Inactivation of non-enveloped viruses such as Parvovirus B19 and Hepatitis E Virus

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Human Non Enveloped Viruses

- Adenoviruses
- Papillomaviruses
- Reoviruses
- Polyomaviruses
- Picornaviruses
  - Hepatitis A Virus
- Parvoviruses
  - B19V, PARV4
- Caliciviruses, Hepeviridae
  - Hepatitis E Virus
- Astroviruses
- Circoviruses
  - (TTV)

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Hepatitis A Virus (HAV)

Asymptomatic viraemic donors, no 'classical' risk group as for HIV, HCV, HBV
Epidemiology is changing (decrease of convalescent donors in Europe, traveling)
Clinical risk: defined (acute hepatitis, non-chronic)

Viraemia: $10^5$ GE/mL (up to $10^7$ GE/mL)

Transmission cases (e.g. Germany 1997)
SD-treated FVIII, no robust step for HAV inactivation/removal

Safety measures:
- NAT (optional, must be ultra sensitive for large plasma pools)
- Inactivation/removal (at least 1 robust step required)
- Vaccination possible
Hepatitis E Virus (HEV)

Asymptomatic viraemic donors, no 'classical' risk group as for HIV, HCV, HBV
High Prevalence/Incidence
Clinical risk: genotypes 3,4: low for immunocompetent (with exceptions)

Viraemia: up to $10^7$ GE/mL

Transmission cases documented with non-inactivated blood components (red blood cells, platelets…)

Safety measures:
• currently no NAT screening
• virus reduction step (as for HAV?)
• no vaccination (vaccine in China)
Parvovirus B19 (B19V)

Asymptomatic viraemic donors, no ‘classical‘ risk group as for HIV, HCV, HBV
High Prevalence/Incidence
Clinical risk: defined, low for immunocompetent

Viraemia: high, up to $10^{12}$ GE/mL

Transmission cases documented (dry-heat treated products)

Safety measures:
• High titer NAT (pool limit $10^4$ Ge/ml) (optional, voluntary)
• Virus reduction step
• no vaccination
PARV 4

Identified in 2005
Asymptomatic viraemic donors assumed risk group as for HIV, HCV, HBV? (unclear)
Moderate/high Prevalence and Incidence
Clinical risk: unclear, few data

Viraemia: few data, up to $10^8$ to $10^{10}$ GE/mL
Transmission cases probable
[Sharp et al., JID, 2009 and Transfusion 2012]

Safety measures:
• No NAT screening
• Virus reduction step (as for other parvoviruses?)
• no vaccination
Methods for inactivation/removal

1. Liquid heat treatment (pasteurization 10h, 60°C)

2. Dry heat treatment

3. Small pore nanofiltration

4. Pathogen inactivation of blood components (psoralene derivatives, methylene blue, riboflavin)

5. (UV-irradiation): not discussed here
Hepatitis A Virus at Pasteurization
(10h 60° in albumin)

PEI-Study (unpublished)

Farcet, Kindermann, Modrof, Kreil, Transfusion 2012

Yunoki et al, Transfusion 2013
Sakai and Yunoki, Poster 2013

LRF = 4.0

VSS 18f LRF = 2.4
HM175: LRF = 4.2

Rec-FR1 LRF ca. 5
KRM003 LRF ca. 2

LRF 4 to 6 usually reported from HAV inactivation at FVIII production
HEV at Pasteurisation (albumin)

From Poster: Sakai and Yunoki. PDA Virus Safety Forum, Berlin, June 2013

HEV inactivation in FVIII, FIX? Data required.
Inactivation of **Parvovirus B19 (B19)** versus **Porcine parvovirus (PPV)** at Pasteurisation of 5% Albumin

Same inactivation kinetics with viruses from various plasma-samples [Blümel et al., (2002). Transfusion 42:1011-1018]

Same inactivation kinetics with Parvovirus B19 genotypes 2 and genotype 3 [Blümel er al., Transfusion 2005, Blümel et al., Transfusion 2012]
Inactivation of B19 in Clotting Factors at Pasteurization

From Poster: Gröner et al., 2010, GTH, 24-257 Feb. Nürnberg, Germany

Parvoviruses at Heating

heat → ad nuclease
PARV 4 at Pasteurization of Albumin

Infectivity assay

Nuclease assay

[Baylis, Tuke, Miyagawa, Blümel, Transfusion, 2013]
Dry Heat Treatment

- Inactivation of a broad range of viruses (HIV, HBV, HCV, HAV); parvoviruses partially inactivated
- 80°C for 72 hours or 100°C for 30 minutes
- Good inactivation of enveloped viruses and some non-enveloped viruses (HIV, HBV, HCV und HAV parvoviruses are not completely inactivated)
- Drying leads to stabilisation of viruses; residual moisture is very important (0.1 to 2%)

Critical parameters:
- Exact temperature
- Exact control of residual moisture

Residual moisture too high: damage/loss of product
Residual moisture too low: no virus inactivation
HAV at Dry Heat Treatment

Initial loss of infectivity at freeze drying
Inactivation at heating phase
HEV at Dry Heat Treatment

80°C in fibrinogen concentrate
Yunoki et al., *Vox Sanguinis* (2008) 95, 94–100

HEV Inactivation in FVII, FIX?
Parvoviruses at Dry Heat Treatment

A 100°C, 0.30-0.35% RM

B 80°C, 0.30-0.35% RM

C 100°C, 0.85-1.19% RM

D 80°C, 0.85-1.19% RM

Log-reduction factor

Time (min)

Time (hours)

Low res. moisture

High res. moisture

B19  PPV  * below detection limit

[ Blümel et al., Vox Sang (2008)]
Virus Filtration

Pressure

Membrane
Efficient removal removal of HAV
Efficient removal of HEV

Parvovirus removal at nanofiltration

<table>
<thead>
<tr>
<th></th>
<th>PPV</th>
<th>B19V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planova 35N pre-filtation</td>
<td>1.7</td>
<td>0</td>
</tr>
<tr>
<td>Planova 20N</td>
<td>&gt;4.3</td>
<td>4.6</td>
</tr>
</tbody>
</table>

in 0.5% albumin infectious virus assay, reduction factor given in log_{10}

Sometimes two filters need to be put in series in order to achieve more than 4 log reduction factors.

Parvovirus filters have been introduced for few ‘high purity’ FVIII products.
4. „Pathogen Inactivation“

- Psoralen derivatives + UVA-light
- Riboflavin + light
- Methylene-Blue

Problem: The nucleic acid modifying substance must penetrate the virus shell in order to get contact to the viral DNA/RNA

Inactivation of Parvovirus B19 possible (complete?)
No inactivation of non-enveloped HAV (and HEV?) and animal parvoviruses
### Typical Log Reduction Factors (LRF) at Manufacture of FVIII

<table>
<thead>
<tr>
<th></th>
<th>HAV</th>
<th>HEV</th>
<th>B19</th>
<th>Parv4</th>
<th>PPV MVM CPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurization</td>
<td>4 (4-6)</td>
<td>?</td>
<td>3-5</td>
<td>2-3?</td>
<td>&lt;1-2</td>
</tr>
<tr>
<td>Dry heat</td>
<td>&gt;5 (4-6)</td>
<td>?</td>
<td>&lt;1-5</td>
<td>?</td>
<td>&lt;1-4</td>
</tr>
<tr>
<td>Small virus filtration</td>
<td>&gt;5</td>
<td>&gt;5*</td>
<td>3-5</td>
<td>3-5</td>
<td>3-5</td>
</tr>
</tbody>
</table>

* (expected)

**Additional LRF from purification steps:**
- Intermediate purity: 1-3
- High purity: 3-5
Thank you!