Simplified & Stabilised FMD Diagnostic Kits

Brocchi, Ferris et al: IZS-Brescia & IAH-Pirbright

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Institute for Animal Health
Aims

Generate “next generation” ready-to-use kits

For FMDV antigen detection / serotyping

✓ MAb based – sandwich ELISA
✓ Lateral flow devices (LFDs)

For antibody detection

✓ Ab to FMDV serotypes O, A, Asia 1 → MAb based – SPCE
Test Purposes

Antigen detection
- Rapid detection and serotyping of FMDV
- Direct testing of lesion materials
  - Vesicular epithelium or fluid
- Indirect testing of all samples after amplification in cell cultures

Structural protein serology
- Diagnosis of suspect cases
- Estimating prevalence and substantiating freedom
- Estimating vaccine efficacy and population immunity
- Trade certification
Current ELISA formats

- **Serotype specific sandwich ELISA kits (antigen detection)**
  - Rabbit polyclonal capture antibody
  - Guinea pig polyclonal indicator antibody
  - Control antigens are inactivated cell culture grown virus

- **Serology ELISA kits (antibodies to structural proteins)**
  - Some alternative simplified tests using mAbs
Supply of FMD ELISA kits in 2011
**FMDV antigen detection/serotyping kit sandwich ELISA – Monoclonal Antibodies**

**DETECTOR MAb** PO-conjugated

**FMDV Antigen (positive sample)**

**CATCHING MAb** (coated)

<table>
<thead>
<tr>
<th>Catching MAb</th>
<th>Unique conjugated MAb pan-FMDV</th>
<th>FMDV specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>type O</td>
<td>1F10</td>
<td>O</td>
</tr>
<tr>
<td>type A</td>
<td>1F10</td>
<td>A</td>
</tr>
<tr>
<td>type Asia 1</td>
<td>1F10</td>
<td>Asia 1</td>
</tr>
<tr>
<td>Type C</td>
<td>1F10</td>
<td>C</td>
</tr>
<tr>
<td>pan-FMDV</td>
<td>1F10</td>
<td>FMDV</td>
</tr>
</tbody>
</table>

- ✔ Stable at 5°C (> one year)
- ✔ FMDV control antigens already present in relevant wells
- ✔ All stabilized reagents included (conjugates, buffers, chromogen)
## Selection of typing MAbs

<table>
<thead>
<tr>
<th>FMDV Serotype</th>
<th>N. isolates tested</th>
<th>Period covered</th>
<th>Final selection of catching Monoclonal Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A</td>
<td>130</td>
<td>1943-2010</td>
<td>MAbs 4D12 5F6 1C11 N. isolates reactive 122 112 113</td>
</tr>
<tr>
<td>Type O</td>
<td>108</td>
<td>1950-2010</td>
<td>MAbs 3B11 A8 7E1 3C8 N. isolates reactive 105 105 81</td>
</tr>
<tr>
<td>Type Asia 1</td>
<td>53</td>
<td>1954-2009</td>
<td>MAbs 3D8 1F10 4G6 3C6 5E10 N. isolates reactive 53 51 48 43 35</td>
</tr>
<tr>
<td>Type C</td>
<td>33</td>
<td>'60s-2005</td>
<td>MAbs 3E9 2B1 5C4 4D7 2E5 3E5 4C4 N. isolates reactive 32 32 31 30 27 27</td>
</tr>
</tbody>
</table>
## Results & Interpretation

<table>
<thead>
<tr>
<th></th>
<th>Type O</th>
<th>Type A</th>
<th>Type Asia 1</th>
<th>Type C</th>
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<tr>
<td></td>
<td>MAb 3B11</td>
<td>MAb 4D12</td>
<td>MAb 5F6</td>
<td>MAb 3D8</td>
<td>MAb 3E9</td>
</tr>
<tr>
<td>Sample 1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Sample 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 3</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Sample 4</td>
<td></td>
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<td></td>
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<tr>
<td>Sample 5</td>
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<tr>
<td>Sample 6</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>POS contr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEG contr</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Sample 1 is positive for Type O.
## Results & Interpretation

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<td>MAb 3E9</td>
</tr>
<tr>
<td>Sample 1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Sample 2</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Sample 3</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sample 4</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 5</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sample 6</td>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POS contr</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEG contr</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SAT ?
# Sensitivity

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Type-specific MAbs-based ELISAs

<table>
<thead>
<tr>
<th>FMDV types</th>
<th>N.</th>
<th>POS</th>
<th>Sens.</th>
<th>Polyclonal</th>
<th>POS</th>
<th>Sens.</th>
<th>Pan-FMD ELISA based on MAb 1F10</th>
<th>POS</th>
<th>Sens.</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>136</td>
<td>116</td>
<td>85%</td>
<td>129</td>
<td>95%</td>
<td></td>
<td>118</td>
<td>87%</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>103</td>
<td>67</td>
<td>65%</td>
<td>41</td>
<td>40%</td>
<td></td>
<td>67</td>
<td>65%</td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>30</td>
<td>22</td>
<td>76%</td>
<td>26</td>
<td>90%</td>
<td></td>
<td>21</td>
<td>72%</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>29</td>
<td>19</td>
<td>66%</td>
<td>20</td>
<td>69%</td>
<td></td>
<td>20</td>
<td>69%</td>
<td></td>
</tr>
<tr>
<td>TOTAL Epithelium suspensions</td>
<td>298</td>
<td>224</td>
<td>75%</td>
<td>216</td>
<td>72%</td>
<td></td>
<td>226</td>
<td>76%</td>
<td></td>
</tr>
</tbody>
</table>
FMDV antigen detection/serotyping
Evaluation on field samples

External evaluation (field samples)
- Kimron Veterinary Institute, Israel (April 2011)
- Indian Immunologicals Limited, India (October 2011)
- SAP Institute, Turkey (May 2011)

Demonstration/training (Kees van Maanen)
- Kenya
- Egypt

Supply (FAO, EUFMD programs)
- Afghanistan, Pakistan, Tajikistan
- Iran, Armenia, Azerbaijan, Georgia
Work in progress:

Development of similar kits for FMDV SATs

- At IZSLER: several MAbs specific for SAT 1 and SAT2
- At IAH: several MAbs specific for the three SAT serotypes
- Identified at least two candidates MAbs for each serotype
- Candidate MAbs purified and peroxidase-conjugated
- Sandwich ELISAs with anti-SAT 1, SAT2, SAT3 selected MAbs
- Specific and efficient reactivity with reference viruses
LFD Test Procedure

Results read after 10-30 minutes

A line in the "C" position indicates a valid test

No line in the "T" position:
NEGATIVE test result

A line in the "T" position:
POSITIVE test result
LFD Development and Application

- **Pan-specific test** (based on pan-FMDV Mab 1F10)
  - Used in UK 2007 outbreaks

- **SAT2 specific test** (based on a pan-SAT2 specific Mab)

- **Asia 1 specific test** (based on a pan-Asia1 specific Mab)
  - Validated on 160 epithelium samples at IAH
  - Available through EUFMD

- **Other type specific tests**
  - Under development
ELISA Kits for SP-Ab detection
FMDV serotypes O, A, ASIA 1

Objective: convert in-house MAbs based SPCE-ELISA into “ready to use” ELISA kits

Desired properties for these kits:

✓ Fast and easy
  • All inclusive
  • Use of a single protocol for the three different serotypes
  • Test run in only 2-2.5 hours (Room Temperature)
  • Specific skill not required

✓ Stable
  • Shelf-life ≥ 1 year

✓ Reliable
  • Diagnostic performance under evaluation
Starting point → in-house SPCE, MAbs-based

✓ Conditions for stabilization of inactivated FMDV antigens trapped by homologous MAbs defined
  → >1 year the shelf-life of plates sensitized with FMDV captured by Mabs

✓ Conditions for stabilization of conjugated MAbs (competition phase) defined
  → shelf-life ranging from 1,5 to 3 years

✓ Drawback : FMDV antigens tend to degrade to 12S during the stabilization process → need of initial excess of reagents

✓ Prototype kits developed and under evaluation

✓ Possible improvement: use of recombinant capsid particles with stabilized structure
Test results: FMDV O (strain O Manisa)

- 1064 naive cattle sera: 4 false positive & 4 borderline = 0.75% FP
- 72 naive pig sera: 0% false positive
- 72 naive goat sera: 0% false positive
**Test results: FMDV O (strain Manisa)**

**104 sera from 7 vaccine potency tests**
*(courtesy of CODA-CERVA)*

<table>
<thead>
<tr>
<th>Vaccine dose</th>
<th>Number sera</th>
<th>N. POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VNT</td>
<td>in-house LPBE</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>32 (91%)</td>
</tr>
<tr>
<td>1/4</td>
<td>35</td>
<td>26 (74%)</td>
</tr>
<tr>
<td>1/16</td>
<td>34</td>
<td>12 (35%)</td>
</tr>
</tbody>
</table>

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Next steps

✓ Wider $Se$ and $Sp$ validation underway for each FMDV serotype: **O, A, ASI A1**
✓ External evaluation and completion of validation
✓ Analyze field sera from endemic countries
✓ Refine the reaction setup, if necessary
✓ Start the setup of ELISA kit for FMDV SAT 1 and SAT 2 serotypes
Conclusions

- Pan-specific, SAT2-specific and Asia1-specific LFDs launched
- New antigen detection ELISA for Eurasian FMD ready for launch
- Eurasian serotype-specific Ab ELISAs under final validation
- SAT-specific Ab/Ag ELISAs under development
- Other serotype-specific LFDs to follow
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