



TRITON™ X-100: strategy & Alternatives for virus inactivation

International Workshop on “Surveillance and Screening of Blood-borne Pathogens”

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MERCK

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Agenda

- 1 Triton™ X-100 Background**
- 2 REACH (Registration, Evaluation, Authorization and Restriction of Chemicals)**
- 3 Application for Authorisation of Triton™ X-100**
- 4 Selecting an alternative to Triton™ X-100**
- 5 Summary**

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Triton® X-100 Applications

Industrial/Commercial

- Paints & Emulsifiers
- Textile & Paper
- Fuel Processing
- Construction Materials
- Cleaners
- Cosmetics

Agriculture

- Pesticides & Herbicides
- Dust Binding Agents
- Wool Processing

Life Sciences

- Diagnostics
- Protein Analysis & Molecular Biology
- Medical Device Lubrication
- Bioprocessing
 - Vaccines
 - Plasma
 - mAbs

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

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REACH

Registration, Evaluation, Authorisation 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 2018
181 chemicals

Triton™ X-100 & Authorisation 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

2021

Sunset:
January 4
2021

Triton™ X-100 REACH Exemption

- R&D applications
- Medicinal products manufactured outside the EU

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REACH

Application on authorisation

Info on applicant

- Substance ID
- use

Socio-economic analysis

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



Analysis of alternatives

- Possibly substitution plan

Chemical Safety Report

- Use information

18 months for preparation of application documents

Experience and thoughts on the application

Background and strategy:

- We had expertise from previous application for authorisation
- We plan to submit an application for authorization for the GMP production of Triton™ X-100 (Not for downstream users)
- Follow-up and monitoring of new developments within authorisation topics by our regulatory expert team

Some Tips:

- Start early (challenging to perform in 18 months)
- Ensure to apply early before the latest application date
- Use the support of a consultant
- Presubmission information session offered by ECHA (info needs to be provided 1 month in advance to the session)
- Process is a refinement (start with an idea and then finetuning)

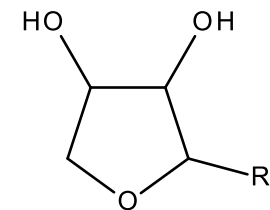
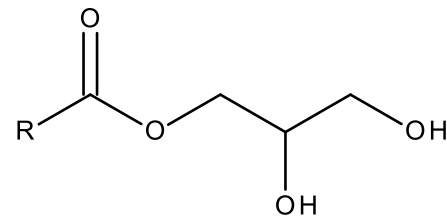
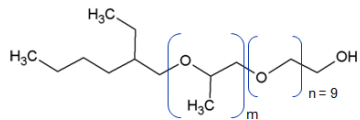
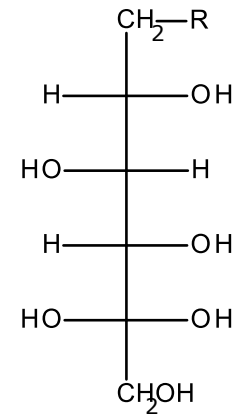
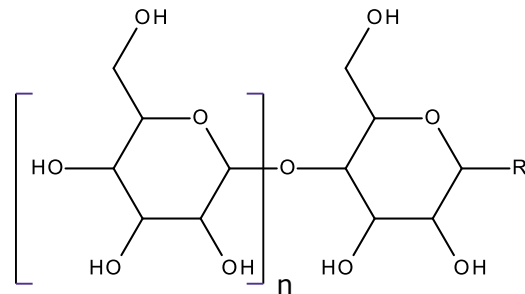
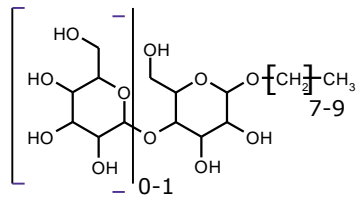
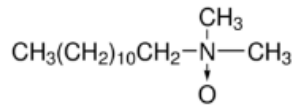
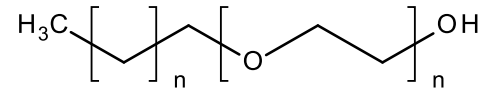
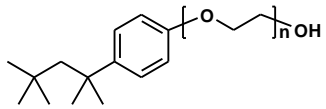
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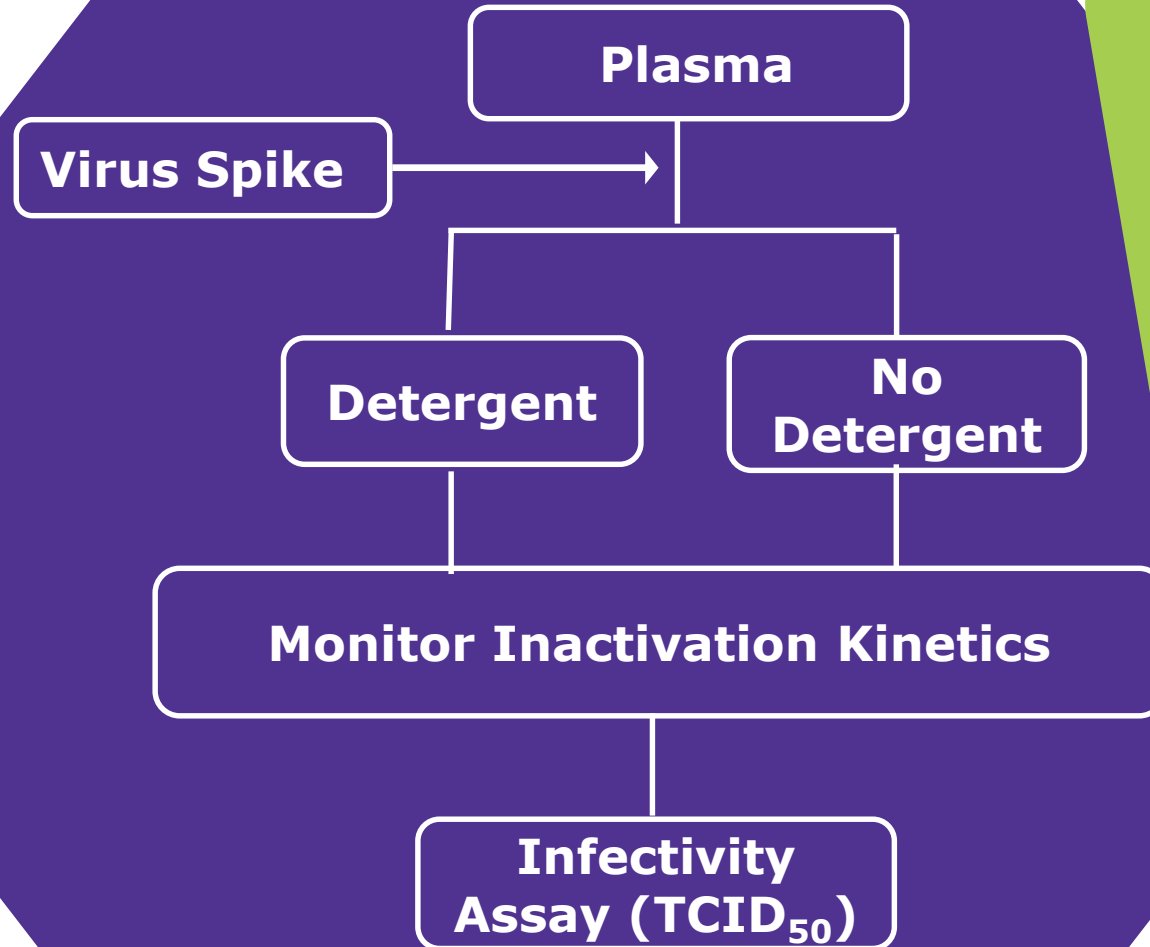
Considerations

- 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

Spectrum of Detergent Structures



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

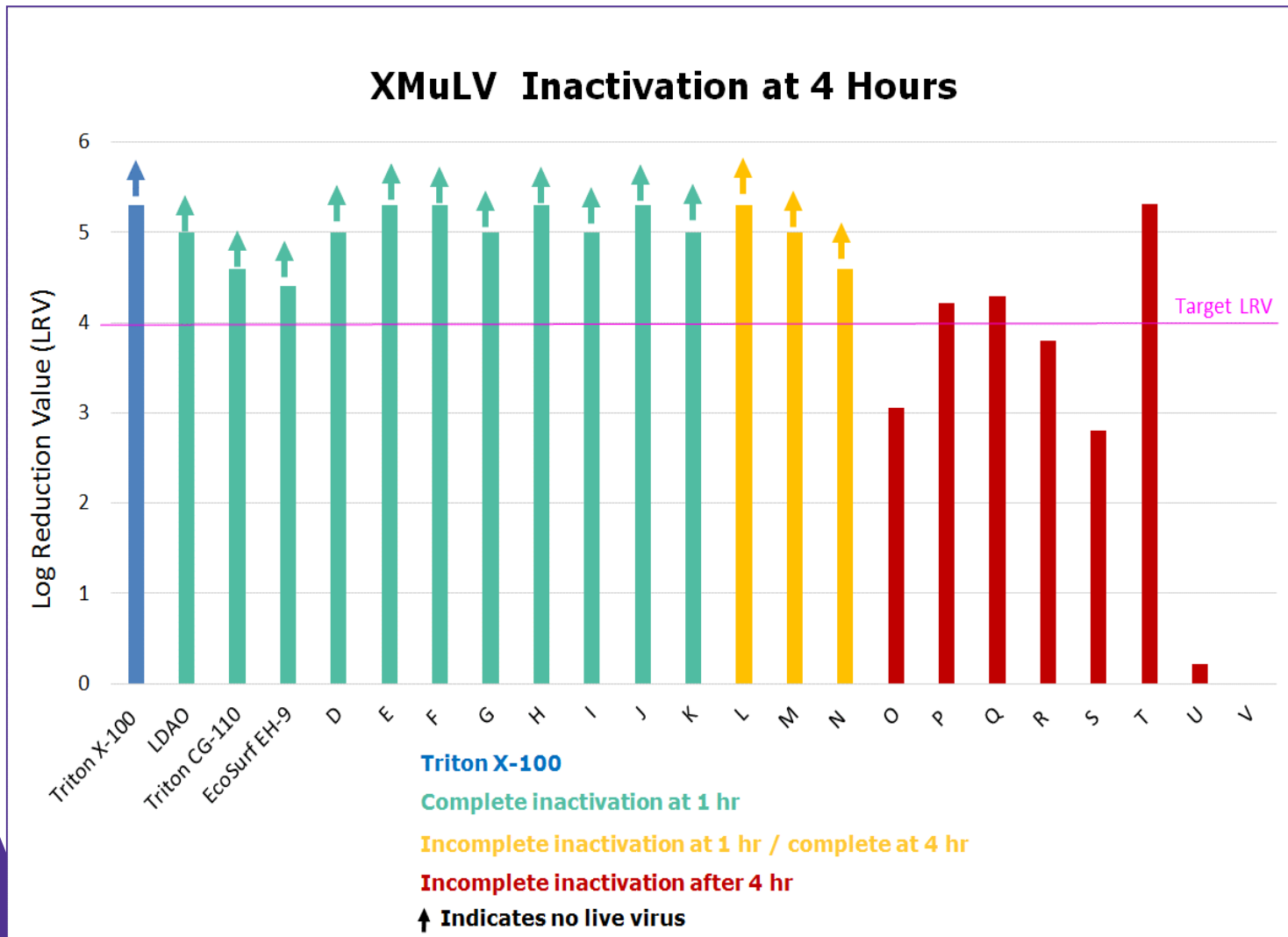
Murine Leukemia Virus (XMuLV)

- ~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

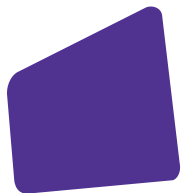
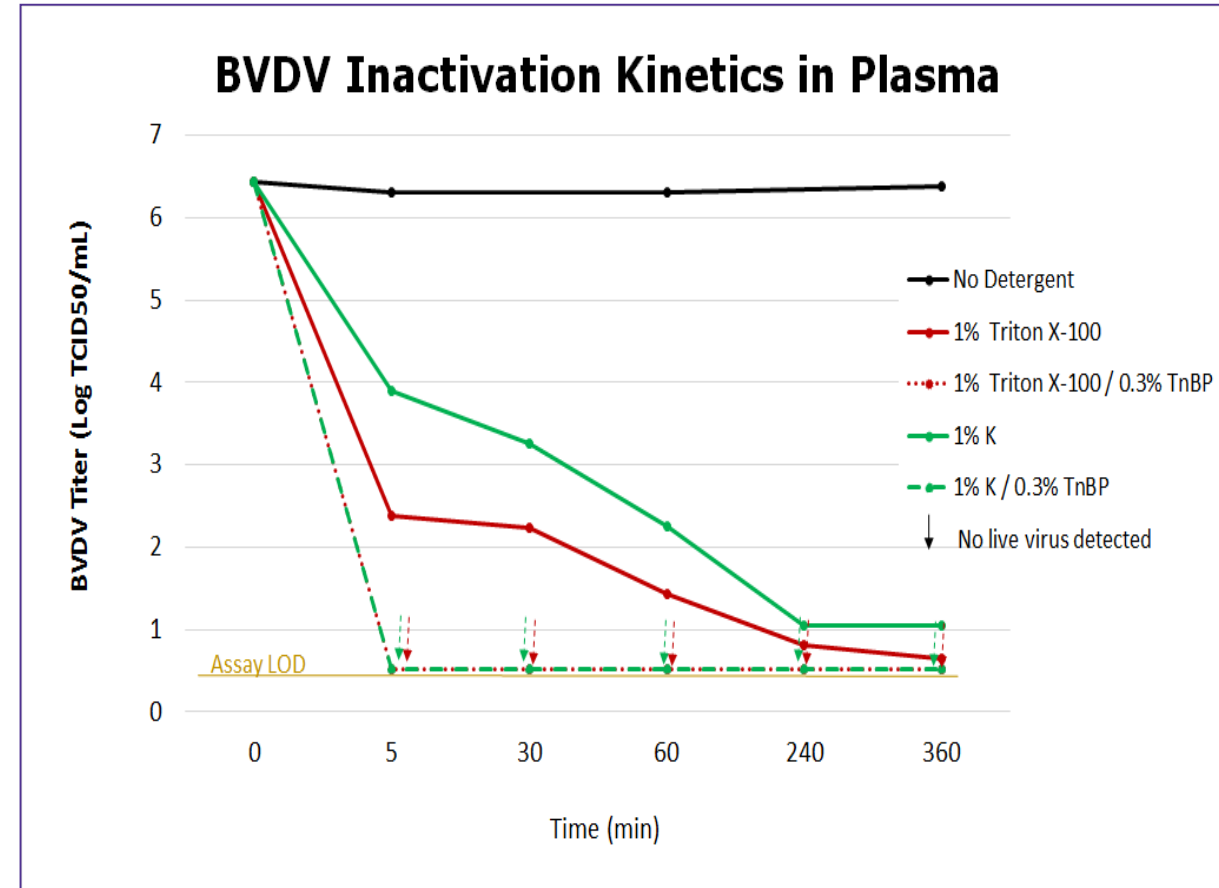
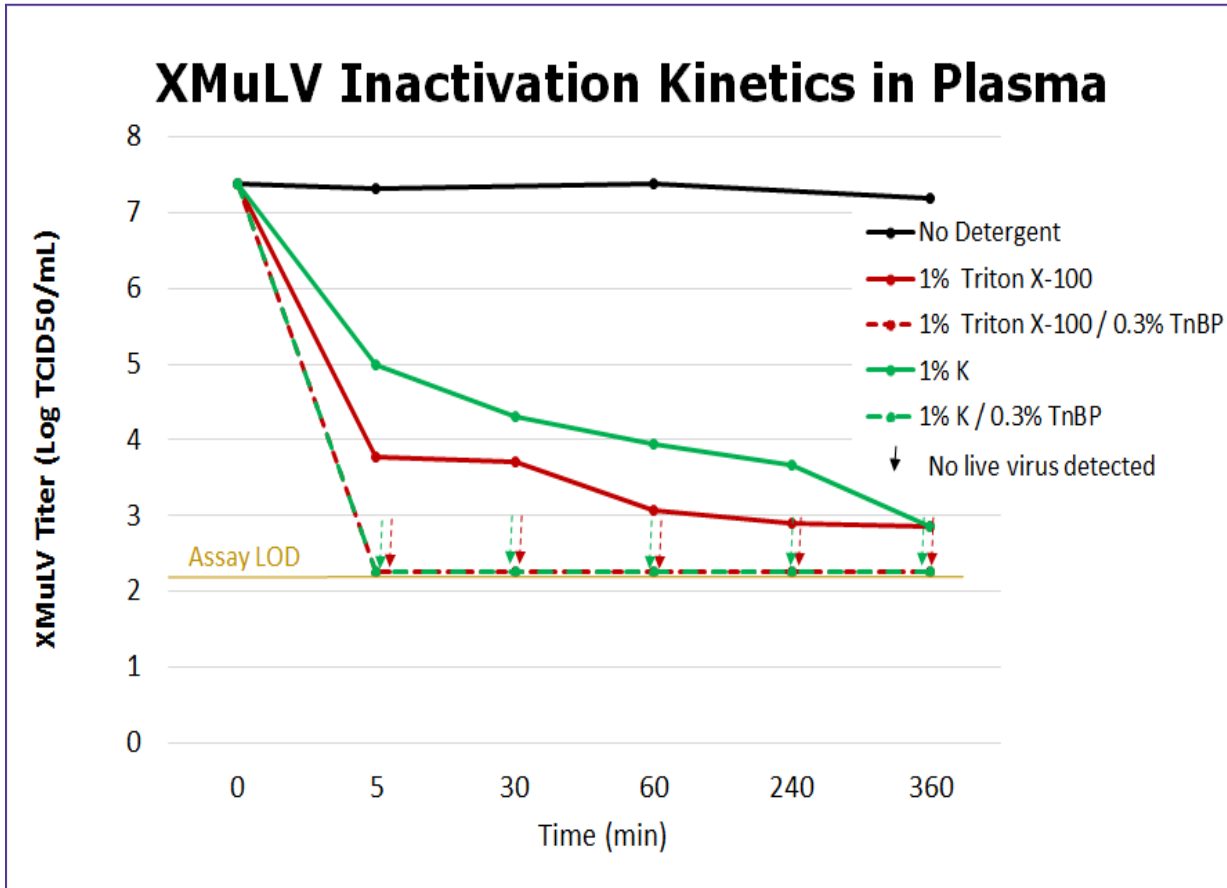
- XMuLV in 10g/L IgG
- 1% (w/v) detergent at 22°C
- 1 and 4 hr incubation

Criteria for further investigation

- Virus inactivation capability
- GMP manufacturability

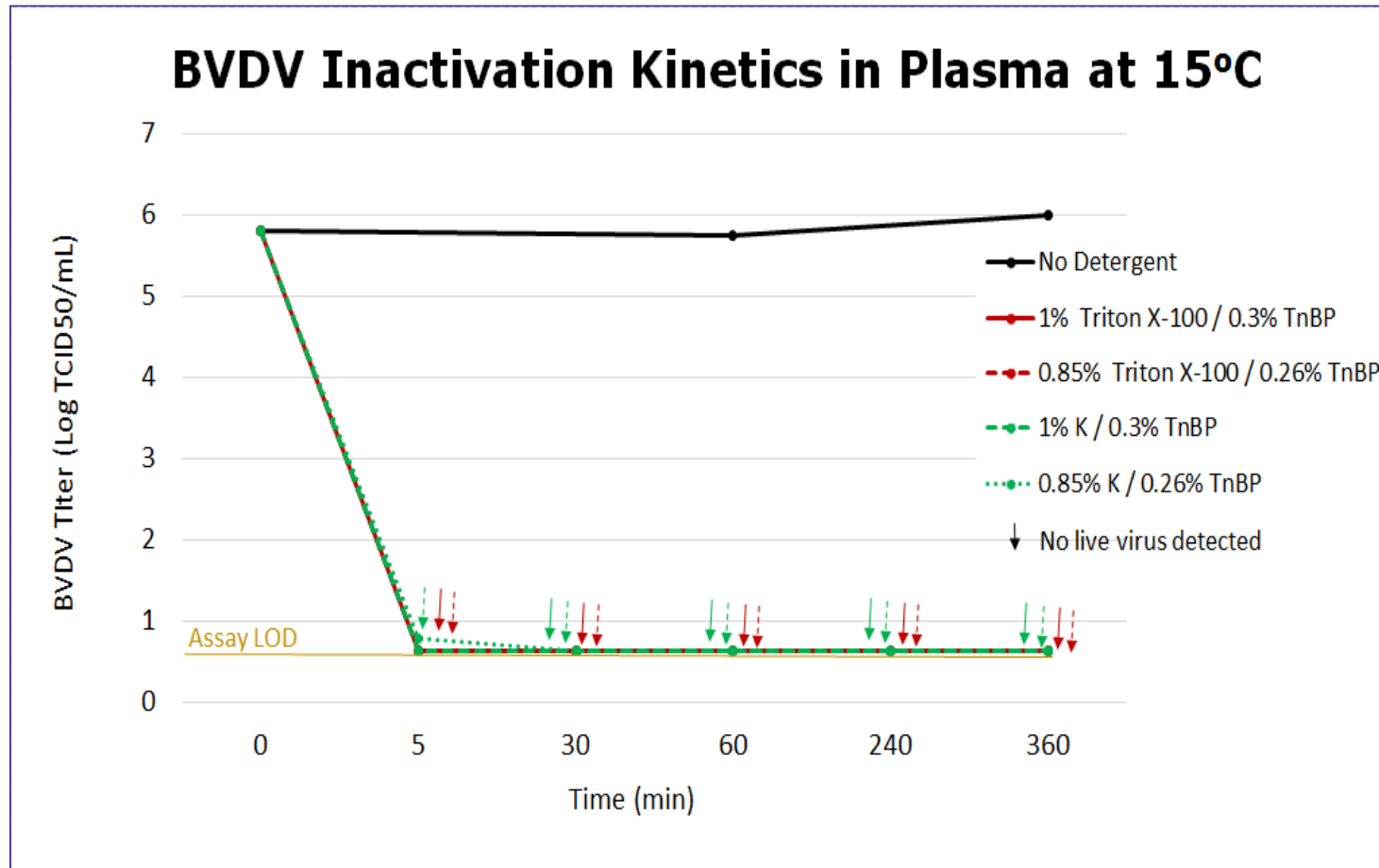
Several candidates pass the inactivation screening, let's review the other studies with candidate K as an example

Example for Virus Inactivation Kinetics in Plasma at 22°C



Detergent & solvent combination is more effective than detergent alone for virus inactivation in plasma

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 confirmed

Exemplary Detergent Removal

Cation Exchange Chromatography

1% wt/v Detergent K + Phosphate Buffer

9141 µg/mL



Flow-Through Waste

9035 µg/mL

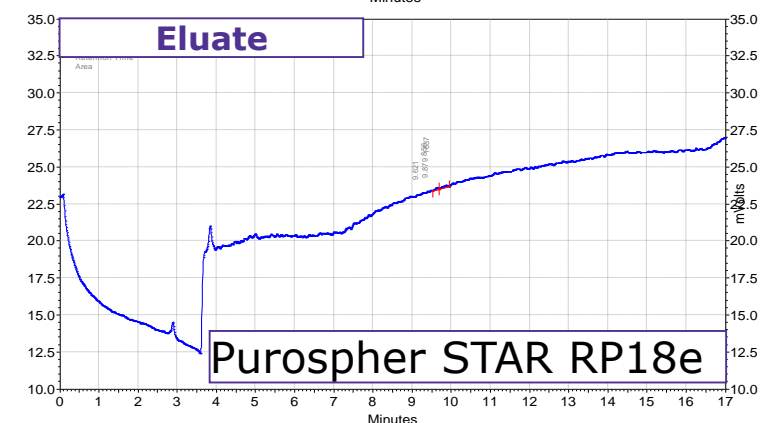
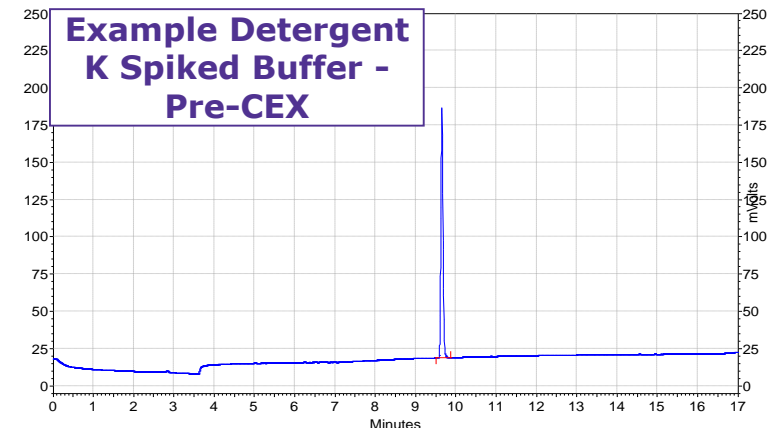
Fractogel® EMD SO₃⁻ Resin



Eluate

Not detectable (<0.1 µg/mL)

➤ CEX: effective for detergent removal



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

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

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- ❑ 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
- ❑ We have identified potential substitutes and we will apply for REACH-registration.



Thank you!
questions?

contact details

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