Overview of Biologics Testing and Evaluation: Regulatory Requirements and Expectations

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Definition of Biologics: PHS Act, section 351

“Virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, protein, or analogous product”

“Applicable to prevention, treatment, or cure of a disease or condition of human beings”

Biologics differ from Traditional drugs:

high MW, made with live cells (presenting an inherent and contamination risk), multiple critical process steps, less well characterized, complex heterogeneous mixtures, optimal rather than maximal dose, multiple or even unknown mechanisms of action, often immunogenic

Vaccine – flu, mumps
Monoclonal antibody
Gene therapy vectors

Complex processes for manufacture, product testing and evaluation of safety and efficacy: all which are regulated by government authorities worldwide
Historical Safety Incidents – Highlights from the Biologics Chapter

- 1901: Diphtheria antitoxin contaminated with tetanus from an infected milk horse (led to the Biologics Control Act of 1902)
- 1945: Yellow Fever Vaccine contaminated with Human Hepatitis B from HSA isolated from human blood
- 1955: Poliovirus vaccine made in monkey primary cell lines contaminated with SV40
- 1970+: Human blood and plasma derived products responsible for numerous cases of HIV, Hep A, B, C, vCJK transmission to patients

The advent of producing biologics using Continuous cell culture technology is the single most important reason why there are no cases today.
Regulatory Agencies

- US Food and Drug Administration (FDA)
  - Center for Drugs Evaluation and Research (CDER)
    - Recombinant proteins, monoclonal antibodies, protein hormones
  - Center for Biologics Evaluation and Research (CBER)
    - Specialized biologics - vaccines, gene therapy, cell therapy
- Japan
  - Pharmaceuticals and Medical Device Agency (PMDA)
- EU European Medicines Evaluation Agency (EMEA)
  - Product marketing applications

*Biological Safety Testing is Mandated by Regulatory Agencies Worldwide*
US Regulatory Framework: Three-Tiered System

Statutes (Laws):
- Passed by Congress and signed by the President
  - Food, Drug & Cosmetic Act (FD&C Act)
  - Public Health Service Act (PHS Act)

Regulations (details of the law):
- Written by FDA and approved by the Executive Branch
  - 21 CFR (Code of Federal Regulations)

Guidance (FDA’s interpretation of the Regulations):
- Written and approved within FDA
  - Advice non-binding on FDA or sponsor
BioSafety Testing Categories

Biological Product

- Identity/Product Characterization
- Purity
- Bacteria, fungi – sterility
- Mycoplasma
- Viruses
Biologics Safety Testing: Multiple Laboratory Disciplines

- Sterility
  - Bioburden
  - Mycoplasma
  - Microbial Identity

- Microbiology
  - Electron Microscopy
  - FISH for genetic stability
  - Karyology
  - Histology

- Immunology
  - ELISA
  - Cell Based Assays
  - Electrochemiluminescence
  - Western Blots

- Cell Biology
  - Stability
  - Degradation
  - Identity

- Analytical
  - Potency
  - Efficacy

- Molecular Biology
  - In Vivo Virus Screening
  - In Vitro Virus Screening

- Virology
  - MP-Seq™
  - DNA/RNA Sequencing
  - Pinnacle Q-PCR™
  - Standard PCR

- Biological Safety Testing
  - Biodistribution

- BioReliance® by SAFC

- Biologics Safety Testing
  - Analytical
  - Immunology
  - Microbiology
Generic Biologics Production and Testing Points


CLC = cell line characterization and LRT = lot release testing
**In Vitro Cell Culture Virus Assay**

Sample

Detector cells passaged at day 14

Cell Monolayer

Passaging of cells

28 Days

Uninfected Vero cells

HSV-infected Vero cells
Detection of Adventitious Viruses Using the In Vitro Adventitious Virus Assay

20 Years of BioReliance (US) Testing – Summary

• Cell Lines (MCB, WCB, EPC) - No viruses detected
• For non-CHO cell production - No viruses detected

• For CHO cell production - The following viruses were detected in unprocessed bulk:
  - Reovirus – Two positive studies; attributed to serum*
  - Cache Valley virus – Four positive studies; attributed to serum*
  - Calicivirus – Two positive studies

• Over a twenty year period, BioReliance testing (over 15,000 in vitro viral assays), there were only eight positive studies for adventitious viruses, which represent 0.05% of assays performed

• That means 14,992 negative results reported
Confidence in evaluating result is based on Assay Validation

“Validation of an assay method is the process of establishing by laboratory studies, that the performance characteristics of the method meets the requirements for the intended applications”

A regulatory requirement

- Validation of Compendial Methods <1225>, United States Pharmacopoeia, Volume 35. Effective date: 01-Dec-2012.
Question: Why Validate?

• Regulatory requirement

• Assay validation is part of overall quality assurance program
  – Quality Control of critical reagents/standards
  – Quality Assurance of conduct and evaluation of test

• Validation provides confidence that a result is reliable

• Good science requires well-planned, well executed, well documented assays with meaningful interpretation of data

• Sound Study Design – meaningful system suitability controls

• Ongoing process: risk assessment and maintaining validation
**How much testing is enough? Are we doing the right tests?**

Recent Instances of Viral Contamination of Continuous Cell Culture in Biological Products

<table>
<thead>
<tr>
<th>Virus</th>
<th>Possible Source</th>
<th>Material Tested/Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minute Virus of Mice</td>
<td>Medium/unknown</td>
<td>CHO cells/bulk</td>
</tr>
<tr>
<td>Human Rhinovirus</td>
<td>Unknown</td>
<td>BHK bulk</td>
</tr>
<tr>
<td>Bovine Viral Diarrhea Virus</td>
<td>Bovine serum</td>
<td>Various Cells</td>
</tr>
<tr>
<td>Bovine Polyomavirus</td>
<td>Fetal Bovine serum</td>
<td>Raw Material (FBS)</td>
</tr>
<tr>
<td>Epizootic Hemorrhagic Disease Virus</td>
<td>Bovine Serum</td>
<td>CHO bulk</td>
</tr>
<tr>
<td>Reovirus</td>
<td>Bovine Serum</td>
<td>CHO and BHK cells/bulk</td>
</tr>
<tr>
<td>Nodavirus</td>
<td>Latent infection</td>
<td>Insect cells</td>
</tr>
<tr>
<td>2117 Calicivirus</td>
<td>Unknown</td>
<td>Bulk</td>
</tr>
<tr>
<td>Porcine Circovirus</td>
<td>Trypsin</td>
<td>Final product -vaccine</td>
</tr>
</tbody>
</table>
Challenges Facing Industry and Regulators

- Continuing contamination incidences
- Discovery of new viruses
- Use of new cell substrates
  - New transformed cells for vaccine production, insect cell lines
- New product types
  - Advanced therapies: gene therapy, cell therapy including stem cells, tissue engineering
  - Biosimilar products
- New production processes
- New endpoints for clinical trials
  - Biomarkers, pharmacogenomic analysis

- Development of new, rapid methods to detect contaminants
  - How and when to use, interpreting results
    - PCRs, rapid microbiology methods, immunological assays, Massively Parallel Sequencing
Thank You!

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