Solvent-Detergent for Virus Inactivation: An Evolving Technology

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Background
Enveloped Viruses

• Defining property-inactivation by
  • Detergent or
  • Organic solvent
• A property later shown to be due to the presence of an outer lipoprotein layer
• Main target viruses in plasma products
  • HIV, HBV, HCV, WNV
The SD Technology

• Uses a combination of solvent and non-ionic detergent to destroy NEV
• Developed by NYBC & widely used since 1985 for plasma products
• Proven by laboratory, animal & clinical use
• General applicable to full range of plasma and other biologicals eg mAbs
Method Variations

- **Solvent**
  - Ether > Tri-n-butyl phosphate (TnBP)
- **Detergent**
  - Sodium cholate >
  - Polysorbate (Tween) 80
    or Triton X-100
Conditions

- **TnBP**: 0.3% or 1% for plasma
- **Temperature**: 20, 25 or 37°C (or 4°C)
- **Time**:
  - 6 hr for Tween 80
  - 1 hr for Triton X-100 or Tween 20
Virus Validation

• Relevant Virus models (CPMP Guidelines)
  • HIV
  • HCV/ WNV: BVDV or Sindbis
  • Herpes: PSR, IBR or HSV
• Tested at standard manufacturing conditions and limits
Virus Inactivation
Virus Inactivation and Resistant Viruses

Factor VIII: SD containing Triton X100

<table>
<thead>
<tr>
<th>Virus</th>
<th>0.5hr</th>
<th>1hr</th>
<th>2hr</th>
<th>6hr</th>
<th>24hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sindbis</td>
<td>4.0</td>
<td>6.1</td>
<td>7.1</td>
<td>7.1</td>
<td>7.1</td>
</tr>
<tr>
<td>HSV-1</td>
<td>&gt;5.6</td>
<td>&gt;5.6</td>
<td>&gt;5.6</td>
<td>&gt;5.6</td>
<td>&gt;5.6</td>
</tr>
<tr>
<td>VSV</td>
<td>4.1</td>
<td>&gt;5.0</td>
<td>&gt;5.5</td>
<td>&gt;5.5</td>
<td>&gt;5.6</td>
</tr>
<tr>
<td>HIV-1</td>
<td>6.3</td>
<td>6.3</td>
<td>6.3</td>
<td>6.3</td>
<td>6.3</td>
</tr>
<tr>
<td>VacV</td>
<td>0.3</td>
<td>0.9</td>
<td>1.0</td>
<td>2.4</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Resistant Viruses

- Using a more vigorous detergent increases the vaccinia inactivation level
  - Triton X-100 or Tween 20
- However the VI rate remains slower
- Relevance of vaccinia as a model virus
  - Very unusual structure
  - Of concern in specific situations eg Vac IgG
Resistant Viruses

• Other viruses reported to be more resistant:
  • VSV Vesicular stomatitis virus
  • PRV Pseudorabies herpes virus
• Not found to be true for all products
• Product, conditions, virus strain may influence
Some Manufacturing Issues
Some Manufacturing Issues

• Mixing SD+product to homogeneity
  • Validation to identify minimum time
  • Two vessel method or static mixer
• Segregation to prevent ‘re-contamination’
  • By material from an early stage
  • ‘Virus secure area’
• Removal of Solvent/detergent
  • Placement in the process or by a column
Mixing and Segregation

**Primary Mixing Stage**
In First Vessel

- SD added to product

**Formal Inactivation Step**
In Second Vessel

- Transfer
- VIRUS SECURE AREA
Alternative detergents
Changing the Solvent/Detergent Chemistry

- Greater/more rapid virus inactivation
- Concerns over toxicity
- Advantages to the process/product
  - Easier to remove
  - More compatible with product formulation
Formulation of Optivate

• Factor VIII/VWF Optivate® final product has poor solubility
• Requires a non-ionic detergent
• Preferred detergent based on safety profile and stabilising properties
  • eg Pluronic F127, F68; Tween 20
Detergent Selection

Solvent/Detergent Step

Detergent
Tween 80, Triton X-100
or Tween 20

Final Formulated Product

Detergent
Tween 20
Detergent Selection

- Advantages to one detergent only for S/D step and formulation
  - Reduces QC activities
  - Removing the S/D-detergent by the process is less critical - it is present in the final product (0.2%)
# Virus Inactivation by Different SD Detergents

<table>
<thead>
<tr>
<th>Detergent</th>
<th>Virus (Sindbis) Inactivation (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 min</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td>0.7</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>&gt;5.1</td>
</tr>
<tr>
<td>Polysorbate 20</td>
<td>&gt;6.0</td>
</tr>
<tr>
<td>Pluronic F127</td>
<td>0.0</td>
</tr>
<tr>
<td>Pluronic F68</td>
<td>-0.1</td>
</tr>
</tbody>
</table>

# Inactivation of a Range of Viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>2 min</th>
<th>10 min</th>
<th>30 min</th>
<th>1hr</th>
<th>5 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>5.1</td>
<td>5.3</td>
<td>5.5</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Sindbis</td>
<td>6.5</td>
<td>6.5</td>
<td>6.5</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>SFV</td>
<td>6.8</td>
<td>6.8</td>
<td>6.8</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>HSV-1</td>
<td>4.6</td>
<td>4.6</td>
<td>4.6</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>VSV</td>
<td>5.7</td>
<td>5.7</td>
<td>5.7</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>VacV</td>
<td>2.0</td>
<td>3.3</td>
<td>3.8</td>
<td>4.0</td>
<td>4.6</td>
</tr>
<tr>
<td>EMC&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

<sup>A</sup> non enveloped virus
Conclusions

• Some alternative non-ionic detergents can be used in SD
  • But not all
• Polysorbate 20 is effective
• Virus inactivation is rapid and comparable to Triton X-100
Detergent Alone
Using Detergent Alone

• Enveloped viruses defined as sensitive to both solvent or detergent
• Detergent alone is used for
  • Viral glycoprotein purification
  • As (part) of viral vaccine preparation
Using Detergent Alone

- Less vigorous detergents such as cholate or Tween 80 are enhanced by TnBP in the original SD procedure.
- Later variations used faster acting detergents eg Triton X100 or Tween 20.
- Is solvent needed in these cases?
## Using Detergent Alone

<table>
<thead>
<tr>
<th>Detergent</th>
<th>Virus</th>
<th>Inactivation (log)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Triton X-100</td>
<td>HSV-1</td>
<td>&gt;5.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MuLV</td>
<td>&gt;3.8</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>RSV</td>
<td>&gt;5.4</td>
<td>2</td>
</tr>
<tr>
<td>0.5% Tween 80</td>
<td>HSV-1</td>
<td>0.4</td>
<td>1</td>
</tr>
</tbody>
</table>

1: J Virol 1987; 61; 3688-3693
2: Devel Biol Stand 1991; 75: 159-169
Using Detergent Alone

• A vigorous detergent alone is likely to be effective but data is limited
  • Full data on virus range, robustness etc required
• The traditional SD approach is so well established now
Robustness of the process and limits
Virus Inactivation in Different Batches

<table>
<thead>
<tr>
<th>Batch</th>
<th>Virus (Sindbis) Inactivation (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 min</td>
</tr>
<tr>
<td>303</td>
<td>nd</td>
</tr>
<tr>
<td>305</td>
<td>nd</td>
</tr>
<tr>
<td>368</td>
<td>nd</td>
</tr>
<tr>
<td>378</td>
<td>nd</td>
</tr>
<tr>
<td>254</td>
<td>nd</td>
</tr>
<tr>
<td>247</td>
<td>5.7</td>
</tr>
<tr>
<td>245</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Optivate and SD with Tween 20
## Effect of Process Parameters on Virus Inactivation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conditions&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Virus Inactivation (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 min</td>
</tr>
<tr>
<td>TnBP/Poly 20</td>
<td>0.12/0.62 %</td>
<td>&gt;5.3</td>
</tr>
<tr>
<td></td>
<td>0.46/2.46 %</td>
<td>5.3</td>
</tr>
<tr>
<td>Protein (&lt;A&lt;sub&gt;280&lt;/sub&gt;)</td>
<td>0</td>
<td>&gt;5.6</td>
</tr>
<tr>
<td></td>
<td>5.4</td>
<td>&gt;5.4</td>
</tr>
<tr>
<td></td>
<td>20.5</td>
<td>&gt;5.2</td>
</tr>
<tr>
<td>Temperature</td>
<td>20ºC</td>
<td>&gt;5.2</td>
</tr>
<tr>
<td></td>
<td>30ºC</td>
<td>&gt;5.2</td>
</tr>
</tbody>
</table>

<sup>A</sup> Standard conditions: TnBP/Poly 20 0.3/1.0%, protein 8-11, at 25ºC

Virus used: Sindbis
## Effect of Process Parameters on Virus Inactivation

### Combined Worst-Case Conditions

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Virus Inactivation (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 min</td>
</tr>
<tr>
<td>Sub-Optimal(^{A})</td>
<td>3.1</td>
</tr>
<tr>
<td>Standard(^{B})</td>
<td>&gt;6.7</td>
</tr>
</tbody>
</table>

\(^{A}\)High protein (20.5 A\(_{280}\)), low SD (0.4/0.59%) at 20°C

\(^{B}\)Standard protein (10 A\(_{280}\)) and SD (0.3/1.2 %) at 25°C

Virus used: Sindbis
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Virus Validated Limits</th>
<th>Manufacturing Control Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>TnBP/poly 20</td>
<td>0.12-0.46%</td>
<td>0.25-0.40%</td>
</tr>
<tr>
<td></td>
<td>0.59-2.46%</td>
<td>0.88-1.45%</td>
</tr>
<tr>
<td>Protein A$_{280}$</td>
<td>0-20.5</td>
<td>&lt;20.5</td>
</tr>
<tr>
<td>Temperature°C</td>
<td>20-30</td>
<td>23-27</td>
</tr>
<tr>
<td>Time min</td>
<td>2-10</td>
<td>&gt;60</td>
</tr>
</tbody>
</table>
PPTA Robustness Data Set

- Data collected for a range of products, SD systems and process parameters
- Confirmed to consistently inactivate a wide range of viruses
- Most parameters had no effect except for SD concentration

Conclusions
Conclusions

- Long history of >20 yrs in clinical use with various plasma products
- Effectiveness with a wide range of enveloped viruses shown by virus modelling studies
- Robustness to manufacturing variables shown
Conclusions

• SD procedure was developed as a toolbox of associated procedures

• Some standard procedures in regular use but
  • Further modifications are possible where an advantage conferred
  • Evaluation and validation required
Summary
Acknowledgments

Thanks to staff at BPL that have worked on the SD VI over the years inc:
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