Virus Safety Studies: From Model Viruses to Verification, from Classical to Molecular Virology

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Global Pathogen Safety

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• CPMP BWP/268/95
Note for Guidance on Virus Validation Studies
  - Any virus used in a validation study is actually a model virus
  - HIV must be evaluated

• CPMP BWP/269/95
Note for Guidance on Plasma-Derived Medicinal Products
  - Should include at least ... HIV
  - Model for hepatitis C virus
  - Enveloped DNA virus
  - Non-enveloped virus
Virus Validation Studies: Standard Virus Panel

- HIV
- PRV → "enveloped DNA virus"
- BVDV → HCV model
- HAV
- MMV → B19V model
HIV and Model Viruses

- **HIV: mandatory use**
  Has dictated the use of BSL-3 conditions for virology labs, at
  - considerable efforts,
  - biosafety concerns, and
  - expenses

- HIV model viruses (retroviruses)
  Never considered, despite availability ...
HCV & HBV Model Viruses

• **HCV** model viruses: **many**, and all used to some degree
  - BVDV  Pestivirus, Flaviviridae
  - TBEV  Flavivirus, Flaviviridae
  - SFV  Alphavirus, Togaviridae

• **HBV** model viruses: **none** widely used ... (quite impractical)
  - Duck hepatitis virus  Avihepadnavirus, Hepadnaviridae
    • primary hepatocytes or in vivo titration \(\rightarrow\) NAT readout
  - Woodchuck hepatitis virus  Orthohepadnavirus, Hepadnaviridae
    • primary hepatocytes or in vivo titration \(\rightarrow\) NAT readout
• Removal of HCV by Cohn III precipitation
  - SFV 4.6 versus BVDV 1.6
  - HCV 4.0
    Yei, Yu & Tankersley, Transfusion [1992] 32/9: 824
  - TBEV >4.7 versus BVDV 2.0 (GPS, unpublished)

• SUMMARY
  - HCV more similar to SFV, TBEV (?)
  - BVDV standard in reduction studies (= worst case)

→ Use of a deliberately dissimilar model virus (?)
HCV and Model Viruses: Summary

- **Standard model = BVDV**
  reason: “worst case” (?)

- Model virus data: generally accepted

- Newer HCV tissue culture models: not widely used ...
  - “Production of infectious hepatitis C virus in tissue culture ... “

• Occasional transmissions, e.g. S/D-treated FVIII

A New Cluster of Hepatitis A Infection in Hemophiliacs Traced to a Contaminated Plasma Pool

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Hepatitis A Virus Infection in Tamarins: Experimental Transmission via Contaminated Factor VIII Concentrates

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HAV: Different Laboratory Strains

- HAV cell culture infectivity system have been available, since 1987 also with a cpe-based read-out (= gold standard)
  - Strain: pHM175, Cromeans et al

```
  HM175 wt
  ↓  6x marmoset, 6x BSC1, 10x AGMK
 p16 HM175
  ↓  persistent infection in BSC1, >1 year; 4x FRhK4
 pHM175 stock (cpe)  (Daemer et al., 1981; Binn et al., 1984)

  1. HM175/43c (cpe)
  1. HM175/24a (cpe)
  2. HM175/18f (cpe)
```

1. BSC1 passage
2. FRhK4 passage  

(Cromeans et al., 1987; Lemon et al., 1991)
But: depending on the specific laboratory isolate used, very different HAV inactivation has been observed

→ Variant **24a is more heat sensitive** than variant 18f / 43c-18f

<table>
<thead>
<tr>
<th>HAV variant</th>
<th>Mean log_{10} reduction factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.5% HSA</td>
</tr>
<tr>
<td>HM175/24a (ABL)</td>
<td>nd</td>
</tr>
<tr>
<td>pHM175/24a (BRL)</td>
<td>&gt;5.4</td>
</tr>
<tr>
<td>HM175/24a, previous study</td>
<td>5.2 ±0.9</td>
</tr>
<tr>
<td>HM175 43c-18f</td>
<td>nd</td>
</tr>
<tr>
<td>HM175 18f</td>
<td>nd</td>
</tr>
</tbody>
</table>
• Occasional transmissions (upstream products)

• Emerging Disease Association
  - Fifth disease of childhood  mild
  - Hydrops fetalis  at risk groups
  - Fulminant hepatitis  rather rare
  - Myocarditis  rather frequent (TBC)
    (prevalence of the B19V genome in endomyocardial biopsy specimens)
• **Selection**
  Not efficacious: viremia \( \rightarrow \) mostly asymptomatic
  - Frequency of highly viremic donations
    \( \rightarrow 1:800 - 1:8,000 \)
  - Concentration
    \( \rightarrow \) up to \( 10^{12} \) genomes / ml (!)

• **Testing**
  Significant contribution (!)

• **Reduction**
  - Model parvovirus data, only (MMV, PPV)
  - Generally: very resistant (!)

**Average reduction of viral load by > 5 log10, i.e. 100,000-fold (!)**
B19V Infectivity Assay

- UT7 / Epo-S1 cells: EPO-dependent differentiated cell line → VP 1&2 staining of permeabilized UT7 / Epo-S1

- Readout:
  RT-PCR quantification of spliced B19V RNA
Infectivity Assay for B19V

- Readout: RT-PCR quantification of spliced B19V RNA
**Pasteurization** of Human Serum Albumin (HSA)
Higher than expected heat sensitivity of B19V vs. animal parvoviruses

<table>
<thead>
<tr>
<th></th>
<th>B19V</th>
<th>PPV</th>
<th>MMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSS</td>
<td>-</td>
<td>7.6</td>
<td>9.2</td>
</tr>
<tr>
<td>SSM</td>
<td>6.3</td>
<td>6.0</td>
<td>7.7</td>
</tr>
<tr>
<td>5 min</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 min</td>
<td>&lt; 2.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 hour</td>
<td>-</td>
<td>5.2</td>
<td>7.5</td>
</tr>
<tr>
<td>10 hours</td>
<td>-</td>
<td>3.8</td>
<td>6.2</td>
</tr>
<tr>
<td>RF</td>
<td>&gt; 3.8</td>
<td>2.2</td>
<td>1.5</td>
</tr>
</tbody>
</table>
**B19V Reduction**

- **Vapor Heating** of FEIBA
  Higher than expected heat sensitivity of B19V vs. animal paroviruses

<table>
<thead>
<tr>
<th></th>
<th>MMF RF</th>
<th>VH1 #1</th>
<th>VH1 #2</th>
<th>VH2 #1</th>
<th>B19V mean RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive control</td>
<td>-</td>
<td>11.2</td>
<td>11.8</td>
<td>n.d.</td>
<td>-</td>
</tr>
<tr>
<td>spike control</td>
<td>-</td>
<td><strong>10.3</strong></td>
<td><strong>10.5</strong></td>
<td><strong>10.5</strong></td>
<td>-</td>
</tr>
<tr>
<td>after Lyo</td>
<td>0.6</td>
<td>9.8</td>
<td>10</td>
<td>9.8</td>
<td>0.6</td>
</tr>
<tr>
<td>3h 60°C</td>
<td>n.d.</td>
<td>7.7</td>
<td>7.6</td>
<td>n.d.</td>
<td>2.7</td>
</tr>
<tr>
<td>505 min 60°C</td>
<td>0.6</td>
<td>6.1</td>
<td>6.5</td>
<td>6.5</td>
<td>4.1</td>
</tr>
<tr>
<td>55 min 80°C</td>
<td><strong>0.9</strong></td>
<td>5.7</td>
<td>5.5</td>
<td>5.8</td>
<td><strong>4.8</strong></td>
</tr>
</tbody>
</table>

B19V and Model Viruses

- Most complicated virus model, at the time
  - EPO-dependent differentiated cell line
  - RT-PCR quantification of spliced RNA

- Disappointment for the model virus concept
  - B19V is a lot more sensitive to e.g. heat than earlier-used parvovirus models
  - Worst case (!)
Recent Concerns: **West Nile Virus - a Learning ...**

- **1930-ies:** discovered **blood** of a febrile woman ...
- **1999:** first in the US
- **2002:** unprecedented  
  - ~3,500 cases  
  - ~240 deaths
- **Blood transfusion → transmission**
WNV: Safety Tripod

• **Selection**
  
  → 20% or $0.3 \log_{10}$ risk reduction
  
  - ~81% viremic donors → asymptomatic (654 of 818)

• **Testing**
  
  → 93% of $1 \log_{10}$ risk reduction
  
  - MP PCR: only 1/2 to 2/3 of viremic donations identified
    (only 8-15% of viremic donations are infectious:  
    → high WNV load, w/o IgM)

• **Inactivation**

→ investigated using a WNV infectivity assay (BSL-3):
  - NY zoo snowy owl isolate / Vero
  - Solvent detergent, IVIG & F VIII
  - Vapor heating, FEIBA
  - Low pH incubation, IVIG
  - Pasteurization, HAS

→ all effectively inactivate WNV
Model Viruses → “Verification Studies”

• Lots of model virus data available: TBEV, BVDV, SINV ...

• “Verification” studies required, to support differentiation between transfused & manufactured products
  - PCR testing for transfused blood components
  - No PCR testing for plasma for fractionation

• Other flaviviruses: Should (?) be covered SLEV, DENV ...
Virus De Jour - Verification ? !?
More Recent Yet ...

- SARS
- H5N1
- CHIKUNGUNYA
Memories of SARS

• 2003 Outbreak
  - Origin: Guangdong, China (civet cats ?)
  - Global distribution, very rapid spread: 8422 infections 9% lethality (!)

• SARS Coronavirus (CoV)
  - spherical, 120-160 nm, lipid enveloped
  - ss linear RNA, 27-31 kb
  - short pre-clinical incubation period

Coronavirus Inactivation

- CoV model: murine hepatitis virus
  - Vapor heating, Pasteurization
- SARS CoV (CBER, US FDA)
  - Pasteurization, S/D, UV-C

Pasteurization of HSA

- Vapor Heating of FEIBA, Fibrin Sealer, F VII

- CoV model: murine hepatitis virus
  - Vapor heating, Pasteurization
- SARS CoV (CBER, US FDA)
  - Pasteurization, S/D, UV-C
A Recent Reminder: “Novel Coronavirus”

- Emergence: Middle East
- September 2012 – May 2013
  - 34 laboratory confirmed cases
  - 18 deaths
- Person-to-person transmission: likely, though limited
- Source: High sequence homology with bat and porcine coronaviruses; highest with viruses carried by Pipistrellus bats, widely prevalent in Middle East

- BUT: Should equally be covered by Coronavirus models ...
• Influenza history: recurring pandemic episodes ...
  - 1918: Spanish flu, H1N1
  - 1957: Asian flu, H2N2
  - 1968: Hong Kong flu, H3N2

• H5N1 emergence (?)
  - Preclinical viremia: does occur ...
  - Experimental studies: BSL-3 plus (!)
Influenza H5N1: Results

- Vapor heating
- Solvent-detergent
- Pasteurization
- Low pH @ elevated temperature

→ effectually inactivate H5N1 influenza
  similar to other lipid-enveloped viruses tested

- Re-assurance of safety margins for plasma derivatives

- Against ALL pandemic influenza candidates (H7N9 ... )
A Current Challenge: Chikungunya

• **Emergence**
  - French Overseas, India: Epidemics of substantial size
  - United States: large parts infested competent mosquito vector, i.e. Aedes albopictus
A Current Challenge: Chikungunya

• **Emergence**, Europe / Italy:
  - 205 cases of infection with CHIKV between July 4 and Sept 27, 2007
  - Presumed index case: a man from India who developed symptoms while visiting relatives.
  - Phylogenetic analysis: high similarity between the strains found in Italy and those identified during an earlier outbreak on islands in the Indian Ocean.
  - The disease was fairly mild in nearly all cases, with only one reported death.
Chikungunya: Inactivation

- Pasteurization
- Vapor heating
- Solvent-detergent
- Low pH @ elevated temperature

→ effectively inactivate CHIKV similar to other LE-viruses tested

- Re-assurance of safety margins for plasma derivatives
Porcine Circovirus (PCV): Not a lot of experience ...

- FDA, EMA
  - "extraneous virus detected ...",
  - DNA from porcine circovirus 1 (and infectivity, too)
  - no evidence ... safety concern

Infectivity study: Rotarix

- Mock-infected Swine testis (ST) cells yielded negative results
- Cells inoculated with Rotarix (GSK1 and GSK 2) showed increasing PCV1 DNA quantities, measured by 529 nt qPCR
- Particle-associated PCV1 DNA was also produced
- (Not shown) Particle-associated PCV1 DNA was also detected in cell culture supernatants at day 3 and day 6
- (Not shown) Inoculation of cell lysates from day 6 of vaccine cultures onto fresh ST cells revealed 2-4 log increases in PCV1 DNA quantity after 3 additional days in culture
- We have not formally evaluated the sensitivity of this assay

European Medicines Agency statement on new information on Rotarix oral vaccine

European Medicines Agency

22 March 2010
EMA/189050/2010
Press Office

Press release

European Medicines Agency statement on new information on Rotarix oral vaccine

U.S. Food and Drug Administration

Home > News & Events > Newsroom > Press Announcements

FDA NEWS RELEASE

For Immediate Release: March 22, 2010

Media Inquiries: Shelly Burgess, 301-796-4651; shelly.burgess@fda.hhs.gov
Consumer Inquiries: 888-INFO-FDA

Components of Extraneous Virus Detected in Rotarix Vaccine; No Known Safety Risk
Porcine Circovirus

- Circoviridae, Circovirus; PCV1, PCV2
- 17 nm – smallest viruses of eucaryotes
- ss ambisense DNA, approx. 1,800 nucleotides, encodes for two ORFs, rep and cap
- non-lipid enveloped, icosahedral
- BSL 1 organism (apathogenic)
- **Model?** → **NOT** closely related to anything
- PCV: not easily available (w/o antibody !) either
• Transfection of PS cells with PCV-1 genome

• Cells incubated (11 days, 2 passages) harvested (2 x ultracentrifugation); pellet resuspended and analyzed by PCV PCR: **PCV-1 @ >10^{10} geq/ml**

• **PCV-1 infectivity ?**
  - Harvest of supernatant after 1, 3 and 6 days of incubation
  - Low speed spin for removal of cell debris
  - Pelleting of virus by ultracentrifugation (UC)

![PCV-1 stock](image)
Model & target virus: **comparable** inactivation kinetics

![Low pH Inactivation](image)
Model & target virus: clearly different inactivation capacity / kinetics
• Model & target virus: \textbf{totally different} inactivation kinetics

![UV Inactivation](image)

\begin{itemize}
  \item Reduction Factor: $\log_{10} \pm \text{SEM}$
  \item UV dose: mJ/cm$^2$
  \item PCV-1: black line
  \item PPV: gray line
  \item MMV: blue line
\end{itemize}
PCV and Model Viruses

• Molecular virology
  - de novo expression of virus
  - RT-PCR quantification

• Disappointment for the model virus concept (another ... )
  - Little is known, when little is known.
    (no studies with even related viruses available for predictions.)
Emerging Concerns: HEV

- Non-enveloped ss RNA virus (7.8 kb), 27-34 nm in size
- Clinical: similar to Hepatitis A; risk groups: pregnant woman (?)
- Transfusion transmission demonstrated (Japan), blood donor RNA-positivity detected (Germany)
- Plasma Donors (Sweden, Germany, US)
  - RNA ~ 1:5,000, <5.68 log geq/ml
- Plasma Manufacturing Pools (EU, US, Middle East, SE Asia)
  - RNA ~ 10%, <3 log geq/ml

Source: RKI

**Human HEV cases notified in Germany**

![Graph showing HEV cases notified in Germany](image)

• Swine Fecal Isolates, Genotype 3 or 4

• Infectivity assay: A549 cell culture & RNA detection by RT-PCR

• BUT: Results – quite variable …
  - Matrix effects
  - Isolate differences (35N: RF = 1.1 to >3.6)

**ORIGINAL PAPER**

Extent of hepatitis E virus elimination is affected by stabilizers present in plasma products and pore size of nanofilters
• Transfection of rHEV genome (~4x the size of PCV !), into HepG2/C3A or PLC/PRF/5 cells

• Propagation of recombinant HEV, → biosafety level III

• Infectivity readout by PCR assay
Alternative: Model Viruses

- HEV: Taxonomically between (capsid protein alignment)
- Feline Calicivirus (FCV), Calciviridae
  - Propagation on CRFK cells, clear cpe: 100% after 3d
- Hepatitis A virus (HAV) / Picornaviridae
  - Propagation on BSC-1, titration on FRhK-4 (14d assay)

[Image: Phylogenetic tree of Caliciviridae showing HEV and FCV in relation to other viruses]
Results and Conclusions

• **MODEL VIRUSES**
  - Robust concept, from and for the early days

• **VERIFICATION STUDIES**
  - Risk-based approach: difficult decisions need support

• **MOLECULAR VIROLOGY**
  - A quite different skill set; but quite different opportunities, too

• **CONCLUSION**
  - After many (!) years – still no dull moments around viral safety