Pen-side tools for the rapid detection of foot-and-mouth disease virus

Donald King

Molecular Characterisation and Diagnostics Group,
Institute for Animal Health, Pirbright, UK
Reference Laboratories at IAH Pirbright Laboratory

- World Reference Laboratory of FAO
- Regional Reference Laboratory of OIE
- Community Reference Laboratory of EU

- Bluetongue
- Foot and Mouth disease
- Rinderpest
- Swine Vesicular disease
- Peste des petits ruminants
- African Horse sickness
- African Swine Fever
- Sheep and Goat pox
- Lumpy Skin disease
- Molecular Characterisation & Diagnostics Group
Foot-and-Mouth Disease

- Variable severity
- Affects cloven-hoofed livestock and various wildlife species
- Highly contagious
  - Rapid replication and short incubation
  - Massive virus release
  - High susceptibility of hosts
- Difficult to control and a major international trade issue
- Not a significant zoonosis

(Pictures by E. Ryan, J Gloster)
The cull widens as the contagion grows

DEADLY VIRUS THAT HAS SHUT DOWN THE COUNTRYSIDE

Foot-and-mouth crisis: The tragedy unfolds
Background: why do we need field tests?

• FMD spreads very rapidly

• Lessons from 2001
  • Rapid decision required
  • Average time to receipt of samples >24hrs

• Devolved and POC formats offer potential to significantly decrease assay time
  • Support of diagnosis based on clinical signs
New technologies / new opportunities

1. Lateral-flow devices for FMDV antigen
2. Mobile PCR
3. Isothermal assays
Field detection: assay considerations

- Reliability
  - Simple-to-use
  - Non-specialist
- Performance
  - Limit of detection
  - Ability to correctly identify infection due to diverse FMDV strains
- Speed
- Scalability
- Cost?
- Disposable?
Lateral-flow devices for FMDV Antigen detection

- Rapid, disposable, inexpensive and simple to use
- Clearview ® Technology (Unipath)

Clinical sample Containing FMDV

FMDV Pos

TEST LINE (anti-FMDV ab)

CONTROL LINE (anti-lg reagent)

Colloidal gold latex beads coated with FMDV-specific Ab

Release pad

Absorbent pad

FLOW

FLOW
Lateral-flow devices for FMDV Antigen detection

- Developed by IAH in collaboration with Brescia and Svanova
- Quick and simple to perform
- Pan-serotypic
- During 2007: used for rapid (<10 mins) confirmation of FMD in the field
- Also useful in the Lab
- LFD marketed by Svanova

FMDV Pos
## Validation data for pan-reactive LFD

- Sensitivity data generated for archived clinical samples

<table>
<thead>
<tr>
<th>Virus serotype</th>
<th>ELISA Fraction</th>
<th>%</th>
<th>1F10 (gold-particle) Fraction</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMDV type O</td>
<td>121/126</td>
<td>96</td>
<td>119/129</td>
<td>92.2</td>
</tr>
<tr>
<td>FMDV type A</td>
<td>32/41</td>
<td>78</td>
<td>36/41</td>
<td>87.8</td>
</tr>
<tr>
<td>FMDV type C</td>
<td>14/24</td>
<td>58.3</td>
<td>15/24</td>
<td>62.5</td>
</tr>
<tr>
<td>FMDV type SAT 1</td>
<td>13/24</td>
<td>54.2</td>
<td>16/24</td>
<td>66.7</td>
</tr>
<tr>
<td>FMDV type SAT 2</td>
<td>28/32</td>
<td>87.5</td>
<td>18/32</td>
<td>56.3</td>
</tr>
<tr>
<td>FMDV type SAT 3</td>
<td>9/10</td>
<td>90</td>
<td>7/10</td>
<td>70</td>
</tr>
<tr>
<td>FMDV type Asia 1</td>
<td>36/40</td>
<td>90</td>
<td>39/40</td>
<td>97.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>253/297</strong></td>
<td><strong>85.2</strong></td>
<td><strong>250/300</strong></td>
<td><strong>83.3</strong></td>
</tr>
</tbody>
</table>

Data from Nigel Ferris (IAH)
Potential scenario for field diagnosis of FMD

Veterinary visit → Clinical diagnosis

Lateral-flow devices (LFDs)
- Sensitivity ~80%

• Rapid confirmation of positives?

Off-site confirmation using mobile or laboratory RT-PCR
Portable/Handheld PCR platforms

- Cepheid GeneXpert®
- Idaho Technology RAZOR™
- Enigma Diagnostics PCR-light/FL
- Smiths Bio-Seeq™
Rapid detection of FMDV in the field: Portable PCR platform

- Non-specialist user
  - Nucleic acid extraction
  - PCR set-up
  - Analysis
- 5 independent modules
- Battery operated
- Decontaminate by immersion
- Field trial (Turkey)
- Platform for other livestock diseases

Smiths Bio-Seeq Vet™
Molecular Characterisation & Diagnostics Group
Alternative amplification technologies

Isothermal amplification (RT-LAMP)

• Nucleic acid amplification at a single temperature
• No need for fragile precision instrumentation
• Basis of disposable device / cost effective
• More suitable for use in the field
• Very rapid, similar sensitivity to rRT-PCR

Dukes et al., 2006
LAMP assays: use in the field?

- LAMP products can be detected using LFD-FITC and Biotin labelled oligos

- Now: incorporate this with simple and rapid nucleic methods

- Simple – rapid – sensitive disposable test?
What might a disposable mol. test look like?

Concept picture from LLNL/IAH collaboration
Opportunities for non-invasive sampling

• FMDV excreted in air
• Air-samplers (MesoSystems)
• Hand held and simple-to-use

<table>
<thead>
<tr>
<th></th>
<th>BioCapture</th>
<th>BioBadge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle 3 dpi</td>
<td>10.24</td>
<td>11.23</td>
</tr>
</tbody>
</table>

Log10 FMDV copies detected by rRT-PCR after 5 minute collection near animals infected with FMDV (serotype Asia-1) (Ryan et al., 2007)

• Integrated with FMDV detector?
• Static located in high-risk areas?
Field testing vs centralised testing

• Use of rapid field-based tests to support diagnosis based on clinical signs
  • Positives confirmed using LFDs
  • Support diagnosis (typing) using mobile rRT-PCR
  • How sample testing arranged during active surveillance programmes (screening for pre-clinical animals)?

• Role of NRL and International Reference laboratories
  • Confirmation of 1st case (in a FMD-free country)?
  • Strain characterisation (sequencing and antigenic properties)
Summary: Future organisation of tests?

Infected animal

LOCAL CLINICAL OBSERVATION

Rapid confirmation and negation (?) of clinical signs for secondary cases

supporting LABORATORY DIAGNOSIS
(Local or NRL)

First cases in FMD-free countries?
Surveillance screening
Strain typing
Molecular epidemiology (VP1 & CG)

supporting INTERNATIONAL REFERENCE LABS

Use for:

Future prospects:

Serotyping LFDs
Serotyping RT-PCRs
LFDs for serology
Isothermal assays

Microarrays? Others?
Future challenges

- Rapid development of technologies
- Key role of commercial partners
  - Is the market viable?
  - expectation of stakeholders
- Availability of technology
  - Freely available vs control of local diagnosis/reporting for notifiable diseases
- Use in FMD-endemic countries
Acknowledgements:

- Scott Reid
- Katja Ebert
- Bartek Bankowski
- Nigel Ferris
- Geoff Hutchings
- Neil Boonham
- Partners on LAB-ON-SITE (FP6)
- Partners on EPIZONE (FP7)