Novel Virus Clearance and Virus Detection Technologies and Applications

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Three Pillars of Product Virus Safety – ICH Q5A

CHO cell derived therapeutic protein products have not been implicated in virus transmission

• Virus clearance
  – Eco friendly detergent
  – Small virus retentive filtration
  – CHO RVLP removal

• Virus risk of CHO cell lines
  – CHO Retrovirus-like Particles (RVLP) non-infectivity

• Recent industry trend
  – NGS for virus detection
  – Virus clearance validation for continuous processing

• Proposal of ICH Q5A revision
Triton CG-110 – Genentech/Roche Non-ionic Eco-friendly Detergent

- Alkyl polyglucoside
- Non-ionic detergent
- Natural ingredient from corn and coconut
- Used in baby shampoo and other consumer products
- Readily biodegradable

- LDAO – Biogen Eco Friendly Zwitterionic (Amphoteric) Detergent

Conley, L. et al., Biotechnol and Bioeng 2017 114(4):813-820

<table>
<thead>
<tr>
<th>Triton CG-110 Concentration (%)</th>
<th>LRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>0.10</td>
<td>1.1</td>
</tr>
<tr>
<td>0.15</td>
<td>2.3</td>
</tr>
<tr>
<td>0.20</td>
<td>3.6</td>
</tr>
<tr>
<td>0.25</td>
<td>5.8</td>
</tr>
<tr>
<td>0.30</td>
<td>≥ 5.4</td>
</tr>
<tr>
<td>0.35</td>
<td>≥ 5.4</td>
</tr>
<tr>
<td>0.40</td>
<td>5.8</td>
</tr>
</tbody>
</table>

X-MuLV inactivation in HCCF at 12°C 1 hour

Inactivation of other enveloped viruses HCCF, 0.3%, 1 hour, 20°C

<table>
<thead>
<tr>
<th>Virus</th>
<th>Time (min)</th>
<th>Triton X-100 Run 1</th>
<th>Triton X-100 Run 2</th>
<th>Triton CG 110 Run 1</th>
<th>Triton CG 110 Run 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRV</td>
<td>30</td>
<td>&gt; 5.8</td>
<td>&gt; 5.3</td>
<td>5.7</td>
<td>&gt; 6.3</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>&gt; 5.8</td>
<td>&gt; 5.3</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>BVDV</td>
<td>30</td>
<td>5.4</td>
<td>5.5</td>
<td>&gt; 6.4</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>&gt; 5.4</td>
<td>&gt; 5.5</td>
<td>&gt; 6.4</td>
<td>&gt; 6.5</td>
</tr>
</tbody>
</table>

- Effective inactivation of enveloped viruses
- Comparable inactivation by Triton X-100 and Triton CG-110
• Parvovirus passage has been reported in select virus filters post de-pressurization or reduced flow conditions
  – Virus diffusion out of pore in absence of flow
  – Low pressure (low flow) has been correlated with reduced LRV
  – Virus passage may be filter dependent
    • Dishari et al., 2015; LaCasse et al., 2016; and Yamamoto et al., 2014

LaCasse and Brorson lab, Biotechnol. Prog., 2016
MMV Worst Case Run for Filter 1

- Worst case conditions (high and low flux, pause, high mass throughput)
- Constant flux mode
- Stepwise reduction of flux:
  - 300 L/m²h (LMH) → 140 LMH → 20 LMH
  - Pause → Buffer chase at 140 LMH
- 2x volume and mass throughput
- MMV LRVs ≥ 6.40, ≥ 6.28 by plaque assay
- All studies performed with 1% MMV spike
- High flux/high pressure followed by low flux/pressure and 1 hr process pause
- Fractionate load pre and post process pause
- No impact of low flux and pause on MMV removal

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Volume Throughput (L/m²)</th>
<th>Mode of Operation</th>
<th>MMV LRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,300</td>
<td>Constant Flux</td>
<td>≥ 6.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>≥ 6.28</td>
</tr>
<tr>
<td>2</td>
<td>2,100</td>
<td>Constant Flux</td>
<td>≥ 7.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>≥ 7.79</td>
</tr>
<tr>
<td>3</td>
<td>2,100</td>
<td>Constant Pressure</td>
<td>≥ 5.62</td>
</tr>
<tr>
<td>4</td>
<td>1,450</td>
<td>Constant Pressure</td>
<td>≥ 6.74</td>
</tr>
</tbody>
</table>
Filter 1 DoE: Volume Throughput, Filter Fouling and MMV Removal

Orange squares represent MMV log reduction factors (LRFs).
Coloured bars represent volume throughput.
Runs terminated due to >90% flow decay are marked with an asterisk.

Results: filter fouling does not impact MMV removal
Parvovirus LRV for larger viruses for BLA

- Evaluation of parvovirus removal at worst case conditions for process understanding
- Applying MMV LRV to larger viruses

### Table

<table>
<thead>
<tr>
<th></th>
<th>MMV LRV</th>
<th>SV40 LRV</th>
<th>X-MuLV LRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vpro LRV</td>
<td>≥ 6.40</td>
<td>≥ 2.91</td>
<td>≥ 3.24</td>
</tr>
<tr>
<td></td>
<td>≥ 6.28</td>
<td>≥ 2.94</td>
<td>2.94</td>
</tr>
<tr>
<td>Pre-filter Loss</td>
<td>0.35</td>
<td>1.68</td>
<td>3.21</td>
</tr>
</tbody>
</table>

- In this study, X-MuLV and SV40 LRVs were under-estimated for virus filter capacity due to significant pre-filtration loss
Both Genentech and industry experience showed that virus clearance LRVs are maintained after extended resin reuse with proper cleaning.

Virus clearance by used protein A resin can be supported by platform data.

High Titer RVLP Stock for Spike Studies

### Sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lot 1</th>
<th>Lot 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein A Flowthrough</td>
<td>9.1</td>
<td>8.3</td>
</tr>
<tr>
<td>$\log_{10}(\text{particles/mL})$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVLP stock</td>
<td>11.6</td>
<td>10.7</td>
</tr>
<tr>
<td>$\log_{10}(\text{particles/mL})$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>62%</td>
<td>93%</td>
</tr>
<tr>
<td>LRV by 0.22μm filter</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>LRV by NFR</td>
<td>6.2</td>
<td>5.4</td>
</tr>
</tbody>
</table>

High RVLP stock titer
- QPCR: $10.7 \log_{10}$ particles/mL
- TEM: $10.7 \log_{10}$ particles/mL
- Q-PERT: $10.7 \text{nU/mL}$ (1 nU $\approx$ 1 pc, Collaboration with Scott Lute and Kurt Brorson)

Low RVLP aggregation & Particles integrity demonstrated
- TEM morphology
- NFR removal near QPCR assay LOQ

Comparable chromatography removal of RVLP and X-MuLV

<table>
<thead>
<tr>
<th>Sample</th>
<th>RVLP</th>
<th>X-MuLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>QSFF F/T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Load (total copies)</td>
<td>11.0</td>
<td>10.6</td>
</tr>
<tr>
<td>Pool (total copies)</td>
<td>≤4.6</td>
<td>4.8</td>
</tr>
<tr>
<td>LRV</td>
<td>≥6.4</td>
<td>5.8</td>
</tr>
</tbody>
</table>

| QSFF B/E     |      |        |
| Load (total copies) | 11.0 | 10.7   |
| Pool (total copies) | ≤4.5 | ≤4.6   |
| LRV          | ≥6.5 | ≥6.1   |

**CHO RVLP Stock TEM**

3.4 nm

0.34 nm

Hydrogen

Phosphate backbone

CHO RVLP Stock TEM
RVLP and X-MuLV Removal by Chromatography

- Use real particles of concern instead of model retrovirus
- Experiments can be performed in regular process development labs
- Overall comparable RVLP and X-MuLV removal

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Resin Type</th>
<th>RVLP LRV (QPCR)</th>
<th>Experiment Type</th>
<th>XMuLV LRV (TCID$_{50}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Multimodal AEX</td>
<td>1.99* 4.48</td>
<td>Process optimization</td>
<td>1.02* 5.06</td>
</tr>
<tr>
<td>B</td>
<td>Multimodal AEX</td>
<td>1.24* ≥3.31</td>
<td>Process optimization</td>
<td>- ≥5.78</td>
</tr>
<tr>
<td></td>
<td>AEX</td>
<td>1.08* ≥5.54</td>
<td></td>
<td>0.56* ≥6.42</td>
</tr>
<tr>
<td>C</td>
<td>CEX</td>
<td>4.13</td>
<td>Pre-validation/confirmation</td>
<td>≥5.72</td>
</tr>
<tr>
<td>D</td>
<td>Multimodal AEX</td>
<td>4.55</td>
<td>Pre-validation/confirmation</td>
<td>≥6.13</td>
</tr>
</tbody>
</table>

CEX: cation exchange; AEX: anion exchange
*LRVs before and after process optimization

Data by Roche Penzberg Downstream Purification

CHO RVLP spike is an alternative to X-MuLV spike studies
Virus Clearance Platform for mAbs and non-mAbs

- 3 dedicated, robust virus clearance steps
  - Operate within robust parameter ranges
  - Modular validation
  - Decreased reliance on chromatography steps

- Clinical purification process

<table>
<thead>
<tr>
<th>Dedicated Step</th>
<th>Modular X-MuLV LRV</th>
<th>Modular MMV LRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detergent</td>
<td>6.6</td>
<td>--</td>
</tr>
<tr>
<td>Low pH</td>
<td>7.1</td>
<td>--</td>
</tr>
<tr>
<td>Virus Filtration</td>
<td>5.7 ← 5.7</td>
<td></td>
</tr>
<tr>
<td>XMuLV LRV</td>
<td>19.4</td>
<td></td>
</tr>
</tbody>
</table>
Molecular Characterization of CHO RVLPs

• A full length CHO RVLP provirus sequence
  – Multiple nucleotide substitutions, insertions, and deletions disrupt both the gag-pol and env open reading frames
  – Can not produce type C particles from this locus
    – YS Lie et al, 1994

• cDNA clones from CHO RVLP
  – No functional endonuclease due to multiple interruptions of ORF
    – K Anderson et al, 1991

• Chinese hamster genome sequencing
  – Protein BLAST identified 403 hamster proteins with homology to retrovirus protein domains.
    – RNA-seq detected minimal env transcripts
      – NE Lewis et al, 2013
Industry Experience: RVLP Infectivity Never Observed

- **Industry experience**
  - No single case of CHO RVLP infectivity observed
  - 185 manufacturer cell banks of mouse, rat or hamster origin were tested, infectious retrovirus was not detected in cell lines of hamster or rat origin
    - *AJ Shepherd et al, 2003*

- **Genentech experience with CHO cell lines**
  - Testing the presence of infectious retroviruses
    - Cocultivation with indicator cell lines (of multiple species) with S+L- Focus assay and Reverse Transcriptase assay as endpoints
    - Extended S+L- Focus Assay
    - ~30 years of virus testing history
    - >90 cell lines tested
    - >50 molecules tested

CHO RVLP infectivity has never been observed
### Virus Clearance Target

<table>
<thead>
<tr>
<th>Virus in starting material</th>
<th>CHO RVLP</th>
<th>NS0 Retrovirus</th>
<th>Plasma-Derived</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^4-9 vp/mL</td>
<td>Up to 10^{10} vp/mL (~10^{1-2} ffu/mL)</td>
<td>≤ LOQ</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infectious virus</th>
<th>No</th>
<th>Yes</th>
<th>HIV, HCV, HBV, HAV, B19, ...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus pathogenesis</td>
<td>No</td>
<td>Unknown</td>
<td>Yes</td>
</tr>
<tr>
<td>Virus transmission history</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Virus clearance target</td>
<td>≤ 1 vp/10^6 doses (ICH Q5A Appendix 5)</td>
<td>≤ 1 vp/10^6 doses (ICH Q5A Appendix 5)</td>
<td>≤ 1 vp/10^6 doses (EMA/CHMP/BWP/706271/2010)</td>
</tr>
</tbody>
</table>

- ICH Q5A states that each of the three overlapping approaches for product virus safety (cell bank testing, in process testing and virus clearance) is to reduce risks of INFECTIOUS viruses.
- Q5A also acknowledges that CHO RVLPs are well characterized and non-infectious.
- Yet, same virus clearance target applies for products with distinctly different virus risk profiles.
- CHO retrovirus clearance target could be reduced due to its non-infectivity.
Why Next Generation Sequencing?

- Current assays (*in vivo, in vitro*, virus specific PCR assays) have been generally effective for demonstrating the absence of adventitious viruses in biological products.
- Emergence of advanced nucleic acid based technologies such as NGS for broad virus detection
  - Contamination of rotavirus vaccine by Porcine Circovirus 1 (PCV1) detected by NGS (2010)
  - Detection of a novel rhabdovirus in the Sf9 insect cell line (2014)
  - Higher sensitivity and speed than *in vivo* tests
  - Broad virus detection
  - Poor detection of viruses *in vivo* assays are expected to detect – Gombold et al., Vaccine, 32:2916-2926, 2014
- EU directive on the protection of animals used for scientific purposes (2010)
  - Replacement, Reduction and Refinement of animal testing (3Rs)
Progress So Far

- NGS application in adventitious virus testing
  - 1 assay for all tests, eg. cell banks, virus seed lots, bulk harvests

- Advanced Virus Detection Technologies Interest Group (AVDTIG), an FDA/industry group
  - Subgroup A: sample selection/preparation/processing
  - Subgroup B: Virus standards and reference materials – 5 reference virus stocks to evaluation of HTS platforms
  - Subgroup C: Development of a complete and correctly annotated, publicly available virus reference database -- RVDB
  - Subgroup D: Bioinformatics pipelines analysis
  - Subgroup E: follow up strategies to confirm the identity of a “hit”

- Multiple studies to demonstrate NGS capability to detect viruses
  - Detection of spiked virus panels

- Challenges
  - Assay standardization and validation, as the methods are complex and evolving
  - Bioinformatics expertise
  - False positives
  - Consistent global regulatory guidance
5.2.3. Cell substrates for the production of vaccines for human use

• Novel, sensitive molecular techniques with broad detection capabilities are available, including Massive Parallel Sequencing (MPS) methods, degenerate PCR for whole virus families or random-priming methods (associated or not with sequencing), hybridisation to oligonucleotide arrays and mass spectrometry.

• These methods may be used either as an alternative to in vivo or specific NAT tests or as a supplement/alternative to in vitro culture tests, in agreement with the competent authority.

2.6.16. Tests for extraneous agents in viral vaccines for human use

• Introduction of molecular biology methods for specific extraneous agents

• Introduction of broad molecular methods (such as high throughput sequencing) for broad detection of all viruses

5.2.14. Substitution of in vivo method(s) by in vitro method(s) for the quality control of vaccines

• Detection of viral extraneous agents by novel molecular methods

• Novel, sensitive molecular techniques with broad detection capabilities are available, including deep sequencing or high throughput sequencing methods.

• The use of these new broad molecular methods has highlighted the gaps with the existing testing strategy by identifying previously undetected viral contaminants in final product, the cell banks from which it was produced and intermediate manufacturing stages.

• High throughput sequencing detects genomes while the existing in vivo methods are based on observations of the effects viruses have on experimental animals.

• The implementation of such new broad molecular methods as substitutes for in vivo methods requires a comparison of the specificity (breadth of detection) and the sensitivity of the new and existing methods. For this purpose, an appropriate panel of representative well-characterised model viruses should be used to assess the ability of the new method to detect viruses that are (or are not) detected by the in vivo methods and, to determine if the sensitivity is at least equivalent to the sensitivity of the in vivo methods.

The rapid progress made in adventitious virus detection by NGS may also enable its application for virus testing for cell line derived products and other novel biologics modalities.
Continuous Processing

- Process Intensification has shown the potential for significant improvement in manufacturing.
- Implementation for biopharmaceuticals production will lead to reduced equipment size and higher productivity.
- Various technology options exist or are in-development to enable continuous processing or connected processing.
- How to ensure virus clearance?

Konstantinov 2015
Virus Inactivation

• Continuous virus inactivation by low pH
  – In-line adjustment of pH
  – System designed to ensure incubation time and mixing
• Batch virus inactivation has been shown to have comparable results to continuous

Johnson, 2017

Gillespie 2018
Virus Filtration

- Virus filtration can be operated as continuous or batch mode
  - Continuous virus filtration with multiple in-line filters
  - Batch post connected chromatography steps
- Mechanism of action will not change for virus retention by filtration, upstream variation could be minimized by surge tanks
- Integrity of filter must be ensured

Johnson, 2017
Summary and Looking into the Future

• ICH Q5A was published in 1998. CHO cell derived therapeutic protein products have not been implicated in virus transmission

• Tremendous progress has been made in developing effective and robust virus clearance unit operations, platform purification processes, as well as virus assays and virus clearance study approaches

• New technologies such as NGS provide better virus detection tools and may replace *in vivo* and *in vitro* virus testing, while continuous processing require novel approaches to ensure virus clearance capacity and validation

• Advances in technology and industry experience may warrant revision of ICH Q5A
  – CHO retrovirus clearance target could be reduced due to its non-infectivity
  – Parvovirus removal validation applied to larger viruses
  – No protein A used resin virus clearance validation based on prior knowledge
  – NGS to replace *in vivo* virus testing
Genentech Process Virology and Purification Development
Roche Penzberg Downstream Purification
Chugai Downstream Purification