



strategy & Alternatives for virus inactivation of plasma-derived Therapeutics -Triton X-100

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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

Setting Your Strategy

How can you assure viral safety of your plasma therapeutic?





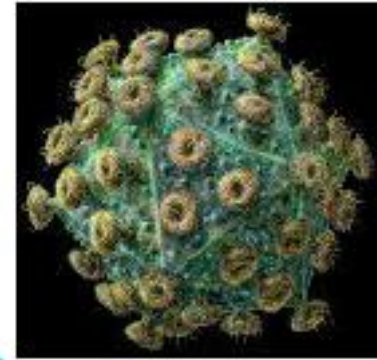
regulatory expectations for human plasma protein products

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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

Virus Group Choices	Types	Details
Enveloped Viruses	HIV 1 Model for Hepatitis C	Pestivirus BVDV could be considered a “worst-case” for HCV.
Enveloped DNA Viruses	Herpes Virus Hepatitis B (duck model)	A validation study should be performed with an appropriate enveloped DNA virus, e.g. a herpesvirus such as pseudorabies.
Non-enveloped Viruses	Hepatitis A B19V	HAV and B19V should be used for validation studies for coagulation factors (History of transmission).
Model viruses for virus reduction filtration (nanofiltration)	HIV BVDV PPV	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.





virus safety: Multi-Layer strategy

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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.



Emprove®: Raw Materials

Our Emprove® bio raw materials for use in biopharmaceutical production offer specifications, as well as a Certificate of Analysis adjusted to your fractionation processes. Selected Emprove® bio products comply with American Chemical Society standards. With Emprove® bio, you benefit from our proven expertise and reputation for uncompromising reliability and safety — so you can be certain that your documentation is comprehensive.

Emprove®: Ready-to-use Documentation

The Emprove® bio dossiers are specifically compiled to support material qualification for a safe biopharmaceutical process. Emprove® bio dossiers contain comprehensive information on manufacturing processes, testing procedures, and purity and are structured in conformance with international standards (Common Technical Document [CTD] or comparable format). Emprove® bio greatly reduces your workload and costs, considerably accelerating your qualification and registration processes.

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**



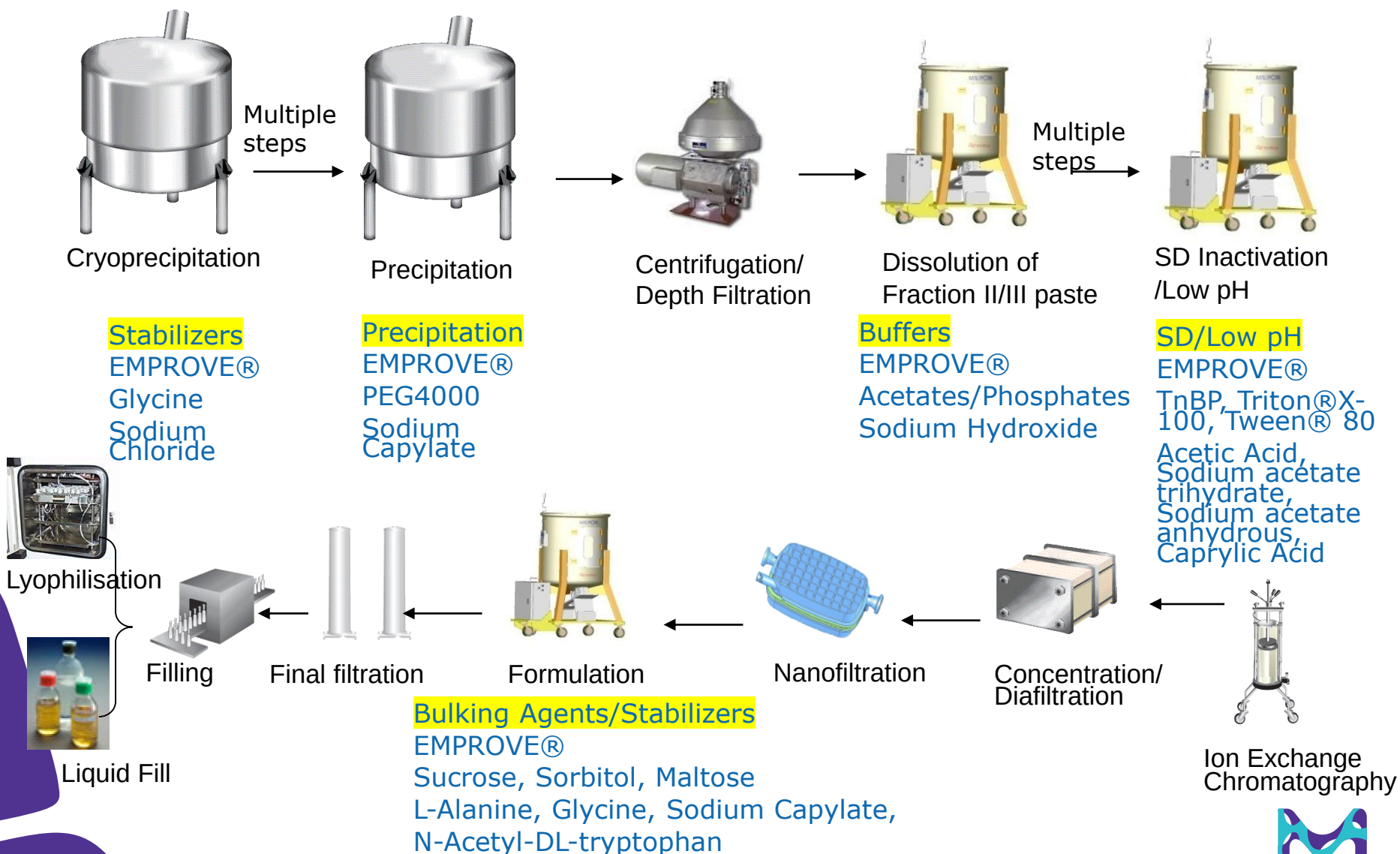
Operational Excellence Dossier

- 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



A Merck Perspective

Virus Safety: Multi-Layer Strategy



Prevent

Virus Safety of Raw Materials



Remove

Virus Clearance Technologies



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

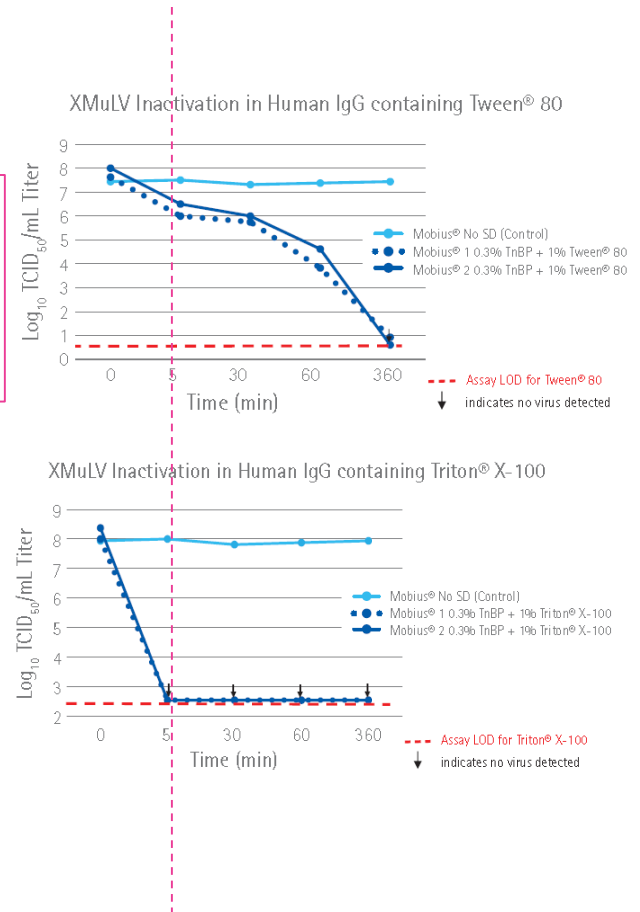
Spiked viruses		ENVELOPED VIRUSES				NON-ENVELOPED VIRUSES			
		HIV-1	Sindbis	BVDV	PRV	SV-40	EMCV	HAV	PPV
Model for		HIV	HCV		Enveloped DNA virus (HBV)	Highly resistant virus	HAV		Parvovirus B19
Specific steps	S/D treatment	e 4.4	e 5.4	NT	e 4.3	N/A	N/A	N/A	N/A
	Nanofiltration through 20 nm filter	5.6 ^⓪	5.6 ^⓪	5.6	5.6 ^⓪	5.4 ^⓪	e 5.2	NT	4.3
Contributive steps	Caprylic acid fractionation	e 4.0	NT	5.1	e 5.0	NT	e 5.6	e 5.6	3.7
	Anion-exchange chromatography	NT	NT	NT	NT	NT	1.3	NT	3.8
	Final product: LowpH incubation in the final container	4.0	NT	3.2	NT	NT	NT	NT	NT
	Final product: Virus-neutralising capacity by the antibodies	N/A	N/A	N/A	N/A	N/A	N/A	3.3 ^⓪	N/A
Overall viral reduction capacity		e 18.0	e 11.0	13.9	e 14.9	5.4	e 12.1	e 8.9	11.8



Efficacy of Solvent Detergent Virus Inactivation Using Model Enveloped Viruses in Mobius® Single-Use Processing Containers

Matrix	Virus	Solvent/ Detergent	Replicate	LRV at 6 hr	Time to Inactivation (min)
IgG	XMuLV	TnBP/ Tween® 80	Mobius® 1	≥6.5	≥360
			Mobius® 2	6.8	≤360
		TnBP/ Triton® X- 100	Mobius® 1	≥5.4	≤5
			Mobius® 2	≥5.5	≤5
Plasma	BVDV	TnBP/ Tween® 80	Mobius® 1	≥5.6	≤5
			Mobius® 2	≥5.6	≤5
		TnBP/ Triton® X- 100	Mobius® 1	≥4.5	≤5
			Mobius® 2	≥4.5	≤5
	XMuLV	TnBP/ Triton® X- 100	Mobius® 1	≥5.4	≤5
			Mobius® 2	≥5.5	≤30
Plasma	BVDV	TnBP/ Triton® X- 100	Mobius® 1	≥4.5	≤5
			Mobius® 2	≥4.5	≤5

Table 3: XMuLV & BVDV Inactivation with 1% (v/v) TnBP/1% (v/v) Triton® X-100 Data in Human Plasma.



*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**

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Triton™ X-100 in Bioprocessing

- 1 WHO recommended detergent for virus inactivation¹**
- 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 step³**

¹World Health Organization TR924 (2004) Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human plasma products

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

³Dichtelmuller, et al (2009) Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion 49: 1931



REACH

Registration, Evaluation, Authorization and Restriction of Chemicals

- European Union Legislation (2007)
- 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

**June
2017**

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

**Dec
2012**

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



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



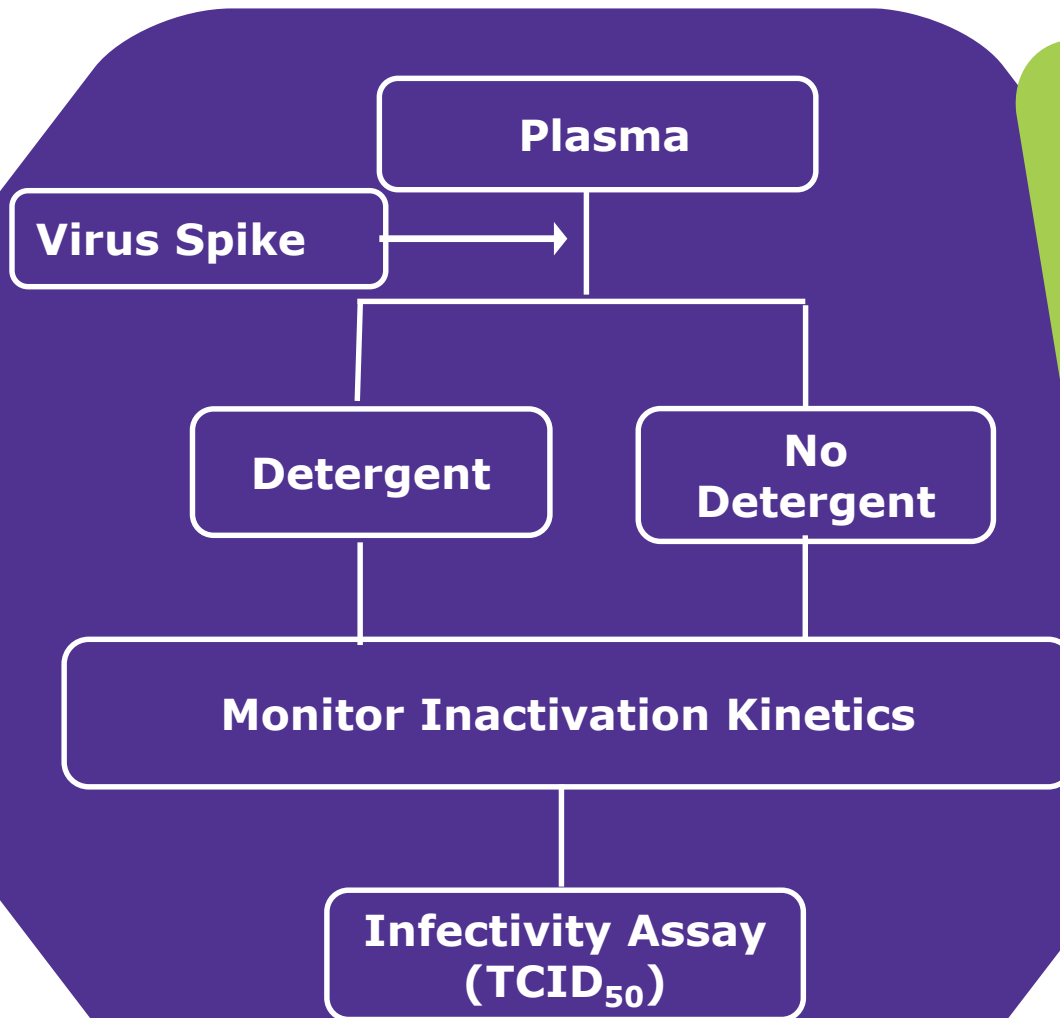
Selecting an alternative to Triton™ X-100 Alternatives

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



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

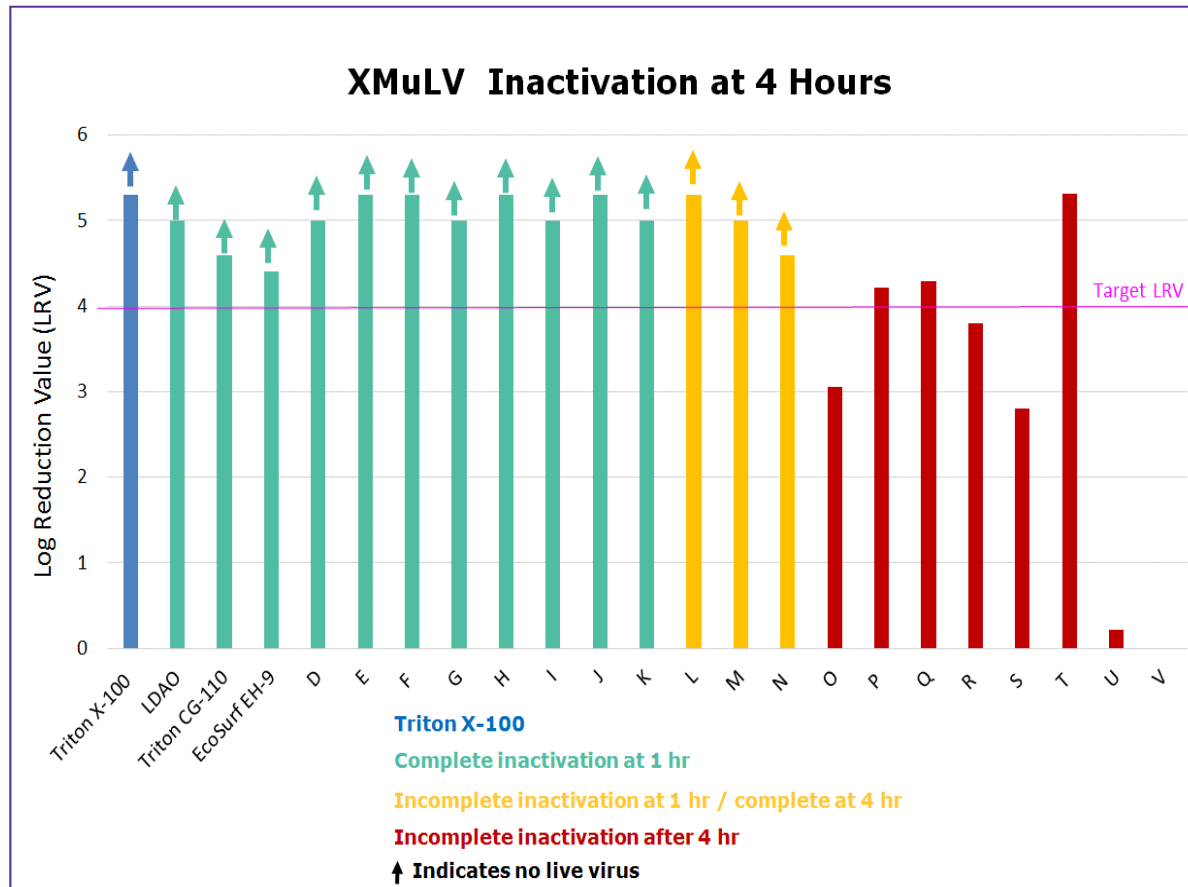
Murine Leukemia Virus (XMuLV)

- ~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

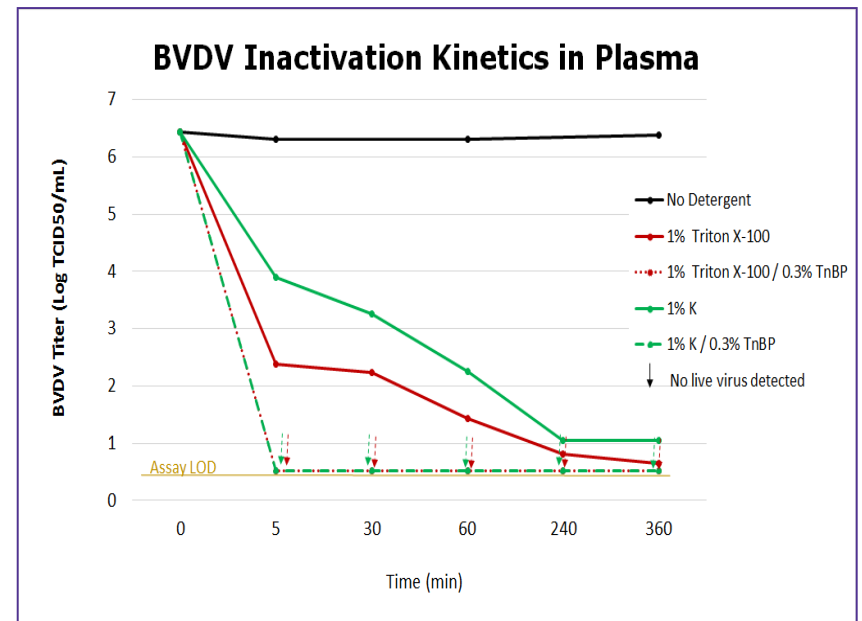
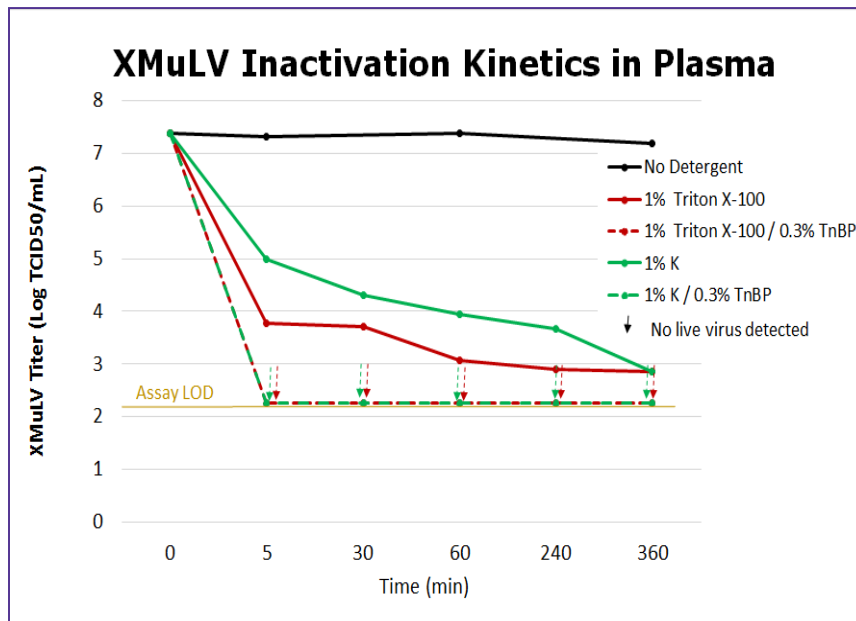
- XMuLV in 10g/L IgG
- 1% (w/v) detergent at 22°C
- 1 and 4 hr incubation

Criteria for further investigation

- Virus inactivation capability
- GMP manufacturability

Several candidates identified, let's exemplary review candidate K in an evaluation study

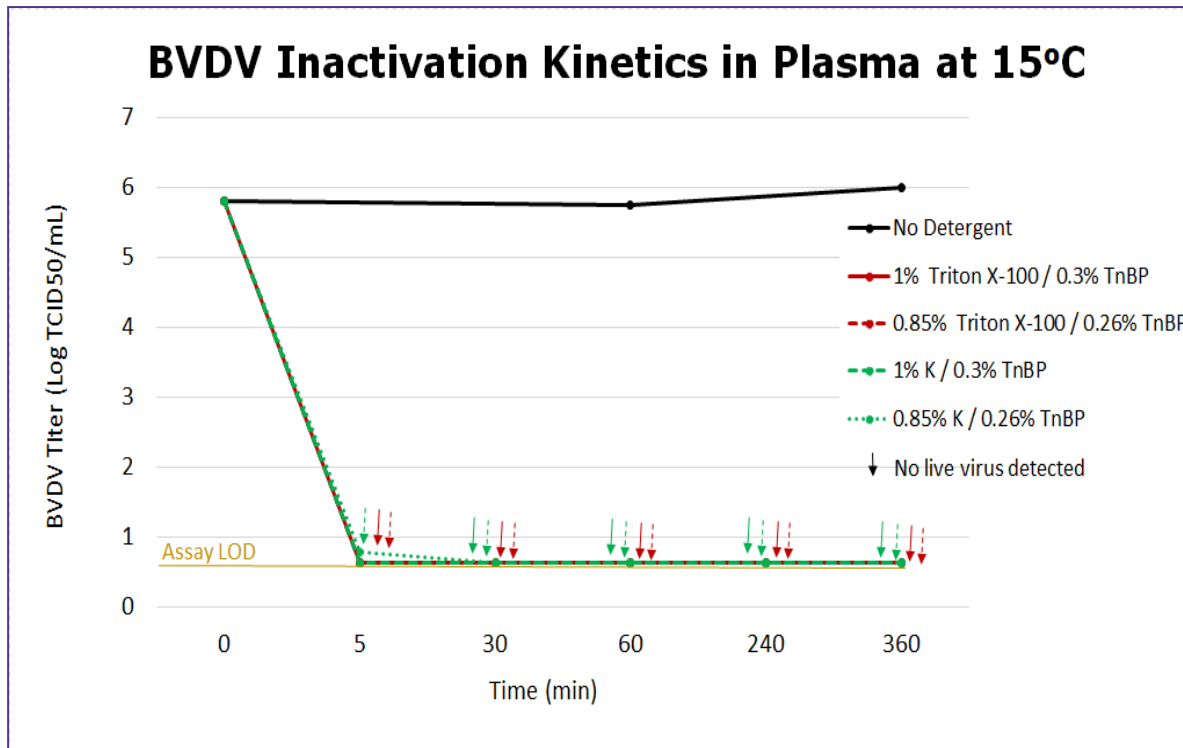
Detergent K – Virus Inactivation in Plasma at 22°C



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



Worst Case Conditions

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

Exemplary example using candidate K

Robust performance under 'worst case' conditions needs to be evaluated



Detergent K – Exemplary Detergent Removal

Cation Exchange Chromatography

1% wt/v Detergent K + Phosphate Buffer

9141 µg/mL



Flow-Through Waste

9035 µg/mL

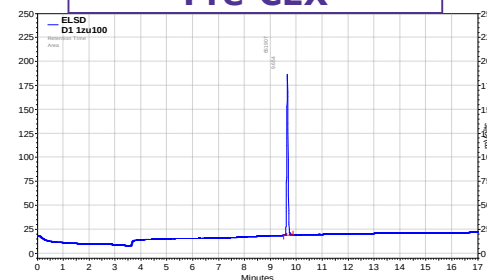
Fractogel® EMD SO₃⁻ Resin

Eluate

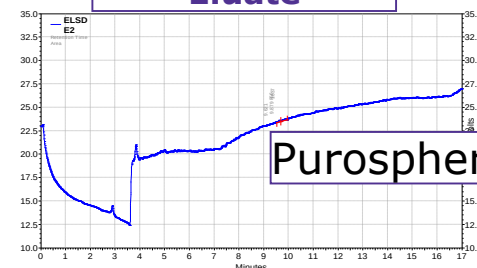
Not detectable (<0.1 µg/mL)

➤ **CEX: effective for detergent removal**

Example Detergent K Spiked Buffer - Pre-CEX



Eluate



CEX chromatography – Fractogel® SO₃⁻ Resin

Fraction	Triton® X-100	Detergent K
Load	8264 µg	9141 µg
Flow through waste	6973 µg (84%)	9035 µg (99%)
Eluate	ND	<0.1 µg/mL



Detergent K – Foaming



Detergent K

Triton™ X-100

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

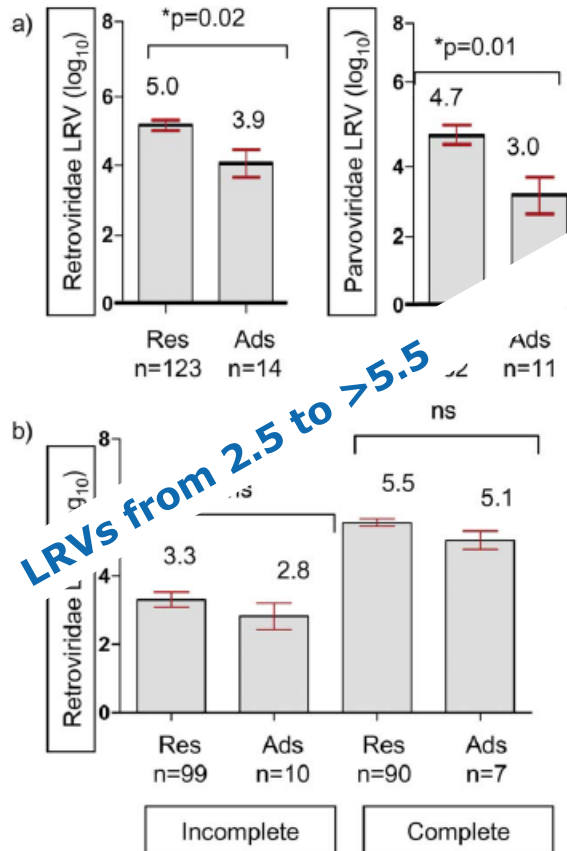
- Official launch of new detergent as a potential substitute product for Triton™ X-100

Virus adsorption using chromatography

AEX: Flow Through/WP Virus Removal

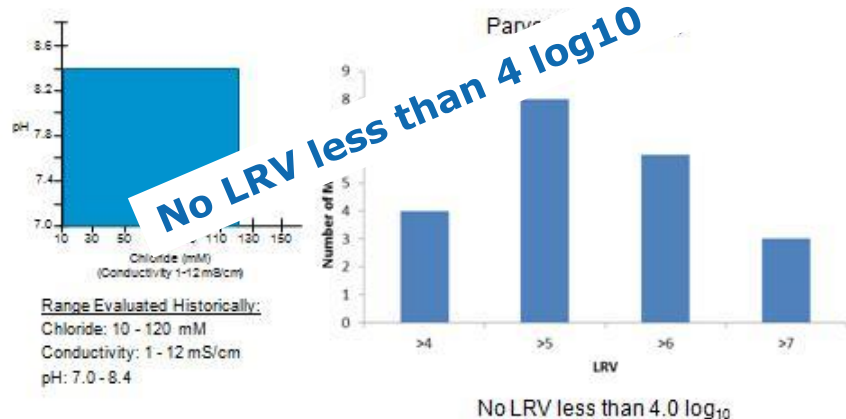


Remove



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



Timothy Iskra *et al* PDA Virus Safety 2014

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

Viral Clearance by Flow-Through Mode Ion Exchange Columns and Membrane Adsorbers

G. R. Miesegans, S. C. Lute, E. K. Read, and K. A. Brorson
Office of Biotechnology Products, CDER/FDA, 10903 New Hampshire Ave., Silver Spring, MD 20903

DOI: 10.1002/btpr.1832
Published online October 26, 2011 in Wiley Online Library (wileyonlinelibrary.com)





Remove

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

Protein (or product)		Enveloped viruses (≥ 40 nm)				Non-enveloped viruses	
						Small (< 35 nm)	Very small (< 25 nm)
		PRV	HIV-1	WNV	BVDV	HAV/EMCV	PPV
A		≥ 4.6	≥ 4.8	≥ 3.6	≥ 4.7	≥ 5.9	4.6
B		≥ 6.1	≥ 5.6	≥ 4.5	≥ 4.5	5.2	4.4
C		≥ 6.0	≥ 4.0	≥ 5.4	≥ 4.9	6.6	6.1
D	D1	≥ 5.5	≥ 5.9	≥ 5.8	≥ 4.1	≥ 4.2	4.1
	D2	≥ 5.2	≥ 6.9	n.d.	≥ 4.7	≥ 5.5	4.2
E	E1	≥ 4.2	≥ 3.8	≥ 6.2	≥ 3.7	≥ 6.4	5.0
	E2	≥ 5.4	≥ 5.4	n.d.	≥ 4.8	≥ 4.2	6.5
F	F1	≥ 6.0	≥ 6.8	≥ 7.0	≥ 6.3	≥ 5.3	3.9
	F2	n.d.	≥ 4.5	≥ 6.0	4.6	≥ 4.4	3.8
G		n.d.	≥ 6.3	n.d.	≥ 5.1	≥ 5.6	4.6

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 al, Grifols 2014



Virus Safety: Multi-Layer Strategy

Virus Clearance Validation | Lot Release testing

1



Prevent

Virus Safety of Raw Materials

2



Remove

Virus Clearance Technologies

3



Detect

Testing of In-Process and Final Product

 **BioReliance**[®]
by **SAFC**

- 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:
- Regulatory Experts, Virus Clearance Team, Process Validation Experience, Plasma Fractionation Process Knowledge and Leading Technologies.

Setting Your Strategy

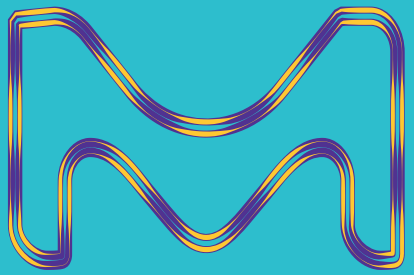
How can you assure viral safety of your plasma therapeutic?





Thank YOU!

contact:
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