Strategy & Alternatives for Virus Inactivation of Plasma-Derived Therapeutics - Triton X-100

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Merck Ltd. Taiwan
Content

Regulatory Expectations for Plasma-Derived Therapeutics

Multi-Layer Strategy for Virus Safety

- Prevention - Raw Materials and Chemicals
- Removal – Inactivation/ Clearance Technologies
- Triton X-100 alternative project
- Detect – In-Process and Final Product Testing
- BioReliance Testing Services

Summary
Regulatory Expectations for Human Plasma Protein Products
Virus Contamination in Plasma

Plasma Product Contamination

- 1980s: HIV contaminated Coagulation Factor
- 1994: Hepatitis C contaminated IgG
- Other threats more recently... Hepatitis E, West Nile Virus, Ebola, Zika...
Regulatory Expectations for Human Plasma Protein Products

✓ Incorporate **at least 2 orthogonal, effective** virus inactivation/removal steps with **at least 4 log virus reduction in each step** is desirable
✓ Virus Inactivation methods/Virus removing filters

<table>
<thead>
<tr>
<th>Virus Group Choices</th>
<th>Types</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enveloped Viruses</td>
<td>HIV 1</td>
<td>Pestivirus BVDV could be considered a “worst-case” for HCV.</td>
</tr>
<tr>
<td></td>
<td>Model for Hepatitis C</td>
<td></td>
</tr>
<tr>
<td>Enveloped DNA Viruses</td>
<td>Herpes Virus</td>
<td>A validation study should be performed with an appropriate enveloped DNA virus, e.g. a herpesvirus such as pseudorabies.</td>
</tr>
<tr>
<td></td>
<td>Hepatitis B (duck model)</td>
<td></td>
</tr>
<tr>
<td>Non-enveloped Viruses</td>
<td>Hepatitis A</td>
<td>HAV and B19V should be used for validation studies for coagulation factors (History of transmission).</td>
</tr>
<tr>
<td></td>
<td>B19V</td>
<td></td>
</tr>
<tr>
<td>Model viruses for virus reduction</td>
<td>HIV</td>
<td>For small pore size filters designed for removal of small non-enveloped viruses, HIV and BVDV should still be part of the virus panel, but robustness studies may focus on small non enveloped viruses. For medium pore size filters, BVDV is appropriate for robustness studies.</td>
</tr>
<tr>
<td>filtration (nanofiltration)</td>
<td>BVDV</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td></td>
</tr>
</tbody>
</table>
Virus safety: Multi-Layer strategy
A Merck Perspective

Virus Safety: Multi-Layer Strategy

1. Prevent
   Virus Safety of Raw Materials

2. Remove
   Virus Clearance Technologies

3. Detect
   Testing of In-Process and Final Product
The Merck EMPROVE® Concept

a package of benefits:

- Strong process safety and reliability – High quality raw materials with GMP production
- Strong regulatory support – EMPROVE dossier in CTD format allows the customer to save time and money
- Full and permanent access to main documents in our website;
- Top level Quality Control procedures, covering the manufacturing and the supply chain steps.

Quality, Safety and Stability are the key words in the EMPROVE® brand products for blood plasma fractionation
Emprove® Dossier library

Material Qualification Dossier*

- In line with CTD chapter 3 quality (adapted for excipients) [2.3*]
  - General information
  - Manufacture
  - Characterization
  - Control of drug substance
  - Reference standard
  - Materials
  - Container closure system
  - Stability
- Information to begin material qualification

Quality Management Dossier

- Quality Self Assessment [2.6*]
- Audit report summary [2.6*]
- Supply chain Information [2.3*]
- Stability data [2.3*]

- Answers questions during risk assessment

Operational Excellence Dossier

- Product quality report [4.1*]
- ICH Q3D Elemental impurity information
- Analytical procedures
- Supports process optimization

Available only in the Dossier

[*] No. of supported section of Guideline on formalized risk assessment for excipients EU/C 95/210
General Fractionation Process Steps with EMPROVE® Chemicals

- **Stabilizers**
  - EMPROVE®
  - Glycine
  - Sodium Chloride

- **Precipitation**
  - EMPROVE®
  - PEG4000
  - Sodium Capylate

- **Buffers**
  - EMPROVE®
  - Acetates/Phosphates
  - Sodium Hydroxide

- **SD/Low pH**
  - TnBP, Triton® X-100, Tween® 80
  - Acetic Acid, Sodium acetate trihydrate, Sodium acetate anhydrous, Caprylic Acid

- **Bulking Agents/Stabilizers**
  - EMPROVE®
  - Sucrose, Sorbitol, Maltose
  - L-Alanine, Glycine, Sodium Capylate, N-Acetyl-DL-tryptophan

- **Steps**
  - Cryoprecipitation
  - Precipitation
  - Centrifugation/Depth Filtration
  - Dissolution of Fraction II/III paste
  - SD Inactivation/Low pH
  - Lyophilisation
  - Filling
  - Final filtration
  - Formulation
  - Nanofiltration
  - Concentration/Diafiltration
  - Liquid Fill
  - Ion Exchange Chromatography
A Merck Perspective
Virus Safety: Multi-Layer Strategy

1. Prevent
   Virus Safety of Raw Materials

2. Remove
   Virus Clearance Technologies

3. Detect
   Testing of In-Process and Final Product
Types of Process Steps

EMA/CHMP/BWP/706271/2010 Guideline on plasma-derived medicinal products  
“For all plasma-derived medicinal products, it is an objective to incorporate effective steps for inactivation/removal of a wide range of viruses of different physico-chemical characteristics.”

**Inactivation**

- Heat Treatment
  - Pasteurization (60°C, 10 hours, Albumin)
  - Lyophilization / dry heat

- Solvent/Detergent (TnBP, Triton X-100, Tween 80)

- Low/High pH (Caprylic acid, Acetic acid)
  - Column Elution/Sanitization

**Partitioning**

- Precipitation
  - EtOH
  - PEG
  - Caprylic Acid

- Chromatography
  - Ion Exchange
  - Affinity
  - Gel Filtration

- NanoFiltration
# Viral Reduction Capacities by Contributive Steps

<table>
<thead>
<tr>
<th>Spiked viruses</th>
<th>ENVELOPED VIRUSES</th>
<th>NON-ENVELOPED VIRUSES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV-1</td>
<td>Sindbis</td>
</tr>
<tr>
<td>Model for</td>
<td>HIV</td>
<td>HCV</td>
</tr>
<tr>
<td>Specific steps</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S/D treatment</td>
<td>e 4.4</td>
<td>e 5.4</td>
</tr>
<tr>
<td>Nanofiltration through 20 nm filter</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>Contributive steps</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caprylic acid fractionation</td>
<td>e 4.0</td>
<td>NT</td>
</tr>
<tr>
<td>Anion-exchange chromatography</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Final product: LowpH incubation in the final container</td>
<td>4.0</td>
<td>NT</td>
</tr>
<tr>
<td>Final product: Virus-neutralising capacity by the antibodies</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Overall viral reduction capacity</td>
<td>e 18.0</td>
<td>e 11.0</td>
</tr>
</tbody>
</table>

Source: Production of ClairYg, Christophe Segard, LFB
### Efficacy of Solvent Detergent Virus Inactivation Using Model Enveloped Viruses in Mobius® Single-Use Processing Containers

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Virus</th>
<th>Solvent/ Detergent</th>
<th>Replicate</th>
<th>LRV at 6 hr</th>
<th>Time to Inactivation (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>XMuLV</td>
<td>TnBP/ Tween® 80</td>
<td>Mobius® 1</td>
<td>≥6.5</td>
<td>≥300</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mobius® 2</td>
<td>6.8</td>
<td>≤300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TnBP/ Triton® X-100</td>
<td>Mobius® 1</td>
<td>≥5.4</td>
<td>≤5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mobius® 2</td>
<td>≥5.5</td>
<td>≤5</td>
</tr>
<tr>
<td>Plasma</td>
<td>XMuLV</td>
<td>TnBP/ Triton® X-100</td>
<td>Mobius® 1</td>
<td>≥5.4</td>
<td>≤5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mobius® 2</td>
<td>≥5.5</td>
<td>≤30</td>
</tr>
<tr>
<td></td>
<td>BVDV</td>
<td>TnBP/ Tween® 80</td>
<td>Mobius® 1</td>
<td>≥5.6</td>
<td>≤5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mobius® 2</td>
<td>≥5.6</td>
<td>≤5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TnBP/ Triton® X-100</td>
<td>Mobius® 1</td>
<td>≥4.5</td>
<td>≤5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mobius® 2</td>
<td>≥4.5</td>
<td>≤5</td>
</tr>
</tbody>
</table>

*Source: Single-use technology for solvent/detergent virus inactivation of industrial plasma products, T. Burnouf et al, TRANSFUSION Volume 56, 2016*
Solvent detergent for virus inactivation: Triton X-100 alternative project progress
Triton™ X-100 in Bioprocessing

1. WHO recommended detergent for virus inactivation

2. Rapid & robust inactivation kinetics (ASTM®2 E3042-16 > 4 LRV)

3. Easily removed during downstream purification

4. No adverse effects on product recovery & quality

5. Well established & approved viral clearance step

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2. ASTM E3042-16 Standard Practice for Process Step to Inactivate Rodent Retrovirus With Triton® X-100 treatment

**REACH**

**Registration, Evaluation, Authorization and Restriction of Chemicals**
- Authority: European Chemicals Agency
  - Register (≥ 1 metric ton)
  - Evaluate (substance and dossier)
  - Restrict (uses)
  - Authorize (uses): Sunset Date

**Substances of Very High Concern (SVHC)**
- Carcinogenic (c),
- Mutagenic (m)
- Toxic to Reproduction (r)
- PBT (persistent, bioaccumulative, toxic)
- vPvB (very persistent, very bioaccumulative)
- EC (substances of equivalent concern)

Jan 2019
197 chemicals
Triton™ X-100 & Authorization under REACH

Triton™ X-100 degradation product
(4-tert-octylphenol ethoxylate)

“Environmental source” of 4-tert-octylphenol, a SVHC, due to endocrine effects

Dec 2012
Triton™ X-100: Inclusion in Candidate List (SVHC)

June 2017
Triton™ X-100: Inclusion in Annex XIV

June 2019
Triton™ X-100: CSR, SEA filing deadline

Q3 2020
Estimated Info on transition-period granted by ECHA

Jan 4th 2021
Sunset: January 4, 2021

Triton™ X-100 REACH Exemption
➢ R&D applications
➢ Medicinal products manufactured outside the EU
REACH

Application on authorisation of Triton™ X-100

Info on applicant
- Substance ID
- Use

Info on applicant

Analysis of alternatives
- Possibly substitution plan

Socio-economic analysis
- Depending on your strategy you are using, SEA might be the one where effort should be put

SEA

CSR

Chemical Safety Report
- Use information

Some Advise:
- Start early (challenging to perform in 18 months)
- Ensure to apply early before the latest application date
- Use the support of a consultant
Summary Triton X-100 under REACH

- We, as a raw material supplier, plan to submit an application for authorization of the GMP production of Triton™ X-100
- We ensure supply to customers holding an own authorization for uses that are not covered by an exemption (e.g. R&D exemption)
- So far the longest possible transition period granted from REACH was 12 years
- We are in the phase of R&D product development of a detergent candidate that is safe for humans & environment to replace Triton™ X-100
Selecting an alternative to Triton™ X-100 Alternatives

Considerations

- Safe for humans & environment
- No/little impact on product recovery and quality
- Comparable virus inactivation potential
- Easily removed in DSP
- No/low foaming
- No impact on other unit operations
- Sensitive analytics
- GMP manufacturability
- Cost-effective
Experimental Approach

Virus Spike

Infectivity Assay (TCID$_{50}$)

Plasma

Monitor Inactivation Kinetics

Study Conditions:

- Virus: <10% spike
- Temp.: ~22°C, 15°C or 6°C
- Detergent conc. (w/v): 0.5% - 1%
- Sampling times: 5 - 1440 min
Panel of Enveloped Viruses

Pseudorabies Virus (PRV)
- 120-200 nm
- ~140 kb, dsDNA
- Model virus

Murine Leukemia Virus (XMuLV)
- ~80-110 nm
- ~8 kb ssRNA
- Model retrovirus

Bovine Viral Diarrhea Virus (BVDV)
- ~40-70 nm
- ~1 kb, ssRNA
- Model for HCV
Screening in Purified IgG

Screening Conditions
- XMuLV in 10g/L IgG
- 1% (w/v) detergent at 22°C
- 1 and 4 hr incubation

Criteria for further investigation
- Virus inactivation capability
- GMP manufacturability

Several candidates identified, let’s exemplary review candidate K in an evaluation study
Detergent K – Virus Inactivation in Plasma at 22°C

Detergent & solvent combination is more effective than detergent alone for virus inactivation in plasma.
Detergent K – Exemplary Virus Inactivation in Plasma at Worst Case Conditions

Robust performance under ‘worst case’ conditions needs to be evaluated

Worst Case Conditions
- 85% of target [detergent] & [solvent]
- 15°C

Exemplary example using candidate K
Detergent K – Exemplary Detergent Removal
Cation Exchange Chromatography

1% wt/v Detergent K + Phosphate Buffer
9141 µg/mL

Flow-Through Waste
Fractogel® EMD SO₃⁻ Resin
9035 µg/mL

Eluate
Not detectable (<0.1 µg/mL)

➢ CEX: effective for detergent removal

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Triton® X-100 (µg)</th>
<th>Detergent K (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load</td>
<td>8264</td>
<td>9141</td>
</tr>
<tr>
<td>Flow through waste</td>
<td>6973 (84%)</td>
<td>9035 (99%)</td>
</tr>
<tr>
<td>Eluate</td>
<td>ND</td>
<td>&lt;0.1 µg/mL</td>
</tr>
</tbody>
</table>
Detergent K – Foaming

Conditions

- 1% detergent in water in baffled shake flasks
- Vigorous shaking for 5 minutes at 340 rpm

Similar foam heights observed for Detergent K and Triton™ X-100
Where Are We Now?

- **Detergents with virus inactivation potential**
- **No/little impact on product recovery and quality**
- **Easily removed in downstream purification**
- **Sensitive analytics**
  - No/low foaming
  - No impact on other unit operations
- **Safe for humans & environment**
  - GMP manufacturability
  - Cost-effective
Summary – Status of development at Merck

Timelines for launch of new potential Triton™ X-100 alternative

### Potential Alternative Detergent

**History**
- Detergent K stop due to economic reasons

**Now**
- Product Re-Development
- Process Re-Development for GMP manufacturing

**Q2 2019**
- First samples in Q2 2019
- GMP Samples for product qualification Q3 2019

**Q4 2019**
- Official launch of new detergent as a potential substitute product for Triton™ X-100
Virus adsorption using chromatography

**AEX: Flow Through/WP Virus Removal**

- AEX can be a robust and reliable VC unit operation
- Removes enveloped and non-enveloped virus
- Parameters affecting operation should be understood

*Platform AEX Viral Clearance: Robust Removal of Retrovirus and Parvovirus for over 20 mAbs*

- Virus removal tested extensively over the course of numerous projects using the AEX chromatography step operated in weak partitioning mode

**LRVs from 2.5 to >5.5**

- No LRV less than 4 log10

*Timothy Iskra* et al. *PDA Virus Safety 2014*
Virus Removal by Nanofiltration

- Virus Filtration performed on 10 different Grifols plasma proteins/products

- Conclusion of Nanofiltration being extremely effective step in virus reduction

- Minimal influence of process parameters like pH, temperature, Conductivity, during NF

---

<table>
<thead>
<tr>
<th>Protein (or product)</th>
<th>Enveloped viruses (≥40 nm)</th>
<th>Non-enveloped viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRV</td>
<td>HIV-1</td>
</tr>
<tr>
<td>A</td>
<td>≥4.6</td>
<td>≥4.8</td>
</tr>
<tr>
<td>B</td>
<td>≥6.1</td>
<td>≥5.6</td>
</tr>
<tr>
<td>C</td>
<td>≥6.0</td>
<td>≥4.0</td>
</tr>
<tr>
<td>D</td>
<td>≥5.5</td>
<td>≥5.9</td>
</tr>
<tr>
<td>D1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>≥5.2</td>
<td>≥6.9</td>
</tr>
<tr>
<td>E</td>
<td>≥4.2</td>
<td>≥3.8</td>
</tr>
<tr>
<td>E1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>≥5.4</td>
<td>≥5.4</td>
</tr>
<tr>
<td>F</td>
<td>≥6.0</td>
<td>≥6.8</td>
</tr>
<tr>
<td>F1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>n.d.</td>
<td>≥4.5</td>
</tr>
<tr>
<td>G</td>
<td>n.d.</td>
<td>≥6.3</td>
</tr>
</tbody>
</table>

BVDV: bovine viral diarrhea virus; EMCV: murine encephalomyocarditis virus; HAV: hepatitis A virus; HIV: human immunodeficiency virus; n.d.: not determined; PPV: porcine parvovirus; PRV: pseudorabies virus; WNV: West Nile virus.

*Source: Robustness of Nanofiltration for increasing the viral safety margin of biological products
S Caballero et all, Grifols 2014
Virus Safety: Multi-Layer Strategy

**Virus Safety of Raw Materials**

**Remove**

Virus Clearance Technologies

**Detect**

Testing of In-Process and Final Product

- Besides **Viral Clearance and TSE Clearance** studies, **Mycoplasma, DNA and Bacterial Clearance** studies can also be performed.
Conclusions

- Three-Pillar Approach for Total Virus Safety Considerations in Plasma.
- Merck has developed Eco-friendly Triton X-100 alternatives planned to launched soon.
- Merck Key Competencies in Plasma:
Thank You!

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