Risk Mitigation Strategies for Raw materials used in the Production of Biologics

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Content

• Viral Risk mitigation in Biotech products
  – Regulatory expectations
  – Sources of risk

• Control strategies
  – Testing requirements

• Quality by Design approach
  – Use of new technologies
  – Case studies
  – Virus control strategies for raw materials
Risk of Virus Contamination in Biotech Products

- Risk to patients from direct inoculation
  - Risk from human pathogens
  - Risk from pathogens with potential to cross species barrier
    - >60% of human pathogens are zoonotic. May not be detected by human cell line culture.
    - >175 pathogenic species associated with emerging diseases
- Risk to product availability for patients with life threatening illness
  - Lifesaving products for unmet medical needs
  - Sole source providers
- Risk to other products in the facility
- Risk to product quality
  - Infected/stressed cells:
    - Types and levels of post-translational modifications may be altered by cellular stress/infection
    - Assays must be comprehensive and sensitive to detect variants or impurities that might have clinical consequences

Rosenberg AS 2012
Sources of recent viral contamination

• Virus Contaminants
  – Cache Valley Virus
  – Bluetongue Virus
  – Epizootic Haemorrhagic Disease virus
  – Minute Virus of Mice
  – Calicivirus 2117
  – Porcine circovirus
## Recent Instances of Viral Contamination of Continuous Cell Culture Biological Products

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

- Selecting and testing raw materials (cell lines and media components) for the absence of viruses
- Testing the product at appropriate steps of production for absence of contaminating viruses
- Assessing the capacity of the production processes to clear viruses
Detection of Adventitious Viruses in Unprocessed Bulk using the *in vitro* Viral Screening Assay

15 Years of BioReliance 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

- ~20,000 In Vitro viral assays have been performed. These eight positive studies for adventitious viruses represent 0.04% of assays performed.
Issues with Detection Assays

• You may not find what is truly there…

  – Sensitivity of assay limits detection
    • All assays have a LOD
    • Sample volume limitations
    • Cell lines may not be permissive for some known or novel viruses

  – Interference/ matrix effects
    • Anti-virus antibodies in FCS used in in vitro assays
    • Cytotoxicity of indicator cells
    • Inhibition of PCR assay enzymes

• Adapted from K Brorson, US FDA
Issues with Detection Assays (2)

• You only find what you are looking for….
  – Screening assays are designed to assess known/past pathogens
    • Source animal surveillance and testing of animal materials by suppliers is limited
    • Screening for specific current/past pathogens
      – Requires assumptions about the type and strain of infectious agent, limiting detection to a small number of known pathogens
      – Ignores emerging, novel viruses
    • Cell line panel used in infectivity assays may not be permissive for novel virus

• Routinely update for detection of prevalent infections or use broad based screening methods
  – Vesivirus identified in 2003 as bovine contaminant but found to be widespread in cattle in USA in 2006
  – New bovine viruses identified by new nucleic acid based technologies

• Adapted from K Brorson, US FDA
Quality by Design

• Quality by design (QbD) principles are laid down in ICH Guidance document Q8 (R2) Pharmaceutical development (2009)

• Implicit within QbD is a common strategy designed to ensure that “a product of required quality will be produced consistently. Elements of the control strategy focus on input materials and the design space that affects control of these materials”
A Quality by Design approach

- Approach to raw material quality control involves 3 or 4 specific steps:
  - Understanding the universe of potential contaminants in the raw material e.g. by massively parallel sequencing
  
  - Develop specific, quantitative assay for those viruses, taking account of the statistical limitations of sampling from the raw material pool
  
  - Relating the potential viral load in a given batch of raw materials to inactivating procedures like gamma-irradiation or HTST
  
  - Where no inactivating steps are in place for the raw material adding monitoring assays later in the process to ensure the viruses are eliminated
Technology Options

- **New technologies:**
  - Virochip DNA Array
  - Degenerate PCR: detection by mass spec ID plex or by sequencing
  - Massively parallel (deep) sequencing

Cell Bank

Virus Seed

Bulk Product

- Greater breadth of detection
- Reduced assay time
Massively parallel sequencing

• Massively parallel sequencing (MP-Seq™) or Next generation Sequencing (NGS) is a recent technology enabling:
  – identification of all adventitious viruses, mycoplasma, bacteria and fungi within a cell line
  – characterisation of the transcriptome.

• BioReliance have applied NGS in regulatory submissions with
  – Bioreactor contaminations
  – Characterising new vaccine cell substrates
  – Characterising raw materials (serum, plant peptones and fish protein used in media)
  – Characterising virus seeds
Sanger Cycle Sequencing vs Next Generation Sequencing

**ABI 3730xl DNA Analyzer**
- 96-capillaries simultaneously
- 3840 samples per day

*Source: Cloned material*

**Roche/454 GS FLX Titanium**
- 1 Million reads
- 600 – 800 bp

**Illumina MiSeq**
- 15 Million reads
- 200 bp

**Illumina HiSeq**
- 3 Billion reads
- 200 bp

**Ion Torrent Proton**
- 150 - 600 Million reads
- 250 bp

*Source: Uncloned material*

To detect unknowns: first, do not bias the input library.
NGS for catching a virus in a cell free matrix

- **Substrate**
  - Cell-free fluids
- **Process**
  - Treat with nuclease
  - Concentrate
  - Extract capsid nucleic acids
  - Amplify
- **Characteristics**
  - Low complexity
  - High S/N
- **Detects**
  - All productive infections
  - Herd contaminants

Cell-free Amplicon MP-Seq™
Case Study as example of detection of new viral agents: New parvovirus contaminant

• In the past few years 4 new bovine parvoviruses have been detected using NGS methods.
• BioReliance has discovered a new parvovirus in bovine serum (BAAV-2)
• It is able to infect human cells and cells of other species
  • It can establish latent infections. Therefore cells that have been exposed in the past need to be screened.
• It is a dependovirus (AAV) and is likely to be mobilised by adenovirus and herpesviruses
  • These viruses are not detectable by 9CFR assays
Need to consider past exposure of cells to serum & trypsin for newly described viruses

- **Bovine polyomavirus**
  - Probably zoonotic and belongs to oncogenic family
- **BPV2 & 3**
  - BPV 2 & 3 only described in past few years ago little known about their biology
- **New parvovirus**
  - New parvovirus identified by BioReliance in 2009, evidence of infection of human cells
- **Bovine & porcine hokovirus**
- **Bovine porcine circoviruses**
  - Radioresistant may establish persistent infections
- **Bovine & porcine anelloviruses**
It doesn’t affect me does it, I have an Animal Origin Free (AOF) process

This is where plant peptones come from

Parvoviruses are shed in feces and are amongst the most resistant viruses in the environment!
Quality by Design
Raw Material Screening Programme

GMP Validation of Inactivation Procedures

- HTST
- γ-irradiation

Develop Quantitative Assays

- PCR
- Infectivity

Determine Viral load in pool or Sub-pool

Sub-pool 1
Sub-pool 2

Compare Virus load to validated Inactivation capacity

Pass/Fail
Virus Control Strategy for Raw Materials

• Raw Materials
  • Source and type
  • Remove/replace naturally sourced raw material from cell culture when possible

• Testing of selected high risk raw materials
  • Maintain current knowledge of prevalent and emerging infections of source animals or associated with plants
  • Use the most sensitive tests to screen raw materials from known potential contaminants

• Processing of raw materials
  • Should include a specific inactivation step

• Does virus propagate in the cell line?

• Testing at production stage for virus
  • General screen (e.g. *in vitro* virus assay)
  • Specific assays (e.g. specific PCR assays)
  • Validated inactivation/removal of virus in downstream process
Viral Inactivation

Virus inactivation between media prep & culture steps lowers by several orders of magnitude chances of infection

- Product treatment options
  - HTST
  - Nano-filtration
  - UV-C inactivation
  - Gamma Irradiation (Serum)
## Virus Inactivation by Gamma Irradiation

<table>
<thead>
<tr>
<th>Type of raw material</th>
<th>Typsin powder</th>
<th>Typsin liquid</th>
<th>BSA powder</th>
<th>FBS</th>
<th>FBS</th>
<th>FBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine adenovirus type 3</td>
<td></td>
<td></td>
<td>&gt; 6.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BVDV</td>
<td>&gt; 6.5</td>
<td>&gt; 5.1</td>
<td>6</td>
<td></td>
<td></td>
<td>&gt; 6.7</td>
</tr>
<tr>
<td>Parainfluenza type 3</td>
<td></td>
<td>&gt; 5.0</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBR</td>
<td></td>
<td>&gt; 4.5</td>
<td>5</td>
<td></td>
<td></td>
<td>&gt; 7.8</td>
</tr>
<tr>
<td>Bluetongue virus</td>
<td>&gt; 6.0</td>
<td></td>
<td>&gt; 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine/porcine parvovirus</td>
<td>&gt; 3.0</td>
<td>&gt; 5.3</td>
<td>&gt; 6.0</td>
<td>&gt; 4.9</td>
<td>1</td>
<td>&gt; 7.0</td>
</tr>
<tr>
<td>Reovirus</td>
<td></td>
<td></td>
<td>&gt; 6.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine circovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Pseudorabies virus</td>
<td></td>
<td>&gt; 7.0</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMC</td>
<td></td>
<td></td>
<td>&gt; 5.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRRSV</td>
<td></td>
<td>&gt; 4.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feline leukaemia virus</td>
<td></td>
<td></td>
<td>&gt; 3</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Minute virus of mice</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Canine adenovirus</td>
<td></td>
<td></td>
<td>&gt; 6.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV40</td>
<td></td>
<td></td>
<td>3.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Virus inactivation by UV-C

<table>
<thead>
<tr>
<th>Virus</th>
<th>mJ/cm²</th>
<th>LRF</th>
<th>Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feline calicivirus</td>
<td>30</td>
<td>4</td>
<td>Protein solution</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>21.5 – 40</td>
<td>4</td>
<td>Waste water</td>
</tr>
<tr>
<td>Coxsackievirus</td>
<td>36</td>
<td>4</td>
<td>Serum free media</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>30</td>
<td>4</td>
<td>Factor VIII</td>
</tr>
<tr>
<td>Echovirus</td>
<td>33</td>
<td>4</td>
<td>Serum free media</td>
</tr>
<tr>
<td>SV40</td>
<td>74</td>
<td>4</td>
<td>Alpha 1 proteinase soln</td>
</tr>
<tr>
<td>Reovirus</td>
<td>74</td>
<td>4</td>
<td>Alpha 1 proteinase soln</td>
</tr>
<tr>
<td>EMC</td>
<td>75 – 100</td>
<td>6.5/4.7</td>
<td>Factor VIII soln; plasma</td>
</tr>
<tr>
<td>VSV</td>
<td>100</td>
<td>4.8</td>
<td>Plasma</td>
</tr>
<tr>
<td>Porcine parvovirus</td>
<td>60 – 100</td>
<td>4.8/5/4</td>
<td>Serum, plasma</td>
</tr>
<tr>
<td>Bovine parvovirus</td>
<td>100</td>
<td>8</td>
<td>10% serum</td>
</tr>
<tr>
<td>Canine parvovirus</td>
<td>50</td>
<td>4.5</td>
<td>10% plasma</td>
</tr>
<tr>
<td>MVM</td>
<td>15 - 100</td>
<td>&gt; 6.11 – 6.57</td>
<td>Serum free media*</td>
</tr>
<tr>
<td>FMDV</td>
<td>100</td>
<td>8</td>
<td>10% serum</td>
</tr>
<tr>
<td>IBR</td>
<td>100</td>
<td>6</td>
<td>10% serum</td>
</tr>
<tr>
<td>Adenovirus type 2</td>
<td>100-160</td>
<td>4</td>
<td>Serum free media; water</td>
</tr>
</tbody>
</table>

Wang et al., 2004; *Weaver & Rosenthal, 2010
## Heat Inactivation of Viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>56°C/30 min (serum)</th>
<th>56-60°C/0.25-18h (serum)</th>
<th>HTST (serum) 85°C/0.5 sec</th>
<th>HTST (media) 102°C/10 sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPV</td>
<td>&lt; 1</td>
<td>-</td>
<td>&lt; 1.5</td>
<td>3</td>
</tr>
<tr>
<td>MVM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&gt; 5.2</td>
</tr>
<tr>
<td>BVDV</td>
<td>4.8</td>
<td>6</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>IBR</td>
<td>7.2</td>
<td>6</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Reovirus-3</td>
<td>5.5</td>
<td>4.7</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Parainfluenza-3</td>
<td>5.5</td>
<td>-</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>-</td>
<td>5.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CAV</td>
<td>6.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polio</td>
<td>-</td>
<td>4.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CMV</td>
<td>-</td>
<td>5.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>X-MuLV</td>
<td>-</td>
<td>3.9</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Risk Mitigation for Viruses in Biotech Products

• Evaluate production culture for adventitious agents *early* in production cycle to mitigate product loss and spread to facility
• Incorporate robust viral inactivation steps and virus removing filter in product purification
• Use advanced techniques (e.g. massively parallel sequencing) to assess contamination in raw materials/production culture especially when inactivation is not an option
  – Develop new biomarkers for infected cultures
Quality Risk Management System and Virus Risk

• Quality risk management system as discussed in ICH Q9
  – Used to assess and document risks associated with viral contamination
• Companies should undertake periodic reassessments of the adequacy of risk mitigation afforded by existing viral contamination strategies:
  – Evaluation of production history
  – Viral controls
  – New analytical and production technologies
  – Relevant literature
  – Evolving standards
  – Current surveys of infectious agents potentially prevalent in raw material sources
Changing Regulatory agency expectations

• Adaptation to Changing Paradigms and technologies:
  “Since the most appropriate techniques may change with scientific progress, proposals for alternative techniques when accompanies by adequate supporting data, may be acceptable”
  *ICH Q5a, 1997*

• “All firms need to meet cGMP and we will be inspecting to ensure firms meet contemporary standards for viral clearance”
  *Rick Friedman, Director, Division of Manufacturing and Product Quality, US FDA, CDER*
Conclusions

• Incidences of contamination continue to occur
  – Safety is maintained in industry through the principles of good manufacturing practice and ongoing testing

• Current technologies allow detection of wide range of contaminants
  – Limited by limit of detection of assays, volume tested

• New approaches are required
  – Technologies such as MP-Seq provide ability to detect all contaminants
  – Methodology detects viral genomes not infectious virus

• Infectivity assays not currently available for all new viruses detected by these techniques

• Industry should be aware of the consequences of detecting new viruses in raw materials and cell lines used for production
  – Ongoing debate related to when and how should these new technologies be used

• As a regulated industry we need to respond to the results generated by this new technology
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