Safe and cost-effective shipment of samples using lateral flow devices for laboratory diagnostic

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Towards global control and eradication of FMD:
- Identification of circulating virus strains in endemic regions
- Implementation of adequate control measures
- Determination of adequate vaccine strain to be used

There is a need to improve regular submission of samples to reference laboratories

One of the main barriers

UN3373
Infectious materiel

UN1845
Dry ice

Cost
Banned by some airlines
Delay (shipping company, authorisations...)

95th Executive Committee EuFMD, 6-7 March- Budapest, Hungary
Use of lateral flow device for safe and low cost shipment of FMDV suspected samples (FMDVINACT)
The lateral flow device (LFD): a support for shipment

Early diagnosis method routinely used on field: immunodetection method on strip

SVANODIP® FMDV-Ag
Boehringer Ingelheim Svanova

http://www.cytodiagnostic.com
How ensure safety from positive LFD?

LFD with positive signal is not safe.

Chemical inactivation of live FMDV

- Citric acid $\text{C}_6\text{H}_8\text{O}_7$ (0.1 to 1%)
- Contact time: 15 sec to 15 min

Incubation of LFD+ in 0.2% $\text{C}_6\text{H}_8\text{O}_7$ bath for 1min is sufficient to inactivate FMDV.

CPE on ZZ-R-127
Validation in the laboratory
Safety and FMDV detection?

Applications:
- 7 reference strains
- 3 field samples

Controls:
- Viral suspension not treated
- Viral suspension inactivated
- LFD+ in H₂O

Soaking **15min** in 0.2% C₆H₈O₇ or in H₂O

Elution

Inoculation on cells

RNA extraction

RT-rtPCR 3D & IRES

rtPCR VP1 & sequencing

Chemical transfection

Virus Rescue

Ag ELISA typing

95th Executive Committee EuFMD, 6-7 March, Budapest, Hungary
### Evaluation on reference strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Virus titre (TCID₅₀/ml)</th>
<th>LFD result</th>
<th>Soaking solution</th>
<th>CPE on cells after inoculation</th>
<th>3D Ct</th>
<th>IRES Ct</th>
<th>VP1 amplification</th>
<th>CPE on cells after RNA transfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>O/IRN/13/2012</td>
<td>10⁷.23</td>
<td>++</td>
<td>H₂O</td>
<td>+24 hpi</td>
<td>19.41</td>
<td>16.50</td>
<td>1155 bp</td>
<td>+24 hpt</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>C₆H₈O₇ 0.2%</td>
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<td>20.65</td>
<td>17.53</td>
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<td>A/IRN05</td>
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<td>H₂O</td>
<td>+24 hpi</td>
<td>19.60</td>
<td>21.76</td>
<td>846 bp</td>
<td>+24 hpt</td>
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<tr>
<td></td>
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<td></td>
<td>C₆H₈O₇ 0.2%</td>
<td></td>
<td>19.15</td>
<td>20.46</td>
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<tr>
<td>C1 Noville</td>
<td>10⁷.72</td>
<td>+++</td>
<td>H₂O</td>
<td>+24 hpi</td>
<td>18.01</td>
<td>24.01</td>
<td>837 bp</td>
<td>+24 hpt</td>
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<td></td>
<td>C₆H₈O₇ 0.2%</td>
<td></td>
<td>17.29</td>
<td>23.21</td>
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<tr>
<td>SAT1/KEN/2/2011</td>
<td>10⁸.82</td>
<td>++</td>
<td>H₂O</td>
<td>+5 hpi</td>
<td>18.48</td>
<td>21.42</td>
<td>1023 bp</td>
<td>+24 hpt</td>
</tr>
<tr>
<td></td>
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<td>C₆H₈O₇ 0.2%</td>
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<td>16.91</td>
<td>20.71</td>
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<tr>
<td>SAT2/LIB40/2012</td>
<td>10⁸.36</td>
<td>+</td>
<td>H₂O</td>
<td>+24 hpi</td>
<td>14.12</td>
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<td>1255 bp</td>
<td>+24 hpt</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>C₆H₈O₇ 0.2%</td>
<td></td>
<td>12.73</td>
<td>37.75</td>
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<tr>
<td>SAT3 Zim 4/81</td>
<td>10⁶.95</td>
<td>++</td>
<td>H₂O</td>
<td>+5 hpi</td>
<td>19.75</td>
<td>29.49</td>
<td>1254 bp</td>
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<tr>
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<td>C₆H₈O₇ 0.2%</td>
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<td>18.24</td>
<td>26.88</td>
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<tr>
<td>Asia/ISR/3/89</td>
<td>10⁶.69</td>
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<td>H₂O</td>
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<td>C₆H₈O₇ 0.2%</td>
<td></td>
<td>30.76</td>
<td>26.61</td>
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</tr>
</tbody>
</table>

FMDV, foot-and-mouth disease virus; LFD, lateral flow device; –, no cytopathic effect after two passages on cells; hpi, hours post-inoculation; hpt, hours post-transfection.

*+++ = strong, ++ = intermediary, + = weak.*
Evaluation on archival epithelium field samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Virus titre (TCID₅₀/ml)</th>
<th>LFD result</th>
<th>Soaking solution</th>
<th>CPE on cells after inoculation</th>
<th>3D Ct</th>
<th>IRES Ct</th>
<th>VP1 sequence homology</th>
<th>CPE on cells after RNA transfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMDV/TUN/1771/2014</td>
<td>10⁵.₉⁵</td>
<td>+</td>
<td>H₂O</td>
<td>+24 hpi</td>
<td>25.56</td>
<td>NA</td>
<td>100%</td>
<td>+24 hpi</td>
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<tr>
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<td></td>
<td>C₆H₈O₇ 0.2%</td>
<td></td>
<td>25.00</td>
<td>NA</td>
<td></td>
<td>+24 hpi</td>
</tr>
<tr>
<td>BEN/1/2011</td>
<td>10³.₄₈</td>
<td>+</td>
<td>H₂O</td>
<td>+48 hpi</td>
<td>25.41</td>
<td>36.46</td>
<td>100%</td>
<td>+48 hpi</td>
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<tr>
<td></td>
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<td>C₆H₈O₇ 0.2%</td>
<td></td>
<td>23.58</td>
<td>33.14</td>
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<td>+48 hpi</td>
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<tr>
<td>O/FRA/DPT77/2001</td>
<td>10⁴.²³</td>
<td>+++</td>
<td>H₂O</td>
<td>+48 hpi</td>
<td>19.98</td>
<td>21.45</td>
<td>100%</td>
<td>–</td>
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<tr>
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<td>C₆H₈O₇ 0.2%</td>
<td></td>
<td>20.23</td>
<td>20.95</td>
<td></td>
<td>–</td>
</tr>
</tbody>
</table>

FMDV, foot-and-mouth disease virus; LFD, lateral flow device; –, no cytopathic effect after two passages on cells; hpi, hours post-inoculation; hpt, hours post-transfection; NA, not applicable.

a+++ = strong, + = weak.
bBased on comparison of the 639 bp of the serotype O VP1.

The protocol is applicable on archival field samples (virus inactivation, genome detection, VP1 sequencing and rescue of live virus)

Needs to be validated on more field samples and in real situation
Evaluation in field conditions of a safe and cost-effective protocol for shipment of samples from FMD suspected cases for laboratory diagnostic (FIELD_EVAL_INACT)

- Anses, France (coordinator)
- Technical University of Denmark (DTU)
- FMD Research Centre of Nigeria (NRVI)
- FMD Institute of Turkey (SAP)
- University of Malakand in Pakistan (UM)
- Merial- Boehringer Ingelheim (BI)
The aims of the project:

- Evaluate/validate the performance of the protocol in real situation through application under field conditions.
- Assess biosafety on fresh samples expected to contain large quantity of virus (vesicular fluid if available).
- Evaluate the impact of storage conditions of the inactivated LFD on the detection of FMDV.
- Optimization of RNA transfection to rescue live virus

In addition, the project will allow characterization of FMDV strains
Example
Example of procedure to apply on field...

In decontamination area:
- Decontamination solution
- S1, S2, S3
- 0.2% citric acid (or 5%)
- At least 15 min
- 30 min

In clean area:
- Triple packaging
- At room T°
- Discard citric acid

In farm:
- In decontamination area
- In clean area
1. Establish the feasibility of engaging paraveterinarians, private animal health service providers or other non-state actors in FMD sample collection and submission to the national laboratories/authorities;

2. A study on the demand of livestock keepers and other stakeholders for services for prevention or management of FMD, to establish if a market potential exists for services (including early warning of risk) and which will identify what will need to change if the demand is to be met and/or the service to be introduced.
CONCLUSION

- Incubation 15 min in 0.2% citric acid is sufficient for inactivation of FMDV on LFD
- FMDV RNA can be extracted from LFD and FMDV detected by rtRT-PCR, VP1 sequenced and live virus rescued after RNA transfection
- Validation of the protocol on the field is ongoing (FAR 2017 & 2018)
- The protocol should facilitate the transport of samples and thus increase the submissions
- Useful for sending samples to laboratories not allowed to use live virus
- The safety needs to be evaluated and validated by the Biorisk Working Group of the EuFMD
Acknowledgement
Thank you for your attention