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Implementation and characterization of solvent detergent viral inactivation in Single Use bags

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Solvent Detergent Viral Inactivation in Mobius® Process Containers

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Introduction

Blood-borne virus transmission (HIV, HBV, HCV) by plasma-based biologics is a pathogen safety risk

Virus filtration is not a viable risk mitigation strategy, especially for plasma products of large molecular mass that cannot be filtered

Solvent detergent (S/D) virus inactivation mitigates pathogen safety risk by inactivating lipid-enveloped viruses plasma and other processing fluids

Cleaning validation requirements for S/D viral inactivation in stainless steel equipment makes evaluation of single-use technologies an attractive processing option

Study Objective

Evaluate feasibility of employing Mobius® mixing solutions and EMD Millipore chemicals for S/D viral inactivation application

Study designed to ask three fundamental questions:

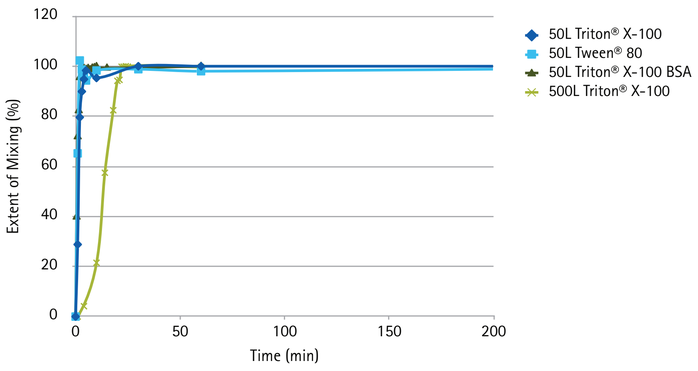
1. Are Mobius® single-use assemblies compatible with chemicals used in the application?
2. Are the Mobius® bags compatible with the requirements for executing SD inactivation operation?
3. What best practices recommendations can be provided in this application?

Characterization of Solvent/Detergent Mixing in Mobius® Single-Use Process Containers

MIX50 and MIX500 containers are generated using PureFlex™ film
Mixing characterized using the following conditions:

	0.3% TnBP 1% Triton® X-100 in PBS	0.3% TnBP 1% Tween® 80 in PBS	0.3% TnBP/1% Triton® X-100 80 5% BSA in PBS	Agitation (RPM)	VI Hold Temperature (°C)
MIX50	X	X	X	200	23-25
MIX500	X			100	31

- Mixing speeds chosen to avoid vortex generation
- Mixing completed in primary vessel; solution transferred to secondary vessel for VI hold (6 hours)
- Samples collected from side sampling ports
- Efficiency of mixing evaluated by measuring Triton® X-100 or Tween® 80 concentration by UV-HPLC
- Samples collected from secondary vessel after 6 hours for leachables testing (in PBS conditions)



- Mixing essentially completed by 10 min at 50 L; 22 min at 500 L
- 50 L mixing efficiency similar in PBS and BSA
- Mixing profile similar between Triton® X-100 and Tween® 80
- Characterization of leachables profile in progress

PureFlex™ Film and Mobius® Mixer Chemical Compatibility

Table of Solutions for Chemical Compatibility Testing

	Study Test			
Solution (in PBS Background)	ESCR	Mechanical Stress	Non-specific Binding	Leachables
PBS Control	X	X		X
1% Triton® X-100	X	X		X
1% Tween® 80	X	X		X
0.3% TnBP/1%Triton® X-100	X	X	X	X
0.3% TnBP/1% Tween® 80	X	X	X	X

- All solutions made up in RO water
- 1 L gamma-irradiated PureFlex™ containers incubated at RT for 24 hours with each test solution

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A. PureFlex™ Tensile Strength Testing:

- Film sample taken from 1 L container post 24 hour incubation with each test solution
- Strength testing results reported as percentages of internal PureFlex™ reference standards

Mechanical Property	PBS (%)	1% Triton® X-100 (%)	1% Tween® 80 (%)	0.3% TnBP 1% Triton® X-100 (%)	0.3% TnBP 1% Tween® 80 (%)
% Elongation at break	125	157	141	149	135
Tensile strength (psi)	102	121	108	114	107
Secant Modulus (psi)	175	93	127	92	123
Toughness (lbf-in/ in^3)	126	172	141	157	135

- PureFlex™ tensile strength is not negatively impacted by exposure to chemicals used in solvent detergent viral inactivation

B. Non-Specific Binding Characterization:

- Fluid sample taken from 1L container post 24 hour incubation with each test solution
- Residual Tween® 80 and Triton® X-100 measured in solutions by UV-HPLC

Solution	% Non-specific binding
0.3% TnBP/1% Triton® X-100	2
0.3% TnBP/1% Tween® 80	1

- Negligible non-specific binding of the S/D mixtures to the PureFlex™ containers

C. PureFlex™ Leachables Testing:

- Fluid sample taken from 1 L container post 24 hour incubation with each test solution
- These conditions represent worst case (largest surface area to volume ratio relative to likely scale of application in 50-500 L range)
- Samples tested for volatile organic compounds (VOCs), semi-volatile VOCs (sVOCs) by gas chromatograhy - mass spectroscopy (GC-MS)

Solution	Total Leachables	
	VOCs (ppm)	sVOCs (ppm)
PBS	0.09	0.47
1% Triton® X-100	0.98	0.55
1% Tween® 80	1.32	0.12
0.3% TnBP 1% / Triton® X-100	0.46	1.2
0.3% TnBP/1% Tween® 80	1.86	0.32

D. Environmental Stress Crack Resistance Test (ESCR):

- ESCR measures susceptibility of plastics to cracking in stressed configuration after exposure to liquid chemicals (ASTM protocol D1693-08)

3 gamma-irradiated resins representing bag components, impeller and impeller cup used in testing

Results of ESCR Testing	
Reagent	% Cracking post 48 hr Exposure
PBS	0
1% Triton® X-100	0
1% Tween® 80	0
0.3% TnBp 1%/Triton® X-100	0
0.3% TnBP/1% Tween® 80	0

1. No aesthetic defects were found on plastics after reagent exposure (pre-stress)
2. Samples did not fail stress test after 48 hours (32 °C)

- Mobius® container components are compatible for use in solvent detergent viral inactivation application

Efficacy of Solvent/Detergent Viral Inactivation in Single-use Process Containers

The objective was to demonstrate the effectiveness of S/D virus inactivation in plasma feeds using single-use Mobius® process containers.

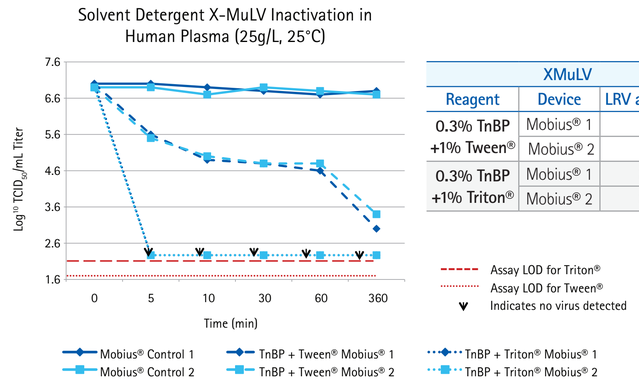
TEST PLAN

- Scale: Mobius® 50 mL process containers
- Solvent/detergent formulations:
  - 0.3% TnBP + 1% Tween® 80
  - 0.3% TnBP + 1% Triton® X-100
- Model viruses:
  - Xenotropic Murine Leukemia Virus (X-MuLV) as a model for HIV retrovirus
  - Bovine Viral Diarrhea Virus (BVDV) as a model for Hepatitis C virus
- Feedstream: Human plasma

PROCEDURE

1. Add plasma, solvent detergent mix, and virus and mix by vortexing then add to Mobius® process containers
2. Mix containers on rocker at 25 °C and ~2 mL sample removed at appropriate times for titer assay.
3. Procedure repeated twice on two separate days
4. Inactivation performed in Polypropylene tubes (Tube control) and Mobius® containers.

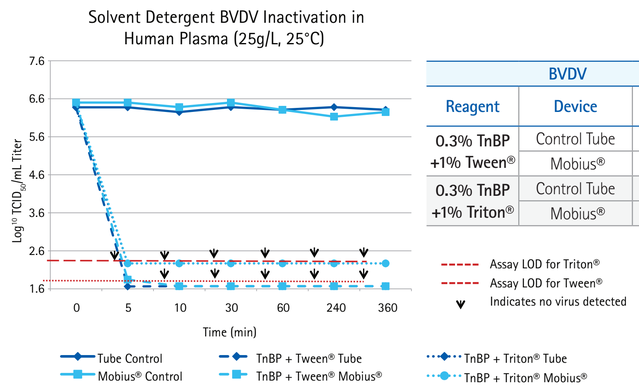
XMuLV Virus Inactivation from Solvent/Detergents in Human Plasma



XMuLV		
Reagent	Device	LRV at 6 hours
0.3% TnBP +1% Tween®	Mobius® 1	3.4
	Mobius® 2	3.3
0.3% TnBP +1% Triton®	Mobius® 1	≥4.5
	Mobius® 2	≥4.4

- Results with tubes & process containers were equivalent
- Tween® inactivation: Although more than 3 logs XMuLV was inactivated in the Tween mixture, it was not complete after 6 hours.
- Triton® immediately inactivates X-MuLV

BVDV Virus Inactivation from Solvent/Detergents in Human Plasma

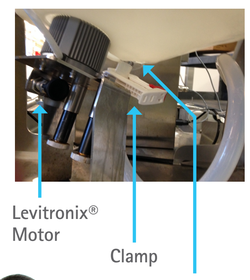


BVDV		
Reagent	Device	LRV at 6 hours
0.3% TnBP +1% Tween®	Control Tube	≥4.3
	Mobius®	≥4.5
0.3% TnBP +1% Triton®	Control Tube	≥4.0
	Mobius®	≥4.0

- Results with both process containers were equivalent
- Kinetics of Tween® inactivation: inactivation of BVDV in Tween was rapid; complete inactivation was observed after 10 mins
- Triton® immediately inactivates BVDV

Initial Recommendations for Hands-On Application

- Add solvent detergent solution through bottom drain port to minimize foaming
- Before mixing, attach clamp on drain line as close as possible to vessel (top right) to minimize dead leg
- Choose minimal speed that facilitates mixing (mitigate foaming or product quality risks).
- Detailed SOP currently in development



Summary

- Mobius® process containers are compatible with chemicals relevant to solvent detergent virus inactivation
- Solvent detergent virus inactivation is effective in Mobius® process containers, as shown using two enveloped virus models (BVDV, XMuLV)
- Implementation of single-use system reduces process contamination risks and requirements for cleaning validation
- Implementation is feasible with lower capital investment and minimal equipment qualification
- Testing to characterize the impact of solvent detergent viral inactivation in Mobius® process containers on plasma product activity and quality attributes is in progress.