Deployment of simple and rapid diagnostic tests away from centralised laboratories

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Changing paradigms: the future of diagnostics?

Suspect case of disease

LOCAL CLINICAL OBSERVATION

LABORATORY DIAGNOSIS (Local or NRL)

Portable tests
Field tests
On-site tests
Pen-side tests
Point-of-care tests

Purpose: faster (more rapid detection)
• Point-of-decision tests
Cost benefit?

TIME TO RESULT

Quicker tests

COST

More expensive tests
Do we really need rapid tests?

Example:

- For highly infectious exotic livestock diseases
  - Foot-and-mouth disease
- Decisions need to be made during veterinary investigation
  - Positive or negative
  - Clinical diagnosis (particularly in small ruminants) can be challenging
- Delays impact upon the potential size of an epidemic
- In the UK (2001) transport time to the lab could be up to 24 hours
The UK case for FMD

“No evidence of FMD virus, antibody or nucleic acid was found in approximately 23% (390/1730) of farms“. Ferris et al., (2006) Vet Record

modern diagnostic methods including pen-side tests – need to be developed that can shift the burden of diagnosis to veterinarians on the farm (2002).
### Where should the testing be carried out?

<table>
<thead>
<tr>
<th>'Lab' location</th>
<th>Transport time</th>
<th>Assay time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Central Lab</td>
<td>Up to 24 hours</td>
<td>5 hours (approx.)</td>
</tr>
<tr>
<td>2: Regional lab</td>
<td>1-2 hours</td>
<td>5 hours (approx.)</td>
</tr>
<tr>
<td>3: Mobile lab or local lab*</td>
<td>Less than 1 hour</td>
<td>Less than 1 hour</td>
</tr>
<tr>
<td>4: On-farm</td>
<td>-</td>
<td>Less than 1 hour</td>
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</tbody>
</table>

*Foci of infection, dairy, abattoir, vet. clinics
Centralised testing: what do we lose?

Uses established laboratory-based assays

Advantages
- Uses trained/proficient staff
- High through-put capability
- Established systems with SOPs
- Accredited to QA standards
- Links to national reporting systems

Limitations/disadvantages
- Transport time
Field tests: considerations

Advantages

Rapid tests that can support local decisions

Do these limitations matter?

• Probably operated by “non-specialists”
• Low throughput (<20 samples)
• Assay performance may not be equivalent to lab-based methods

• Cost
Largely driven by advances in human “personal” medicine

• Immunoassays
  o Lateral-flow devices
  o Bio-sensors

• Molecular tests
  o Mobile PCR assays
  o Isothermal tests
Test characteristics and properties

- Performance
  - Limit of detection
  - Use for different sample types
- Speed (<60 mins?)
- Scalability
- Cost (<$50/test?)
- Simplicity
  - Uses available resources
  - Interpretation of results
- Robust
  - At ambient temp
  - No safety/biosafety concerns
Lateral-flow devices FMDV Antigen detection

- Developed collaboration with international partners
- Quick and simple to perform
- Used in the UK (during 2007)
  - Rapid (<10 mins) confirmation of FMD in the field
  - Also useful in the Lab for triage of samples
- Recognises all 7 FMDV serotypes
- Similar assay performance to lab-based Ag-ELISA
- LFD marketed by

Ferris et al., 2009: J. Virol. Methods
Use of rapid tests in developing countries

- LFDs are suitable for deployment into FMD endemic settings
- Ideal for use in countries where lab infrastructure is still being developed
- Support local diagnostic capacity
- Field evaluation on-going in Tanzania

Paulo-Fupi Raphael in Tanzania
Potential scenario for field diagnosis of FMD

Suspect case of FMD

Lateral-flow devices (LFDs)
Sensitivity ~80%

Rapid confirmation of positives?

Clinical diagnosis

Off-site confirmation using mobile or laboratory RT-PCR

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Potential scenario for field diagnosis of FMD

Suspect case of FMD

Lateral-flow devices (LFDs)
Sensitivity ~80%

“Rapid” Molecular assays
Sensitivity >95%

Rapid confirmation of positives?
• Ability to confirm negatives
• Additional surveillance use
• Strain characterisation?

Clinical diagnosis
Detection of FMDV by real-time RT-PCR

- Non-specialist user
  1. Nucleic acid extraction
  2. PCR set-up
  3. Analysis
- 1-6 independent modules
  - Battery operated
- Location on/or near farms
- Platform for other (livestock) diseases
- Uses mature and established technologies
  - Equivalent to lab-based methods
  - but is relatively expensive

Madi et al., 2012: *Vet. Journal*
Alternative amplification technologies

• Nucleic acid amplification at a single temperature (isothermal)
  o No need for fragile precision instrumentation
  o More suitable for use in the field
  o Portable formats being developed/evaluated

• **LAMP** – Loop-mediated isothermal amplification

• Very rapid and similar performance to rRT-PCR

• Other formats (NASBA, RPA etc..)

• Assays developed for many livestock pathogens

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Dukes et al., 2006: *Arch. Virol.*
Chen et al., 2011: *Virol. J.*
Yamazaki et al., 2013: *J. Virol. Methods*
RT-LAMP detection of FMDV

- Handheld instrument
  - Built-in GPS
  - 1-8 samples
  - Launch July 2013

- Dry-down reagents also in development

- Pilot data for FMDV

- Similar systems have been deployed for Ash dieback (fungus: *Chalara fraxinea*)

- Equivalent performance to lab-based real-time RT-PCR
RT-LAMP: simple formats/minimum equipment

• Simple sample prep protocols
• LAMP products can be detected using LFD
  - FITC and Biotin labelled oligos
• Amplification can be performed using a water bath
  o Simple
  o Rapid
  o Inexpensive?
  o Basis of a disposable test?
• Addresses limitations of “equipment-based” assays that cannot be deployed to multiple sites
How samples will be collected?

- Air-samplers (MesoSystems)
- Hand held
- non-invasive
- Simple-to-use

- Integrated with FMDV detector?
- Static located in high-risk areas?

BioCapture 650

BioBadge 100

<table>
<thead>
<tr>
<th></th>
<th>BioCapture</th>
<th>BioBadge</th>
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<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)
Current status

• A range of different innovative technologies are available for real-time or rapid diagnosis of livestock diseases
  ○ New chemistries and platforms are inevitable

• These technologies have different characteristics which will define *where* and *how* tests will be used
  ○ Testing in local labs, abattoirs, dairies, ports-of-entry may be more suitable than on-farm (pen-side) testing

• In addition to the performance of the test (se and sp)..... evaluation should consider:
  ○ Requirements of complete assay pipeline (from sample collection and processing to reporting of results)
  ○ Biosecurity implications of equipment decontamination

• In the short term... cost/benefit may preclude use of these assays widely for many endemic as well as exotic diseases
Use in endemic countries

- Obvious scope to deploy rapid tests to provide diagnostic capability in FMD endemic countries
- Different drivers in these setting compared to FMD-free countries where most of these tests are developed
- Tailored tools may be required?
  - Serotyping and characterisation
- Cost/per test may govern how widely these technologies are used
- How do we defer these costs?

Training

Luc Aplogan and Lorraine Edwards

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Challenges

• Selecting the “winning” technology
• Bridging the gap between assay development and marketing of tests
  o Is the market big enough to warrant investment?
  o Freedom to exploit IP
  o Partnerships between commercial and academic sectors
  o Engagement of end-users
  o Validation under field conditions
• How do you ensure tests are available for sporadic outbreaks?
  o Diagnostics bank?
Policy decisions

• Acceptance of tests (and their results) by the policy-makers
  o How are the results used?
  o Links into national control systems
  o Incorporation into national contingency plans
  o Involvement of OIE?

• Who uses the tests? – particularly for notifiable diseases
  o Expectation of stakeholders
  o Freely available vs loss of control of local diagnosis/reporting
  o Importance of veterinary expertise
Control of exotic livestock diseases through co-ordinated testing

Future prospects:

Use for:

Secondary cases:
- Rapid confirmation of positives
- Negatives: hold off cull?

First cases in FMD-free countries/zones?
- Surveillance screening
- Strain typing
- Molecular epidemiology

Use for:

- Supporting laboratory diagnosis (Local or NRL)
- Supporting international reference labs
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