High Performance Virus Filtration

Scale-up and Manufacture of a new 20nm filtered Intravenous Immunoglobulin

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Virus Filtration of IVIG

- Objectives & Background
- Selection of Virus Filter
- Process considerations
- Virus clearance
- Scale-up & accelerated implementation
Objectives

License new IVIG formulation incorporating virus filtration for improved safety:

- Regulatory Guidelines
- Non-enveloped virus [=NEV] clearance (HAV, Parvovirus B19)
- Selection of most suitable virus filter
- No/minimal impact on product (function/yield)
- Minimum impact on process time
- Scalable & automatable
- Economically viable
Background

- BPL’s current licensed IVIG (‘Vigam’) has 2 virus reduction stages (SD and low pH incubation)
- Reformulating for improved stability etc and undertaking clinical trials in PID & ITP
- Opportunity to enhance safety by introduction of 3rd virus reduction step
- Review of options – virus filtration viable
- Strategy to introduce virus filtration into all IgG products (IVIG, SCIG & Hyperimmunes)
CHMP Guidelines

‘Note for Guidance on Virus Validation Studies: CPMP/BWP/268/95 (1996):

6.1.(iii): ‘Log reductions of the order of 4 logs or more are indicative of a clear effect’


3.3 d: ‘Virus Removal by Virus Filtration’ – critical parameters for virus removal…..aggregation of virus spike

5.2.1.e: ‘Immunoglobulins: …. addition of a specific virus inactivation/removal step for non-enveloped viruses is …. an objective.’
Why Choose Virus Filtration?

• Orthogonal virus reduction step

• Performed under mild conditions (pH, pressure, osmolality, temp.)

• Doesn’t require stabilisers or other chemical agents

• Doesn’t compromise biological integrity & adverse biologic or immunological reactions unlikely
Virus Filtration of IgG: 15/20nm

- Ortho: anti-D IMIG: Viresolve 180 [PPV 3.3, EMC 4.1 & HAV > 5.1log] - TFF format

- ZLB: Rhophylac anti-D IVIG: Planova 15

- Cangene: WinRho SDF anti-D: Planova 20

- Sanquin/FRC: Low pH/pepsin + Planova 15N [>6 B19 & HAV, >3.4/≤4.3 CPV]

*Clearance of small Non-Enveloped Viruses [NEV] in Hyperimmune IgGs & Medium Scale Processes*
Virus Filtration of IgG: 35/50nm

- Massachusetts Biologic Labs: - 75/35/35nm

- ZLB - Sandoglobulin/Carimune NF – 50nm

- Baxter – Kiovig/Gammagard NF – 35nm

**Clearance of small Non-Enveloped Viruses [NEV] dependent on presence of antibody to specific viruses – ie. removal of antibody-virus immune complex.**
But…………………………

• Incomplete clearance of HCV by 35nm virus filtration in IgG:
  “Nanofiltration of immunoglobulin with 35nm filters fail to remove substantial amounts of HCV”
Virus Filtration - Considerations for Licensing

- Selection of Virus Filter – knowledge of media / recognised supplier
- Availability of ‘generic’ virus validation data
- Target viruses – emphasis on non-enveloped viruses
- Availability of model viruses and assays for quantitation
- Availability of suitable scale-down process models
- Product characterisation - analytical tests
- Product recovery & process consistency
- Confirmation of filter integrity
- Clinical trials .......... (if required ....)
## Selection of Virus Filter #1: Supplier’s Claims

<table>
<thead>
<tr>
<th>Company</th>
<th>Filter</th>
<th>Prefilter</th>
<th>Claims (LRV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asahi-Kasei</td>
<td>Planova 15N</td>
<td></td>
<td>&gt;6.2 parvovirus</td>
</tr>
<tr>
<td></td>
<td>Planova 20N</td>
<td>Planova 75N</td>
<td>&gt;6.7 polio</td>
</tr>
<tr>
<td></td>
<td>Planova 35N</td>
<td></td>
<td>&gt;4.3 parvovirus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;5.4 EMC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;5.9 BVDV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;7.3 HIV</td>
</tr>
<tr>
<td>Millipore</td>
<td>Viresolve NFP</td>
<td>0.1μ</td>
<td>&gt;4 (\phi)X-174 bacteriophage</td>
</tr>
<tr>
<td></td>
<td>Viresolve NFR</td>
<td></td>
<td>&gt;6 Retrovirus</td>
</tr>
<tr>
<td>Pall</td>
<td>Ultipor DV20</td>
<td>DVD 0.1μ</td>
<td>&gt;3 PP7 bacteriophage</td>
</tr>
<tr>
<td></td>
<td>Ultipor DV50</td>
<td>0.1μ</td>
<td>&gt;6 PR772 bacteriophage</td>
</tr>
<tr>
<td>Sartorius</td>
<td>Virosart CPV</td>
<td>0.1μ</td>
<td>&gt;4 PR772 bacteriophage</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;6 Retrovirus</td>
</tr>
</tbody>
</table>

Manufacturer’s literature + Burnouf T & Radosevich, M., Haemophilia (2003), 9, 24-37
Selection of Virus Filter #2

Other Factors:
- Cost / m²
- Automatable process system
- Filter format:
  - Pleated membrane vs hollow fibre
  - Tangential flow vs ‘Dead end’
  - Disposable capsule etc
- High surface area cartridges
- Integrity test: convenience & automatable
- Sanitary/sterile operation
Preliminary Selection

- Pall DV50 [50nm] filter selected initially on basis of process performance and economics
- Proven clearance of LEV with potential clearance of NEV such as HAV and B19 dependent on immune complexes
- Borderline clearance of HAV of $\leq 4$ logs in presence of high levels of HAV antibody
- Poor clearance of CPV of $\sim 2$ logs
- Inconsistent clearance of NEVs for which antibodies present in product (HAV & B19)
- Clearance not predictable for other adventitious viruses

**Regulatory advice:** Clearance of non envelope viruses not sufficiently robust with $\leq 4$ logs
# Non-enveloped virus clearance by DV50

<table>
<thead>
<tr>
<th>Virus</th>
<th>Intermediate</th>
<th>Virus Elimination (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DVD Prefilter</td>
</tr>
<tr>
<td>HAV</td>
<td></td>
<td>1.3 - &gt;1.9</td>
</tr>
<tr>
<td>CPV</td>
<td>Post SD</td>
<td>ND.</td>
</tr>
<tr>
<td></td>
<td>purified IgG</td>
<td>0.1</td>
</tr>
<tr>
<td>Polio-1</td>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>EMC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND: not done
Selection of Alternative Virus Filter

• Review of alternative filters for enhanced Non Enveloped Virus clearance

• Operational performance & process economics

• *Pall DV20* filter selected……
## Performance Comparison of Virus Filters

<table>
<thead>
<tr>
<th>Virus Filter</th>
<th>Initial Flux (L/m²h)</th>
<th>Vmax (L/m²)</th>
<th>Scale-up prediction S.A./batch (m²)*</th>
<th>Cost Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pall DV20</td>
<td>8</td>
<td>450</td>
<td>~40</td>
<td>1.0 x</td>
</tr>
<tr>
<td>Millipore NFP</td>
<td>64</td>
<td>12.2</td>
<td>157</td>
<td>4.2 x</td>
</tr>
<tr>
<td>Asahi Planova 20N</td>
<td>30</td>
<td>93</td>
<td>44</td>
<td>1.5 x</td>
</tr>
<tr>
<td>Pall DV50</td>
<td>46</td>
<td>1200</td>
<td>~9</td>
<td>0.3 x</td>
</tr>
</tbody>
</table>

All tested on Post SD purified IgG intermediate  
* 8 hour process time
Selection of Process Step

Protein conditions
- Optimal protein concentration
- Ionic strength/pH/temperature
- High purity
- Highly monomeric (low aggregate)

Process/operational issues
- Prefiltration
- Product segregation
- Process time and hold stages
Optimal Protein Concentration

Protein concentration Optimum for Virus Filtration: Pall DV20 & Millipore data

Estimated membrane area (m²)

IgG concentration (g/L)

* Adapted from Kern & Krishnan, Pharm Tech Europe, Dec 2006, 29
Selection of Process Step

Virus filter step

Plasma

Ethanol Fractionation

Fraction II

DEAE Sephadex adsorption

SD Incubation

CM Sepharose chromatography

Ultrafiltration

Formulation

Filling

Terminal low pH incubation
IVIG Manufacturing Process + VF

Plasma

Ethanol Fractionation

Fraction II

DEAE Sephadex adsorption

SD Incubation

CM Sepharose chromatography

Virus Filtration

Ultrafiltration

Formulation

Filling

Terminal low pH incubation

Process segregation
Post-CM Sepharose

- CM Sepharose contributes to virus safety (low pH and clearance)
- Column/elution conditions make a very effective prefilter!
- High purity and monomeric state of eluted IgG
- Suitable protein concentration of eluted IgG @10-15g/L
- Appropriate buffer conditions (ionic strength, pH)
- Low pH helpful for reduced dimer/high monomer
# Process Robustness:
## Design of Experiment Study Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.5-5.0</td>
</tr>
<tr>
<td>Protein concentration (g/L)</td>
<td>9-15</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>10-25</td>
</tr>
</tbody>
</table>

DoE design: 3 factors, 10 runs, 1 centrepoint

Using Umetrics Modde (ver 7) software
**DoE Contour Map for DV20 Virus Filter**

Robustness studies

Investigation: Repeat DV20 robustness Oct06 (PLS, comp.=3)

Response Surface Plot

**Process time DV20**

Optimal conditions: pH no effect

pH = 4.73
Flux rates of DV20 Virus Filter (lab-scale)
Flux rates during DoE Robustness Testing

![Graph showing flux rates over time](image-url)
Flux rates during Robustness and Virology Studies

![Graph showing flux rates over time for different viruses (CPV and HAV).](image-url)
DV20 Virus Filtration: Flux v LRV

- HAV > 3.5 LRV
- B19 > 5.0 LRV
- CPV > 4.3 LRV

Filtrate flux (kg.m\(^{-2}\).h\(^{-1}\))

Virus log reduction value (-)

Time (h)
Comparison of Non-Enveloped Virus data for 50nm and 20nm filters

<table>
<thead>
<tr>
<th>Virus</th>
<th>Virus Elimination (Log)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50nm (DV50)</td>
</tr>
<tr>
<td>Polio</td>
<td>3.0 – &gt;4.9</td>
</tr>
<tr>
<td>HAV</td>
<td>2.2 - &gt;4.3</td>
</tr>
<tr>
<td>CPV#</td>
<td>1.8 – 2.1</td>
</tr>
<tr>
<td>PPV#</td>
<td>Not tested</td>
</tr>
<tr>
<td>B19</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*Data from plaque infectivity assays, except Parvovirus B19 which was by Q-PCR

# Animal virus - no antibodies present
## Comparison of DV50 and DV20 intermediates

<table>
<thead>
<tr>
<th>Test</th>
<th>Unfiltered Intermediate</th>
<th>DV50 Intermediate</th>
<th>DV20 Intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomer (%)</td>
<td>90.9</td>
<td>91.1</td>
<td>91.5</td>
</tr>
<tr>
<td>Dimer (%)</td>
<td>9.0</td>
<td>8.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Aggregate (%)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Fragment (%)</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>ACA [C1q] (CH50/mg)</td>
<td>0.56</td>
<td>0.34</td>
<td>0.54</td>
</tr>
<tr>
<td>Subclass (%1:2:3:4)</td>
<td>63, 33, 3.6, 0.4</td>
<td>64, 31, 4.1, 0.4</td>
<td>63, 32, 4.1, 0.4</td>
</tr>
<tr>
<td>IgA (µg/ml)</td>
<td>1.17</td>
<td>1.24</td>
<td>1.19</td>
</tr>
<tr>
<td>IgE (ng/ml)</td>
<td>15.5</td>
<td>14.8</td>
<td>15.1</td>
</tr>
<tr>
<td>Plasmin/ogen (IU/ml)</td>
<td>0.07/ND</td>
<td>0.06/ND</td>
<td>0.08/ND</td>
</tr>
<tr>
<td>Fc function / Antibody Specificity</td>
<td>Rubella SRH, Measles, Diphtheria, Hib, Pneumonia (PCP) : all showed equivalent titres</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Manufacturing Process Scale-Up

BPL Manufacturing Facility
Accelerated Programme
Disposable Virus Filter system:

**Pros:**
- Simple manifold design
- Simple operation (pump, pressure gauges, vessels)
- No Capital expenditure - lower risk
- Rapid and straightforward scale-up
- Rapid licensure and time-to-market
- Sanitary operation option
- Minimal process validation requirements

**Cons:**
- Manual operation
- Manual integrity testing of individual capsules – pre and post use is time consuming
- Cost – marginal difference
Scale-up Considerations

- Process time/flux rates
- Feed concentration
- Pre-filtration
- Pre-use sterilisation/sanitary operation
- Membrane wetting
- Integrity testing arrangements
- Conditioning of filter media
- Product recovery (post-use flush)
- Space & product segregation requirements
Comparison of process parameters for 20nm filtration at laboratory and production scale

<table>
<thead>
<tr>
<th>Scale</th>
<th>Area (m²)</th>
<th>Protein flux (g/m².h)</th>
<th>V_{max} (L/m²)</th>
<th>Initial Flux (L/m².h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab</td>
<td>0.0014</td>
<td>115</td>
<td>446</td>
<td>8</td>
</tr>
<tr>
<td>Process</td>
<td>40</td>
<td>160</td>
<td>500</td>
<td>13</td>
</tr>
</tbody>
</table>

Scale-up Factor: 28,500 x (Area)
Virus Filter Capacity vs Process time

* Batch size 1600L
No. of DV20 Cartridges (20’’) v Batch Size and Process Time
Sterile Operation of Virus Filters

- Industry trend to sanitize or sterilise virus filter to reduce bioburden
- Options:
  - Gamma-irradiation
  - Autoclaving
  - Steam-in-place
- Opted not to sterilise filters as no risk to sanitary operation
  - Very low bioburden after CM Sepharose column
  - Pre-use flushing (20% ethanol, buffer etc)
  - Pre & post monitoring of bioburden/LAL testing

Is sterile/sanitary operation necessary?
IgG Recovery across Virus Filter: Full scale production process

<table>
<thead>
<tr>
<th>Virus Filter System</th>
<th>% Average Recovery (n)</th>
<th>Protein Flux (g/m².h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVD / DV50</td>
<td>99.0 (3)</td>
<td>380</td>
</tr>
<tr>
<td>DVD / DV20</td>
<td>97.7 (2)</td>
<td>160</td>
</tr>
</tbody>
</table>
IVIG Manufacturing Process + VF

- Plasma
- Ethanol Fractionation
- Fraction II
- DEAE Sephadex adsorption
- SD Incubation
- CM Sepharose chromatography

**Virus Filtration**

- Ultrafiltration
- Formulation
- Filling
- Terminal low pH incubation
CM Sepharose column (400L)

DVD/DV20 Disposable Virus Filter System

High Area DV20 Filters

DVD Prefilters
Virus Filtration of IVIG: automated system
# Overall Virus Reduction for new IVIG including DV20 filter

<table>
<thead>
<tr>
<th>Virus</th>
<th>Virus Elimination (Log)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solvent/Detergent</td>
<td>20nm virus filtration</td>
</tr>
<tr>
<td>HIV-1</td>
<td>&gt;6.8</td>
<td>&gt;5 *</td>
</tr>
<tr>
<td>Sindbis [HCV model]</td>
<td>&gt;6.7</td>
<td>&gt; 7.4 *</td>
</tr>
<tr>
<td>BVDV [HCV model]</td>
<td>pending</td>
<td>&gt; 4.6</td>
</tr>
<tr>
<td>IBR [Herpes model]</td>
<td>pending</td>
<td>&gt; 5.8 *</td>
</tr>
<tr>
<td>HAV</td>
<td>NT</td>
<td>&gt;4.1</td>
</tr>
<tr>
<td>CPV [PPV]</td>
<td>NT</td>
<td>&gt;4.3 [&gt;4.8]</td>
</tr>
<tr>
<td>B19</td>
<td>NT</td>
<td>&gt;5.0</td>
</tr>
</tbody>
</table>

* Extrapolated from DV50 data – DV20 results pending

NT: not tested
Virus Filtration: Conclusions

• Enhanced product safety of IVIG by 20nm Virus Filtration with >4 logs HAV/parvovirus B19
• Reduced risk of ‘new’ small viruses & adventitious agents
• Not dependent on ‘antibody enhanced mechanisms’ and presence of relevant Abs
• Implemented for large scale manufacture of IVIG (~6000L)
• Good flux rates @ 160g per m² per hour
• Economic financial case & realistic process time
• Accelerated implementation possible - disposable systems
• Clinical trials requirements? - ICH Q5E guidelines etc
• New IVIG with triple Virus Reduction steps
Acknowledgements

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- Co-authors: Shirley Stagg & Peter Roberts
- Tammy Lang, Amy Shackell, Julia Burrows