USE OF SPECIFIC LLAMA ANTIBODIES FOR QUALITY CONTROL TESTING OF FMD VACCINES

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FMDV particles: quantification and monitorization

Neutralizing antibodies

146S ✓
75S ✓
12S ✗

Quantification methods:

- Affinity chromatography, S-E chromatography, UV spectrophotometry, thermo fluor assay, sucrose density gradient:
  - 146S (inactivated vaccines)
  - Final production of a vaccine

- DAS ELISA based on VHH antibodies:
  Quantification of the content of antigen and integrity:
  - 146S (inactivated vaccines)
  - 75S (subunit vaccines)
  - 12S
  - in different steps of production (optimization of expression, storage conditions)
  - detection of antigen expressed at small scale: quick identification of new vaccine candidates
VHH llama Ab (from CVI, Lelystad)

Camel Heavy-Chain antibody

VHH or Nanobody

Conventional antibody

75 KDa

13 KDa

150 KDa
VHH llama Ab (from CVI, Lelystad)

Camel Heavy-Chain antibody

VHH or Nanobody

Conventional antibody

O1 Manisa

M170: 146S/75S
M3:12S
(Harmsen MM et al., 2011)

Asia 1

M332: 146S/75S
M3:12S
M98: 146/75S + 12S

SAT2

M377: 146S/75S
M311: 146/75S + 12S
**VHH llama Ab** (from CVI, Lelystad)

**Camel Heavy-Chain antibody**
- VHH or Nanobody
- 75 KDa

**VHH or Nanobody**
- 13 KDa

**Conventional antibody**
- Fab 50 KDa
- Fc
- ScFv 25 KDa
- 150 KDa

**O1 Manisa**
- M170: 146S/75S
- M3: 12S
- Harsen MM et al., 2011

**Asia 1**
- M332: 146S/75S
- M3: 12S
- M98: 146/75S + 12S

**SAT2**
- M377: 146S/75S
- M311: 146/75S + 12S

**Homologous or heterologous double antibody sandwich elisa**
- **Standard**: Sucrose purified antigen with a known quantification (UV260nm)
  - 146S elisa: intact antigen
  - 12S elisa: heat treated at 56°C for complete dissociation to 12S
- **Screening of antigen**: quantification by interpolation
O1 Manisa binders: M170 (146S) and M3 (12S)

Standard: O1Manisa BEI (0.5ng/ml)

Homolog sandwich elisas:
- M170 (146S) antigenic site I (GH-loop), trypsin-sensitive, neutralizing ab (Mateu and Verdaguer, 2004)
- M3 (12S) antigenic site II of VP2? (Mateu and Verdaguer, 2004)

- Signal in M170 (146S) elisa disappears when antigen is dissociated
- Signal in M3 (12S) elisa increases when antigen is dissociated

✓ Quantification of antigen by 146S elisa coincides with quantification by UV absorbance
✓ 50% of antigen is 12S but still, it is a good vaccine antigen (146S)

Batch discarded
Detection of O1Manisa VLPs by VHH:

Thermal stability

- %146S
- M170 (146S/75S)
- M3 (12s)
Detection of O1Manisa VLPs by VHH:

**Thermal stability**

![Thermal stability graph showing the stability of O1Manisa BEI, O1M mutant VLPs cell lysate, and O1M mutant VLPs sucrose purified at various temperatures and pH values.]

**pH stability**

![pH stability graph showing the stability of O1Man-BEI and O1Manisa mutant VLPs at different pH levels.]

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Asia1 VHHs binders (M332): 146S and 75S VLPs

qing

M332 146S

- M332 VHHs are subtype specific: 146S
- No cross-reaction with other serotypes

VLPs: cell lysates

- 75S Asia1 Shamir
  - Mut VLPs > wt VLPs
  - T.ni > sf9 insect cells

M3 12S

- 75S Asia1 Shamir
SAT2 VHHs binders (M377)

**M377 (146S)**

**Polyclonal antiSAT2**

Thermal stability: SAT2 wt and mutants
SAT2 VHHs binders (M377)

Cell supernatant: Eritrea and ZIM isolates

- 146S (M377F)
- Polyclonal ab

Detection of 146S SAT2 ZIM > Eritrea SAT2

SAT2 Egypt?
Other applications of VHHs

Control (no primary)  IB11 Mab  M3 VHH (12S)  M170 VHH (146S)

Immunofluorescence on epithelial tissue: Cattle infected with O1Manisa FMDV intradermalingually at 3 dpi  (Nick Juleff)
(Green: O1 Manisa)
(Blue: DAPI)

Rabbit antiO1Manisa  M3 VHH (12S)  M23 VHH (12S+146S)

IBRS2 cells  IBRS2 cells  Goat ZZR cells

IPMA on IB-R2 and goat epithelial ZZ-R cells infected with O1Manisa FMDV at MOI 0.5 for 3.5h
Other applications of VHHs

Immunofluorescence on monolayer

Sf9 cells infected with O1Manisa-His baculovirus at MOI 0.1

Red: O1Manisa
Blue: DAPI
Green: His

M23 VHH (12S+146/75S)

Anti-His Mab
<table>
<thead>
<tr>
<th>Serotype ab binders</th>
<th>Isolates</th>
<th>146S/75S VLPs</th>
<th>12S</th>
<th>Content and integrity of FMDV antigen</th>
</tr>
</thead>
</table>
| O                   | O1 Manisa
O Turkey
O UKG
O BFS          | M170 VHH     | M3 VHH         | ☐ Inactivated vaccines
☐ Subunit vaccines |
| Asia1               | Asia1 Shamir            | M332 VHH     | M3 VHH         | ☐ Antigen detected during the whole process of production
☐ Antigen detected even when produced at small-scale:
  ▪ Optimization of expression (cells, buffers, time of infection)
  ▪ Test the viability of modifications/mutations of antigen |
| SAT2                | SAT2 Zim
Eritrea SAT2
Egypt?        | M377 VHH     | -              | ☐ Storage conditions |
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