

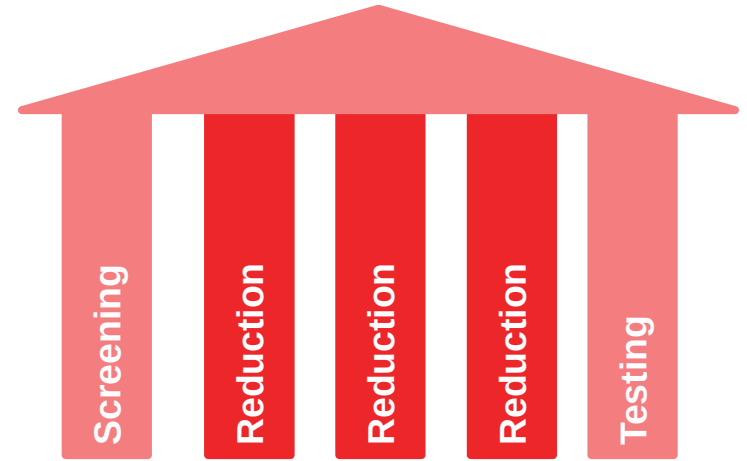


Inactivation/reduction of non-enveloped viruses during manufacture of PDMPs

Nathan J Roth, PhD
Executive Director, Global Pathogen Safety

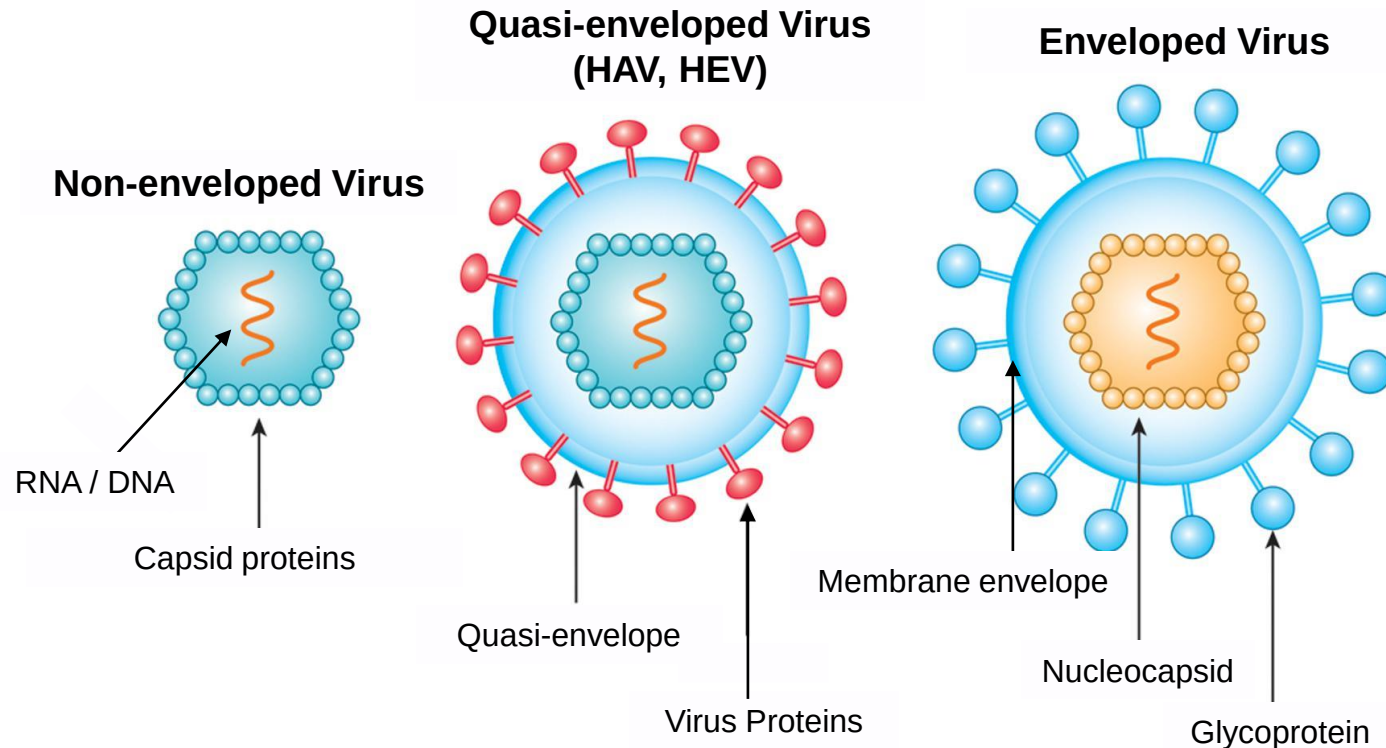
Pathogen Safety Tripod: PDMPs

Reduction capacity plays a major role – but other pillars can significantly contribute



- Screening (~1 log)
 - only healthy, qualified donors / epidemiology
- Testing (NAT & Serology) (0 to 5 log)
 - donations and manufacturing pool for relevant blood borne virus
 - HBV (~1 log safety), HIV (~3 log), HCV (~5 log)
 - HAV (~2 log), B19V (~5 log at the 10^4 pool limit), emerging (0)
- Virus Reduction (~6 to >15 logs)
 - Multiple orthogonal steps
 - Designed to proactively address emerging viruses

Enveloped vs Non-enveloped Viruses



- For PDMPs relevant non-enveloped viruses are HAV, B19V, and HEV (for SD plasma only)
- HEV virus is an emerging non-enveloped virus of concern for all other PDMPs

Regulatory Guidance

- EMA/CHMP/BWP/706271/2010 - Guideline on plasma-derived medicinal products
 - ... *it is desirable... to incorporate two distinct effective steps which complement each otherat least one of the steps should be effective against non-enveloped viruses*

- EMA/CHMP/BWP/360642/2010 rev. 1 - Guideline on the warning on transmissible agents in summary of product characteristics and package leaflets for PDMPs
 - *The warning statements make specific reference to the viruses that have been transmitted in the past by PDMPs. The measures taken to prevent the transmission of enveloped viruses such as HIV, HBV and HCV are considered effective for all marketed products.*
 - *Non-enveloped viruses, such as HAV and parvovirus B19, are more difficult to inactivate / remove and the effectiveness of measures for non-enveloped viruses differs among marketed products.*
 - *Therefore, the information given in the SmPC and user Package Leaflet should highlight the remaining potential risk of transmission of the non-enveloped viruses, HAV and parvovirus B19, taking into account the characteristics of the safety measures taken and the results of the viral inactivation / removal studies performed by the Marketing Authorisation Holder.*

Regulatory Guidance (2)

➤ FDA current thinking ⁽¹⁾

- Two orthogonal viral clearance steps for relevant enveloped and non-enveloped viruses
- For enveloped viruses, one of the clearance steps must be inactivation. The other step could be either inactivation or removal
Total log reduction for enveloped viruses should be ≥ 10 logs
- For non-enveloped viruses, the total log reduction should be ≥ 6 logs

• Japan MHLW ⁽²⁾

- With regard to virus inactivation and/or removal, regardless of whether or not the virus is enveloped, it is preferable to include two or more distinct procedures with high robustness for virus clearance.

1) Basil Golding M.D., Division of Plasma Protein Therapeutics, CBER, FDA. CMC Review Responsibilities for Biologics License Applications. PPTA Plasma Proteins Industry Summit Beijing, September 6-7, 2017

2) MHLW Draft revision of Guideline of Securing Viral Clearance of PDMP

Virus Inactivation / Removal Methods

**DEDICATED
EFFECTIVE**

Inactivation

- Solvent Detergent
- Octanoic acid (caprylate)
- Low pH
- **UVC**
- **Heat treatment**
 - Pasteurization
 - Freeze dry / Dry heat
 - Vapor

Removal

- **Virus Filtration**
 - Small pore (20 nm)
 - Medium pore (35 nm)
- **Cold EtOH Fractionation**
 - FrIV to FrV (Albumin)

Purification

- **Cold EtOH Fractionation**
- **Column chromatography**
- **Ammon Sulfate, PEG, OA precipitation, etc, + separation**

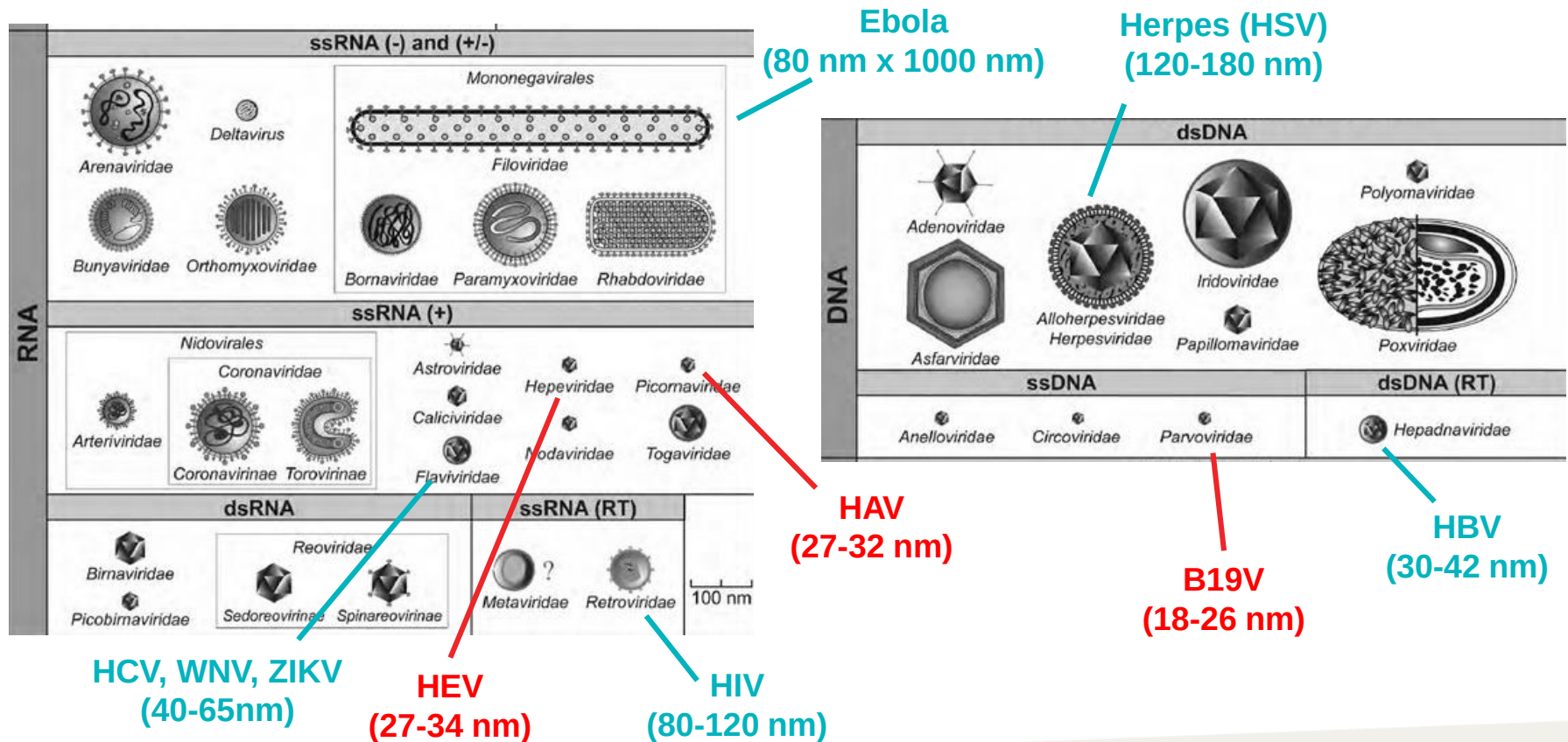
CONTRIBUTING

Methods in **red** *may* provide effective or contribute to non-enveloped virus reduction

Virus Filtration - Virus Size

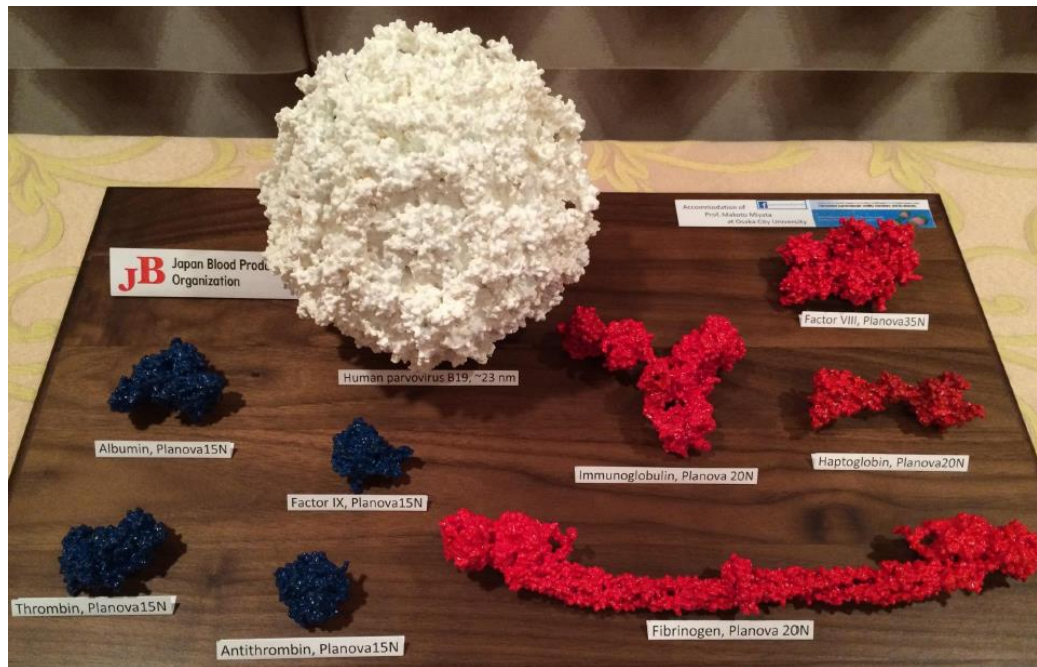
➤ Retention of virus based on size exclusion

- Small pore virus filters (~20 nm can provide effective retention of parvovirus and larger viruses)



Virus Filtration (nanofiltration)

- Retention of virus based on size exclusion
 - filters with pore sizes of nanometer sizes that are specifically designed to remove viruses
 - can be used when the virus is larger than the therapeutic protein(s)



A J Kubo 2014 Asahi Planova Workshop

Virus Filters

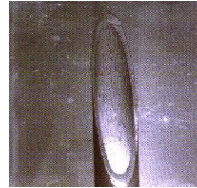
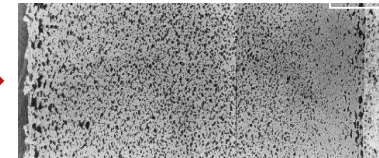
Housing

Membrane

Structure

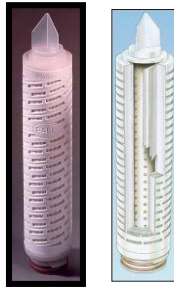
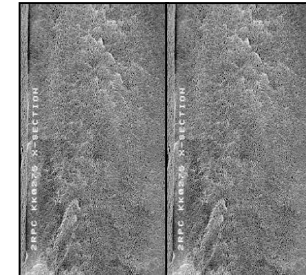
Flow →

Asahi Kasei
Planova 20N



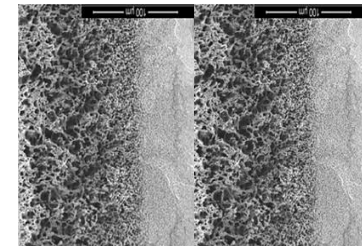
single layer
hollow fiber

Pall
DV20



dual layer

Millipore
Viresolve Pro



dual layer

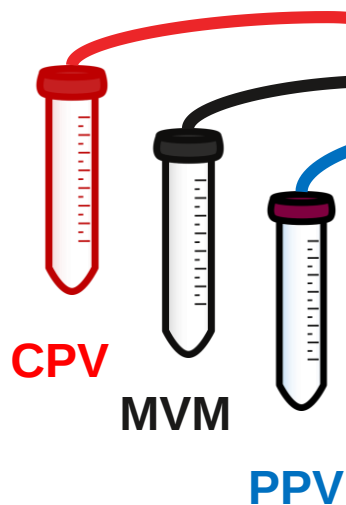
Removal of Small NEV by Virus Filtration

Product Class	Virus Filter	HEV LRV by VF	
		CPV/MMV/PPV	HAV/EMCV
IgG	20 nm	≥ 5.5	≥ 5.4
IgG	20 nm	≥ 6.2	≥ 4.9
FVIII	20 nm	3.4	≥ 5.5
FIX	20/20 nm	≥ 8.2	≥ 6.7
FXIII	20/20 nm	5.6	≥ 6.1
PCC	20/20 nm	6.5	≥ 6.1
C1-INH	15/20 nm	≥ 7.2	≥ 5.3
ATIII	15/15 nm	≥ 4.8	≥ 7.6
A-1 PI	20/20 nm	≥ 6.4	≥ 5.3

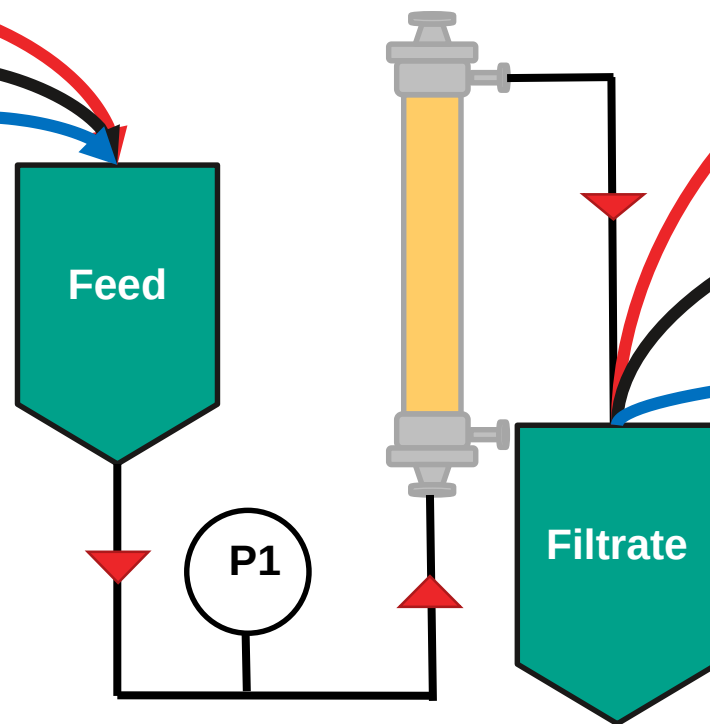
- Demonstrated reduction factors for parvoviruses (B19V) and picornaviruses (HAV) are considered to be relevant for HEV
- 15 or 20 nm virus filtered products have a robust and high safety margin against HEV

Study Design – Triple Parvovirus Spike

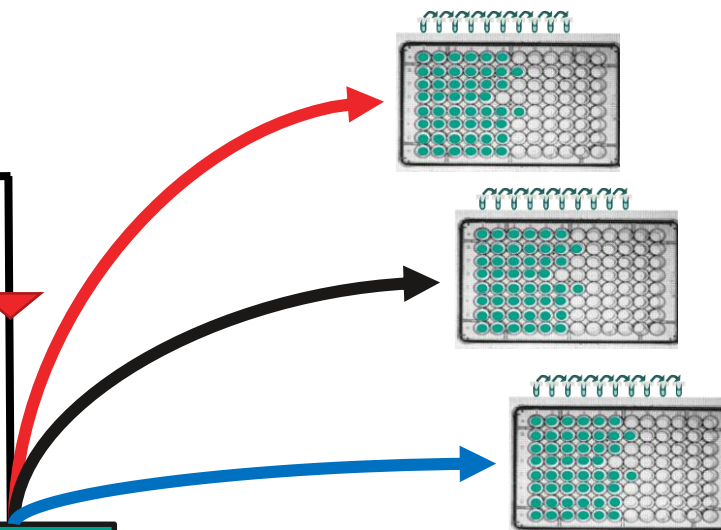
1) Spike



2) Run Virus Filtration



3) Assay for each Virus



Feed A: PCC, 2 mg/mL, pH 6.5, 15 mM NaCl 50 L/m²,
Feed B: Albumin, 4 mg/mL, pH 5.5, 225 mM NaCl, 900 L/m²

Commercial Small Pore Virus Filters Tested

Manufacturer	Filter	Composition
Asahi	Planova 20N	Regenerated cellulose, hollow fiber
	Planova BioEX	Modified PVDF, hollow fiber
Sartorius	Virosart CPV	Hydrophilic PES, double layer
	Virosart HF	Hydrophilic PES, hollow fiber
	Virosart HC	Hydrophilic PES, double layer
Pall	DV20	Hydrophilic PVDF, double layer
	Pegasus SV4	Hydrophilic PVDF, double layer
	Pegasus Prime	Hydrophilic PES, double layer
Millipore Merck	Viresolve NFP	Hydrophilic PVDF, triple layer
	Viresolve Pro	Hydrophilic PES, double layer

Filter Generation

1st

2nd

3rd

Differential Retention of Parvovirus – Feed A

Nowak et al., 2019 Biologicals accepted

Filter	Vol (%)	LRF		Δ LRV (MVM-CPV)		
		CPV	MVM	PPV		
A	100	4.2	≥ 6.5	≥ 5.6	> 2.3	Filter Gen 1st
B	100	4.3	≥ 6.0	≥ 4.4	> 1.7	
C	100	≥ 5.7	≥ 5.8	≥ 4.6	unknown	
D	68	2.3	5.2	≥ 3.9	2.9	2nd
E	67	0.7	3.5	4.9	2.8	3rd
F	100	1.8	≥ 5.8	≥ 4.6	> 4.0	
G	[-]	[-]	[-]	[-]	[-]	
H	100	≥ 6.9	≥ 6.8	≥ 5.7	unknown	
I	100	5.0	≥ 7.0	≥ 5.5	> 2.0	
J	100	≥ 5.4	≥ 6.1	≥ 4.9	unknown	

Feed A: PCC, 2 mg/mL, pH 6.5, 15 mM NaCl 50 L/m²

Feed B: Albumin, 4 mg/mL, pH 5.5, 225 mM NaCl, 900 L/m² (Similar results observed)

Parvovirus Model for B19V

Virus Spike	LRF ($\log_{10}$)*		
	CPV	MVM	B19V
CPV	3.8		
MVM		≥ 7.3	
B19V			≥ 4.4
Co-spike CPV+MVM	3.4	≥ 7.1	
Co-spike CPV+MVM+B19V	3.5	≥ 4.8	≥ 4.3

Feed: PCC, 2 mg/mL, pH 6.5, 15 mM NaCl 50 L/m²

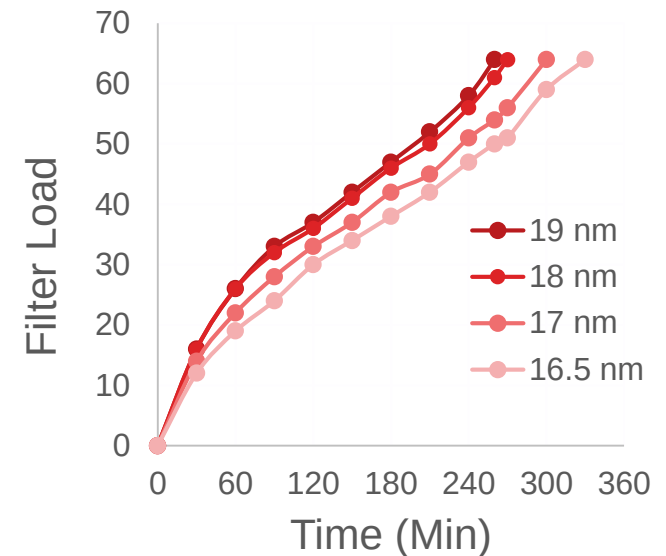
* All data from infectivity assays

- MVM (and PPV) are appropriate model for B19V
- CPV is a worst case model parvovirus for virus filtration validation studies

Size of Canine Parvovirus (CPV)

Custom made research filters provided by Asahi Kasei
- varying pore size using Planova 20N process as base

Pore Size (nm)	LRF ($\log_{10}$)		
	CPV	MVM	PPV
19	4.2	≥ 7.1	≥ 6.0
18	4.6	n.d.	n.d.
17	≥ 6.7		
16.5	≥ 6.8		



- CPV behaves smaller than MVM and PPV
- a worst case model parvovirus for VF studies

VF: Process Interruption / Low Flow VF

Virus Breakthrough after Pressure Release during Virus Retentive Filtration

27-Jun-2011 PDA, Barcelona
Dr. Marcel Asper



- Pressure release / low flow can result in small virus breakthrough
- Larger virus (enveloped) not affected
- Not Universal: Filter A $\neq$ Filter B
- Can lead to significantly reduced LRFs
- Low P not high P is worst case

➤ Care should be taken to **understand, validate, and control** for these events during manufacturing and virus validation studies

VF: Low Pressure / Pressure Release

Meeting Report:

Joint BWP/QWP workshop with stakeholders in relation to prior knowledge and its use in regulatory applications

23 November 2017, European Medicines Agency, London

With regards to virus filters at low pressure/pressure release, low pressure or pressure release is considered a *potential worst case parameter that could be critical under certain circumstances*. More knowledge from parvovirus retention under worst case conditions is desired and *product specific validation runs considering worst case conditions such as filter loading and pressure* are expected.

NEV Inactivation - Heat Treatment

- Stabilizers are added to protect the product
- Stabilizer can also protect virus, influencing rate of inactivation and LRF
- Highly effective against enveloped virus
- Effectiveness against NEVs varies case by case
 - Often provides significant inactivation of a broad range of NEV
 - Compare to low pH or S/D which have no (or very specific) effects on NEV



Pasteurization Vessel

Pasteurization / Human Albumin

- Human Albumin produced according to e.g. the European Pharmacopoeia is heated at least 10 hours at 60°C
- Stabilizers included are sodium caprylate and N-acetyl-tryptophan
- Albumin produced according to an established ethanol fractionation process including a terminal pasteurization has an excellent virus safety record



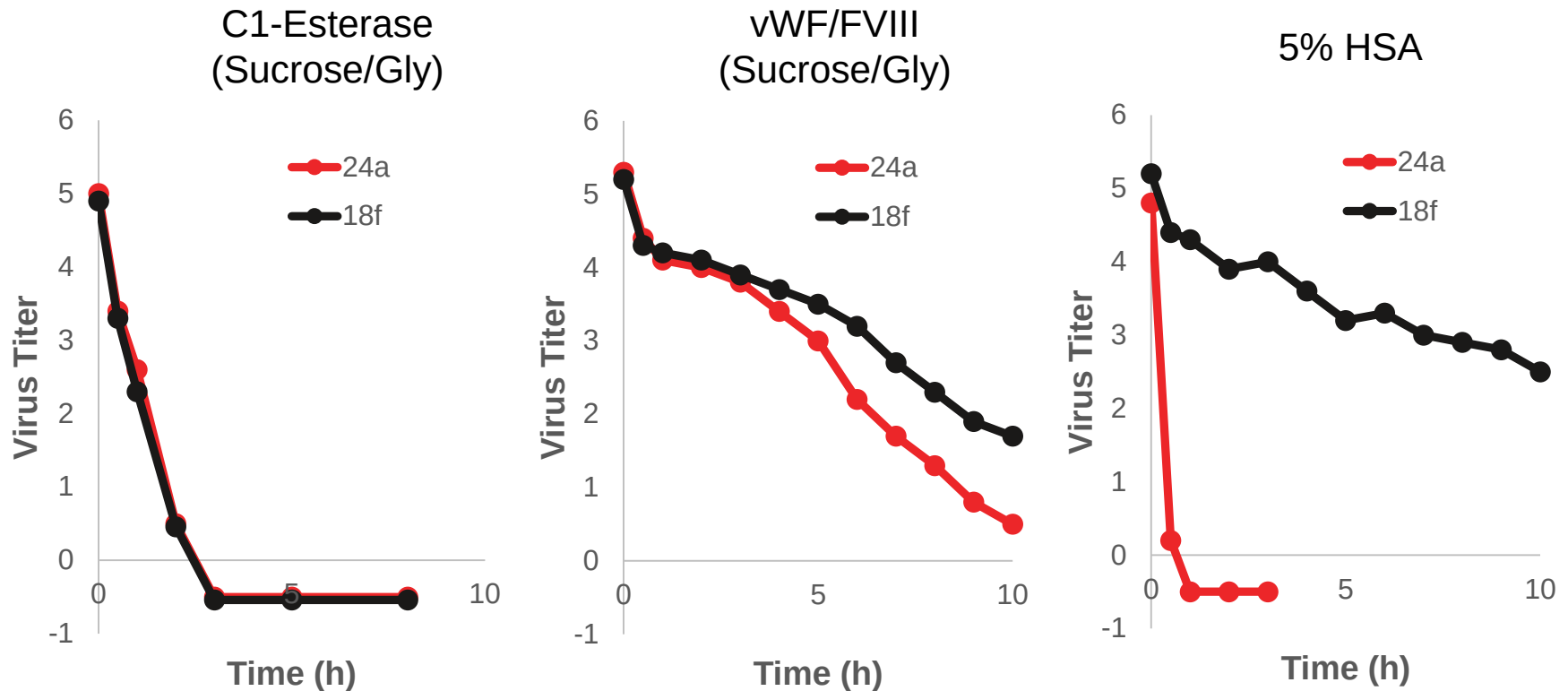
HSA Pasteurization – Non-enveloped virus

Albumin concentration			
	4%/5%	20%	25%
Virus	Virus reduction factor [log] / time to no detectable infectivity [h]		
BVDV	≥9.0/6	≥9.1/4	≥8.2/2
HAV _{HM175/24a}	≥6.9/2	≥6.9/6	≥6.6/4
HAV _{HM175/18f}	2.7/10	≥4.3/9	4.3/10
HEV _{ΔORF3}	ND	ND	3.7/10
B19V	≥4.3/1	≥4.0/1	ND
CPV	1.7/10	1.2/10	1.7/10

ND = not determined.

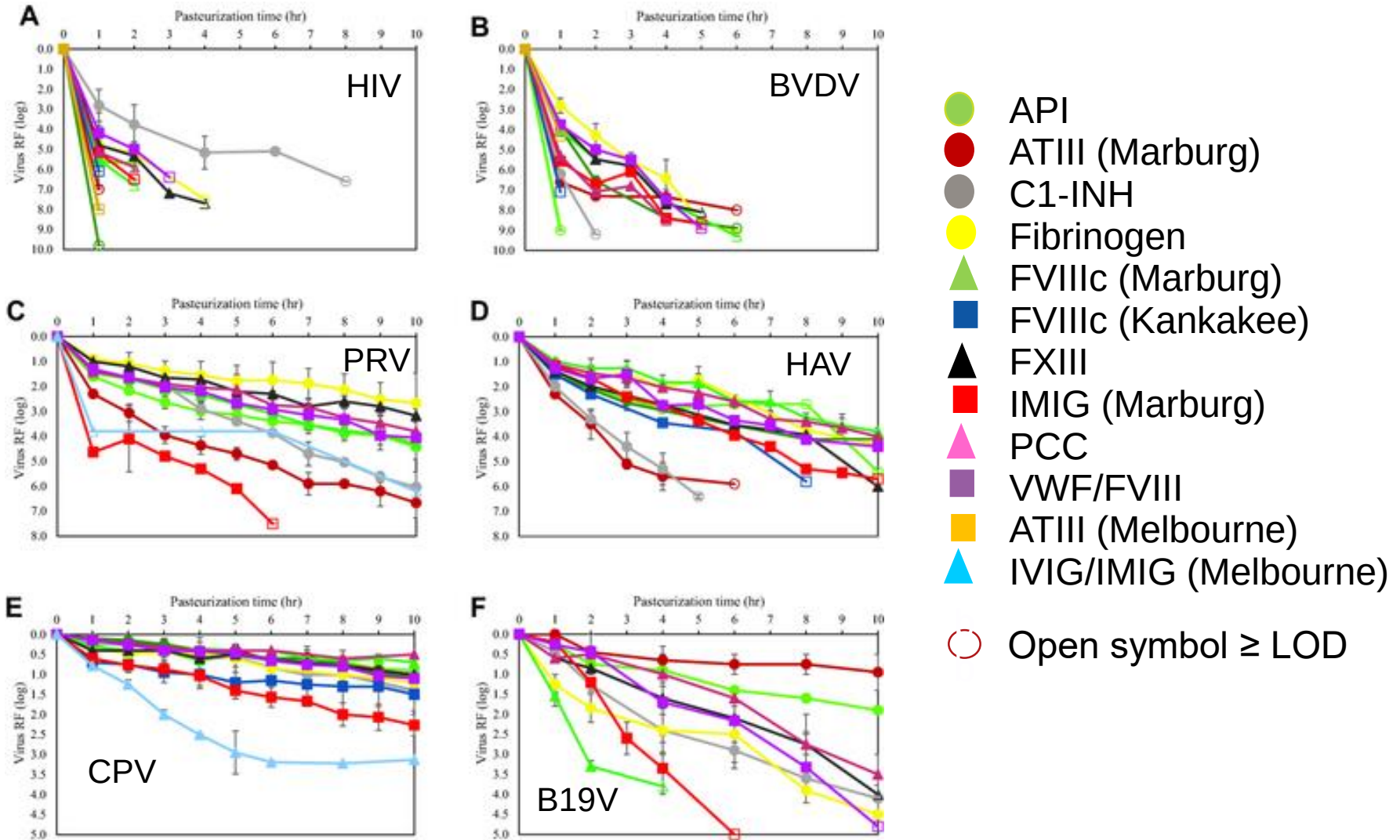
- Non-enveloped viruses more resistant to inactivation than enveloped
- B19V much more labile than animal parvovirus models (CPV, PPV, MVM)
- Pasteurization is generally effective against non-enveloped viruses

HAV Strain Differences - Pasteurization



- HAV strain variants can show significantly different inactivation by heat treatment
- Inactivation differences influenced by stabilizers and protein matrix (sucrose vs albumin, caprylate, hTrp)

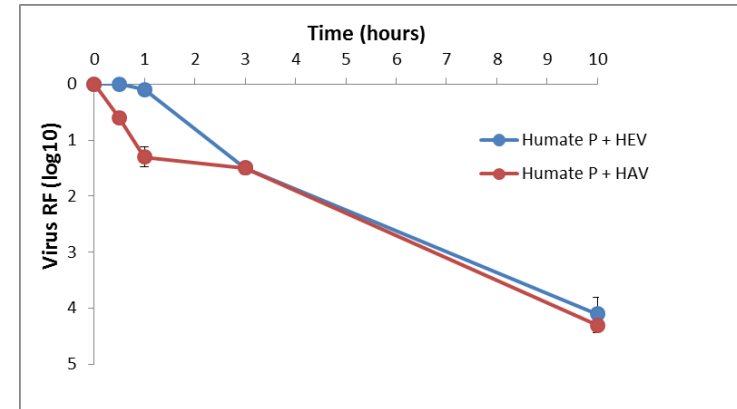
Pasteurization / Virus Inactivation / Diverse Compositions



Transfusion, 2018 58, 1, 41-51

Pasteurization vWF/FVIII - HEV

In vitro



In vivo



FLI / CSL Behring collaboration

Assay System	vWF/FVIII Pasteurisation LRF (log ₁₀)		
	HEV (ΔORF3)	HEV (wild boar)	HAV (18f)
Piglet <i>in vivo</i> (FLI/CSL)		4.0 (n=1)	
<i>In vitro</i> infectivity (CSL)	4.1 (n=2)		4.2 (n=5)

[BMC Vet Res.](https://doi.org/10.1186/s12917-018-1713-8) 2018 doi: 10.1186/s12917-018-1713-8

➤ High correlation between *in vivo* and *in vitro* results increases confidence

Freeze Dry / Dry Heat Treatment

Heating the final container in a lyophilized form

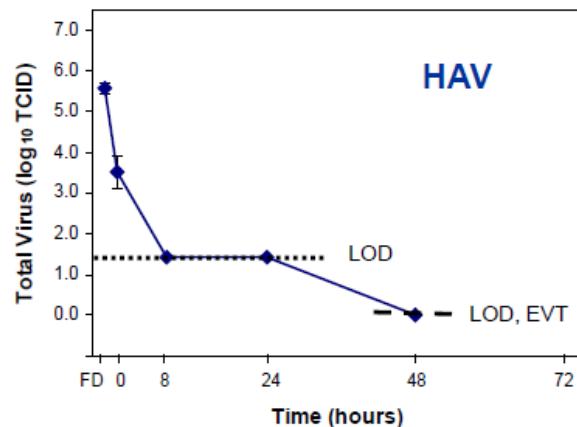
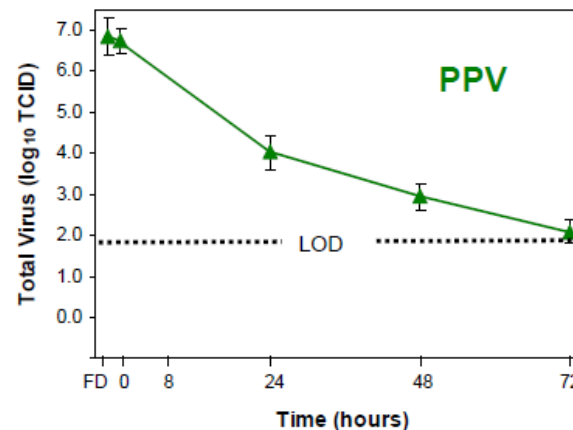
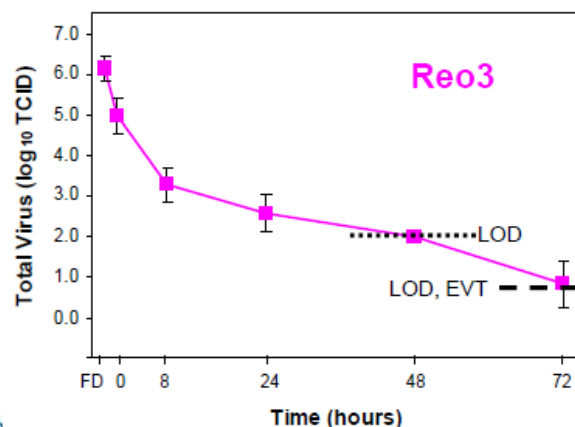
- Stabilizer composition
- Freeze dry (lyophilization) cycle
- Residual moisture
 - Residual moisture critical to evaluate
 - May impact virus inactivation kinetics and LRF
 - May negatively impact product quality (activity and stability)
 - Stopper composition and control
 - Moisture may be reintroduced to cake during heating
- Heating temperature/time
 - Typically 72°C for 72 h OR 100°C for 30 min



FD/DH Treatment



Non-Enveloped Virus Inactivation



Moisture Level 0.5 - 0.8%

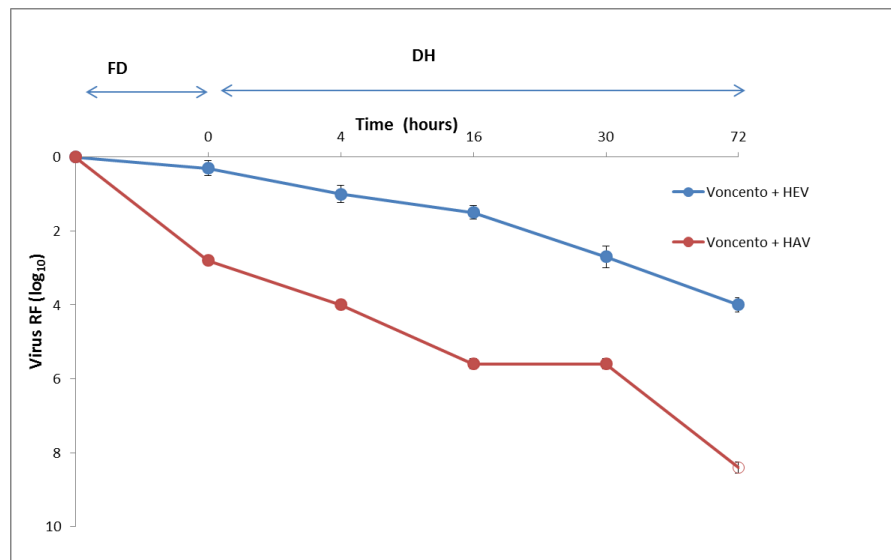
Virus	LRV
HAV	≥5.6
Reo3	5.3
PPV	4.8

Roth et al, Viral Inactivation during Dry Heat Treatment, Grifols, PDA 2012 3 DA Virus and TSE Safety Forum, Berlin

Freeze Dry/Dry Heat – FVIII/vWF

	FD	DH	Total
HEV (log ₁₀) (n=3)	0.4	3.6	4.0
HAV (log ₁₀) (n=2)	2.8	≥5.6	≥8.4

HAV 18f
HEV ΔORF3

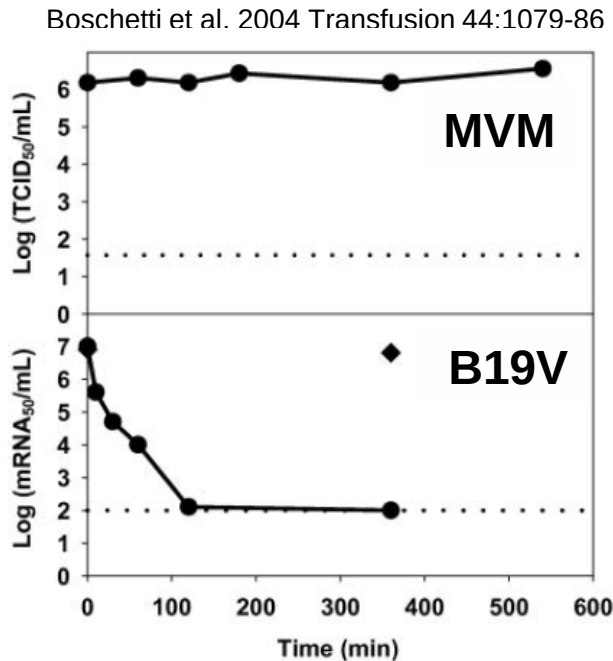
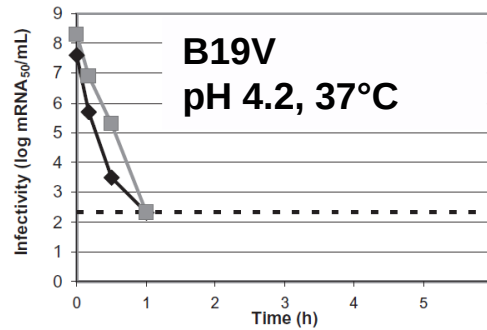
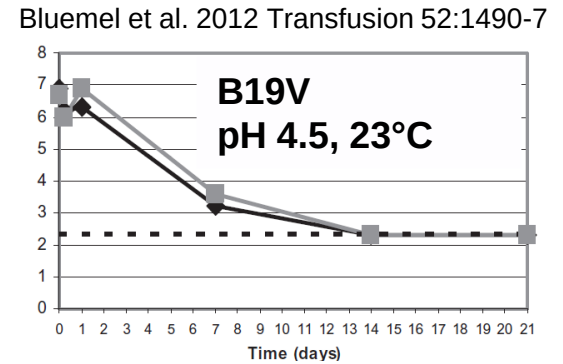


○ Assay LOD reached
● Assay LOD not reached

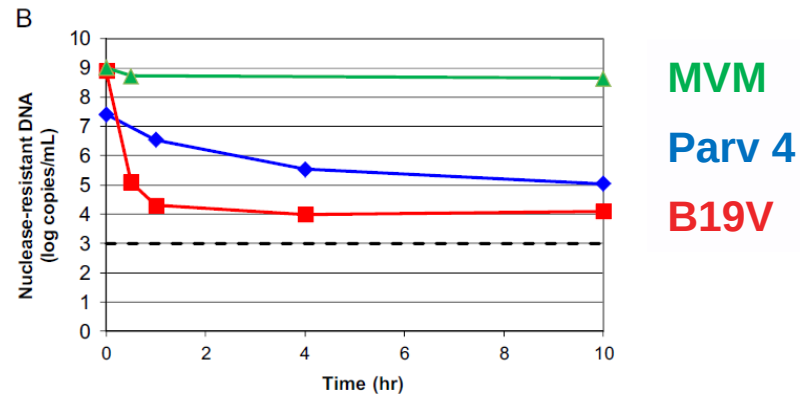
➤ FD/DH conditions: 72 hours at 80°C, 1% albumin stabiliser with a moisture of 0.3-0.6% (w/w)

➤ HEV is more resistant over the FD/DH process than HAV, with little or no inactivation during lyophilisation

Low pH Inactivation – B19V



◆ Genotype 1 ■ Genotype 3 - - - Detection Limit



Baylis et al. 2013 Transfusion 53:2585-92

- Not a broadly effective step for NEV but can contribute significantly to the final container safety of B19V and other parvoviruses

Purification Steps / Partitioning of NEV

Cold EtOH Fractionation Fr IV step

Virus reduction	CPV (n = 5)	B19V (n = 1)	HAV (n = 18)	MEV (n = 2)	PPV (n = 14)	MMV (n = 12)	REO (n = 42)
Lower limit of range	2.50		3.50	6.11	1.93	2.30	3.43
Mean	3.90	5.50	5.10	6.13	3.16	4.72	5.80
Median	3.50		5.20		3.60	4.50	5.99
Upper limit of range	5.40		7.03	6.15	4.60	7.00	8.15
>4 log ratio			16/18		4/14	10/12	39/42

Adapted from Dichtellmuller et al. (PPTA) 2011 Transfusion 51:1412-1430

- **Significant contributor to non-enveloped virus safety of Albumins (along with Pasteurization)**

Purification Steps / Partitioning of NEV (2)

Caprylate (Octanoic Acid) Precipitation & Removal

	Nonenveloped viruses		
	Reo3	HAV	PPV
Original (cloth filtration)	2.1 ± 0.4	2.6 ± 0.2	2.2 ± 0.4
Modified (depth filtration)	≥3.5	≥3.6	4.0 ± 0.1

Adapted from Barnette et al, Biologicals 40:247-53

IVIG – Process A

IVIG – Process B

	EMCV	PPV
Model for	HAV	Parvovirus B19
Caprylic acid fractionation	≥5.6	3.7

Adapted from Goussen et al, Biodrugs 2017; 31: 251–61

- Can significantly contribute to Non-enveloped virus safety of final product

Summary

NEV Inactivation / Removal in PDPMs

- Virus removal filtration is a highly effective method, based primarily upon size, for assuring NEV safety
 - Filters keep improving – direct implications to more robust NEV safety
 - Care must be taken to understand and control for low flow/P breaks
- Heat treatment can provide significant inactivation of NEV
 - Stabilizers affect efficacy of step
 - Strain considerations during virus validation studies
- Cold ethanol fractionation
 - FrIV step a key contributor to albumin safety (other steps also contribute)
- Purification steps
 - Can have significant contributions to final container safety of NEV
- Emerging non-enveloped viruses e.g. HEV
 - Should be evaluated against knowledge base and confirmation studies performed when necessary

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