Vaccine efficacy of FMD virus-like particles produced by the baculovirus expression system

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Improving VLP stability

PROTECTIVE

NON-PROTECTIVE

Storage or heat or low pH
Example: Stabilization of the VP2-VP2 interface

- **Not stabilized**: VP2 (wt)
- **Stabilized**: VP2-93Cys, VP2-93Phe

- Cysteine bridge
- Electrostatic interactions
Optimization of VLP production

• High yield of VLPs important to get
  • affordable vaccines
  • multivalent vaccines

• How to improve the yield
  • Optimization of the baculovirus expression system
  • Stabilization of the capsids

• Yield of FMDV protein increased $\sim 10^2$ fold
Generation of stabilized VLP based on 4 serotypes

Preparation of VLPs

Empty capsids

Inactivated FMD virus

A  O  Asia1  SAT2

200 nm
Efficacy of VLP vaccines in cattle

- 5-6 cattle per group
- VLP vaccine contains same adjuvant as MSD current vaccine (double oil emulsion)
- Homologous challenge at 3 wpv

<table>
<thead>
<tr>
<th>Serotype / vaccine</th>
<th>VN titres $\geq 1.5\log_{10}$</th>
<th>Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>O VLP (stabilized)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>O classic</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>A VLP (stabilized)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>A classic</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Asia1 VLP (stabilized)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Asia1 classic</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>SAT2 VLP (stabilized)</td>
<td>Yes</td>
<td>Trial scheduled</td>
</tr>
<tr>
<td>SAT2 classic</td>
<td>Yes</td>
<td>Trial scheduled</td>
</tr>
</tbody>
</table>
Product development aspects

- VLP vaccine
  - Product registration
  - Cell and virus seeds (EA test)
  - Batch release test (potency)
  - Upstream & downstream processing
  - Vaccine stability
  - Animal safety and efficacy
Large scale production of VLP

1. Insect cells
2. Baculovirus
3. Cell cultivation
4. Baculovirus inoculation and VLP expression
5. Harvest culture
6. Inactivation of recombinant baculoviruses
7. Concentration
8. (multivalent) vaccine formulation
What is the product profile of a VLP vaccine?

<table>
<thead>
<tr>
<th>Target Product Profile</th>
<th>Current Killed Virus vaccine</th>
<th>New VLP vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coverage</strong></td>
<td>Multi-strain formulation</td>
<td>Multi-strain formulation</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>2-8°C</td>
<td>2-8°C</td>
</tr>
<tr>
<td><strong>DIVA (marker vaccine)</strong></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Response to new strains</strong></td>
<td>Slow</td>
<td>Fast</td>
</tr>
<tr>
<td><strong>Production in low containment</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Batch release</strong></td>
<td>Cattle potency</td>
<td><em>In vivo or in vitro</em> potency?</td>
</tr>
</tbody>
</table>
Benefits *in vitro* potency test compared to batch release test using animals:
- Cheaper
- Quicker (1 day vs weeks/months)
- Fullfils the 3R principles of animal testing (replacement, reduction, refinement)
- Less variation (risk of batch failure is reduced)
- No need for high containment

Current *in vivo* potency test:

\[X \, \mu g \text{ of } A \text{ strain}\]
\[Y \, \mu g \text{ of } O \text{ strain}\]
\[Z \, \mu g \text{ of } \text{Asia1 strain}\]

Requirement for high containment and cell-adapted viruses

- VN test A
- VN test O
- VN test Asia1
In vitro potency test

Requirements of an *in vitro* potency test:
- Can differentiate between 146S and 12S
- Can differentiate between serotypes

Options:
- HPLC
- ELISA
- Other?

**PROTECTIVE**

**NON-PROTECTIVE**

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<table>
<thead>
<tr>
<th>OD 450 nm</th>
<th>Dilution</th>
</tr>
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<tbody>
<tr>
<td>146S particles</td>
<td></td>
</tr>
<tr>
<td>12S particles</td>
<td></td>
</tr>
</tbody>
</table>

[Graph showing OD 450 nm for heated and unheated samples]
Summary

- Virus-like particles can be produced by baculovirus expression
- Yield and stability of capsids can be significantly improved
- Technology works for at least serotypes A, O, Asia1 and SAT2
- To maximize the benefit of the VLP technology (i.e. no high containment, quick response to market, affordable), an \textit{in vitro} batch release test is desirable.

\textit{\rightarrow} Virus-like particles have the potential to be a commercially viable alternative to conventional killed virus vaccines
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