of understanding what the results mean needs to be taught to the next generation of quality and regulatory personnel.

There needs to be a training center or two where industry staff can be trained in these components alongside clinical virologists who get this type of training routinely. I will talk with Carl Hanson from the CA Public Health labs in Berkeley, CA and a friend at the CDC to see if they have any suggestions on how we could develop such a training course of 2 months at these facilities. The people who finish this training could receive a certificate saying they have been through this training and are "Master Virologists". I also propose that we get these two institutions involved in providing a proficiency panel of viruses to be sent to participating companies. I see so many people who claim they are virologists and senior management who believe them because they have no way to evaluate them.

NOTES:
Analytical Challenges Associated with New Molecular Modalities and Combination Products

There are many well established analytical technologies utilized for the structural elucidation of antibodies, proteins, vaccines and blood products. However, when it comes to new molecular modalities and combination products a variety of analytical challenges are faced requiring modification or novel applications of well-established methods, as well as implementation of new analytical techniques. These new molecular modalities include novel mAb products (mAb cocktails, bispecific mAbs and mAb-related scaffolds), fusion proteins, virus like particles, non-mAb scaffold proteins, and an array of conjugates. Each poses its own analytical challenges and often pushes analytical methodologies to the limits of performance. For example, analytical tools for conjugates must be capable of monitoring the incoming components, final conjugate as well as clearance of unreacted components and conjugation by-products. There are also combination products that consist of multiple mAbs, proteins, and conjugates. These products are not only more heterogeneous (complicating product definition), but require the detection of different molecular entities mixed or linked together (while avoiding interference of one by the other). This plenary will include industry case-studies summarizing strategies for addressing these challenges.

For this plenary, presentations addressing the following questions will be targeted:

- What Low and High resolution techniques are used in the industry for these complex molecular modalities?
- What are the expectations for development and application of high resolution techniques for monitoring heterogeneity? (Heterogeneous mixes of heterogeneous biomolecules………infinite number of potential species).
- Since conjugates are usually heterogeneous, when is it appropriate to use low resolution techniques?
- How are potency and activity measured?
- What in-process controls are commonly used?
- Is each component treated as an intermediate, a reagent or a DS? Do all components require cGMP manufacturing? (e.g. linkers)

NOTES:
Analytical Challenges for Multiple Glycosylated Proteins

Hansjoerg Toll

Sandoz GmbH, Kundl, Austria

Glycosylation is an important parameter when assessing biopharmaceutical product quality. It can impact drug safety and efficacy and, in addition, is an independent indicator for manufacturing consistency. For proteins with multiple glycosylation sites, results from a toolbox of methods need to be combined to obtain a comprehensive picture of the glycosylation pattern.

The talk will compare different methods for the determination and quantification of glycans from proteins with multiple glycosylation sites, focusing on chromatographic methods in combination with mass spectrometry as methodologies for the identification and structural characterization of glycan species.

NOTES:
Analytical Challenges in the Analysis of Peptide Bioconjugates

Jeffrey Schneiderheinze

*Pfizer Biotherapeutics, St. Louis, MO USA*

Abstract was not available at time of printing.

**NOTES:**
Analytical and Development Challenges with Recombinant Antibody Mixtures for the Treatment of Infectious Disease and Cancer

Torben Frandsen

Symphogen A/S, Lyngby, Denmark

Mixtures of antibodies are presently being evaluated and developed to treat serious indications within infectious disease and oncology. We have e.g. developed Sym004, a product consisting of two monoclonal antibodies targeting EGFR and we are currently evaluating Sym004 in clinical trials. Alternative oncology targets and infectious disease targets are also being tested using our platform technologies aiming at developing recombinant antibody mixtures. This presentation will focus on the challenges with manufacturing and characterization and present strategies on how to use different assays to evaluate the identity of antibody mixtures in terms of polyclonality.

NOTES:
Blood and gene/cell therapy therapeutic products are frequently classified as “complex” based on their composition (e.g., low purity complex molecular mixture), structural and functional properties of an active ingredient (e.g., multifunctional high molecular weight protein or cellular product), or both. In either case, their characterization poses significant analytical challenges. It frequently relies more on biological/functional assays than a thorough structural characterization due to the limitations of the analytical methods. Incomplete structural characterization makes it difficult to acquire the comprehensive product and process knowledge and to clearly link specific quality attributes with the clinical performance.

The goal of this plenary session is to further our understanding of characterization strategies and explore the challenges faced in the manufacture and licensure of these products. The session will include case-studies illustrating strategies used for plasma derived and recombinant clotting factors, viral vectors for gene therapy, and cell/tissue products. Concepts of Quality by Design paradigm and Risk-based approaches will be discussed when applicable.

NOTES:
rFVIII and rVWF: Strategies to Meet the Challenge of Structural and Functional Characterization of the Most Complex Therapeutic Proteins

Peter Turecek

Baxter Bioscience Division, Vienna, Austria

Abstract was not available at time of printing.

NOTES:
Critical Parameters for the Characterization of Human Embryonic Cell Based Therapies

Jane Lebkowski

Geron Corporation, Menlo Park, CA USA

Many cell therapies involve the administration of cell populations containing a range of cell types and varying degrees of maturation. During the development of such cell-based therapeutics, it is critical to investigate multiple characteristics of these cell populations to potentially predict their safety and efficacy in humans. Especially important parameters for characterization include: 1) the presence of adventitious agents; 2) the phenotype and cellular composition of the products; 3) their production of growth factors or other active agents; 4) the immunogenicity of the cells; and 5) their potential for ectopic tissue growth. Techniques such as gene expression arrays; immune-phenotyping; qRT-PCR, protein arrays, and other technologies should be used collectively to provide an integrative view of the properties of the therapeutic cell populations. Correlation of these properties of the cell population with observations from in vivo in animal studies is important. The above considerations will be discussed in the context of the development of human embryonic cell derived oligodendrocyte progenitors, for the treatment of spinal cord injury.

NOTES:
Towards Better Characterization of Recombinant AAV Gene Transfer Vectors: Case Studies Illustrating Challenges with Dose Determining Vector Concentration Methods

J. Fraser Wright

*University of Pennsylvania School of Medicine, Philadelphia, PA USA*

Abstract was not available at time of printing.

NOTES:
Complex Biological Products - The Challenge of Plasma Protein Characterization

Stefan Schulte

*CSL Behring GmbH, Marburg, Germany*

Plasma Protein therapeutics are purified from human plasma and used for treatment of serious and rare diseases. Based on their composition and functional properties these products are complex and their characterization imposes a significant analytical challenge requiring sophisticated in vitro and biological testing. Beriplex P/N is a virus safe Prothrombin complex concentrate (PCC) product comprising of several Vitamin-K dependent coagulation factors and is licensed in Europe/Canada for Warfarin reversal in emergency situations. A major risk of PCC products is potential thrombogenicity caused by the presence of activated coagulation factors in the product. Limitation of analytical methods and due to the fact that this product is a mix of several active protein ingredients makes it impossible to fully characterize this product on a molecular level. Characterization of this product is mainly based on a combination of potency assays for each active ingredient, specific tests for activated coagulation factors and specific animal models to assess efficacy and thrombogenicity. The thorough evaluation of the results from these assays guarantees a safe, efficacious and consistent product with an excellent clinical performance.

NOTES:
ICH Q11 - An Overview of the Current Draft Guideline

Brian Withers

Abbott Laboratories, Kent, United Kingdom

ICH Q11 is currently under development for Active Pharmaceutical Ingredients (APIs) to harmonize the scientific and technical principles relating to the description and justification of the development and manufacturing process (CTD sections S 2.2 – S 2.6) of Drug Substances including both chemical entities and biotechnological/biological entities. It is intended to provide guidance on what information should be provided in S.2.2 – 2.6 and provide clarification on the applicable principles and concepts described in ICH guidelines Q8, Q9 and Q10.

This talk will focus on providing some background and providing an overview of the structure and content of the current draft of the guideline.

NOTES:
Manufacturing Process Development for Biotechnology Products: Concepts Under Consideration for ICH Q11

Patrick Swann

CDER, FDA, Bethesda, MD USA

Current guidance on Manufacturing Process Development (CTD S.2.6) can be found in ICH M4Q but, as stated in that guidance, applicable text is intended to provide guidance on format. “The text following the section titles is intended to be explanatory and illustrative only”.

ICH Q11 is intended to provide guidance on what information should be provided in S.2.2 – 2.6 and provide clarification on the applicable principles and concepts described in ICH guidelines Q8, Q9 and Q10. This talk will focus on principles and concepts from the Manufacturing Process Development section of the current draft of ICH Q11 which are important for biotechnology products. These include:

- Use of prior knowledge (platform manufacturing)
- Identification of CQAs
- Linking material attributes and process parameters to drug substance CQAs
- Risk ranking and categorization of process parameters to support lifecycle management

NOTES:
Process Validation for Biotech: Concepts Under Consideration for ICH Q11

Brendan Hughes

Pfizer Global Manufacturing, Andover, MA USA

Process Validation is defined within the scope statement for ICH Q11 and is a specific chapter in the guideline. Q11 contains both small molecules and biotech molecules within its scope and, because of some differing requirements, the guideline section addressing PV for biotech products is addressed in a separate sub-section. The text refers the reader to the ICH Q5 series for specific technical guidance which is still valid. The guideline refers to the contribution of data from small-scale studies to the overall PV dataset and acknowledges that data from these studies can reduce the dependence on full scale runs. The requirement for scientific justification of all small-scale models is stated. The guidance also recognizes the potential for manufacturers to leverage proprietary data from platform systems and notes the requirement for specific control strategies to be created in these circumstances.

NOTES:
Workshop Descriptions

Workshop Session 1
Monday, January 10, 2011
16:00 – 17:15

Small Company Key Issues: Early Product Development Strategies
Michelle Frazier-Jessen, Micromet, Inc.; Lotte K. McNamara, LKM CMC Consulting; Ilona Reischl, AGES PharmMed; Barbara Rellahan, CDER, FDA; Ramjay Vatsan, CBER, FDA

Workshop Description
Small companies are limited by tight timelines, small budgets and limited technology while needing to move ahead quickly to ensure continued funding. However, regulatory requirements do not delineate with regard to these challenges, which can result in delays for introduction of new products into the clinic or stall programs later in development. This workshop will focus on the identification and analysis of common strategy gaps in early phase development programs. Discussion will focus on the prioritization of key IND-enabling activities that aid program advancement with global regulatory authorities and/or potential business partners. The following potential topics will be touched upon:

- Sufficient/appropriate characterization early in development
- Preliminary formulation studies early in development
- Adequate documentation
- Adequate reference standards and retain samples
- Sufficient stability protocol
- Designing appropriate release assays and setting appropriate specifications
- Knowing when to seek help from consultants, industry colleagues and regulators

Discussion Topics:

1. With regard to product development and keeping the end goal of successful commercialization in mind, what are the must-have activities versus the nice-to-have activities that will allow for initiation of First-In-Human studies?
2. What are the challenges – similarities, differences – with regard to new product introduction from a global perspective?
3. From a partnering perspective, what are the “deal killers” and “deal sealers” that have been observed on diligence assessments?
4. What resources are needed and/or currently lacking for small companies and early development groups to improve early product development and lifecycle management?

NOTES:
Modernizing Analytical Methods for Legacy Products
Gabriel Bikah, Merck & Co., Inc.; Rajesh Gupta, CBER, FDA; Karen Lee, Genzyme Corporation; Ashutosh Rao, CDER, FDA; Peter Vandeberg, Talecris Biotherapeutics, Inc.

Workshop Description
The rapid evolution of analytical sciences and our understanding of the complexity of biological products have generated opportunities for the industry and regulatory agencies into seeking and adapting newer analytical methods for legacy products. However, development and implementation of new methods present significant scientific, regulatory and business challenges. Industry and regulatory agencies need to work together to address these challenges. This workshop will discuss the specific needs and challenges that arise from these new ventures with a view to generating ideas to implement modern analytical methods for legacy products.

NOTES:
Linking Product Quality Attributes to Safety and Efficacy
Anthony Lubiniecki, Centocor R & D, Inc.; Iftekhar Mahmood, CBER, FDA; Joao Pedras-Vasconcelos, CDER, FDA; Ziping Wei, MedImmune

Workshop Description
Grand Ballroom
The workshop will discuss tools and approaches which have been or could be used to help to establish the linkage between product quality attributes and safety and efficacy for biopharmaceuticals. The understanding of the linkages is essential to identify critical quality attributes and could improve during the course of the development life cycle. The in vitro tools include laboratory methods as well as information management/literature/in silico approaches. The in vivo tools include animal models and testing samples from human trials. The limitations and challenges of various tools will be discussed:

• How can the information obtained from animal PK, safety, and efficacy be translated to human PK, safety, and efficacy in early drug development? (PK parameters from animals can be extrapolated to humans using interspecies scaling and then a first-in-human dose can be selected. However, extrapolation of safety and efficacy from animals to humans remains an issue).
• What are the in vitro and in vivo tools to predict the immunogenicity of biopharmaceuticals? Can immunogenicity data from animals be extrapolated to humans? What are the limitations and challenges of each tool?
• What is the role of scientific knowledge from the literature and prior knowledge from related products in assessing the structure – function relationships for a new product, and in assessing the importance of variations in structure? At what point is there enough evidence to eliminate the need of additional laboratory studies to re-establish that the generalization from related products also applies to the new product?
• What is the role of product recovery and characterization studies in assessing the functional importance of product structural variations? For example, PK study samples from treated patients are collected, product is extracted and purified, and then characterized in terms of molecular heterogeneity. Can these types of studies gain insight into pharmacological importance of structural variations, e.g., oligosaccharide profiles?

NOTES:
Equipment Cleaning Validation
Alfredo Canhoto, Genzyme Corporation; Bo Chi, CDER, FDA; John O'Connor, Genentech, a Member of the Roche Group; Destry Sillivan, CBER, FDA

Workshop Description
Cleaning validation demonstrates that cleaning processes consistently and adequately remove residues (product related, process related, environmental) from equipment so that it may be used in the production of pharmaceutical and/or biotechnology products. As defined in PDA Technical report No. 49, cleaning validation is the documented evidence that, with a high degree of assurance, that a cleaning process will result in products that meet their predetermined quality attributes.

In this workshop, we will explore various aspects of cleaning process development and validation including design of experiments, the use of small-scale models, setting of limits for product and/or process residues, cleaning agents, biological assays (endotoxin, bioburden) and sampling methods. Furthermore, as there has been an ongoing debate regarding surface monitoring techniques and whether visual inspection, swabbing or rinsing is the preferred method. We will discuss the acceptability and limit determining techniques, how to demonstrate equivalency between the methods, and how to qualify operators on these sampling techniques.

Questions:

1. What are the advantages and disadvantages of the techniques?
2. Can the techniques be considered equivalent?
3. What is the role of laboratory scale studies in supporting commercial equipment?
4. Is using Maximum Allowable Carry-over acceptance criteria for cleaning validation appropriate, and if so, in what instances?
5. Elastomers on equipment are difficult to clean and maintain and could be the source of contamination and cross-contamination. How should the elastomers be evaluated during cleaning validation? How should they be handled during product changeover at a multi-product facility?

NOTES:
Small Company Key Issues – It’s a Risky Business…Or Is It? Managing Risk Associated with Early Product Development
John Frenz, GlobeImmune, Inc.; Michael Havert, CBER, FDA; Kathy Lee, CDER, FDA; Jeffrey Staecker, PPD, Inc.

Workshop Description
Many new initiatives such as QbD at first glance seem out of reach for small companies and early development groups due to resource and time limitations. This workshop will evaluate how risk management might be applied early in development (from the pre-IND stage to End of Phase 2) in a small company with limited resources. Additionally, simple risk management techniques that can apply to early phase programs will be discussed. The following potential topics will be touched upon:

- Use of a Target Product Profile (TPP) in driving early development strategies
- Prioritization of resources in early stage programs
- When to outsource activities
- Balancing short term and long term risks
- Contingency planning (e.g., process retain samples)
- Use of risk assessment tools
- Common issues in working with contract organizations, suppliers, and vendors

Discussion Topics:

1. What risk management tools can be used effectively early on in product development? What risk management tools are people currently using?
2. What risk management activities do you need to perform when making changes (formulation, analytical, manufacturing, raw materials, container-closure, etc.) during early development?
3. How and when should risk assessments be communicated to regulatory authorities?
4. How soon can CQAs be determined? What kind of prior product knowledge might be available for small companies that can be leveraged to develop initial CQAs? Who can help in defining CQAs?
5. Who can lead small companies in performing risk assessments? What resources are currently available that might be leveraged for these activities?
6. What are key considerations in choosing a contract organization?

NOTES:
Workshop Description

The Fc region of therapeutic monoclonal antibodies can play an important role in their safety and efficacy. Although much is known about the structure-activity relationship of antibodies and the factors that influence Fc effector functions, a process has not yet been described to clearly delineate how manufacturers should assess and control Fc functionality during development and manufacturing. This workshop intends to share a proposed strategy by the industrial working group for assessing effector functions of different classes of therapeutic antibodies. A path for routine testing and controls for manufacturers of monoclonal antibody products will also be discussed.

Questions:

1. What, when and how to monitor Fc effector functions during R&D and manufacturing processes of therapeutic antibodies?
2. What are recommended strategies for effector function assessment and control?
3. Should glycosylation testing be routinely monitored and specifications be set for all therapeutic Abs?
4. Is there a product life-cycle approach for assessment & control of Fc effector Functions of therapeutic Abs?

NOTES:
Practical and Innovative Solutions for Applying QbD Principles to Complex Biologics
Ilse Blumentals, GlaxoSmithKline; Michele Dougherty, CDER, FDA; Christopher Joneckis, CBER, FDA; Stefanie Pluschkell, Pfizer, Inc.

Workshop Description

Over the last few years, industry has begun to more consistently and prospectively integrate Quality by Design (QbD) approaches into the development of biotherapeutics. The value of the iterative use of risk management tools, of methodical studies to increase product and process understanding, and of systematic approaches to defining design space and control strategies has been recognized. This is especially the case for well-characterized biotherapeutics such as monoclonal antibodies.

This workshop is intended to go beyond this product category and will explore opportunities and challenges in using innovative QbD approaches in the development of more complex biotherapeutics and biologicals. Topics will include the potential role and value of prior knowledge, practical ideas on how to assess the criticality of quality attributes, and how to develop a design space for the production of more complex biologics. Scientific and regulatory issues will be discussed in the context of how they might (or might not) differ in comparison to monoclonal antibodies.

Questions:

1. What are the challenges in applying QbD to more complex biologics? How might the approaches differ between mAbs and non-mAbs?

2. The limited availability of platform or prior knowledge represents a challenge to applying QbD principles for the manufacture of complex biologics. Can knowledge from similar processes and products be identified that can guide/contribute to risk assessment or process characterization? How could this information facilitate the development of enhanced process and product understanding for complex biologics? What are the opportunities and limitations associated with this approach?

3. An often overlooked area of QbD is raw material assessment and control. Can criticality of raw materials be linked to product CQAs, design space or an overall control strategy for complex biologics? How are risk management principles applied to raw material sourcing and control?

4. Are there any differences in regulatory expectations with respect to QbD between mAbs and non-mAbs?

NOTES:
Characterization of Higher Order Structure in Protein and Monoclonal Antibody Therapeutics
Michail Alterman, CBER, FDA; Sarah Kennett, CDER, FDA; Andrew Kosky, Genentech, a Member of the Roche Group; Alla Polozova, MedImmune

Workshop Description
International Conference on Harmonization guidelines Q6B and Q5E indicate that higher order protein structure characterization data should be collected and provided during product development and comparability assessments. Characterization of higher order structures of proteins became particularly critical with the advent of biosimilars. The focus of this session is on common challenges in characterizing higher order protein structures of antibodies and other therapeutic proteins. Topics for discussion include approaches to characterize higher order structure and advantages and limitations of currently available methodologies. The question of whether there should be different requirements for higher structure characterization between monoclonal antibodies and other therapeutic proteins will also be discussed.

Key questions to address:

1. How do we define the term higher order structure of proteins?
2. What are the best approaches suitable for the characterization of higher order structures? Is a binding or bioassay sufficient to ensure the integrity of the higher order structure?
3. What are the common challenges in characterization of higher order structure?
4. Is there a difference between monoclonal antibodies and other therapeutical proteins in terms of the characterization requirements for the higher order structure?

NOTES:
Workshop Session 3  
Wednesday, January 12, 2011  
11:00 – 12:15

Analytical Challenges Associated with New Molecular Modalities

**Workshop Description**

The complex structural properties and molecular heterogeneity associated with biotechnology products requires application of multiple analytical methodologies to appropriately characterize, assess and control quality. Each analysis technique will have inherent limitations making the task of generating required data for regulatory approval difficult even for well established biopharmaceuticals such as monoclonal antibodies. Growing industry trends, however, indicate an emphasis on increasingly more complicated molecular modalities to address certain diseases. For example, bispecific and multivalent antibodies as well as antibody cocktails are being pursued for cases where multiple mediators contribute to pathogenesis. Covalent derivatization, a process referred to as bioconjugation, is yet another strategy routinely being employed to produce biomolecules having a broad range of pharmaceutical, diagnostic and imaging applications. Depending on the nature of the compound used to derivatize the protein, the ability to detect modifications at the amino acid level may be severely compromised. In the field of vaccines, virus-like particles (VLPs) are being utilized, with one product successfully licensed and others undergoing clinical evaluation. These and other new molecular modalities raise the level of technical complexity for analytical testing associated with control strategies.

The objective of this workshop is to take a deeper look at these challenges in the context of preparing suitable data packages for regulatory submissions. Interactive discussions will explore what is achievable with existing analytical tools and when is it necessary to apply nonstandard testing approaches or more sophisticated technologies. Industry and health authority experiences relative to the analytical requirements associated with structural characterization, in-process and end-product release testing, process monitoring, stability and comparability will be additionally discussed.

**Questions:**

1. Bispecific and multivalent antibodies represent significant challenges for the development of specific and functional *in vitro* cell based bioassays to serve as surrogates for the mechanism(s) of action and to assess product quality:
   a. What bioassay strategies are needed to effectively evaluate product quality for bispecific and multivalent antibodies?
   b. How are considerations for effector functions factored into the assay requirements?
   c. What are the limitations for bioassays to assess product quality (e.g., product release, stability and comparability) for bispecific and multivalent antibodies given the inherent variability associated with such methods?
2. Monoclonal antibody cocktails, defined as two or more mAbs administered at a fixed ratio, may be combined just prior to administration, prior to filling or during the fermentation, purification or formulation process. Analytical considerations for cocktails will vary depending on when the mAbs are combined:

a. For cocktails combined prior to final formulation/filling, what are the analytical challenges for release testing in order to demonstrate identity and some aspect of potency (e.g. binding) of each mAb present in the cocktail? How should stability be monitored?
b. For mAb cocktails where the mAbs are formulated and released individually and combined prior to administration, how should the combined levels of impurities (product and process-related) in the final cocktail mix be evaluated?
c. For cocktails produced by combined fermentation, what are the challenges associated with demonstrating consistency of the cell culture process and the quantity, potency and stability of each antibody in the final mixture? How should comparability be assessed after process changes?

3. Under the “the process defines the product” paradigm for biologics, final testing is focused to a relatively small number of product quality attributes. But there is a very high likelihood that process changes will be required during development:

a. What analytical techniques are useful for process development and characterization of complex molecules such as bioconjugates? If high heterogeneity is observed, are “product profiles” enough to assess impact?
b. What data are expected to characterize the extent of conjugation and to establish acceptance criteria including range of conjugation within a production batch?
c. What assays can be used for determining/detecting product changes due to process changes (including scaling up)?
d. How are the challenges and requirements for analytical comparability addressed? What are the expectations?

4. Bioconjugates tend to exhibit considerable heterogeneity. Depending on the type of conjugates, the degradation pathways for the parent molecule(s), the moiety conjugated to the parent and the resulting conjugate product may exhibit differences during stability storage:

a. What strategies are used to monitor stability?
b. Does collecting stability data for the individual reactants add value? How is this knowledge used?
c. What are the expectations for determining stability profiles?
5. The expression and release of recombinant viral antigens may result in the spontaneous assembly of highly immunogenic virus-like particles (VLPs). VLP vaccines have been licensed for human papillomavirus and are in clinical trials for influenza vaccines. Issues regarding VLP vaccines may include:

a. Size and shape of VLPs may be critical to their immunogenicity. What technologies may be used to characterize size and shape in the range expected for VLPs (~120nm for influenza)?
b. What are stability concerns related to VLPs and how may they be addressed?
c. What role might physico-chemical determinations of antigen content such as HPLC or LC/MS have? How might these complement immunochemical measures of potency such as SRID?
d. There may be differences in immunochemical measures of potency between recombinantly expressed antigens and those obtained from traditional vaccines. What are the issues in using traditional vaccine standardized reference materials, e.g. for influenza virus vaccine, as antigenic references for VLP and other recombinant vaccines?

NOTES:
How to Get Your Comparability On – Understanding Common Challenges During Comparability Studies
Siddharth Advant, ImClone Systems Inc.; Markus Blümel, Novartis Pharma AG; Michael Kennedy, CBER, FDA; Richard Ledwidge, CDER, FDA

Workshop Description
This workshop deals with the design of comparability studies and the CMC requirements needed to ensure consistent product quality during clinical development or post approval. We will elaborate on topics of concern to both sponsors and regulatory agencies.

During this workshop you are invited to contribute your thoughts on topics such as:

- How to determine the number of representative lots to be tested in the comparability study for both characterization and accelerated stability studies?
- Do testing requirements change at different stages of product development? How are acceptance criteria established?
- What implications do tighter comparability acceptance criteria have on overall specification setting strategy?
- Do we always need side-by-side testing? Are statistical tools used to assess analytical comparability?
- Which analytical tests are key for the comparability assessment after drug substance manufacturing changes? What about changes to drug product dosage forms such as a switch from lyophilisate in vial to a liquid formulation in a pre-filled syringe?

You are welcome to raise additional topics during the workshop.

NOTES:
Characterizing Complex Biological Products
Steven Bauer, CBER, FDA; Roman Drews, CBER, FDA; John Frenz, GlobeImmune, Inc.; Lisbeth Palm, Novo Nordisk A/S

**Workshop Description**

Growing technological sophistication in our understanding of the origins of disease, the biological responses to disease and how to modulate those responses has led to greater sophistication in the biological agents that are approved or in clinical development for treatment of a broad spectrum of diseases. These agents represent complex molecular assemblages whose gestalt contributes to the specificity and effectiveness of the induced biological response. These therapeutic approaches include targeted therapy such as gene and cellular therapy, and use biological agents including viral vectors, plasmid vectors, RNAi, activated lymphoid cells, dendritic cells, tumor vaccines, gene-modified tumor vaccines, complex protein products like blood factors, and inactivated whole cell immunotherapies, among others. These complex biological agents pose novel characterization challenges, whether in interpretation of data obtained from conventional analytical methods, development of methods using unconventional or cutting edge technology and the development of new technology for exploring pertinent product attributes. Challenges in linking biological function of these products to the measurable product attributes remain an essential aspect of investigating the appropriate approaches to routine release and stability testing, characterization and comparability following process changes and scale-up. On top of all this, validation challenges for these methods can represent significant investments in time and resources for these complex, novel biologics. Early communication among regulators, biotechnology companies and academic investigators is essential to the rapid implementation of these novel technologies. The workshop participants are encouraged to give voice to the associated challenges and provide examples of analytical and stability issues that they have encountered and how novel technologies can be incorporated in the development of complex biological medical products.

Questions and issues to be discussed include the following:

- **How much is enough?** Many aspects of complex biologics can be measured; the challenge is to decide what should be. Examples include cell-based and blood-derived products and pegylated proteins for which orthogonal methods for size are available.
- **Given that many aspects of complex products can be measured,** which should be included as lot release or in process tests versus as initial characterization techniques?
- **How do the requirements for characterization and release criteria evolve as a product moves through the stages of development (pre-clinical, Phase 1, Phase 2, Phase 3 and commercialization)?**
- **When a useful method is no longer supported by a vendor (eg, Edman sequencing) how does a sponsor deal with less-than-perfect replacement methods (eg, mass spectrometry)?**
- **Some aspects of complex biologics may not lend themselves to ready analytical characterization, how do sponsors deal with inadequate analytical tools? One example of this issue is the monitoring of reversible aggregates, there are many more.**
- What are the tradeoffs in choosing between functional and structural methods of analysis? Does the biological relevance of a functional assay mean that less thorough structural assessment is appropriate? For complex MOAs, functional assays may be expensive and imprecise, are robust structural assays then preferable?
- **Are the expectations of potency assays different for complex products than for more-characterizable products?**
- **For complex products, how important is assessing the relevance to safety and efficacy of potential chemical changes on stability testing? Are there changes that can be understood to be benign?**
- **Is there an approach to judging the incremental value of additional product knowledge gained by more assays in terms of clinical meaningfulness?**

**NOTES:**
Adventitious Agents – Mitigating Risk
Donna Boyce, GlaxoSmithKline; Arifa Khan, CBER, FDA; George Miesegaes, CDER, FDA; Merribeth Morin, Targeted BioStrategies

Workshop Description
All biological products carry an inherent risk of being contaminated with adventitious agents. Due to the nature of the source materials and complex nature of biological products, these risks may not easily be eliminated. The approach to preventing contamination relies on mitigation strategies at all levels of the production process from risk assessments of all biological materials used, to implementation of process controls and design of manufacturing processes. Included at each level are tests for adventitious agents. The tests include generalized tests to detect the presence of a contaminant as well as tests for specific agents. Much of the current regulatory framework surrounding the required testing has arisen from incidents from the past where contaminants were identified in commercial biological products and vaccines. In spite of stringent controls that have been utilized in recent years to avoid such incidents, there have been publicized events of contamination in human drug production in the past couple of years. Attendees of the workshop are encouraged to open a discussion on what approaches can be taken to mitigate the risks associated with biological products and strategies being taken to address recent concerns.

Questions:

In light of recent events:

- What steps can be taken to avoid contamination and to design manufacturing processes?
- Can current assays for detection of adventitious agents be modified or is there a need to adopt new ones?
- How do organizations deal with the constant availability of ever-more-sensitive, more recent adventitious agent assays?
- What analyses are needed to balance risk and benefit when selecting materials and processes for a biological product? Can an algorithm be developed?

NOTES:
Pre-filled syringes are a preferred delivery system for many biopharmaceuticals. Syringes present a number of challenges in developing and producing biopharmaceutical products including the extractable/leachables, choice of container (including plastic versus glass), choice of closure, compatibility of the protein and formulation with the syringe materials, stability of the protein in the syringe, and integrity of the container and closure system. Historical lessons have shown that leachables from the closure, silicone from the syringe, and tungsten from the formation of the glass syringe can cause aggregation and other issues for the product. These issues may be protein specific, and development of a new biopharmaceutical needs to test all of these parameters. In addition, as the industry focuses on sub-visible particles the ability to analytically differentiate and quantitate protein aggregates from silicone particles presents new challenges. This workshop will address the development of biopharmaceuticals in pre-filled syringes and the challenges that industry and regulatory agencies have faced in developing and producing these products.

Questions:

- How are we monitoring and characterizing the subvisible particles?
- Is siliconized glass enough for formulation to prevent adsorption?
- Are there regulatory challenges for new container closure materials?
- Will the Human factors program alter the materials or design?

NOTES:
Biopharmaceutical Reference Standard Establishment and Life-Cycle Management
Anu Bansal, Genentech, a Member of the Roche Group; Sarah Demmon, Eli Lilly and Company; James Kenney, CBER, FDA; Carla Lankford, CDER, FDA

Workshop Description
Colonial Room
Developing adequate reference standard materials, used in the different aspects of biopharmaceutical product development and lifecycle management constitutes a particular challenge. Some of the challenges are with respect to producing and maintaining representative material, compatibility with containers, presentations for long-term storage (e.g. lyophilized vs. liquid), qualification and lifecycle management. Even though international qualified reference standards exist for some biopharmaceuticals (e.g., interleukins, enzymes), for many others such standards do not exist. However, the challenge with some of the international standards is that they may be qualified by methods/assays very different from those used by an innovator company for their product testing (e.g. potency assay). Another challenge is stability of these standards, hence the need for continuous stability testing as well as new lots manufacturing, qualification and comparability with the existing lots. This workshop will focus on the reference standards used in the release and stability testing of drug substance and drug products. Industry experiences and regulatory expectations for development, qualification and maintenance of biopharmaceutical product reference material will be discussed.

Possible topics for discussion:

1. Establishing and qualifying reference standard material for existing and new biopharmaceutical product – what is essential and what is good to have?
2. Material lifecycle management:
   a. Re-qualification of the existing reference material – what is essential?
   b. Reference Standard stability studies – what tests to perform, frequency of testing?
   c. Establishment and qualification of a new lot of primary standard material due to depletion of the existing standard – what tests to perform, comparability?
   d. Comparability with international standard material – is it necessary?
   e. Determining the path and rate of degradation in the intended container closure system vs. product container closure system
3. Establishing primary and secondary standards and their qualification and comparability strategies

4. WHO and USP standards for biopharmaceuticals – are they necessary, how to harmonize testing?

NOTES:
Biosimilars – Regulatory Considerations
Martin Schiestl, Sandoz GmbH; Thomas Schreitmüller, F. Hoffmann-La Roche Ltd.

Workshop Description

Biopharmaceuticals based on recombinant proteins have started to go off-patent, opening the way for other manufacturers to place follow-on biological products or biosimilars to the market. This presents new challenges that are not ordinarily presented by small-molecule generic medicines because a biosimilar can only be proven to be similar and not identical to its reference product. This fundamental difference of biosimilars, has led to the adoption of a specific legal basis and regulatory framework for biosimilars by the regulatory authorities in the European Union (EU). Several biosimilar products have been approved by the CHMP based on these new guidelines and a couple of them are now on the market for more than four years without safety or potency problems. In addition, other applications were rejected by the CHMP or withdrawn by the sponsor, which also indicates a working system. Meanwhile regulatory agencies in various parts of the world (incl. WHO) have established distinct guidance, which follow in essence the principles of the European guidelines, although different names have been used in these guidances as follow-on biological products (FOB’s), second entry biologics (SEB’s) or similar biotherapeutic products (SBP’s).

Nov. 2-3 also FDA held a 2-day public hearing to obtain input on specific issues and challenges associated with the implementation of the Biologics Price Competition and Innovation Act of 2009 (BPCI Act) demanding an abbreviated approval pathway for biological products that are demonstrated to be “highly similar” (biosimilar) to, or “interchangeable” with, an FDA-licensed biological product. In order to fuel the ongoing public discussions after the meeting the following questions should be discussed during this workshop:

- How similar a biosimilar has to be to the reference product?
- What can be leveraged and what are the limits of the classical comparability approach when establishing similarity?
- What role play product specific pharmacopoeial monographs in the context of establishing similarity?
- What should be considered by a biosimilar manufacturer when establishing a control strategy for his product?
- What should be considered by a biosimilar manufacturer when a manufacturing process change requires a comparability assessment for his product?

NOTES:
Methods monitoring is a critical component of quality systems that can often be overlooked. De riguer, this is the implementation and monitoring of assays and their results over the life cycle of a product. In addition, several guidance documents (ICH, USP, etc) state that methods monitoring is important, but give little guidance on how it should be accomplished.

- What else is methods monitoring?
- Can different analytical parameters be more friable?
- Should risk assessment be used to tier methods monitoring? What does your Firm implement? What should the Agency expect to see?
- What else besides the important parameters that include the creation and trending of reference standards, trending of system suitability and failed tests and results should be parameterized?

We will have these questions and more to provoke a lively discussion.

NOTES:
In the Chinese Room
Sponsored by Agilent Technologies

Analytical Characterization of Monoclonal Antibodies: Rapid Glycan Profiling Using the Microfluidic Based mAb-Glyco Chip-LC/MS solution & Analysis of Biotherapeutics Using the Infinity 1260 Bio-inert LC

Ning Tang¹; Martin Vollmer²

¹Agilent Technologies, Santa Clara, CA USA; ²Agilent Technologies, Waldbronn, Germany

Characterization of glycans from antibodies is fundamentally important in biotherapeutics design and disease progression and detection. The ability to characterize glycans rapidly has been limited by the sample preparation steps and structural complexity of the glycoproteins. To address this problem, we have developed a microfluidic chip that performs rapid on-line cleavage of glycans from monoclonal antibodies, captures the released glycans and then separates them prior to nanospray ionization in the mass spectrometer. The entire run time is 12 minutes. A glycan accurate mass database was established allowing quick assignment and identification of the glycans. Further glycan characterization is done by MS/MS experiments on QTOF. Discussion will also be made on the relative glycan quantitation ability of the technique and its role in comparing different batches of antibody glycans.

The analysis of large bio-molecules by HPLC to confirm correct physico-chemical properties with size exclusion and ion-exchange chromatography is still limited by lack of robustness, repeatability, and sensitivity and characterized by very long analysis times. The Agilent 1260 bio-inert quaternary HPLC in combination with the Agilent Bio column portfolio provide a superior solution to improve confidence in analysis and increase productivity for NBE characterization. True bio-inertness eliminates the need for cumbersome passivation procedures. UHPLC capability allows for higher throughput now also for bio-molecules and 10x more sensitivity through new waveguide detector cell technology eliminates the need to waste excessive amounts of valuable samples. This in combination with the bio-inert fraction collection option will add significant flexibility and ease of use for efficient therapeutic protein characterization.

NOTES:
MARKET READINESS INDEX (MRI)
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COME TO OUR
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HELP IMPROVE YOUR MRI.

CONVERGENT BIOSCIENCE
A Cell Biosciences Company
The iCE280, An Excellent Tool for Fast and Efficient Process Development, Product Control and Product Comparability

Chantal Felten

*Alpine Analytical Academy, Vancouver, British Columbia, Canada*

In today’s competitive biopharmaceutical environment, fast and efficient product and process development is critical to a new drug’s success. One of the key product attributes monitored as an indicator for both process and product control is the Charge Variant Profile (CVP).

iCIEF, a common sight in biotech laboratories around the globe, has proven over the last few years to be the tool of choice for charge variant analysis throughout the product’s lifecycle. This presentation will review critical analytical activities during drug development and emphasize the impact of iCIEF on your overall development resources.

Naturally, as the contribution of iCIEF to product development increases so does the need for in depth profile characterization and critical quality attribute evaluation. This is especially true when evaluating product comparability, where iCIEF in general demonstrates much higher resolution then IEC for micro-profile charge variant comparison. A review of current iCIEF profile characterization by alternate techniques will be presented.

NOTES:
In the Senate Room
Sponsored by Bruker Daltonics

From Discovery to the Routine – Mass Spectrometric Workflows for the Characterization of Protein-Based Biopharmaceuticals

Laura Main

*Bruker Daltonics Limited, Coventry, United Kingdom*

Characterization of a biopharmaceutical compound is essential at each stage during its life-cycle. However, characterization of protein-based biopharmaceuticals is challenging in comparison with their small molecule counterparts due to their high molecular weight and heterogeneous nature. Of the analytical tools available, mass spectrometric methods are some of the most powerful. Here we present an overview of Bruker Daltonics solutions including an automated rapid workflow for the routine characterization of biopharmaceutical products; discovery and development tools for more detailed characterization including primary structure and N-terminal sequence determination, determination of post-translational modifications including glycosylation patterns, and determination of disulphide bonds.

**NOTES:**
In the Chinese Room
Sponsored by Waters Corporation

Deploying High Resolution Protein Analytics across Biopharma Organizations: New Technologies, Informatics, and Compliance (Oh My)

Scott J. Berger

Waters Corporation, Milford, MA USA

Waters is a company focused on delivering leading technologies, purposely developed for the biopharmaceutical industry. This targeted Waters focus translates to laboratory and data workflows that increase laboratory efficiency. Waters is looking ahead to the needs of organizations like yours, where multiple departments need to share information, and transfer methods, data, results and their underlying technologies. These efficiencies do not come from innovations in hardware, chemistries, informatics alone, but rather through broad innovation and effective integration of these technologies:

Advances in UPLC combined with the performance of our Peptide Separation Technologies have changed the focus of peptide mapping, from simply achieving enough resolution, to enable balancing of resolution and higher assay throughput.

The combination of biocompatible materials with simplified system operation from AutoBlend Plus technology in the ACQUITY UPLC H-Class Bio have set a new standard for robust and efficient bioseparations method development and execution.

The capabilities of a benchtop TOF MS can now address all the routine biotherapeutic characterization questions, and engender a paradigm-shift where floor-standing MS instruments are more practically differentiated by their advanced modes of operation (e.g. IMS, ETD, MALDI/ESI switching) than by specifications.

BiopharmaLynx heralded the era of biopharma-specific data and informatics processing workflows, standardizing days of manual peptide map and intact protein data processing down to minutes of routine automated processing. Waters new scientific information system offering, UNIFI, will enhance and extend such capabilities across biopharmaceutical organizations.

Going forward, these technologies will further mature, but today’s seminar will focus on how Waters is integrating these technologies to extract the maximum amount of qualitative and quantitative information from biotherapeutic samples, and on how your organization can utilize such high resolution analytical capabilities, from discovery through regulated development and QC environments.

NOTES:
High Throughput / High Resolution Glycosylation Pattern Analysis of Monoclonal Antibodies by Capillary Electrophoresis

Andras Guttman; Zoltan Szabo; Barry L. Karger

The Barnett Institute, Northeastern University, Boston, MA USA

Structural characterization of the glycan moieties of therapeutic monoclonal antibodies is very important during both the clone selection and the production processes. Capillary electrophoresis (CE) in conjunction with laser induced fluorescent (LIF) detection is a sensitive and high resolution separation method that is already broadly utilized and validated in the analysis of complex carbohydrates in the biotechnology industry. By means of capillary electrophoresis, glycans released from subnanomolar amounts of antibodies can be quickly and accurately profiled. This presentation gives an overview of the state of the art of capillary electrophoresis based glycan analysis of monoclonal antibodies, including sample preparation, glycan labeling and cleanup, oligosaccharide profiling, carbohydrate sequencing and glucose unit (GU) value based structural prediction. Rapid (<7 min) separation of 12 key IgG glycans will be shown along with 96 well plate operation for convenient overnight processing for such high throughput applications as clone selection. The relative migration time reproducibility of the optimized separation method was RSD <0.09%. This facilitates peak assignment for individual components in any glycan pool, thus allowing high precision structural predictions using GU value databases. Exoglycosidase based sequence verification of IgG glycans will also be presented.

NOTES:
Higher Order Structure Ad
P-102-M

Development of a CHO-Cell Bioreactor Design Space for Monoclonal Antibody Production

Xinfeng Zhang; Robin Luo; Haiyan Kong; Melissa Schwartz; J. Russell Grove; Chulani Karunatilake

Process and Product Engineering, Amgen Inc., Fremont, CA USA

A streamlined approach to development of a production bioreactor design space for a therapeutic antibody is described. A comprehensive panel of quality attributes was chosen for process characterization (PC) studies based on their relevance to the production bioreactor process performance. A failure mode and effects analysis (FMEA) exercise was performed to prioritize bioreactor operating parameters. Multivariate DOE studies were designed to quantify main effects and interactions of specific operating parameters with respect to the chosen quality attributes. Operating parameters that directly influence quality attributes, such as charge variants, aggregate, fragments and oligosaccharides, were identified and design space boundaries were defined. The operating parameters were categorized based on their impact on product quality and the operational ranges were verified to ensure the process robustness. The design space development of production bioreactor led to achieving operational flexibility while maintaining desired process performance. The process capability knowledge gained from the design space development was used to rationalize the control strategy for the relevant quality attributes.

P-103-M

Use of a Fault Tree Analysis to Evaluate Pipette Verification Intervals

Jeffrey Staecker; Brian Fuller; Nathan Penn

PPD, Inc., Wayne, PA USA

The pharmaceutical industry is increasingly utilizing FMEA (Failure Mode Effect Analysis) risk assessments as part of the decision process for drug development. In addition to the FMEA a Fault Tree Analysis (FTA) is another risk assessment tool that has application to the drug industry. This poster describes application of an FTA risk assessment to determine the frequency for pipette verifications. The longer the interval between pipette verifications the greater the chance that data generated utilizing a failing pipette could be reported. Our analysis concluded that product release testing produced the highest risk because of the combined impact of incorrect results with the usual rapid turn-around from test completion to utilization of test results. The fault tree serves as a visual representation of the potential sequence of events and the probabilities of each. The FTA provides a meaningful platform for decision making and is easily updated when additional information becomes available.
Use of One-Factor-At-a-Time and Multivariate Experimental Design Approaches for Developing Robust Bioassays

Joyce Pasion; Kendall Carey; Xu-Rong Jiang; Robert Strouse; Michael Washabaugh

MedImmune, Gaithersburg, MD USA

Implementation of accurate and robust bioassays early in product development supports process development and product characterization efforts. In order to rapidly implement robust cell-based potency assays to support pre-clinical product development, we have developed a process for optimizing assay parameters. This process combines a One-Factor-At-a-Time (OFAT) assay optimization approach with a multivariate experiment design (DoE) screening assay approach to identify critical assay variables, their interactions, and impact on assay response with a minimal number of experiments. A case study will be presented that demonstrates the superiority of using the combined OFAT-DoE approach over a traditional OFAT approach alone during assay development. The application of the combined OFAT and DoE approach resulted in the rapid implementation of a robust bioassay that is stability indicating early in the lifecycle of a project.

Strategy Used for Product Risk Assessment of Pegylated Interferon B-1a

Mia Kiistala; Andy Weiskopf; Robert Gronke; Kazumi Kobayashi

Biogen Idec Inc., Cambridge, MA USA

For approximately 15 years, interferon β -1a (IFN β-1a) has been an effective treatment option for patients with relapsing multiple sclerosis. PEGylated interferon β -1a (PEG IFN β-1a), now in a Phase III clinical trial, is being developed to improve convenience and compliance by reducing treatment frequency, while maintaining the established safety and efficacy profile of intramuscular IFN β -1a.

A product risk assessment of PEG IFN beta-1a was conducted to identify the critical quality attributes (CQAs) of the product, in order to better focus process characterization activities and increase understanding of the effects of process parameters on product quality. This poster will discuss the product risk assessment conducted for PEG IFN beta-1a

Method Development of Q-PCR DNA Assay Used for Quantification of Residual CHO Cell DNA

Yosuke Ikeda

Chugai Pharmaceutical Co. Ltd., Tokyo, Japan

Q-PCR has been recently used for quantification of residual host cell DNA, which must be removed and its clearance must be verified through manufacturing process of biopharmaceutical products. We have developed a CHO cell DNA assay with Q-PCR technique. A primer/probe set was selected from several designs considering amplicon base length to improve the method sensitivity and applied to our
biopharmaceutical products. To improve the accuracy, DNA extraction method was investigated with three commercial kits and measured DNA concentrations were compared. An internal assay control with non-relevant lambda DNA was introduced to evaluate and assure DNA extraction process for each assay. The internal assay control also contributes to reduce the number of DNA extraction operation when used as System Suitability Test instead of positive sample controls. A method validation of the Q-PCR DNA assay for biopharmaceutical drug substances and an evaluation of DNA removal through manufacturing process were carried out. Linearity of DNA in cell culture supernatants was regarded comparable to that of the CHO genome DNA used as a standard for the assay. The method was proven to have enough ability with respect to specificity, accuracy and precision. In conclusion, the Q-PCR DNA assay will be the company’s standard method for detecting residual CHO cell DNA in cell culture supernatants, other in-process samples and drug substances considering the sensitivity, dynamic range, throughput and costs.

NOTES:
Mass Spectrometric Profiling of Host-Cell Proteins in Biopharmaceuticals

Catalin Doneanu\textsuperscript{1}; Alex Xenopoulos\textsuperscript{2}; Weibin Chen\textsuperscript{1}; Holly Prentice\textsuperscript{2}; StJohn Skilton\textsuperscript{1}; Jeff Mazzeo\textsuperscript{1}

\textsuperscript{1}Waters Corporation, Milford, MA USA; \textsuperscript{2}Millipore Corporation, Bedford, MA USA

Residual host-cell-proteins (HCPs) are a major component of biopharmaceutical process related impurities. They are commonly present in minute quantities in the final biopharmaceutical product (typically expressed as ng/mg of recombinant protein or ppm concentrations), but they can potentially elicit an immune response in patients. Thus, characterization of low-abundance HCP impurities (10-100 ppm) is very important for biopharmaceutical industry.

We recently developed a two-step, generic LC/MS-based assay for fast HCP analysis: the first step involves the HCP identification using a protein discovery MS platform, followed by a second quantification step based on an MRM assay of several peptides from each HCP identified [1].

This analytical methodology was applied for identification and quantification of HCPs from a recombinant IgG1 antibody. A chimeric anti-phosphotyrosine IgG1 mAb (PTG1) was expressed in two CHO cell lines (CHO-S and DG44) and purified by Protein A chromatography using two different protocols. Null cell cultures (expressing no PTG1), were grown under identical conditions and Protein A purified. Five protein standards were spiked in PTG1 in the concentration range of 10 – 1,000 ppm and the resulting protein mixture was denatured, reduced, alkylated and digested with trypsin. The peptide mixture was separated by two-dimensional RP/RP chromatography (high pH/low pH) coupled to a QTOF mass spectrometer to yield information on the identity and quantity of the existing HCPs. Fifty HCPs were successfully identified and quantified using this approach. Twenty HCPs were further selected for MRM monitoring. The results generated across multiple samples derived from Protein A purification schemes as well as different cell lines were compared to provide a better understanding in the variation of purification methodologies. In addition, total HCP concentrations were compared with the traditional ELISA assays.

References:


Application of Molecular Biology Technologies for the Quality Control of Vaccines

Jean-Pol Cassart; Isabelle Ernest; Olivier Verlaeten; Isabelle Piérard; Corine Lecomte; Michel Duchêne

GlaxoSmithKline Biologicals, Rixensart, Belgium

Numerous infectivity assays are used for the quality control of viral vaccines in order to determine the viral potency and to detect potential adventitious agents or process residuals. Most of these assays rely on microscopical or immunological read-outs often requiring long incubation times to achieve clear positive signals with the appropriate controls.
Molecular biology approaches mainly based on the use of the PCR technology offer the possibility to detect net replication activities of infectious material at early stages of the culture period. These new methods are more and more accepted and encouraged by the different regulations.

GSK aims at implementing PCR-based tools in replacement of the current QC protocols of vaccines. Saving of time and the high throughput of the assays represent the major gain in terms of lead time delivery. The availability of quantitative data facilitates the development of the assay and monitoring of its performance.

Two examples of replacement of molecular methods will be presented:

1. The development of an infectivity quantitative RT-PCR assay to demonstrate the effective inactivation of hepatitis A virus vaccine
2. The development of an infectivity quantitative PCR assay to detect Mycoplasma contamination

For both assays, the infectivity part of the procedure remains mostly unchanged: the cell substrate and the culture formats are conserved. Only the current read-outs are replaced by a molecular detection.

NOTES:
P-109-M

A Non-Cell Based Surrogate Measure of ADCC

Aaron Miller

*Genentech, a Member of the Roche Group, South San Francisco, CA USA*

Antibody dependent cell-mediated cytotoxicity (ADCC) is an important mechanism of action (MoA) for many monoclonal antibody therapeutics. As such, quantitative measurement of ADCC activity is key to drug development. We have successfully developed a cell lysis based ADCC assay using FcRIII transfected NK cell line which has acceptable precision and accuracy. This assay is superior to the traditional PBMC based ADCC assay, however, it still requires the maintenance of two cell lines, cell labeling, several washing steps, and transfer of cell lysates. Therefore, a non-cell based ADCC assay was developed that simultaneously measures binding of epitope to antibody and antibody to FcγRIII, both of which are required for initiation of ADCC activity in vivo. This method offers the ease of use of a standard ELISA, is sensitive to antibody fucose levels, and is stability indicating. The non-cell based ADCC assay correlates well with the cell lysis method and has the potential to be used as a surrogate measure of ADCC activity.

P-110-M

Strategies for Qualification of Critical Reagents Used in Cell-Based Potency Assays

Namrata Panday

*MedImmune, Gaithersburg, MD USA*

A reagent is classified as critical when there is a significant risk that by changing the reagent (i.e. new lot, vendor, etc) the performance of the method could be impacted. In cell-based potency assays, changes to critical reagents can affect many aspects of the method, including IC50/EC50 values, upper and lower asymptote values, and asymptote ratios, all of which have the potential to cause the method to fail system suitability criteria. As such, reagents deemed to be critical to a method must be qualified prior to use to ensure the continued performance of the method over time. A case study will be presented for a cell-based potency assay developed to measure ligand-mediated upregulation of a cell surface adhesion molecule on the surface of primary endothelial cells. As part of this complex assay, multiple reagents were identified as critical to the performance of the method and needed to be qualified. As there is no single way to definitively ensure the quality/comparability of different lots of complex biological reagents, we provide details on three different strategies that were evaluated for qualifying critical reagents for this method. Decision trees show the steps required to qualify the reagent and final decisions on the suitability of the new lots of reagents. The principles and strategies developed for qualifying critical reagents for this method may be applicable to the qualification of critical reagents for other bioassays and bioanalytical methods.
Characterization of a Multiple Antibody Drug Product for Neutralization of Botulinum Neurotoxin Serotype A, B and E

Joel Freeberg

XOMA, Berkeley, CA USA

Botulinum neurotoxins (BoNTs) are extremely potent toxins secreted by Clostridium botulinum that can cause mortality upon exposure. Recently, two fully human and one humanized therapeutic monoclonal antibodies were developed and manufactured that, in concert, have been shown to neutralize the effects of the botulinum neurotoxin type A (BoNT/A). Analytical assays were developed for combined drug product to quantify the individual mAb components and measure the impurity profile. Characterization assays included tryptic peptide mapping, CE-SDS, RP-HPLC, HIC-HPLC, CZE, ELISA, and Q-TOF MS. In total, seven assays are capable of identifying and quantifying the individual mAb components in the mixed drug product. The result of this effort was a release test panel that enables accurate quantification of each component as well as measuring all key aspects of the impurity profile for the BoNT/A three-antibody mixture. IND-enabling characterization assays were also developed that yield a comprehensive picture of the quantity, integrity and purity of the mixed drug product. Significant analytical challenges were realized when the separation methods were further developed to enable characterization of a nine-antibody mixture for BoNT/A, B and E neutralization. The sensitivity and benefits of the orthogonality of these release and characterization assays will be shown.

This project has been funded in whole or in part with federal funds from the National Institute of Allergy and Infectious Diseases, National Institute of Health, and Department of Health and Human Services under contract number HHSN266200600008C and HHSN272200800028C.

High Throughput Process-Related Impurity Testing Using a Gyrolab™ Workstation

Meletios Roussis

MedImmune, Gaithersburg, MD USA

In biologics manufacturing, it is necessary to accurately quantify process-related impurities to monitor the performance of the manufacturing process, and to measure the level in the Drug Substance. Typically, process-related impurity testing is performed using a sensitive immunoassay method such as ELISA. Although this method is sensitive, it has the disadvantage of being labor and reagent intensive, and is not scalable for large numbers of samples.

This poster describes the development of a Gyrolab Workstation method to quantify process-related impurities, and compares its performance to an established sandwich ELISA. In addition, advantages of the Gyrolab Workstation over the ELISA platform will be discussed.
Development of Engineered Cell Lines and Bioassays for Monitoring Fc Effector Function of Monoclonal Antibodies

Joyce Pasion; Kendall Carey; Xu-Rong Jiang; Robert Strouse; Michael Washabaugh

MedImmune, Gaithersburg, MD USA

Antibody-dependent cellular cytotoxicity (ADCC) is a major effector mechanism of therapeutic IgG class monoclonal antibodies. Traditional ADCC bioassays that use effector cells isolated from peripheral blood mononuclear cells (PBMCs) are generally not performed in a quality control setting (QC) due to high assay variability. To improve assay performance, we have developed engineered effector cell lines to replace PBMCs in ADCC bioassays used to monitor Fc effector function. We have implemented one of these genetically modified effector cell lines to develop a robust ADCC bioassay using a europium release assay method for monitoring potency in a QC setting. We also describe the construction and implementation of a reporter gene cell line to monitor effector function. Both methods were determined to be more robust and precise than a traditional ADCC bioassay method using PBMCs. Using these cell lines, we have developed a platform to test the Fc effector function of multiple therapeutic antibody candidates, and demonstrate that the potency results using either the europium release ADCC method or the reporter gene ADCC method are equivalent for the therapeutic antibody candidate. In summary, genetic modification of cell lines can be used as an approach to improve bioassay performance and assay robustness of highly variable cell-based assay methods.

A Homogeneous T-cell Mediated Apoptosis Bioassay for Bispecific Antibodies (BiTEs)

T. Shao; H.J. Kim; M. Doh; X. Jiang; R. Strouse; M. Washabaugh

Department of Analytical Biochemistry, MedImmune, Gaithersburg, MD USA

In this poster, we describe the development of a homogeneous bioassay for quantifying the bioactivity of a recombinant Bispecific T cell Engaging antibody (BiTE). BiTE antibodies are composed of two distinct scFv binding domains: one domain binds to CD3 on effector T cells, and the other domain binds to an antigen on target cells. The transient ligation of the effector cell and the target cell via the BiTE antibody activates the effector cell, activates caspasess in target cells and subsequently results in apoptosis. Caspase activation can be detected by a sensitive, commercially-available luminogenic substrate, and the amount of luminescence is directly proportional to the level of apoptosis. This bioassay offers reduced assay time, a simple homogeneous format, and is amenable for use in a regulated environment.
Qualification and Optimization of Micro-Flow Imaging Method to Analyze Particles in Bio-Therapeutics

Dave Thomas; Deepak Sharma; Clark Merchant; Peter Oma; Dave King

Brightwell Technologies Inc., Ottawa, Canada

The subvisible particles that might be present in protein therapeutics have been identified by the regulatory agencies as a potential safety issue. Some of these particles can be highly transparent, fragile and unstable. In addition, for much of the size range of concern, no practical measurement method with adequate sensitivity and accuracy has been available. The need has therefore been identified for new analytical methods which can accurately measure these particle types. Micro-Flow Imaging (MFI) is one such technique that has been shown to provide improved sensitivity and characterization potential over a wide range of sample types. This paper will describe the optimization and qualification of MFI method to characterize the particle size and levels of protein particulates. Case-Studies will be presented showing how the qualified MFI technique can be applied to quantify and analyze particles/aggregates in protein formulations. The MFI assay results showed improved sensitivity to detect subvisible particulates compared to conventional light obscuration detection, presumably due to the translucent nature of the protein particles.

New Method for Detection of Sub-VISIBLE Particles in Protein Solutions by Fluorescent Labeling and Flow Cytometry

Yoen Joo Kim; Alla Polozova; Ziping Wei; Patricia Cash

MedImmune, Gaithersburg, MD USA

It is challenging to characterize the formation of proteinaceous particles in high concentration protein solutions. Analytical methods, based on light obscuration and flow microscopy, are widely used by the industry; however, both approaches have limitations. Methods based on light obscuration require large sample dilution to reduce potential interference from background protein. Flow microscopy-based methods offer good sensitivity and provide information about particle morphology. The flow microscopy technique is also capable of analyzing high concentration protein samples; but requires large sample volumes (> 1 ml).

Here we present a new method for detection of sub-visible particles in high concentration protein solutions by using fluorescent labeling with flow cytometry. This technique has advantages over other sub-visible particle counting techniques in that it: requires small sample volume (50 ul); analyzes high concentration protein samples without dilution; uses a 96-well plate format having high throughput capabilities; classifies different particle types based on their hydrophobicity. In this method protein samples are labeled with Nile Red, a fluorescent dye known for selective binding to protein aggregates and hydrophobic surfaces. A flow cytometer equipped with selective filters for Nile Red fluorescence measures particle counts and relative particle sizes. In addition, different particle types can be classified based on the level of fluorescence intensity of individual particles.
We demonstrate the utility of this new approach with a case study of two different lots of a monoclonal antibody. Changes in particles counts in both lots, after a 0.22 µm sterile filtration step, were tracked over 10 days. One of the lots was prepared by an improved process designed to minimize particles. As expected, this lot showed much lower particle counts over the course of the study than the other lot. The particle counts obtained by the method were in good agreement with observations by the flow microscopy technique.

NOTES:
New Advancement in Hydrogen Deuterium Exchange MS for Biotherapeutic Protein Characterization

Joomi Ahn; John Taraszka; Michael Eggertson; Keith Fadgen; Weibin Chen; Ying Qing Yu

1 Waters Corporation, Milford, MA USA; 2 Novartis Institutes for Biomedical Research Inc., Cambridge, MA USA

Hydrogen deuterium exchange mass spectrometry (HDX MS) has proven to be an indispensable analytical technique for the study of protein interaction and binding. Many leading biopharmaceutical companies utilize this technology to locate the epitope site and protein interaction/binding location at the discovery and development states of drugs. The applications in HDX require a system that can perform rapid chromatographic separations at 0 °C and accurate mass measurements of labeled proteins and peptides with small quantities of material. Such analyses are often challenged due to the complex samples analyzed in short separation time.

In this study, we showed some of the latest advancements in the HDX MS system from Waters Corporation. A nanoACQUITY UPLC system with HDX technology combined with a Synapt G2 HDMSE was used as a robust HDX platform. MSE data acquired on the Q-TOF MS system with high dynamic range gives high sequence coverage for an antigen of interest in the presence of a large antibody. This capability shows promise for future epitope mapping experiments. We also demonstrated that Ion Mobility Separation (IMS) improves the deuterium uptake measurement for some difficult peptides by resolving the peptide of interest from the interfering ions. In addition, a fully automated high-throughput sample handling manager interfaced with the HDX LC-MS system performs robust and reproducible sample preparation, which significantly reduces the amount of time and labor. Last, we showed the new features in the HDX Browser software for deuterium uptake measurement.

An Integrated Workflow for Automatic Mapping of Disulfide Linkages of Therapeutic Proteins Using High-Resolution Mass Spectrometry and Targeted Software

Hongwei Xie; Weibin Chen

Waters Corporation, Milford, MA USA

Disulfide bond formation is critical for establishing three-dimensional folding and maintaining proper function of therapeutic proteins such as monoclonal antibodies (mAbs). Localization and assignment of disulfide bonds are therefore an important aspect in protein structural analysis. However, mapping of disulfide pairing in a protein with multiple cysteine residues is generally more challenging than the determination of a protein sequence due to incomplete disulfide bond formation and disulfide bond scrambling. In this study, an advanced peptide mapping workflow was demonstrated for detection and identification of disulfide linkages (including scrambled disulfide linkages) using a recombinant IgG1 mAb as an example. The integrated workflow includes on-line reversed-phase ultra-performance liquid chromatographic (RP UPLC) separation of endoproteinase Lys-C digests, enhanced mass resolution quadruple time-of-flight (Q-TOF) mass spectrometric (MS) detection and automatic data interpretation and annotation with dedicated peptide mapping software BiopharmaLynx 1.3. Peptides and disulfide linkages were identified by accurate masses of precursors and further confirmed by peptide fragment ions.
spectra, collected by an alternating low and elevated collision energy MS acquisition (MSE) mode. The enhanced mass resolution and mass accuracy were proven to be particularly useful for the assignment of disulfide bond-linked peptides with high molecular masses. Both expected and unexpected (scrambled) disulfide linkages in the mAb were rapidly assigned by the informatics tool using an automatic data processing routine. The identities and abundance of native scrambled disulfide linkages were further differentiated from those potentially formed during sample preparation (artifacts) by comparing digests in parallel prepared with or without alkylation protection of the free sulphydryl groups in the sample prior to digestion. This integrated approach, combining high performance LC-MSE and targeted software, should be applicable for fast mapping and monitoring of disulfide linkages in other mAbs and therapeutic proteins with multiple disulfide linkages.

NOTES:
Characterization of Sub-Micron Protein Aggregates Using Electron Microscopy

Anke Mulder; Joel Quispe; Bridget Carragher; Clint Potter

NanoImaging Services, La Jolla, CA USA

Characterization of sub-micron protein aggregates in drug products is in high demand in the pharmaceutical industry. Transmission electron microscopy (TEM) as a means of assessing protein aggregation has several unique advantages. We demonstrate the method using IgG in a series of controlled experiments. IgG was subjected to repeated freeze-thaw cycles at -80 ºC and -180 ºC and samples from each cycle prepared for visualization by TEM using Uranyl Formate negative stain. Images were acquired at multiple scales of magnification (6,500 – 52,000x) using automated image collection software. Individual IgG monomers (MW 150 kDa) were clearly visible in these images and readily counted using automated particle selection and classification software. The loss of free protein over the course of multiple freeze-thaw cycles can be directly observed and a quantitative analysis will be presented. This study shows the potential of TEM for providing an alternative, orthogonal method for characterizing protein aggregation. The unique advantages of TEM is that it provides a direct means of observing aggregates and discriminating them from contaminate or impurities. It allows for quantitative characterization of both the loss of free protein due to aggregation and the characterization of the aggregates by size, shape, morphology and count in a single experiment.

Development of a Vaccine Based on Candida Albicans Rals3p-N: From Preclinical Proof-of-Concept to Approval for Clinical Studies

John Hennessey; Clint Schmidt

NovaDigm Therapeutics, Inc., Grand Forks, ND USA

NovaDigm is developing a vaccine candidate based on the N-terminal portion of the Candida albicans agglutin-like sequence 3 protein (rAls3p-N). This vaccine antigen protects mice from lethal systemic challenge with Candida albicans as well as Staphylococcus aureus. It has been proposed that the mechanism of protection from systemic challenge by both organisms is based on a Th1/Th17 response. As part of development of this vaccine candidate for clinical studies, we have developed a scalable manufacturing process based on a 100 L production fermentation, analytical methods that reveal the compositional and structural attributes of the recombinant protein, and bioanalytical methods that measure selected B- and T-cell immune responses (including anti-Als3 IgG and IgA1 and Als3-induced T-cell production of IL-17 and IFN-γ) induced by our vaccine in preclinical studies, which will be adapted for use in our human clinical studies. These advances in development of the Als3 vaccine have allowed us to gain approval from the US FDA to initiate clinical studies of this potentially important vaccine. This presentation will present and discuss these aspects of our vaccine development program.
P-121-M

Metabolomics Profiling of Cell Culture Media Leads to the Identification of Riboflavin Photo-Sensitized Degradation of Tryptophan Causing Slow Growth in Cell Culture

L. Zang¹; R. Frenkel¹; J. Simeone²; M. Lanan¹; M. Bryers²; Y. Lyubarskaya¹

¹Analytical Development Department, Biogen Idec Inc., Cambridge, MA USA; ²Manufacturing Sciences Department, Biogen Idec Inc., Cambridge, MA USA

As more protein biopharmaceuticals are produced using mammalian cell culture techniques, it becomes increasingly important for the biopharmaceutical industry to have tools to characterize the cell culture media and evaluate its impact on the cell culture performance. Exposure of the cell culture media to light, temperature stress or adventitious introduction of low-level organisms during preparation can lead to the generation of chemical degradants or metabolites of the media components, which are potentially detrimental to the cell culture process. In this work, we applied a liquid chromatography-mass spectrometry (LC-MS) based metabolomics methodology for the investigation of a media lot used for a mammalian cell culture process that had resulted in low growth rate and failure to meet required viable cell density (VCD). The study led to the observation of increased levels of tryptophan oxidation products and a riboflavin degradant, lumichrome, in the malfunctioning media lot, relative to working media lots. A compound found 7-fold higher in the working media lots appeared to be tetrahydropentoxyline, a condensation product of glucose and tryptophan. A second compound found at an over 50-fold higher level in the malfunctioning media lot remains unknown, although it is confirmed to be a degradant resulting from tryptophan and riboflavin in the media. A study of the cell culture media performed under stress conditions using lab fluorescent light and heat showed that the media powder was highly resistant to light-induced degradation, while solution media could be easily degraded after brief light exposure. It is therefore suspected that inadvertent exposure of the media to light during preparation and storage has resulted in the poor performance of the media causing the low growth and VCD in the cell culture process.

P-122-M

Operators as Sources of Error – Improved Efficiency Through Pipette Technique Training

A. Bjoern Carle

ARTEL, Westbrook, ME USA

Concentrations of biological and chemical components in assays, as well as in the associated dilution protocols, are volume-dependent and inaccurate and/or imprecise pipetting steps will directly impact assay results. Without a way to assess an operator’s pipetting performance, assay results may be unknowingly flawed, even if the pipettes are fully functional and calibrated. While most pipette operators are confident in their pipetting skills and seem to universally believe that they are accurately and precisely pipetting correct volumes, case studies provide clear evidence that operators contribute to assay errors through inconsistent and variable pipetting. Based on the results from numerous case studies, this presentation highlights how operators are introducing a significant amount of error in pipetting steps and discusses the need for pipetting technique training.
The data presented herein show pre-training and post-training pipetting performance assessment for a variety of experienced pipette operators. It will be shown how pipetting technique training improved the inaccuracy and imprecision from over 30% and 60%, respectively, to less than 2% for both values post-training.

Enhancing data integrity and increasing confidence in results is imperative for any laboratory. Inconsistent or flawed results bear usually very costly consequences for laboratories, and will decrease the efficiency of all operative processes within a laboratory.

Minimizing the risk of liquid handling errors and implementing regular pipetting technique training and skills assessment programs is as essential as maintaining properly calibrated equipment. Both measures facilitate compliance with a number of regulatory guidelines, e.g. ISO 17025 (Sec. 5.2.1).

NOTES:
P-123-M

Development of a High-Sensitivity Assay for the Determination of Residual Thrombin Activity

Travis Linkous; Dawn Kernaghan; James McGivney; Robert Strouse

MedImmune, Gaithersburg, MD USA

The determination of residual thrombin activity in recombinant human prothrombin contributes to final product safety, purity, and process consistency. Although physicochemical methods are able to detect levels of residual thrombin, these methods cannot determine if the thrombin is active or inactive. An established activity assay using a chromogenic fibrinogen peptide substrate was unable to detect thrombin activity at low levels due to a lack of sensitivity. Therefore, an assay with higher sensitivity to quantitate active residual thrombin was developed using a fluorogenic peptide substrate. The fluorescent assay resulted in a 60-fold boost in sensitivity over the chromogenic substrate, allowing for the detection of low levels of active thrombin in various in-process samples.

P-124-M

Determination of Polysorbate 80 Content in Protein Samples

Ratnesh Pandey; Kathy Yu; Susanna Bilbulian; Ziping Wei; Patricia Cash

MedImmune, Gaithersburg, MD USA

Polysorbate 80 (PS 80), commercially known as Tween 80, is a nonionic surfactant and emulsifier derived from polyethoxylated sorbitan and oleic acid. PS 80 is widely used as an excipient in protein samples to enhance stability and prevent aggregation and particle formation. In this study, we present a new method for the quantitative determination of PS 80 content in protein samples.

This method is based on a capillary gas chromatography (GC) equipped with a flame ionization detector (FID), to detect ethylene glycol diacetate (EGD) released from PS 80 following hydrolysis and acetylation. The PS 80 concentration is calculated based on the amount of ethylene glycol diacetate (EGD) determined using a regression analysis from PS 80 standard curve (0.005% - 0.05%). The limit of quantitation (LOQ) of the method is 0.005% and the limit of detection (LOD) is 0.0025%. Our results demonstrate that this GC method is sensitive and specific for the determination of PS 80 content in protein samples.

P-125-M

Biopharmaceutical Glycoanalysis Using Nonspecific Pronase Digestion with MALDI-TOF and LC-MS/MS

John Schiel; Jennifer Au; Karen Phinney

NIST, Gaithersburg, MD USA

Glycosylation, the enzymatic addition of carbohydrates to a protein, is one of the most abundant post-translational modifications. The quantity, location, and size of glycans attached can vary, potentially resulting in glycoforms with different stability, toxicity, and activity. This is especially important in the
biopharmaceutical industry where product consistency and safety are vital. Glycoprotein analysis involves numerous mass spectrometry-based techniques, each of which provides various aspects of characterization. One recently developed method utilizes pronase, a mixture of proteases capable of cleaving any peptide bond, to digest the glycoprotein into amino acids and short glycopeptides. Pronase was used in the current study to digest ribonuclease B (a well characterized glycoprotein) and a representative IgG biopharmaceutical. The resulting glycopeptides were concentrated and purified using graphitized carbon solid phase extraction. Compositional matching to the ions observed in MALDI-TOF MS spectra yielded a list of potential glycopeptides. Although this peak list was generated using data with high mass accuracy, more than one possible glycopeptide often resulted for a given m/z. Therefore, glycan identity and glycosylation site were verified using graphitized carbon LC-MS/MS. The glycosylation site and glycan identities of both analytes were found to be in agreement with previously reported literature results, indicating the widespread applicability of these methods for biopharmaceutical characterization.

NOTES:
Sialic Acid Determination in Glycoproteins: Comparison of Two Liquid Chromatography Methods

Deanna Hurum; Jeffrey Rohrer

Dionex Corporation, Sunnyvale, CA USA

Sialic acids are critical in determining glycoprotein bioavailability, function, stability, and eventual catabolism. Although over 50 natural sialic acids have been identified, two forms are commonly determined in glycoprotein products: N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). Because humans do not generally synthesize Neu5Gc and have been shown to possess antibodies against Neu5Gc, the presence of this sialic acid in a therapeutic agent can potentially lead to an immune response. Consequently, glycoprotein sialylation, and the identity of the sialic acids, play important roles in therapeutic protein efficacy, pharmacokinetics, and potential immunogenicity.

This work compares two independent chromatographic assays developed for sialic acids in model glycoproteins. Analyses by both high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) and UHPLC with fluorescence detection (UHPLC-FLD) are evaluated with comparisons of assay performance and total analysis time. Calibration ranges were chosen spanning the expected amounts of Neu5Ac and Neu5Gc in five representative glycoproteins. For both methods, response is linear with correlation coefficients >0.9990 and >0.995 for HPAE-PAD and UHPLC-FLD determination, respectively. Retention time precision (RSDs of <0.12) and peak area precision (RSDs of <3.0) are good for both methods. Glycoprotein hydrolysates were analyzed to evaluate method accuracy and precision. In both cases, method sensitivity easily allows detection of the sialic acids in prepared acid hydrolysates. By UHPLC-FLD, recoveries ranged from 79-114% for Neu5Ac and 81-110% for Neu5Gc. Similarly, HPAE-PAD recoveries ranged from 74-107% and 72-103% for Neu5Ac and Neu5Gc, respectively. Between-day assay precision (RSDs) ranged from 9.5-19% (UHPLC-FLD) and 7.9-14% (HPAE-PAD). Results from the example glycoproteins are used to illustrate method precautions and advantages.

Rapid Monoclonal Antibody Glycan Profiling Using an Integrated Microfluidic Based Mab-Glyco Chip and Quadrupole Time-of-Flight Mass Spectrometry

Ning Tang; Lukas Trojer; Patrick Perkins

Agilent Technologies, Santa Clara, CA USA

Protein biologics now represent a significant share of pharmaceutical sales and future growth potential, particularly in an era of increasing patent expirations. A range of analytical methods is required to determine the purity, identity and integrity of protein biologics at multiple points along the manufacturing process, from cell culture to downstream purification, product characterization and lot release. Characterization of glycans from antibodies is fundamentally important in biotherapeutics design and disease progression and detection. The ability to characterize glycans rapidly has been limited by the sample preparation steps and structural complexity of the glycoproteins. To address this problem, we have developed a microfluidic chip that performs rapid on-line cleavage of glycans from monoclonal antibodies, captures the released glycans and then separates them prior to nanospray...
ionization in the mass spectrometer. The entire run time is 12 minutes. A glycan accurate mass database was established allowing quick assignment and identification of the glycans. Further glycan characterization is done by MS/MS experiments on QTOF. Discussion will also be made on the relative glycan quantitation ability of the technique and its role in comparing different batches of antibody glycans.

NOTES:
P-128-M

CE/LIF and HILIC/LIF-MS of Polysaccharides Labelled by APTS and 2AB

Vérena Poinsot¹; Jaloul Bouajila¹; Audrey Boutonnet²; Pierre Naccache²; Francois Coudere¹

¹Université Paul Sabatier, Toulouse, France; ²Picometrics, Toulouse, France

9-Aminopyrene-1,4,6-trisulfonate (APTS) is a commonly used dye to be used for mono or oligo saccharides (Fraysse et al Electrophoresis. 2003, 24, 3364) that can be excited at 488nm by an Ar ion laser or by a 470nm LED. In the same vein, the dye 2-amino benzoic acid, which is used for the separation of labelled oligosaccharides via HPLC can be employed; it fluoresces using a 325nm HeCd Laser or a 320nm LED. The process to label the sugars is very well known, it consists on the reductive amination of the reductive function of the mono or oligosaccharides. These reactions can be done on very small samples (as small as 5µL).

In this poster we use a variety of different lasers and LED’s to monitor the mono or oligo saccharides and report the lowest detected (LOD) concentration of mono or oligo saccharides The LOD also depends on the stability of the light source.

We used µHILIC/LIF/MS to separate sugars labelled with the above dyes and compared the sensitivity of these two detection means using µHILIC separation. As presented elsewhere, LIF detection is at least 100 x more sensitive than MS even using µHILIC separation. If µHPLC is more familiar than CE for the users, the separation of the 2AB derivatives using µHILIC results in resolution close to CE for APTS sugar compounds.

P-129-M

A New Platform for High Throughput N-Glycan Sample Preparation

Craig Nishida

ProZyme, Inc, Hayward, CA USA

The distribution of N - linked glycans plays a critical role in the pharmacology of therapeutic proteins, potentially affecting immunogenicity, pharmacokinetics and pharmacodynamics. While prompt information is highly valuable for bioreactor control, strain selection, comparability studies and biomarker discovery, the manual, complex and multi - day sample preparation usually limits analysis to only the most critical decision points. With the advent of QbD, there is a growing need for dramatically increased throughput of N - glycan profiling.

ProZyme, Inc. and BioSystem Development, LLC have jointly developed the new GlykoPrep™ platform for rapid, quantitative N - glycan sample preparation. This assay has been implemented on the AssayMAP® platform, which enables microliter - scale separation and enzymatic digestion in a high - throughput format compatible with microplate liquid handling. Using GlykoPrep, fluorescently labeled N - glycans can be produced from up to 192 crude supernatant samples in less than 3 hours, ready for analysis by CE, HPLC or LC/MS.
Detailed characterization of the glycans present on recombinant glycoproteins remains an important challenge in both the development and production of these clinically important biomolecules. Ezose Sciences Inc. has developed a proprietary new high-throughput solution, the GlycanMap #174; platform, which is compatible with complex biological samples and can deliver both qualitative and quantitative data on glycans on purified glycoproteins and in crude protein mixtures. The GlycanMap method for N-linked glycans relies on PNGase release of glycans from the proteins, covalent attachment of the glycans to beads, on-bead washing and chemical derivatization, simultaneous reducing-end labeling and release from the beads, quantitative MALDI-TOF mass spectrometry, and a custom Bioinformatics platform. The key assay steps are integrated into an automated, 96-well robotic assay system and a glycan internal standard is used to enable quantitation. The GlycanMap method has also been adapted to analyze O-linked glycans. Several commercially-available glycoprotein therapeutics were analyzed to further characterize the assay platform. The GlycanMap methodology yielded good reproducibility, linearity and sensitivity compared to conventional glycan analysis methodologies, with clear advantages in quantitation and throughput.

Characterization of Glycation in a Monoclonal Antibody by Electron Transfer Dissociation Mass Spectrometry

Jinhua (Jenny) Feng; Kenneth Moore; Deepti Sharma; Johnson Varghese; Patricia Cash

MedImmune, Gaithersburg, MD USA

Electron-transfer dissociation mass spectrometry (ETD/MS) is a powerful technique that can be used to identify specific glycation sites on monoclonal antibodies. Side chain and post translational modifications such as glycation are left intact with ETD induced fragmentation unlike the conventional collision induced dissociation (CID) fragmentation technique, where the loss of labile side chains is common. A monoclonal antibody (mAb) IgG1 was analyzed by liquid chromatography – mass spectrometry (LC-MS) intact mass analysis. The data for the reduced and PNGase deglycosylated mAb showed a single glycation on the mAb light chain. Tryptic peptide mapping with ETD/MS analysis was able to locate the glycation site on the N-terminus of the light chain. The glycated tryptic peptide fragmented well with both ETD and CID methods. However, the ions retaining the glycan were only present in data generated via ETD/MS technique, resulting in the precise determination of the glycation site at the N-terminal of the light chain.
NOTES:
P-132-M

Separation of Fucosylated, non-Fucosylated, and Complex Carbohydrates Associated with Monoclonal Antibodies using Capillary Electrophoresis

Sushma Rampal¹; Lynn Gennaro²; Mark Lies¹

¹Discovery Business Center, Beckman Coulter, Inc., Brea, CA USA; ²Genentech, a Member of the Roche Group, South San Francisco, CA USA

In order to gain a comprehensive understanding of therapeutic Monoclonal Antibody (MAb) function, it is necessary to critically characterize glycosylation associated with them. Carbohydrates are known to play an important role in the structure, function, and clearance of MAbs and have been shown to be responsible for invoking immune responses in humans. Changes in carbohydrate composition or concentration can significantly impact the overall efficacy of therapeutic MAbs and can also lead to side effects. Because of their link to antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC), accurate analysis of oligosaccharide fucosylation, sialylation, and antennary structure is critical for a complete understanding of MAb microheterogeneity. Capillary electrophoresis (CE) technology has been successfully used to separate major IgG N-linked oligosaccharides G0, G1, and G2 structures from one another. The basis for this separation relies on electrophoresis of oligosaccharides labeled with amino pyrene tri-sulfonic acid (APTS). The complexity of glycans associated with many molecules calls for high resolution separation in order to assess heterogeneity among carbohydrate isomers and co-migrating carbohydrate species. Since CE is already an established technology for automated and quantitative analysis of N-linked oligosaccharides, we set out to develop methodology by which fucosylated, afucosylated, sialylated and complex antennary oligosaccharides can be differentiated from one another. Optimization of chemistry and CE methods enabled separation of these species in a simple, reproducible assay environment.

P-133-M

Comparison of 3 µm snd 5 µm 300 Å Hydrophilic Bonded Silica Gels for the Analysis of Monoclonal Antibodies by Size Exclusion Chromatography

Phu Duong; Taegen Clary; Faizy Ahmed

Agilent Technologies, Wilmington, DE USA

Recently, monoclonal antibodies (mAbs) have gained importance as bio-therapeutic agents in the treatment of serious diseases such as cancer. These proteins undergo chemical and physical changes during manufacturing, formulation and storage. These changes include post-translational chemical modifications, such as oxidation or deamidation or physical changes which include formation of dimers, aggregates and denaturation. These physical changes are known to lead to their immunogenicity as well as reduced therapeutic efficacy.

Size Exclusion Chromatography has been extensively used for the characterization of the physical entities of aggregates and dimers in the mAbs from the earliest stages of their production. Traditionally, 5 µm particles with various pore diameters have been used for the SEC of proteins with 200 Å - 300 Å pore diameter particularly suitable for the analysis of mAb monomer and aggregates. In order to get better separation between these fractions we developed a 3 µm 300 Å silica particle with hydrophilic polymer coated surface and compared it to similarly modified 5 µ 300 Å silica.
Many of the commercially available silica-based SEC columns show interactions with basic proteins because of the presence of surface silanols. We characterized our columns for such interactions using lysozyme, which is a basic protein, under various salt and pH conditions. Several different protein standards were also tested under high and low salt buffers concentrations. Both the 5 µm and 3 µm SEC phases showed excellent linearity for the log MW vs. KD plot. The SEC phases were then used for the separation of a mAb from its aggregates and dimers. We obtained 20 percent better separation of dimers from the native mAb on the 3 µm SEC phase compared to the 5 µm phase.

NOTES:
UNIFI: A Revolutionary Scientific Information System Supporting Biotherapeutic LC/High Resolution MS Studies in Both Non-Regulated and GxP Laboratories

Scott Berger¹; Steve Cubbedge²; Steve Bird²; Ginni Corbin³

¹Biopharmaceutical Sciences, Waters Corporation, Milford, MA USA; ²Data and Informatics Products, Waters Corporation, Milford, MA USA; ³Regulatory Compliance, Waters Corporation, Milford, MA USA

While mass spectrometry has been the empowering technology behind the “well characterized biotherapeutic” regulatory framework, the application of High Resolution Mass Spectrometry (HRMS) for biotherapeutics has hit a virtual “glass wall”, with its use rarely extending past mid-stage development activities. HRMS instrument costs and operator training requirements have significantly decreased over the past decade, but the major remaining objection regarding its use in routine, regulatory-compliant operation has not yet been sufficiently addressed. In this poster, we address this issue, and present the design rationale for the Waters UNIFI™ Scientific Information System, a novel scientific data system that holistically integrates LC/HRMS acquisition, optical and MS data processing, bioinformatics, report generation, and data/result management. This fit-for-purpose biopharmaceutical data and informatics platform delivers high-confidence HRMS results, with methods, data, and results that can be readily transferred between non-regulated and GxP regulated laboratories.

NOTES:
Poster Session Two  
Tuesday, January 11, 2011  
15:05 – 16:05  
East and State Rooms

P-201-T

Identifying Shoulder Peaks of IgG1 from CHO Cell Line Observed in Non-Reduced cSDS Assay

Mingfang Hong; Mike Lewis; Peter Lisi

Johnson & Johnson, Radnor, PA USA

Shoulder peaks of IgG1 derived from CHO cells observed in non-reduced capillary sodium dodecyl sulfate polyacrylamide gel electrophoresis (cSDS) using Beckman-Coulter PA800 were characterized. The first shoulder peak, with a molecular weight of 124K, was identified as an IgG1 fragment, missing one light chain from the intact IgG1 molecule. The second shoulder peak was identified as IgG1 with both heavy chains aglycosylated. The third shoulder peak, which was merely separated from the main peak, was identified as IgG1 with one heavy chain aglycosylated. In addition, for in-process samples, the resolution between shoulder peaks and IgG1 main peak appeared to be greater, due to high salt conc. effect. Furthermore, shoulder peaks 2 & 3 were barely visible for Mabs derived from Sp2/0 cells, consistent with the generally very low aglycosylation level of products from Sp2/0 cell line.

P-202-T

Characterization of Charge Heterogeneity in a Recombinant IgG1 Monoclonal Antibody by Cation Exchange Chromatography and Mass Spectrometry

Jinhua (Jenny) Feng; Deepti Sharma; Kenneth Moore; Niluka De Mel; Methal Albarghouthi; Anthony Shannon; Johnson Varghese; Patricia Cash

MedImmune, Gaithersburg, MD USA

Characterization of the product variants is important in product development. Cation exchange chromatography (CEX) with UV detection is generally used for separating and monitoring the charge heterogeneity of mAbs. However, the off-line characterization through a combination of peptide mapping and liquid chromatography-mass spectrometry (LC-MS) intact mass analysis is often used for determining the identity of the CEX fractionated species. For a typical IgG1 molecule, an array of PTMs was identified utilizing a combination of CEX, peptide mapping and LC-MS. Negatively charged species, primarily due to deamidation, glycation, and sialylation, were shown to elute before the main product peak. Positively charged species, arising from N-terminal un-cyclized glutamine, C-terminal proline amidation and C-terminal lysine, were shown to elute after the main product peak.
LCMS Studies on Oxidation of Monoclonal Antibody Using Micro-Fluidic Based HPLC Chip Coupled to an Accurate-Mass Q-TOF

Ravindra Gudihal; Ning Tang

Agilent Technologies, Bangalore, India

Monoclonal antibodies (mAb) or any other protein drugs can undergo oxidation during production, purification, formulation or storage, leading to altered activity of the protein drug. Therefore, it is very important to analyze and quantify potential oxidation changes during such processes. We have analyzed the forced oxidation of monoclonal antibody with oxidizing agent such as t-butyl hydroperoxide (t-BHP) and hydrogen peroxide to simulate potential oxidative modifications of a mAb that may occur during a manufacturing process. HPLC-Chip coupled to Accurate-Mass Q-TOF LC/MS combined with powerful data analysis software were used to analyze oxidation sites of mAb. Peptide fragments generated by tryptic digestion of a mAb were analyzed by a HPLC-Chip coupled with Q-TOF MS both in MS and MS/MS mode to identify the sites of oxidative modification. The accurate mass measurement in MS only mode of Accurate-Mass Q-TOF LC/MS allowed for easy assignment of peptides, which carry the oxidative modification of sensitive amino acids such as methionine or tryptophan, in the peptide sequence. Furthermore, MS/MS results confirmed the site of modification in the peptide sequence. We have also analyzed Methionine (Met) containing peptides to access the susceptibility of the mAb to forced oxidation. Methionine residues that were susceptible to oxidation were analyzed by generating peptide mapping using trypsin and LCMS analysis. The commonly used DLTMISR peptide sequence was used for quantifying the degree of oxidation in the monoclonal antibody.

Separation and Detection of Protein Charge Isoforms with a Combination of OFFGEL- and Lab-On-Chip Electrophoresis and Mass Spectrometry

Martin Vollmer; Suresh Babu; Christian Wenz; Andreas Ruefer

Agilent Technologies, Waldbronn, Germany

Separation and analysis of charge heterogeneity in recombinant protein or monoclonal antibody (mAb) production is a prime quality control step in the biopharmaceutical industry. This step is often carried out by a combination of separation techniques followed by mass spectrometric detection. Two dimensional gel electrophoresis (2D-GE) is unrivalled in terms of resolution but is a tedious procedure. Here we present a combination of two easy methods that separate proteins in analogy to 2D-GE according to pI and molecular weight (kDa) with high reproducibility for the analysis of mAb’s and recombinant proteins followed by mass spectrometry (MS) analysis.

For the 1st dimension, OFFGEL electrophoresis was used. This method takes advantage of the impressive resolving power of immobilized pH gradient (IPG) gels but in contrast to conventional IEF delivers samples in liquid-phase. Fractions with charged isoforms in solution can directly be analyzed by MS.
For the 2nd dimension, a highly sensitive microfluidic on-chip protein sizing method was employed. This method allows protein separation from 5 to 250 kDa and offers a sensitivity equivalent or better than silver staining and a linear dynamic range across four orders of magnitude.

The charge heterogeneity in a mAb sample was evaluated under native conditions and with addition of a mild detergent (Tween-20). The difference in focusing patterns between the two conditions is clearly visualized with the Bioanalyzer protein assay. The combination of OFFGEL and lab-on-chip protein analysis allows separation and identification of isoforms based on pI and molecular weight. Protein isoforms differing only by a few kDa in apparent molecular weight were successfully separated and enriched by OFFGEL electrophoresis for further downstream analysis by LC/MS-MS. The combination of OFFGEL, Bioanalyzer, and mass spectrometry is thus an efficient combination for detailed characterization of recombinant proteins such as mAb’s.

NOTES:
Capillary Electrophoresis-Mass Spectrometry (CE-MS) Analysis of Glycopeptides in Monoclonal Antibodies

Suresh Babu C V²; Ning Tang¹; Ravindra Gudihal²; Tobias Preckel³; Martin Greiner³

¹Agilent Technologies, Santa Clara, CA USA; ²Agilent Technologies India Pvt. Ltd., Bangalore, India; ³Agilent Technologies R&D and Mktg. GmbH & Co. KG, Waldbronn, Germany

Glycosylation of monoclonal antibodies (mAb) can have impacts on its biological activity and immunogenicity. Due to the importance of mAb as therapeutic agents, there is a growing demand for monitoring the carbohydrate structures attached to mAb. For enhanced separation efficiency, higher resolution, shorter run times, minimal sample/solvent consumption and flexibility, capillary electrophoresis (CE) has an enormous potential for the analysis of biopharmaceuticals. Further, there is growing interest in exploring CE coupled to mass spectrometry (MS) for the higher sensitivity and better compound identification with accurate mass measurements. Improvements in CE technology have made CE-MS a widely used tool for protein characterization. In the present work coupling of an Agilent 7100 CE system to an Agilent 6520 Accurate-Mass Q-TOF was achieved with a coaxial sheath liquid interface. The CE-MS setup equipped with electrospray source and orthogonal sprayer which reduces the risk contamination and improves the MS source cleanliness. We have analyzed the glycopeptides of a mAb using this CE-MS setup. A tryptic digest of the mAb was subjected to CE-MS analysis and the glycopeptides were assigned using accurate mass measurement. Further, CE-MS/MS analysis was performed to search for diagnostic oxonium ions generated from a glycan moiety to identify the glycopeptides. The CE-MS platform, combined with the powerful data processing capabilities of Agilent MassHunter and BioConfirm software, enabled identification of the glycan modification attached to mAb complex.

Multi-Mode Analytical Separations of Proteins

Patricia McConville; Daniel Root; Thomas Wheat

Waters Corporation, Milford, MA USA

The chemical and physical structure of proteins can vary in several ways, affecting even a single amino acid side chain. Many of these alterations profoundly alter biological activity. An analytical method must, therefore, detect and quantitate these small structural changes. Further complications arise because a single biomolecule can harbor more than one such modification. A sample is, therefore, a family of modified structures that are derived from a single parent. To make it more challenging, the modified forms are present in small amounts in the sample. Our methods must detect these changes and provide quantitative information. This complexity requires a different approach to chromatographic analysis than we use for small molecule pharmaceuticals. The most common approach to this analytical problem is the selection of a chromatographic mechanism that is very specific for one kind of chemical property. Depending on the information about the sample that is required for the particular stage of characterization and analysis, the sample may be analyzed using any or all of the techniques of reversed phase, ion exchange, and size exclusion. We will describe an automated system approach that facilitates the free choice of analytical separation method for a particular sample and also permits the analysis of a
single sample with all the techniques in series. A column manager directs the sample to the required column while solvent selection valves match the mobile phase constituents. Auto•Blend Plus™ Technology provides the required pH and salt concentration. The performance of this system approach is tested and demonstrated with a series of protein mixtures that includes monoclonal antibodies of biopharmaceutical interest.

NOTES:
Identification of Both Proteolytic Cleavage and Protease in CHO Cell Production of Glucagon-Like-Peptide-1 Fusion Antibody

Qiaozhen Lu¹; Shiaw-Lin Wu¹; William Hancock¹; Yonghui Wang²; Haimanti Dorai²; Alex Santiago²; Marguerite Campbell²; Qing Mike Tang²; Michael Bond²; Michael Lewis²

¹The Barnett Institute, Northeastern University, Boston, MA USA; ²Centocor R & D, Radnor, PA USA

A glucagon like peptide-1 (GLP-1) fusion antibody was developed for the potential treatment of diabetes disease. In this fusion antibody, the light chain was removed and the heavy chain in the Fab region was replaced with a GLP-1 sequence (CNTO 736). An LC-MS method with MRM approach has been developed in our lab to measure the pharmacokinetics of the fusion antibody in monkey serum and shown that the increase of serum half life with the fusion protein. However, proteolytic degradation (clipping) in GLP-1 portion has been observed for CNTO 736 and also for its closely related generic variant CNTO 3649 when they were expressed in CHOK1SV cell line. Since the cleavages occurred at the active site of the drugs, thus, it is important to understand the nature of the cleavages in order for prevention. In our study, two types of cleavages were identified as one related to chemical degradation (i.e. deamidation), and the other associated with proteases. The proteolytic cleavages were further studied in CHO cell lysate, spent media, and down-stream purification steps by structure characterization as well as by shotgun proteomics techniques. In this study, the LC-MS with multi-enzyme approach identified the major cleavage site at R30 from the N-terminus, and at least one serine-threonine class of protease could be responsible in the host CHO cells by gel separation with shotgun proteomic approach. The proper sample preparation for cellular protein extraction was successfully achieved, and the shotgun proteomics was employed effectively for identification of many CHO proteins, including the proteases. This methodology should provide a new way to trouble-shoot problems in manufacturing processes as well as to examine optimizations in cellular production.

Observation of Cysteine Adducts on Lambda Light Chain Monoclonal Antibodies

Lowell Brady; Becky Scott; Alain Balland

Amgen, Inc., Seattle, WA USA

We investigated the disulfide bonding patterns of lambda light chain containing IgG2 antibodies. We report here the modification of pairs of cysteine residues in the hinge of these antibodies by the low molecular weight thiols cysteine and glutathione. We also observed the presence, at a lower level, of this modification in a kappa light chain containing antibody. Detection of these modifications was made by intact mass analysis following de-N-glycosylation, confirmed by non-reduced peptide map analysis and could be localized by tandem MS techniques. The presence of modified cysteine residues did not appear to result in increased levels of partial antibody molecules. A novel method of detecting hinge-related peptides using tandem MS analysis was developed and found to have great utility for aiding data interpretation. These findings add to the complex story of IgG2 disulfide polymorphisms reported in the literature over the past several years. These data show that modification by low molecular weight thiols can also occur for cysteine residues usually paired in disulfide bonds.
P-209-T

Assessing the Molecular Similarity Between a Candidate Biosimilar and an Innovator Monoclonal Antibody Using an Integrated LC/MS System

Asish Chakraborty; Hongwei Xie; Weibin Chen

Waters Corporation, Milford, MA USA

Biosimilar products are poised to realize its full potential because of the increasing user acceptance. Demonstration of structural comparability between a developing biosimilar product and the innovator product potentially means avoiding the need for extensive clinical trials and shortening the time to bring the product to market. Great efforts are therefore put forth by the biopharmaceutical industry to maximize analytical sciences to fully characterize biopharmaceuticals to show equivalency and structural comparability. In this presentation, we demonstrate the use of a newly-developed and fully integrated biopharmaceutical system, including cutting-edge liquid chromatographic (LC) technologies, accurate mass time-of-flight mass spectrometry (MS) and automated data processing and reporting bioinformatics, for routine characterization of minor differences between a candidate biosimilar and an innovator IgG1 monoclonal antibody (mAb). A two amino acid residue variance in the heavy chain sequence was detected by LC/MS intact protein mass measurement, and located by tryptic peptide mapping with data independent acquisition LC-MS^E. Micro-heterogeneities between the samples caused by N-linked glycosylation and chemical degradation were thoroughly catalogued, measured and compared. The results show that the biopharmaceutical system provides a natural and efficient flow for rapid comparison of molecular similarity between a candidate biosimilar and a commercially available mAb.

P-210-T

A Novel LC-MS System Solution for High Performance Routine Characterization of Protein Biopharmaceutical

Asish Chakraborty; Weibin Chen; Jeff Mazzeo

Waters Corporation, Milford, MA USA

The ability to characterize protein therapeutics throughout the product development cycle is an important requirement for analytical support in the biopharmaceutical industry. Liquid chromatography-mass spectrometry (LC-MS) has played an important role in the ensemble of analytical tools to generate in-depth characterization data for biotherapeutic drugs. All too often in biopharmaceutical characterization, the quest for high-performance MS, when combined with a goal of routine analytical methods, results in compromises in system performance. This poster will present an efficient analytical workflow for protein characterization using LC-MS and SEC-MS. The workflow enables scientists to obtain high-performance characterization in a routine manner without compromises. Results on intact protein analysis and peptide mapping for a humanized monoclonal antibody will be presented. The value of using a logical workflow that combines optimized LC instrumentation, columns, MS, and software for targeted data analysis will also be demonstrated.
A Comparative Study of CE/LIF on Labelled Antibodies Analysis Using Two Running Conditions

Pierre Naccache\textsuperscript{2}; Audrey Boutonnet\textsuperscript{2}; Arnaud Morin\textsuperscript{2}; Francois Couderc\textsuperscript{1}

\textsuperscript{1}Université Paul Sabatier, Toulouse, France; \textsuperscript{2}Picometrics, Toulouse, France

CE-SDS is an important separation technique in biopharmaceutical manufacturing and provides advantages such as improved resolution and the ability for quantification of the compounds of interest, relative to other techniques such as SDS-PAGE and size exclusion chromatography. In the past few years, CE-SDS-LIF has become a popular method for characterization and quantification of MAb’s and dyes such as FITC and TAMRA are widely used for labelling of MAb’s for fluorescence detection.

A commercial kit exists to analyze MAb’s, and a number of different CE methods have been described in the literature (Hunt & Nashabeh, Anal Chem. 1999, 71, 2390-2397; Rodat et al Electrophoresis. 2010, 31, 396-402). In this work, we compared the two methods which mainly differ by the hydrodynamic injection of a water plug followed by the injection of the protein sample diluted in lithium dodecylsulfate.

A Laser Induced Fluorescence (LIF) detector which uses a ball lens to focus the laser beam on the CE capillary was integrated into a Capillary Electrophoresis (CE) system was used to monitor the separation. The analysis of FITC labelled IgG was optimized using a 14 cm effective length capillary and the Rodat method led to an analysis that was ten times more sensitive.

These results were obtained on commercial IgG samples. The integrated LIF detector provides an extremely powerful and convenient tool for antibody analysis for both labelled and native fluorescence analysis of therapeutic monoclonal antibodies in pharmaceutical facilities.

Improved Quantitative Capillary Electrophoresis Sodium Dodecyl Sulfate (cSDS) Method for Quality Control and Stability Monitoring of Monoclonal Antibodies Under Non-Reduced Sample Treatment Conditions

Xiaoying Ji; Simei Shan; Sudhir Burman; John Zhang; Tammy Zingaro; Eric Waltimyer; Erin Wood; Chaomei Lin; Gulnur Elove

Centocor R & D, Inc., Malvern, PA USA

In previous work, we presented the development and validation of a capillary sodium dodecyl sulfate electrophoresis (cSDS) method using the Beckman PA800 with UV detection at 220 nm as part of the quality control release and stability monitoring program of recombinant monoclonal antibodies (X. Ji, WCBP 2008, Validation of a Quantitative Capillary Electrophoresis Sodium Dodecyl Sulfate Method for Quality Control and Stability Monitoring of Monoclonal Antibodies). In that method, the product purity is measured under reduced conditions only, while both reduced and non-reduced conditions are used to measure new impurities. In the current work, a cSDS method under non-reduced sample treatment condition with improved accuracy and precision of purity is described.
It was noticed that in the original non-reducing version of the semi-quantitative method total amount of impurities showed higher variability than expected, due to alkaline pH induced fragmentation caused by sample preparation conditions. Heat and high pH in the presence of SDS used to denature proteins in cSDS can result in disulfide bond breakage and rearrangement, hinge region cleavage, and formation of covalent thioether crosslinks. The optimization of sample buffer pH greatly decreased the alkaline pH induced fragmentation caused by the Tris buffer system at pH 9.0. The implementation of alkylation with N-Ethylmaleimide (NEM) and optimization of the incubation temperature and time minimized the formation of fragments and artifact peaks. In our improved method, a buffer system with 25 mM Bis-tris/citrated at pH 7.0 was chosen as the non-reduced buffer condition. The purity of monoclonal antibodies under non reduced condition was found to increase to approx. 99% similar to the values obtained under reduced condition.

NOTES:
P-213-T

**A New pl Calibration Method in Capillary Isoelectric Focusing (cIEF) that Minimizes the pH Gradient Differences in Different Lots of Carrier Ampholytes (CAs) Used in cIEF**

Jiaqi Wu

*Cell Biosciences, Inc., Toronto, Canada*

Free solution cIEF relies on carrier ampholytes (CAs) to create a pH gradient within the separation column. The determined pl values of a protein may vary depending on the pH gradient within the column and the pl markers used to calibrate the gradient. At Convergent Bioscience, we have over 15 years of experience in using commercial CAs in cIEF. One of the problems of the commercial CAs we observed is the lot to lot variability in the pH gradients. The determined pl value of a protein can have up to ±0.15 pl STD in different lots of CAs when all other conditions are identical. Using multiple pl markers in the pl calibration may minimize the difference. However, the biggest difficulty of using multiple pl markers is the distortions to the pH gradient by salts, as they squeeze the pH gradient created by the CAs. The absolute peak positions of the pl markers shift in the e-grams of different samples with different salt concentration even when other conditions are the same. This shift makes it is impossible to use an external multiple pl marker in the sample pl calibration.

We propose a way of two steps, 5 pl markers, to minimize the difference. In the first step, the distortion of salts to the pH gradient of a sample e-gram is corrected using two internal pl markers in the samples. Then, in the second step, the corrected e-gram is calibrated by an external 5 pl markers blank e-gram. In this method, the 5 pl marker blank e-gram can be used for the calibration of all samples with different salt concentrations as long as the CAs are the same lot. Only two internal pl markers are necessary in all the samples in this method.

P-214-T

**Enhance Separation Resolution of Capillary Isoelectric Focusing (cIEF) for mAbs by Using Servalyts Carrier Ampholytes**

Jiaqi Wu

*Cell Biosciences, Inc., Toronto, Canada*

Although isoelectric focusing has the highest resolution in charge based separation methods for proteins, requirements for higher resolution always exist. For mAb analysis, there is almost no method to enhance the resolution other than using narrow pH range carrier ampholytes. We found that Servalyts, a brand name of carrier ampholytes, have much better resolution for some mAbs, especially, for the acidic variants. Servalyts have 5 times higher UV absorption at 280 nm (the detection wavelength for proteins in cIEF) compared to Pharmalytes, another brand name of carrier ampholytes. One way to reduce the background absorption is using low concentration Servalyts. The Servalyts background can also be reduced by using double wavelength methods. In this poster, several examples of using Servalyts to enhance the resolution of mAbs will be discussed.
P-215-T

Improved Characterization and Quality Testing of Monoclonal Antibodies Through Capillary Isoelectric Focusing of Fab and Fc Fragments Obtained by Papain Digestion

Aldo Hoermann; Alena Böhmova; Maria A. Schwarz

Solvias AG, Kaiseraugst, Switzerland

Capillary isoelectric focusing (cIEF) is commonly used to show charge heterogeneity of intact monoclonal antibodies (mAbs). Usually between 3-6 isoforms are visible. Fab and Fc fragments of monoclonal antibodies are readily obtained by papain digestion. While ion exchange chromatography is usually used in the investigation of charge-related heterogeneity of Fab and Fc fragments, only very limited data using cIEF is available. In this paper we will present data of various commercial and development mAbs to demonstrate the use of cIEF following papain digestion. Two well separated peak families due to Fab and Fc are readily observed. In all investigated mAbs, additional variants were visible that were masked using conventional cIEF with no digestion. The results show the potential of cIEF with papain digestion as a general tool for quality control and characterization of mAbs.

P-216-T

Understand Commercial Ampholytes Used in Capillary Isoelectric Focusing (cIEF)

Jiaqi Wu

Cell Biosciences, Inc., Toronto, Canada

Whole-column detection cIEF is a powerful tool to study the pH gradient of carrier ampholytes in a cIEF separation column because it directly records the sample zone positions within the column instead of, like conventional cIEF, recording retention times of the sample zones. In this poster, the whole-column detection cIEF instrument, iCE280 IEF Analyzer, is used to characterize the linearity of different brand name carrier ampholytes and background noise of different carrier ampholytes.

P-217-T

Platform cIEF Method for Monoclonal Antibody Charge Heterogeneity Analysis

Scott Mack; Ingrid D. Cruzado-Park; Mark Lies; Chitra K. Ratnayake

Discovery Products, Beckman Coulter, Inc., Brea, CA USA

There has been a progressive adoption of charge heterogeneity analysis of mAb’s by cIEF within the Biopharmaceutical Industry. The determination of pI adds a critical dimension to establishing identity, purity, post-translational modification and stability of therapeutic MAb preparations. The charge isoforms of many MAbs are basic in nature with pI points in the pH 7 to 10 range. CIEF separations of these basic compounds present a particular challenge due to the less than ideal nature of CA’s that comprise the basic region and time dependent decay of the pH gradient by isotachophoreis (ITP). The fundamental limitations of cIEF analysis in the basic pH range can be overcome by the incorporation of a cathodic stabilizer in the cIEF sample. The optimization of stabilizer concentration, focusing times and ampholyte concentration was critical for the development of a single universal cIEF method with high
reproducibility in the basic pH range. This poster describes the universal method for cIEF analysis of MAbs and summarizes the results of an inter-day reproducibility study carried out with three unique MAbs during six days using two instruments and two lots of neutral-coated capillaries. The results demonstrate the high reproducibility and robustness of this universal cIEF method.

NOTES:
P-218-T

Analysis of N-Linked Glycans from Recombinant and Human Plasma Derived Coagulation Factor IX Using HILIC LC/FLR/QTof MS

Ying Qing Yu¹; Weibin Chen¹; Anur Kumar²; Cory Sutherland²; Leland Paul²; Jeff Mazzeo¹

¹Waters Corporation, Milford, MA USA; ²CMC ICOS Biologics, Bothell, WA USA

Glycosylation of therapeutic protein drugs is of particular importance because it plays vital roles in the clinical performance of these drugs. In this work, we studied the N-linked glycans from two Coagulation Factor IX biologics that are used for Hemophilia B treatment; one is recombinant (rFIX) and the other one is derived from human plasma (pd-FIX). Both Factor IX proteins are heavily glycosylated. Previous findings on their glycoforms were done using analytical techniques other than mass spectrometry (MS). In this study, we applied Hydrophilic Interaction-Liquid Chromatography (HILIC-LC)/Fluorescent (FLR)/QTof Mass Spectrometry to give a detailed account on the N-linked glycans from both biologics.

Preliminary results showed that more glycoforms were identified from both biologics than previously reported. We also observed that rFIX and the pd-FIX have very different glyco-profiles. Glycans released from the rFIX range from high mannose type to complex core-fucosylated bi-, tri- and tetra-antennary structures, the complex type of glycans showed various degree of sialylation.

N-linked glycans from pd-FIX showed distinct differences. Two sulfated bi-antennary glycans were observed from pd-FIX; no sulfated glycans were found in rFIX. Complex bi-, tri- and tetra-antennary glycans are observed as in fully sialylated forms. The complex type of glycans also exits either with or without fucosylations; unlike the rFIX glycans, which are fully fucosylated at the core.

P-219-T

Obtaining a Constant Product by Applying a Physical Model to an Enzymatic Reaction Step

Janus Krarup

Novo Nordisk A/S, Gentofte, Denmark

The rFVIIa analogue zymogen expressed by the manufacturing cell is converted to an active proteolytic enzyme by cleavage of the peptide bond between Arg152 and Ile153. The activation is autocatalytic and does not require involvement of other proteolytic enzymes. The Arg152-Ile153 bond is by far the preferred substrate for autoproteolysis, but two other sites may be cleaved, however at a much slower rate. Once the activation of the zymogen approaches completion, the unwanted reactions will be dominating. The process is conducted optimally when the activation of the zymogen approaches completion with a minimum of unwanted degradation products generated – If allowed to continue beyond that, degradation products will become a problem. The poster presents how:

- To produce a fully activated rFVIIa analogue molecule containing a minimum of degradation products
- To obtain a consistent and robust output of the activation process step despite a very variable input
- To understand the rFVIIa analogue activation process and gain as much knowledge as possible from the data available
**Characterization and Analysis of γ-Carboxylation and O-Linked Glycans in Long-Lasting Recombinant Factor IX-Fc**

John V. Amari; Qi Lu; Mark Slein; Robert Peters

*Biogen Idec Inc., Waltham, MA USA*

A monomeric recombinant Factor IX-Fc (rFIXFc) fusion molecule, comprising a single Factor IX (FIX) molecule fused to the Fc region of immunoglobulin G1, has demonstrated a 3-fold enhancement in half-life in hemophilia B patients in a phase I/IIa clinical trial compared to published historical data for unconjugated Factor IX (BeneFIX®). rFIXFc is currently in a Phase IIb/III registrational clinical trial.

FIX undergoes a number of post-translational modifications (PTMs), including γ-carboxylation of glutamic acids (Gla), aspartic acid β-hydroxylation, serine phosphorylation, tyrosine sulfation, and N- and O-linked glycosylation. This presentation will describe the analysis of a research lot of rFIXFc with respect to both γ-carboxylation and O-linked glycosylation.

The first 12 glutamic acid residues of mature FIX are potentially γ-carboxylated, and the extent of this modification has a direct impact on biological activity. Three analytical methods were developed to monitor Gla content and included amino acid analysis (total Gla content), peptide mapping by HPLC-MS (Gla distribution) and analytical anion-exchange HPLC (iso-form separation). Biological activity was assessed using the FIX-specific aPTT coagulation assay. These methods were used to characterize an enriched “peak” fraction and a high salt elution “strip” fraction originating from a pseudo-affinity chromatography ion-exchange step, the last column in the 3-column rFIXFc purification.

O-linked glycans located on the EGF domain at serines 53 and 61 of the purified peak fraction were characterized by a Lys-C peptide map using HPLC-MS. O-glycan composition and site identification were determined using CID and ETD MS/MS fragmentation and compared to commercially available products. The rFIXFc molecule was shown to have a population of O-linked glycans that are similar to those of commercially available FIX products.

In summary, a number of analytical methods were developed and utilized to assess post-translational modifications (Gla and O-linked glycans) of rFIXFc.

**New MAbPac Phases for Monoclonal Antibody (MAb) Variant Analysis**

Srinivasa Rao; Yuanxue Hou; Xiaodong Liu; Yury Agroskin; Chris Pohl

*Dionex Corporation, Sunnyvale, CA USA*

MAbs generally exhibit complex heterogeneity including glycosylation, oxidation, phosphorylation, amino terminal modifications, incomplete processing of the C-terminus and asparagine deamidation. These variations in composition could impact their efficacy, stability and safety. Monitoring and reporting of such variations of therapeutic proteins is required by the FDA and other regulatory agencies. Two new MAbPac™ phases were developed to meet these needs.
MAbPac SCX is a newly designed strong cation exchange column for the characterization of heterogeneity of MAb. This is a complimentary addition to the existing ProPac® WCX-10 column providing high resolution and orthogonal selectivity for MAb charge variant analysis. The MAbPac SCX stationary phase is based on nonporous, highly cross-linked styrenic type of polymeric media with a proprietary hydrophilic coating. The sulfonic acid functionality is added through controlled radical polymerization grafting. These particles exhibit a wide range of pH stability with high selectivity and minimal band spreading.

MAbPac SEC-1 is a new size exclusion chromatography (SEC) column specifically developed for characterization of monoclonal antibody (MAb) aggregates, enzyme digested fragments, and other size based separation applications. The MAbPac size exclusion (SEC) column is based on high-purity, spherical, porous (300 Å), 5 μm silica covalently modified with a proprietary diol hydrophilic layer. This stationary phase can handle both high and low salt eluents and mass spectrometry compatible eluents.

The MAbPac column is packed into a non-metallic, biocompatible, PEEK™ column housing to eliminate metal contamination from the column hardware that can compromise MAb separations. The stationary phase is designed to minimize undesired non-specific interactions between proteins and the stationary phase.

Various applications with relevant comparisons along with demonstration of the ruggedness of these new phases are discussed.

NOTES:
**P-221-T**

**Evaluation of the Trinean Dropsense96 Platform for Protein Concentration Analysis**

Wali Malik, Travis Linkous, Dawn Kernaghan, James Mcgivney, Robert Strouse, Michael Washabaugh, Mark Schenerman

*MedImmune, Gaithersburg, MD USA*

The protein concentration in test samples is necessary for subsequent analyses such as potency methods, SEC, and SDS-PAGE. This poster describes the evaluation of the Trinean DropSense96, a high-throughput, microfluidic chip-based UV/Vis platform. A protein concentration determination method using the Trinean was evaluated for linearity, reproducibility, precision, and accuracy for thirteen different monoclonal antibodies. The method linearity was 0.1 - 75 OD units with a coefficient of variation (CV) of replicate samples of ≤ 4.0 % within the linear range. The difference between the Trinean platform and a cuvette-based protein concentration determination method is ≤ 9.5 %. The wide dynamic linear range of the Trinean system required little or no sample dilution prior to analysis, and provided reproducible and accurate results compared to the cuvette method. The Trinean platform serves as an alternative to traditional protein concentration determination methods.

**P-222-T**

**Effect of Membranes on Reproducibility and Recovery in Asymmetrical Flow FFF**

Soheyl Tadjiki; Trevor Havard

*Postnova Analytics, Salt Lake City, UT USA*

Automated asymmetrical Flow FFF has been emerging as a reliable analytical characterization tool for proteins and pharmaceutical products. There have been new studies conducted by the industries on the validation of the separation methodology to explore the possibility of using the technique as a quality control instrument. Run conditions such as channel flow rate, cross flow rate, and focusing time are the parameters that constitute a separation method in the asymmetrical Flow FFF analysis. These parameters may vary from one method to another based on the sample size range and polydispersity. Also, system reproducibility and recovery are crucial to validate a particular method. These values are mostly dependent on the type of the membrane used in the channel.

This presentation demonstrates a general experimental procedure to measure sample recovery in Asymmetrical Flow FFF. Sample reproducibility and recovery are tested using regenerated cellulose, regenerated cellulose-amphiphilic and poly ethyl sulfone membranes for a mixture of protein standards. Relative standard deviation (RSD) of the retention time was measured as less than 0.5% for all of the membrane types. However, the regenerated cellulose-amphiphilic membrane demonstrated the highest recovery and peak area reproducibility.
A New High Throughput Micro-Chromatography Platform for Quantitative Analytical Protein Sample Preparation

Randy Bolger; Scott Fulton; Steven Murphy; Michael Bovee; Jennifer Reich; Zach Van Den Heuvel

BioSystem Development, Madison, WI USA

Liquid chromatography is a very well-developed general technique that can address many of the sample preparation challenges in protein analysis from complex samples such as cell culture supernatant or serum. Even enzymatic digestion can very efficiently be carried out in a column format. However, it has been a major challenge to develop a multiplexed chromatography system which is simultaneously flexible and quantitative with the capacity to operate at the microliter-scale with high-throughput.

This talk will describe a new micro-chromatography platform based on disposable cartridge devices with 5 µL packed bed contained between two insert-molded bed support filters. The packed bed can contain any media in the 15 – 100 µm particle size range. The cartridges can be operated on a 96-channel, microplate-compatible liquid handler with ultra-low dead volume syringes that provide highly precise positive-displacement flow control (to rates << 1 µL/min) through the cartridges in either direction. For applications that do not require precise flow rate control, the cartridges also can be operated as spin columns with a microplate centrifuge and offer a number of design features which enable highly quantitative, single-pass binding and elution.

Applications to be presented include the use of affinity chromatography media to quantitatively capture and purify target proteins (such as MAbs or other biotherapeutic proteins) for either direct quantitation or further structural analysis by other methods such as mass spectrometry. Cartridges are available with a range of pre-immobilized affinity ligands (such as protein A or VHH binding domain proteins) or with streptavidin to enable simple, rapid immobilization of biotinylated antibodies, antigens or other ligands by the end user. Applications demonstrated include purification and quantitation of MAbs or non-antibody proteins from cell culture and purification of human MAbs from animal serum.

Development of an Automated Method for Antibody Purification and Analysis

Gurmil Gendeh1; Wim Decrop2; Remco Swart2

1Dionex, Sunnyvale, CA USA; 2Dionex Benelux, Amsterdam, The Netherlands

The screening and analysis of monoclonal antibodies can be an extremely time consuming task, especially as the many of the workflows used today require many manual steps. This situation is fully remedied by the technology offered by the MAb Analysis platforms from Dionex - a single platform can screen a large number of MAbs and, in a second step, provide detailed analytical information, including charge variant analysis and aggregate assessment. This unique technology allows drug discovery laboratories to develop MAb therapeutics much more quickly than before, and bring these important new therapeutics to market in a shorter time frame.
P-225-T

Characterization of New Biologic Entities (NBE) by Peptide Mapping, SEC and IEX

Martin Vollmer¹; Srividya Kailasam¹; PM Sundarem²; Sriram Bharathi²

¹Agilent Technologies, Waldbronn, Germany; ²Gangagen, Inc, Bengaluru, India

The physico-chemical characterization of therapeutic proteins is required during all clinical phases of drug development. This is important to ensure drug safety and efficacy. Providing proof of protein drug identity can be effectively demonstrated by several LC-based techniques, like peptide mapping, SEC and IEX. Peptide maps are constructed by proteolytic digests of the therapeutic protein followed by high resolving reversed phase HPLC. The goal of the analysis is to chromatographically resolve and detect all protein fragment and thus to achieve complete sequence coverage. Due to the complexity of the analyzed sample containing tenth to hundredth of different compounds, peptide mapping therefore results in very long and time-consuming analytical runs.

SEC is used in contrast to discriminate monomeric molecular species from dimeric or multimeric which can cause allergic adverse responses. IEX is commonly applied to demonstrate the absence or presence of acidic or basic charge variants of the main component.

In this study we demonstrate the complete characterization of the small therapeutic protein P128 by using the Agilent 1260 Infinity bio-inert LC and corresponding column technology. The bio-inert LC features complete bio-inertness, corrosion resistance and high pH compatibility designed for the separation of biomolecules.

P-226-T

Characterizing the Electrophoretic Mobility and Effective Charge of Heparin by MP-PALS, A Novel New Technique

John Champagne

Wyatt Technology Corporation, Santa Barbara, CA USA

Electrical charge is a fundamental property of all macromolecules. In colloidal suspensions, the formulation stability depends critically on the amount of charge developed at the interfaces between particles and their solvent. For most biomolecules—like proteins—electrostatic interactions exercise a profound influence on their conformation, function, and efficacy. Practically speaking, electrophoretic mobility has been the most popular and widely accepted proxy for the molecular charge and interfacial potential, also known as the zeta potential. However, traditional commercial instruments have hit a wall, trying to characterize proteins and other nanoparticles less than 5 nm. The invention of the new Möbiuz instrument, utilizing Massively Parallel Phase Analysis Light Scattering (MP-PALS), shatters this barrier and extends the measurable sample size down to 1 nm. In this poster, we describe the theory of the new Wyatt Möbiuz instrument utilizing MP-PALS and how it is used to characterize the electrophoretic mobility and effective charge of heparin. The results show how effectively this technique is able to differentiate the charge:mass ratio of several heparin-derived polysaccharide samples.
Characterizing Protein-Protein Interactions by Composition-Gradient Multi-Angle Light Scattering

John Champagne

*Wyatt Technology Corporation, Santa Barbara, CA USA*

Macromolecular interactions influence all phases of biopharmaceutical development from drug discovery and target validation to the characterization of stable drug formulations at therapeutic doses. Composition-gradient multi-angle static light scattering (CG-MALS) is a powerful, label-free technique for quantifying reversible interactions and can be applied to many stages of the drug development process. Analysis of CG-MALS data yields a second virial coefficient ($A_2$) for non-specific interactions as well as equilibrium association constants ($K_a$) and stoichiometry for specific self- and hetero-association. Because assays are performed in solution, complex interactions can be observed without being influenced by tags or surface immobilization. This not only provides for absolute characterization of interaction strength and stoichiometry but also enables study of the effects of solvent composition, pH, and molecular conformation on the interaction of interest. In this poster, we describe several applications of CG-MALS characterizing protein-protein binding and other interactions automated by the Wyatt Calypso® system.

Advantages of Asymmetric Field Flow Fractionation for the Analysis of Tau Protein and High Molecular Weight Dextran Samples

John Champagne

*Wyatt Technology Corporation, Santa Barbara, CA USA*

Multi-angle light scattering (MALS) coupled with traditional size exclusion chromatography (SEC-MALS) can provide a wealth of information including both the absolute molar weight (Mw) and radius of gyration (Rg) of macromolecules in solution. In practice, the use of traditional SEC-MALS does have limits due mainly to the column stationary phase which can include a variety of undesirable effects including: shear degradation for high molar mass (HMW) samples (manifests as lower than expected Mw), physical trapping/entanglement of the larger species/aggregates by the stationary phase (manifests as lower recoveries, and/or system/ghost peaks) or the potential for sample-to-stationary phase adsorption (manifests as tailing sample peaks). With the asymmetric field flow fractionation (AFFF) technique, the sample separation occurs entirely within an empty fluid channel and not a packed column and as a result, the AFFF separation technique offers several advantages compared to and can help to eliminate many of the undesirable effects observed with traditional SEC-MALS separations. This poster discusses the advantages of employing MALS-AFFF as compared to MALS-SEC for two specific examples, tau protein and HMW dextran. Analysis of the tau protein sample by AFFF was able to achieve a higher resolution for both the low molecular weight and the high molecular weight species, this was not achievable using SEC-MALS. Analysis of the HMW dextran demonstrated the reduction of shearing due to the column stationary phase and resulted in a significant increase in the calculated Mw by AFFF-MALS compared to the Mw calculated by SEC-MALS.
Advances in Ultrapерformance Size Exclusion Chromatography for the Analysis of Biomolecules

Paula Hong; Kenneth J. Fountain; Damian Morrison

Waters Corporation, Milford, MA USA

Complete characterization and analysis of biopharmaceuticals includes size exclusion chromatography (SEC) to measure protein aggregates and other size variants. Current silica-based HPLC-SEC methods can be time-consuming and unreliable. In this presentation, we will demonstrate how UltraPerformance Liquid Chromatography (UPLC) can now be used in conjunction with sub-2 µm size-exclusion packing materials for improved chromatographic separations.

While size-exclusion chromatography has traditionally been performed on low pressure instrumentation, the introduction of low dispersion, high pressure instruments and sub-2 µm packing materials allows for improvements in size-based separations. The improvement in throughput, robustness, and column reproducibility for UPLC-SEC separations will be shown for biomolecules including monoclonal antibodies. In addition, the recovery and level of secondary interactions of intact proteins will be determined. The use of mass spectrometry in conjunction with UPLC-SEC to assist in antibody confirmation and identification will also be shown. These studies will demonstrate the improvements in impurity detection and/or faster analysis of biomolecules achievable with UPLC-SEC separations.

Determination of Product Quality by Implementing a High-Throughput 96-Well Plate Format Assay

Jasmine Wang; Bhargavi Vemulapalli; Ratnesh Pandey; Kathy Yu; Bediako Kwadwo; Susanna Bilbulian; Ziping Wei; Patricia Cash

MedImmune, Gaithersburg, MD USA

A high-throughput 96-well plate format assay has been developed for the determination of product quality of monoclonal antibodies and His-tagged proteins in conditioned medium. Using the affinity chemistry employed in traditional affinity chromatography, a plate containing Poros MabCapture A high-performance resin was applied for micro-scale purification with volume from 5 µl to 200 µl in each well of multiple subclasses of IgG; and immobilized metal ion affinity chromatography (IMAC) with Cobalt Tips was utilized for the purification of polyhistidine-tagged proteins. Both resins offer a rapid sample processing with high protein recovery in 20 min. The method enables the rapid monitoring of aggregation, fragmentation, oxidation, deamidation, and other chemical degradation pathways of diverse Mabs and His-tagged proteins during upstream process development. Various buffer compositions and different pH values were evaluated for compatibility with this method to demonstrate robustness.
HPLC Separation of Monoclonal Antibody Size and Charge Variants: Comparison of the Agilent Bio SEC-3 and Bio MAb, NP10 to Competitors’ SEC-5 and 10µm Particle WCX Columns Using the Agilent 1260 Bio-Inert Quaternary LC System

Phu Duong

Agilent Technologies, Wilmington, DE USA

Monitoring the purity and stability of monoclonal antibodies (mAb) during manufacturing, the purification process, formulation and under storage conditions requires highly sensitive analytical techniques. The HPLC separation of mAbs from their impurities can be achieved using a variety of chemistries including size exclusion and ion exchange chromatography. The data presented here will show the use of analytical size exclusion (SEC) and weak cation exchange (WCX) for the separation of molecular size, acidic and basic charge variants of a Chinese Hamster Cell derived mAb. Methods are developed on the Agilent SEC-3µm, Bio MAb, NP 10 (non-porous, 10µm particle) and competitors’ SEC-5µm and 10µm particle WCX columns using the Agilent 1260 Bio-inert LC system. Methods were optimized for all columns taking buffer pH, salt concentrations, gradient selectivity, flow rates and separation times into consideration. Buffer pH scouting for cation exchange columns suggests that pH 5.5 buffer provides the most absorption capacity for cation columns. Comparison data showed that the Agilent Bio MAb NP10 column provided better overall separation and peak resolution than the competitors WCX column in all buffer pH conditions. Comparison data from optimized gradient systems shows that the Agilent Bio MAb, NP10 column has a better separation and resolution for this mAb separation in both 60 and 30 minute run time. The Agilent Bio MAb, NP10 column also generated a more stable baseline. The main mAb peak had less tailing using the Agilent Bio MAb, NP10 column and it provided better resolution of the basic variants. Similarly, significant tailing can be observed for the mAb peak using competitor’s SEC-5µm particle column (both no salt and salt additive experiments) whereas analyses with the Agilent Bio SEC-3µm particle column result in symmetrical peak shape and better resolution. The sensitivity of the 1260 Bio-inert LC system for this application is also presented.

Development and Evaluation of a System for Controlling Mobile Phase pH and Ionic Strength for Chromatographic Separations of Proteins

Thomas Wheat; Daniel Root; Patricia McConville

Waters Corporation, Milford, MA USA

The biological properties of proteins directly reflect their structure in solution. That structure and the differences among the structures of the proteins in the mixture determine how the sample may be analyzed. Adjusting the pH and ionic strength of the solution is the most direct way to maximize the differences among the sample components. While screening combinations of these parameters is recognized as one of the best paths to an optimized chromatographic method, the preparation of a series of buffers for experimental evaluation is time-consuming and error prone. To simplify this process, quaternary HPLC pumps have been used to blend buffer stocks in the same way as routinely practiced with buffer preparation tables. Programming such methods as a percentage flow from each bottle is frequently used. A more straightforward system permitting programming directly in units of pH and salt concentration was developed. In its simplest form, the algorithm is based on the Henderson-Hasselbalch
equation. A second type of calibration was developed to accurately adjust the proportions of buffers across the range of ionic strengths commonly encountered in protein separations. The performance of the system was tested by executing methods and buffer systems typical of protein separations in anion exchange, cation exchange, and size exclusion chromatography. Fractions of the system effluent were collected during these experiments and the pH measured with a pH meter. These tests included pH gradients which proved to be very accurate and predictable because the algorithms recalculate the required proportions at each pump stroke during the separation. A formal validation procedure was developed based on these tests and used to demonstrate equivalent performance across several systems.

NOTES:
P-233-T

Systematic Development of Ion Exchange Separations of Proteins

Daniel Root; Thomas Wheat; Patricia McConnville

Waters Corporation, Milford, MA USA

Proteins are analyzed using a variety of separation techniques that each emphasizes a different chemical or physical property. Ion exchange chromatography is the most generally powerful chromatographic technique for these analyses both because of the inherently high resolving power of IEX and because of the many options for manipulating the selectivity of the separation. While some information for planning methods development can be obtained from isoelectric focusing or prior knowledge of the sample type, a more complete and systematic screening of the separation space is generally useful. The first step is usually the selection of a cation or an anion exchange column. However, many samples can be usefully separated in either mode. It is, therefore, desirable to test both modes and to include both strong and weak exchangers. For each of the columns included in this process, the separation conditions must approximate the optimal. Adjusting the pH and ionic strength of the separation is the most direct way to maximize the differences among the sample components. This optimization requires the selection of the correct buffering ion and the screening of a series of pH conditions. This combination of columns, buffers, and pH values is a labor-intensive and cumbersome process. We describe here a system that reduces the number of preparation steps and automates the execution of the test methods. A battery of column chemistries is rotated with a column selection valve, and the appropriate buffers are matched to the column with a built-in solvent selector valve. The pH is adjusted for each experiment using Auto•Blend Plus™ Technology. The performance of this system approach is tested and demonstrated with a series of protein mixtures that includes monoclonal antibodies of biopharmaceutical interest.

P-234-T

A Novel Approach to Comprehensive Characterization of Human Monoclonal Antibodies by Online SEC-MS

Ashley Eastham; Angie Ziebart; Scott Freeman; Yuling Zhang; Robert Bailey; Lowell J. Brady

Amgen, Inc., Seattle, WA USA

We investigated clipping in a fully human monoclonal antibody using small-particle Ethylene Bridged Hybrid (BEH) size-exclusion chromatography (SEC) coupled on-line to a time-of-flight (TOF) mass spectrometer. Clipped moieties had initially been observed by non-reduced (NR) peptide mapping and confirmed using a combined approach involving several methods, including reduced & alkylated peptide mapping and reduced reverse-phase (RP) by LC-MS. We found that online NR-SEC-MS was able to characterize product heterogeneity related to clipping in a single analysis. We report here our findings from this approach and demonstrate a rapid, straightforward online SEC-MS method capable of providing valuable mass analysis with minimal up-front sample handling. This method demonstrates significant promise for evaluating process changes on product heterogeneity, and the ability to separate components of different size with on-line MS detection.
NOTES:
Characterization of a Novel Combination of Two Anti-EGFR Antibodies Showing Superior Anticancer Efficacy

Andrea Porchia

*Symphogen A/S, Lyngby, Denmark*

The epidermal growth factor receptor (EGFR) is involved in the development and maintenance of the malignant phenotype of several human cancers and is therefore an attractive therapeutic target.

We have developed a product, Sym004, which consists of a mixture two anti-EGFR monoclonal antibodies (mAb) against two distinct nonoverlapping epitopes in the extracellular domain III of the EGFR. Similarly to monoclonal anti-EGFR antibodies presently in clinical use, Sym004 inhibits cancer cells by blocking ligand binding, receptor activation/phosphorylation, and downstream signaling. Importantly, and in contrast to mAbs, Sym004 induces rapid and efficient removal of the EGFR from the cancer cell surface by inducing EGFR internalization and degradation.

Here we present an outline of the manufacturing strategy of the two mAbs, 992 and 1024, which is present in Sym004 as well as examples of the release and characterization assays used for analysis of Sym004.

**NOTES:**