



**New tools for rapid field  
diagnostics:**  
opportunities to deploy  
decentralised tests to detect  
**FMDV**

Emma Howson



# The diagnostic “pipeline”

Initial observation



Vet examination



Sample collection

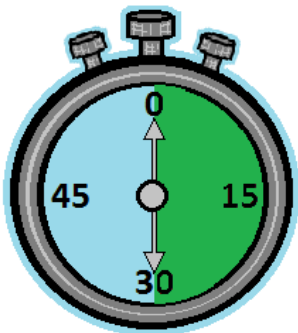


Sample shipment



Processing / testing

Result reporting  
and decision  
making





“modern diagnostic methods including pen-side tests – need to be developed that can shift the burden of diagnosis to veterinarians on the farm (2002)”.



# Desirable Test Characteristics

Rapidity



£

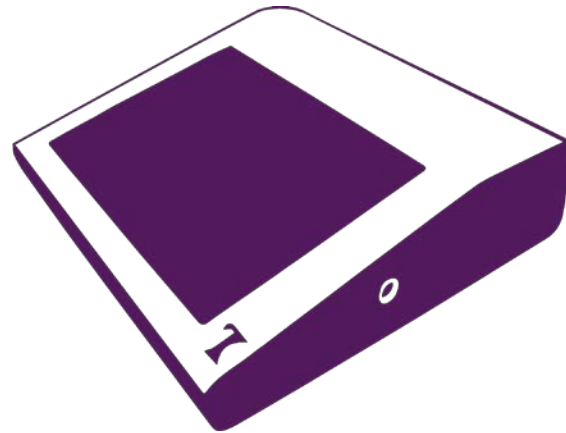
Cost of test

Ease of use



Se

Sensitivity



Decentralised  
test characteristics

Sp

Specificity

Compatibility



# Antigen-lateral flow device

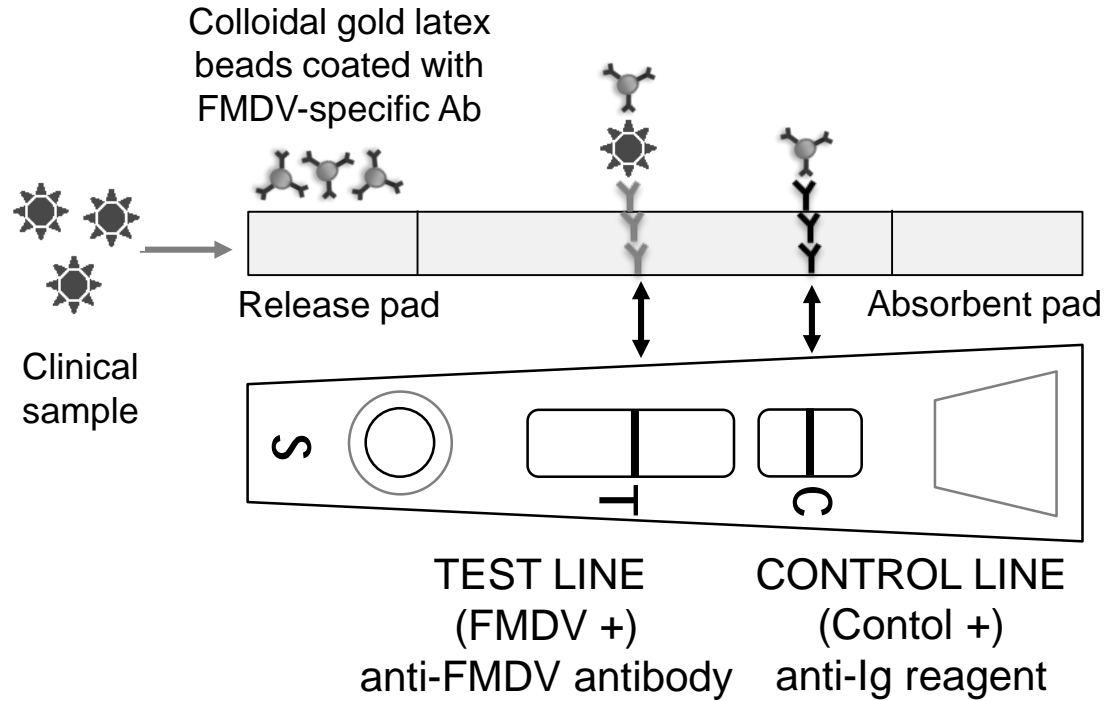


Department  
for Environment  
Food & Rural Affairs

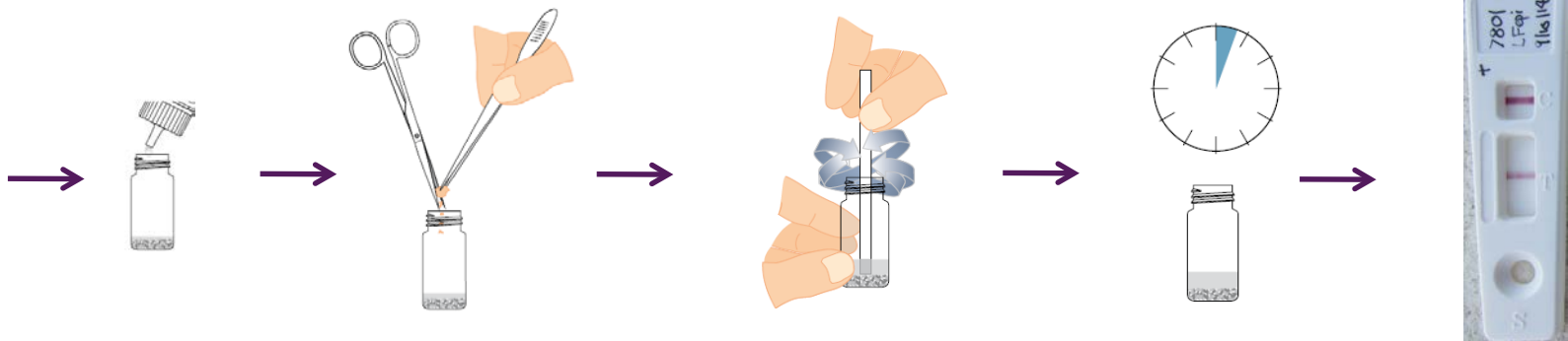


- ✓ Simple to use
  - ✓ Rapid (~10 mins)
  - ✓ Disposable
  - ✓ Highly portable
  - ✓ Inexpensive
  - ✓ Commercially available
- Ideal POCT: rinderpest

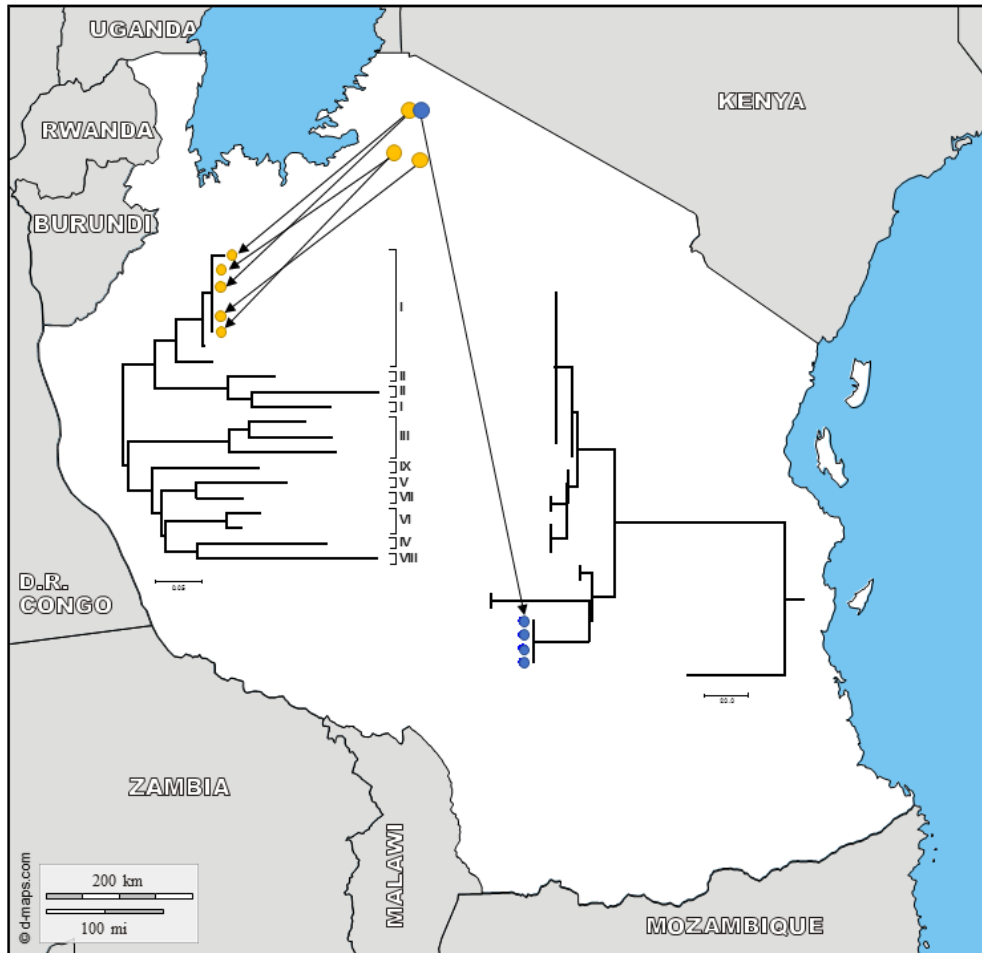
- Sensitivity
- Limited sample types



Ruptured lesion



# Antigen-lateral flow device



Store and transport samples



# Mobile rRT-PCR

- ✓ Sensitive
- ✓ Use of accredited assays  
Established technology
- ✓ Battery operated  
No need for mains
- ✓ Getting quicker  
> 1 hour 30 (rRT-PCR)
- ✓ Lyophilised reagents
- ✓ Serotyping possible

- Cost
- Bio-containment

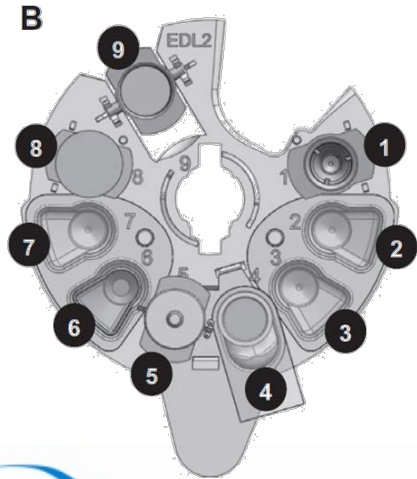
1)



2)



**Tetracore**<sup>®</sup>



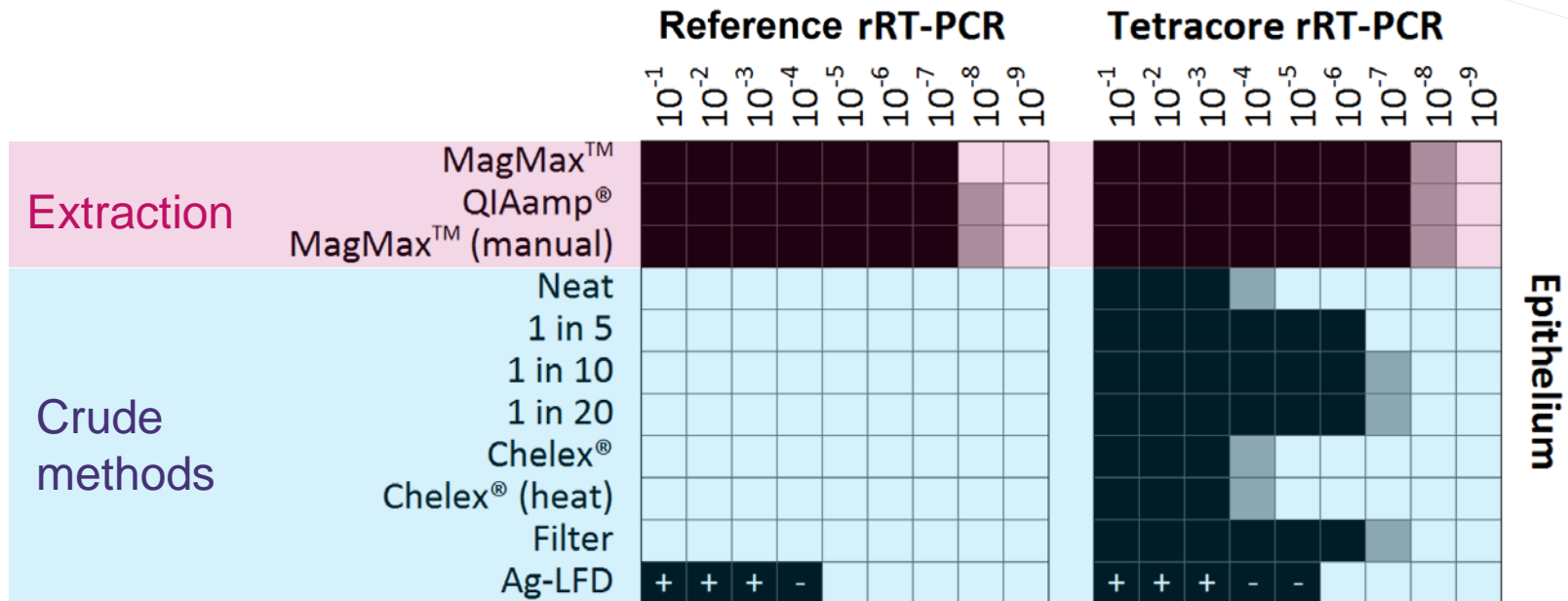
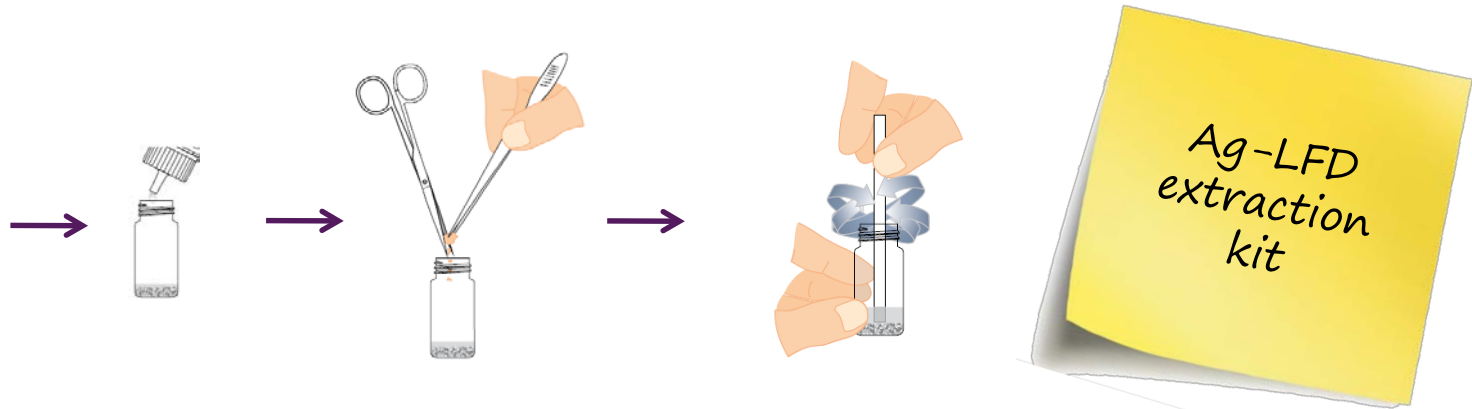
enigmadiagnostics



# Mobile rRT-PCR – simple sample prep



Ruptured lesion

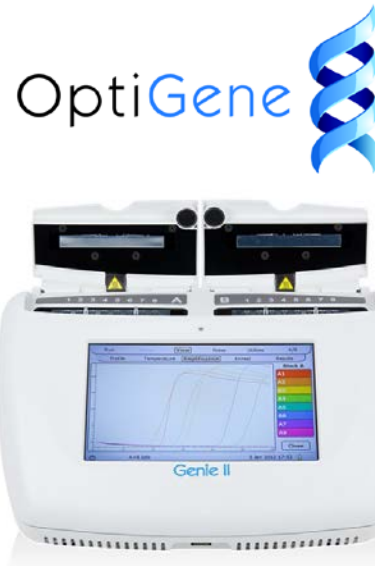




# Isothermal alternatives

- ✓ Simplified machinery
- ✓ Sensitive
- ✓ Battery operated
- ✓ Rapid
  - 10 minutes RPA
  - 30 minutes LAMP
- ✓ Lyophilised reagents
- ✓ LAMP – crude samples

- Serotyping difficult
- Bio-containment

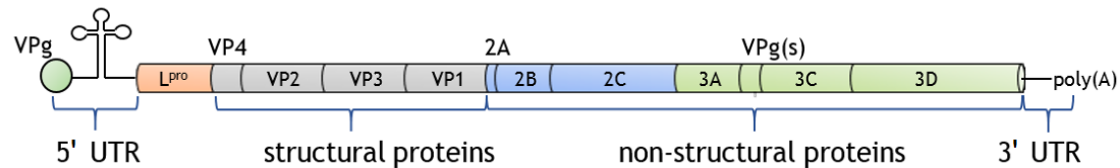


**LAMP**

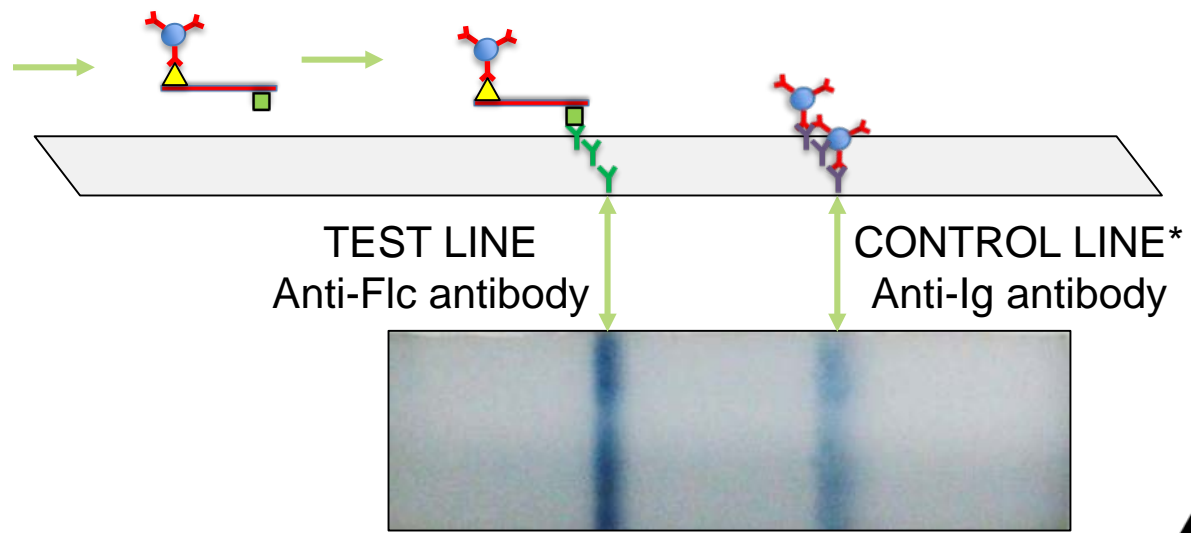
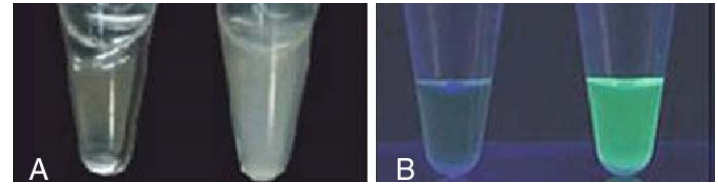
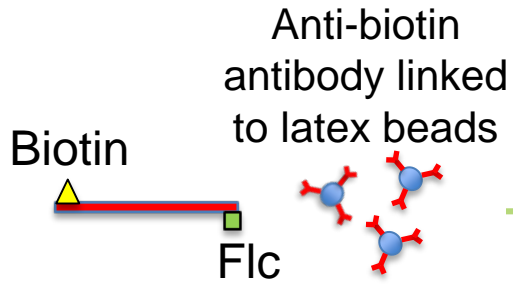
TwistDx



**RPA**

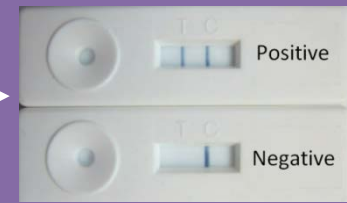


# Isothermal alternatives



*Reducing the cost*

- Water bath
- Heat block
- Molecular LFD



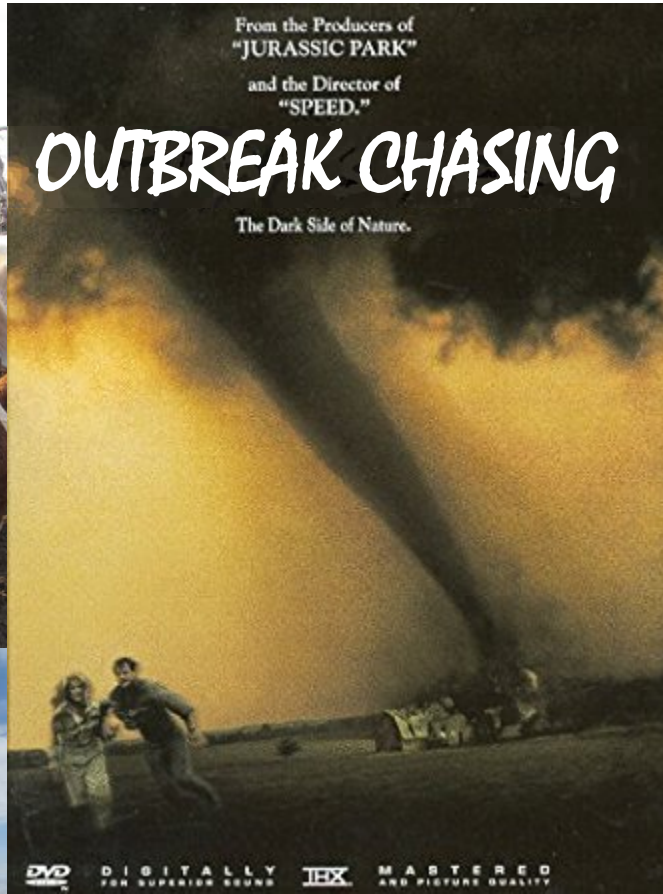
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10

20

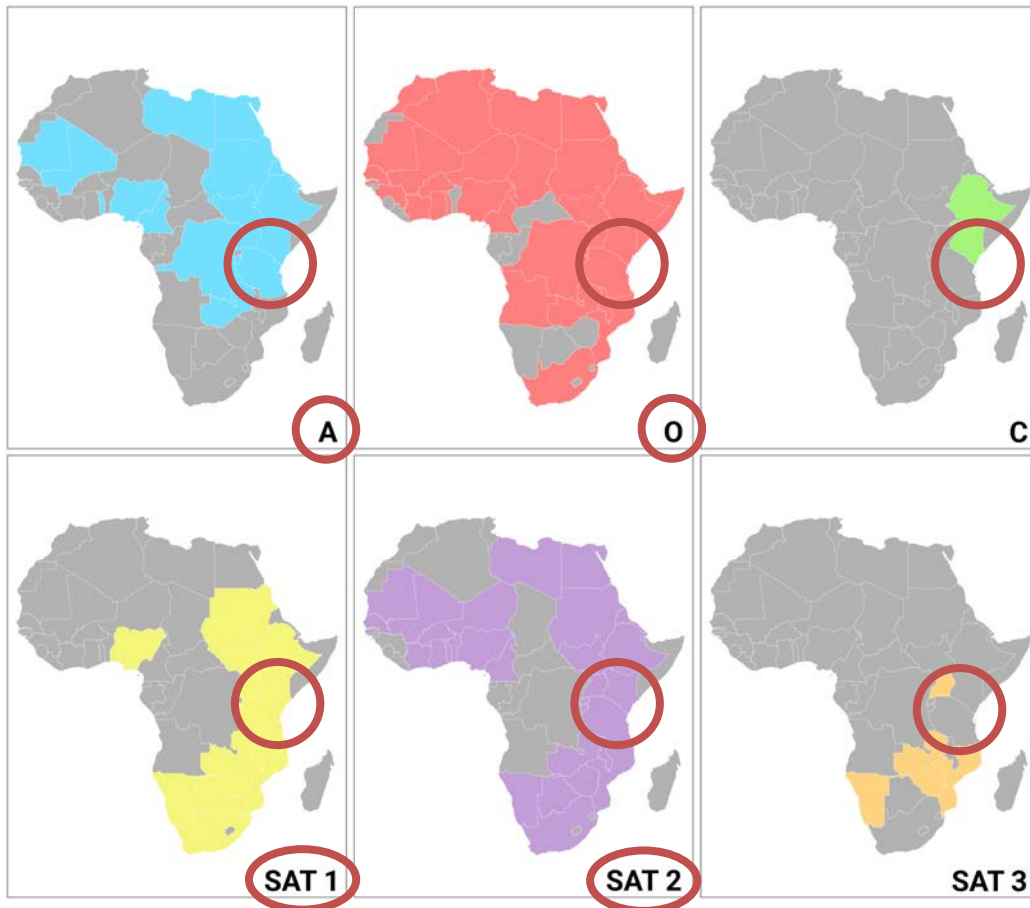
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# Assays in action



# Case study - East Africa

- Seven FMDV serotypes – four commonly circulate in pool 4

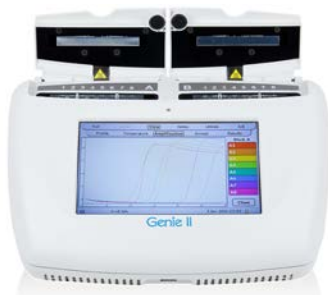
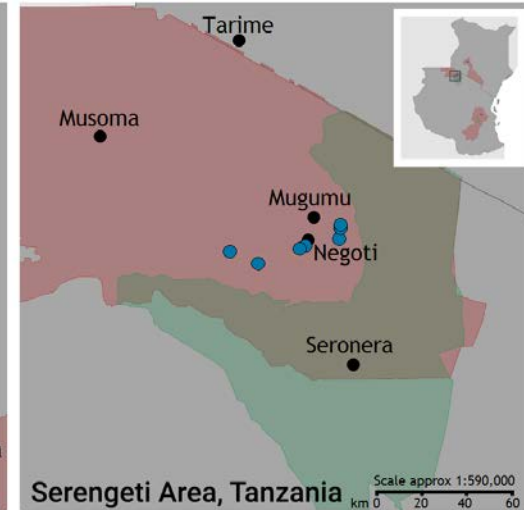


← Last reported 2004 (Kenya)

**Tanzania**  
>99% livestock in smallholdings  
73% in communal grazing (contact & share resources)  
FMDV under-reported  
regular & low mortality

# Field evaluation: rRT-LAMP

- 10 farms
- 60 cattle
- Three sample types
- 145 samples
- Comparison of LAMP against Enigma FL



VS



# Field evaluation: rRT-LAMP

Transboundary and Emerging Diseases

Transboundary and Emerging Diseases

ORIGINAL ARTICLE

## Evaluation of Two Lyophilized Molecular Assays to Rapidly Detect Foot-and-Mouth Disease Virus Directly from Clinical Samples in Field Settings

E. L. A. Howson<sup>1,2</sup>, B. Armonson<sup>1,2</sup>, M. Madi<sup>1</sup>, C. J. Kasanga<sup>3</sup>, S. Kandusi<sup>3</sup>, R. Sallu<sup>4</sup>, E. Chepkwony<sup>5</sup>, A. Siddle<sup>6</sup>, P. Martin<sup>7</sup>, J. Wood<sup>7</sup>, V. Mioulet<sup>1</sup>, D. P. King<sup>1</sup>, T. Lembo<sup>5</sup>, S. Cleaveland<sup>2</sup> and V. L. Fowler<sup>1</sup>

<sup>1</sup> The Pirbright Institute, Pirbright, Surrey, UK

<sup>2</sup> Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary & Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow, UK

<sup>3</sup> Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro, Tanzania

<sup>4</sup> Tanzania Veterinary Laboratory Agency, Dar-es-Salaam, Tanzania

<sup>5</sup> Foot-and-Mouth Disease Laboratory, Ministry of Agriculture, Livestock and Fisheries, Nairobi, Embakasi, Kenya

<sup>6</sup> OptiGene Limited, Horsham, West Sussex, Salisbury, UK

<sup>7</sup> Enigma Diagnostics Limited, Salisbury, Wiltshire, UK

### Keywords:

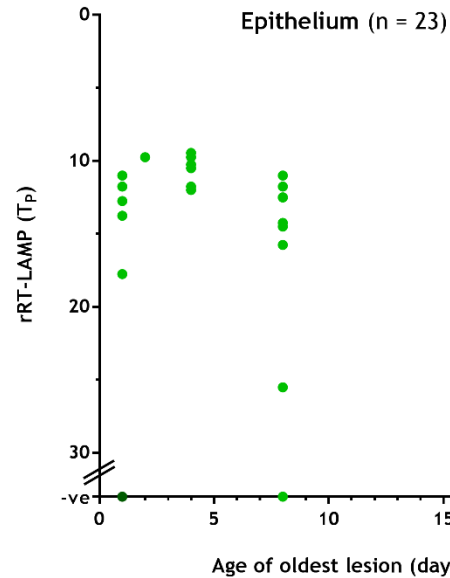
foot-and-mouth disease; foot-and-mouth disease virus; diagnostics; rRT-PCR; RT-LAMP; lyophilized

### Summary

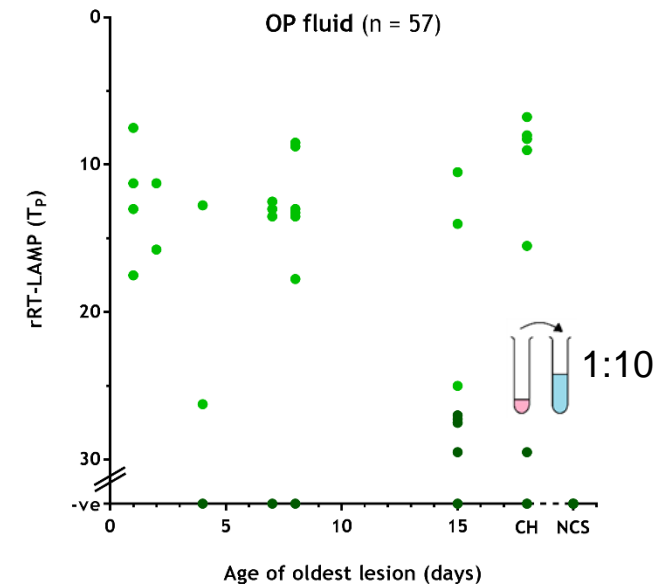
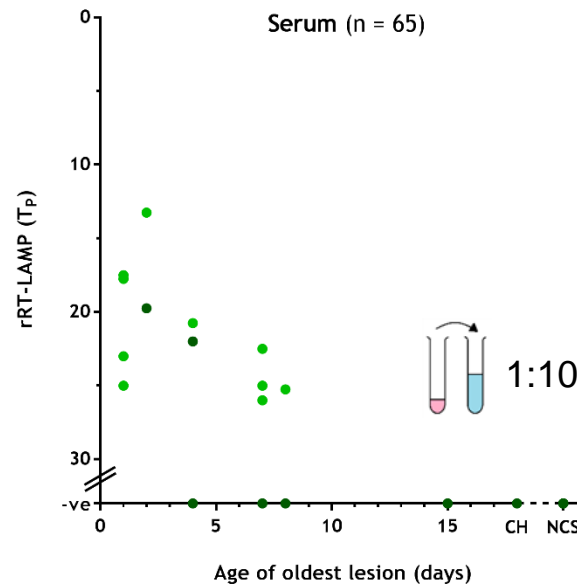
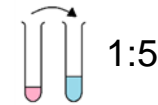
Accurate, timely diagnosis is essential for the control, monitoring and eradication of foot-and-mouth disease (FMD). Clinical samples from suspect cases are normally tested at reference laboratories. However, transport of samples to these centralized facilities can be a lengthy process that can impose delays on critical decision making. These concerns have motivated work to evaluate simple-to-use

### Correspondence:

V. Fowler, The Pirbright Institute, Ash Road,



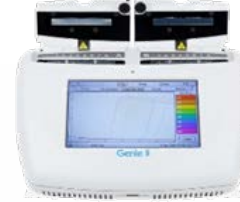
OptiGene



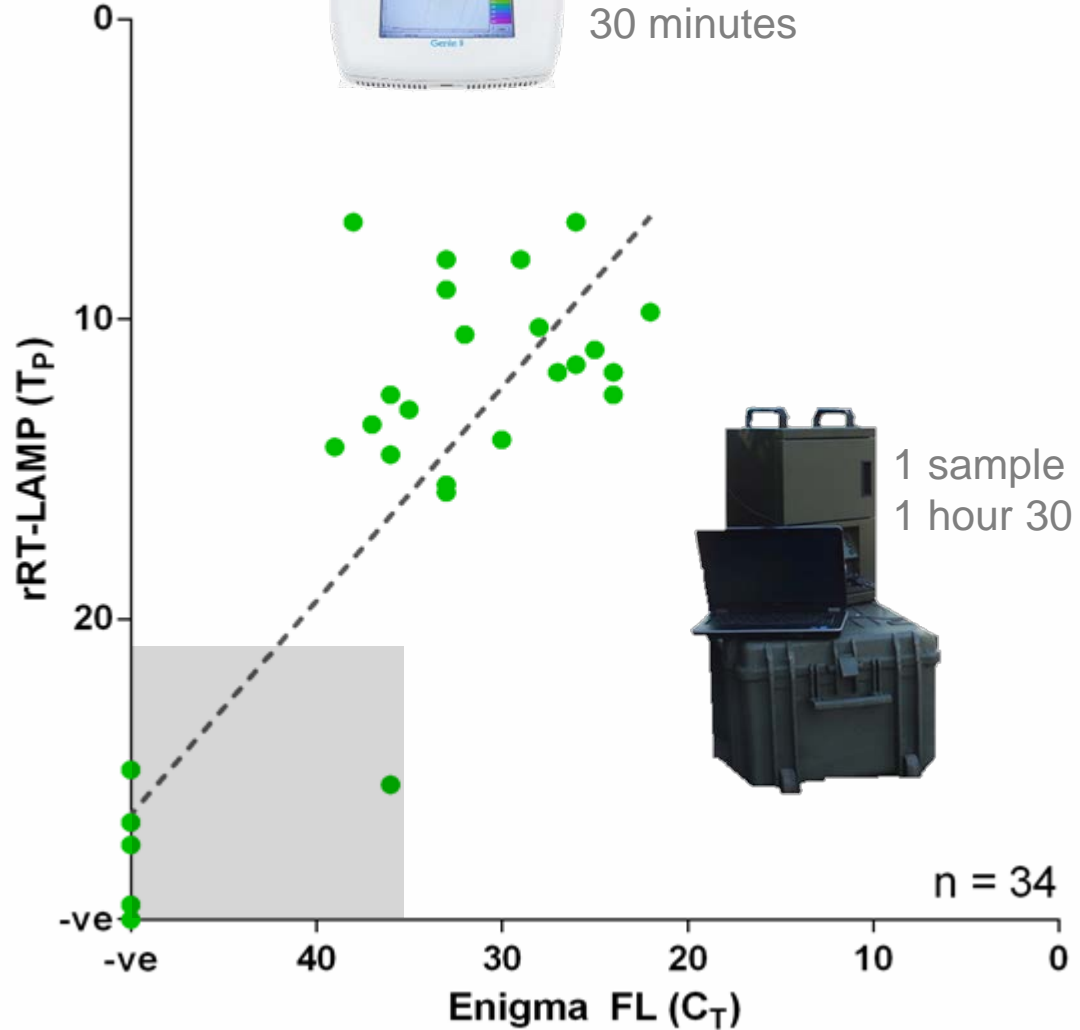
# Field evaluation: rRT-LAMP



Of 13 +ve's (LAMP and PCR), 8 were +ve by Ag-LFD



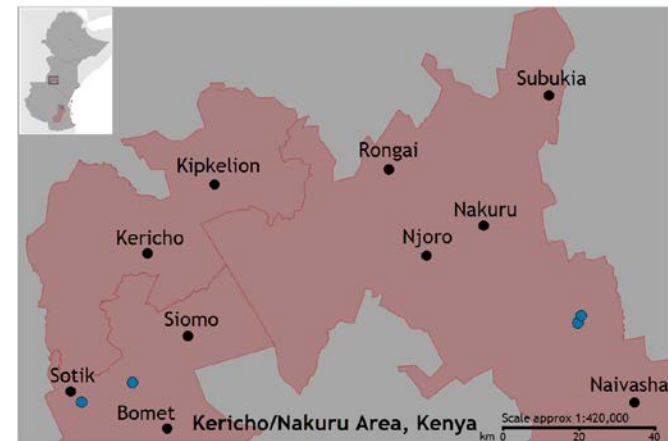
16 samples  
30 minutes



1 sample  
1 hour 30 minutes

# Field evaluation: rRT-PCR

- 78 cattle
- 144 samples
- 13 farms
- Four sample types
- Comparison of field and lab based PCR

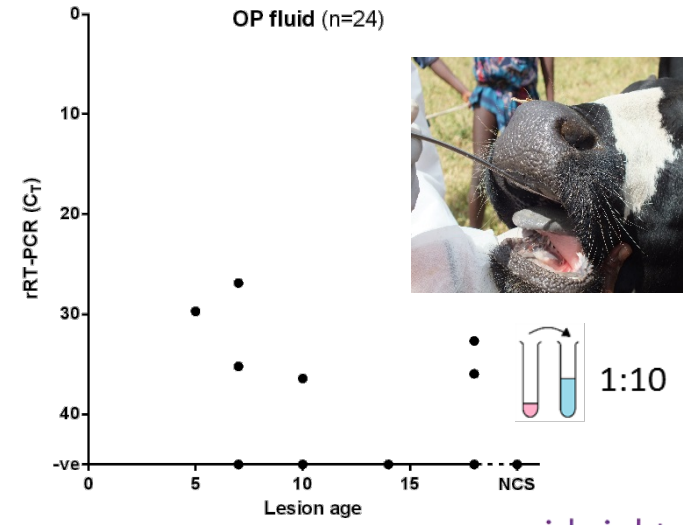
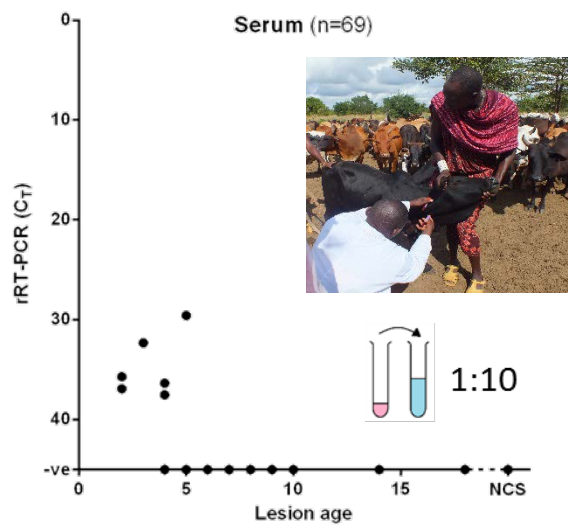
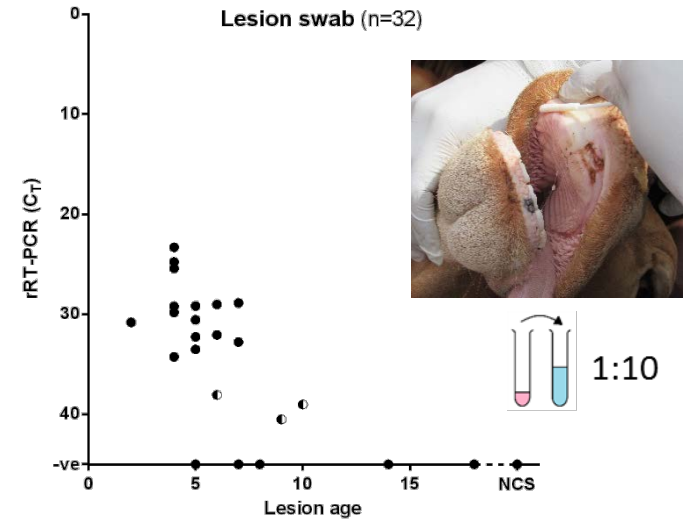
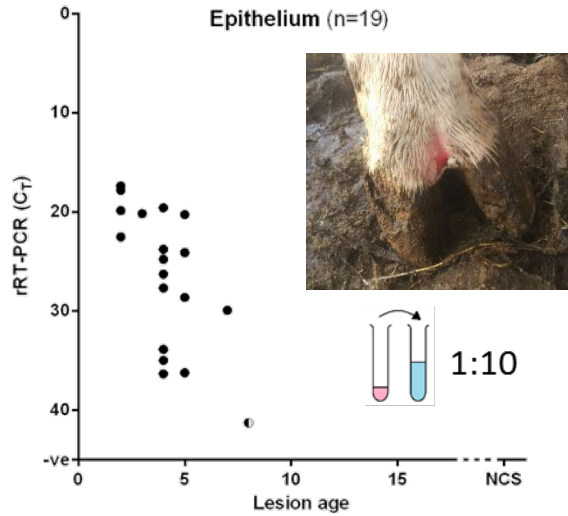


VS

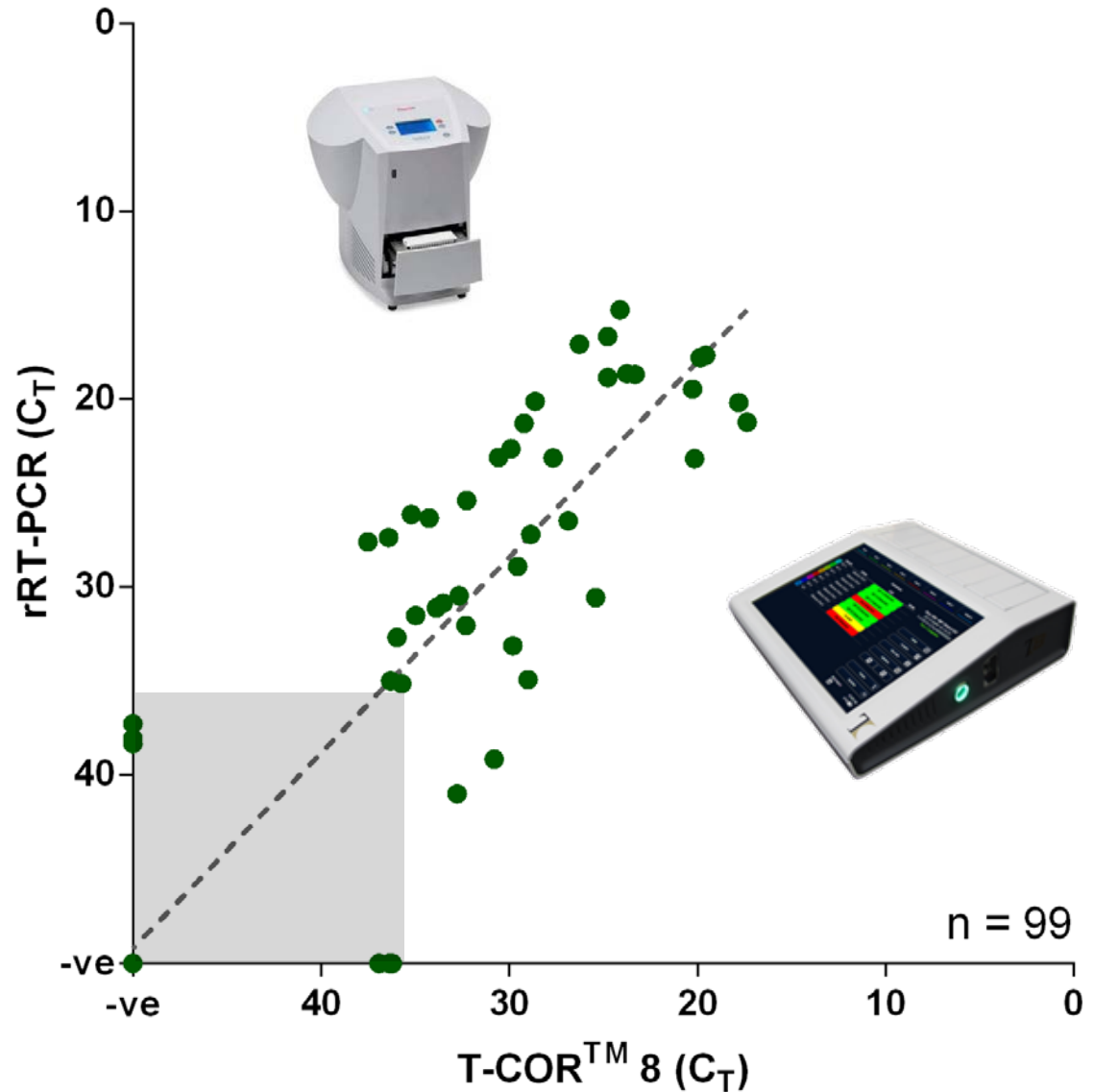




# Field evaluation: rRT-PCR



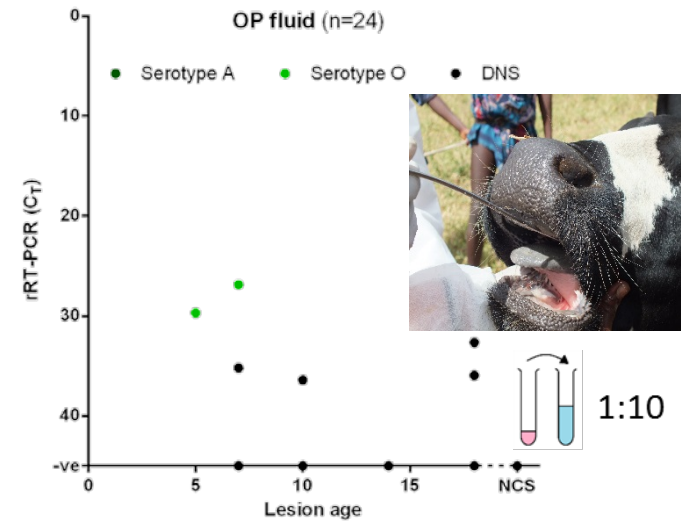
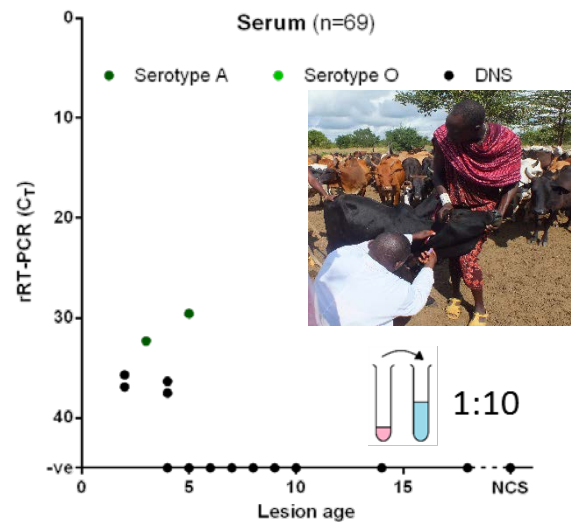
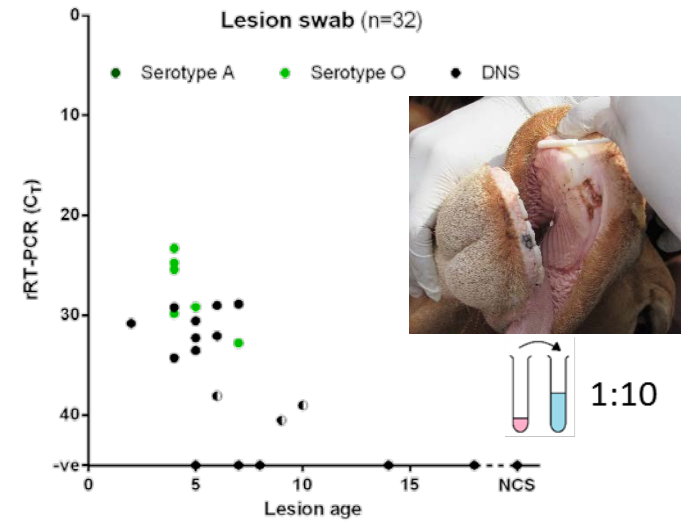
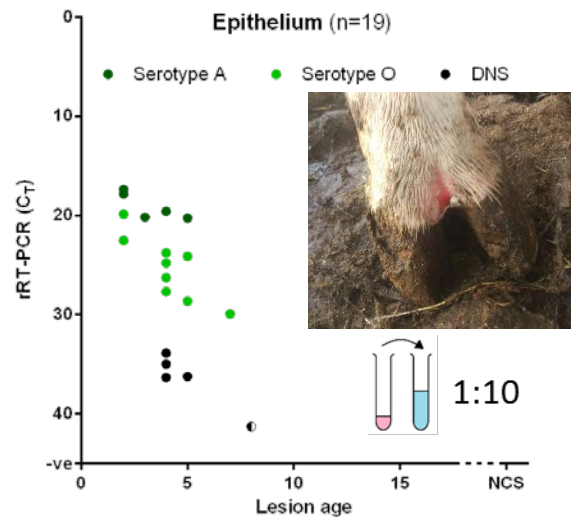
# Field evaluation: rRT-PCR



# Initial evaluation: typing rRT-PCR



4 serotypes; 1 well



Received 21 March 2017

DOI: 10.1111/med.12684

ORIGINAL ARTICLE

WILEY

**Direct detection and characterization of foot-and-mouth disease virus in East Africa using a field-ready real-time PCR platform**

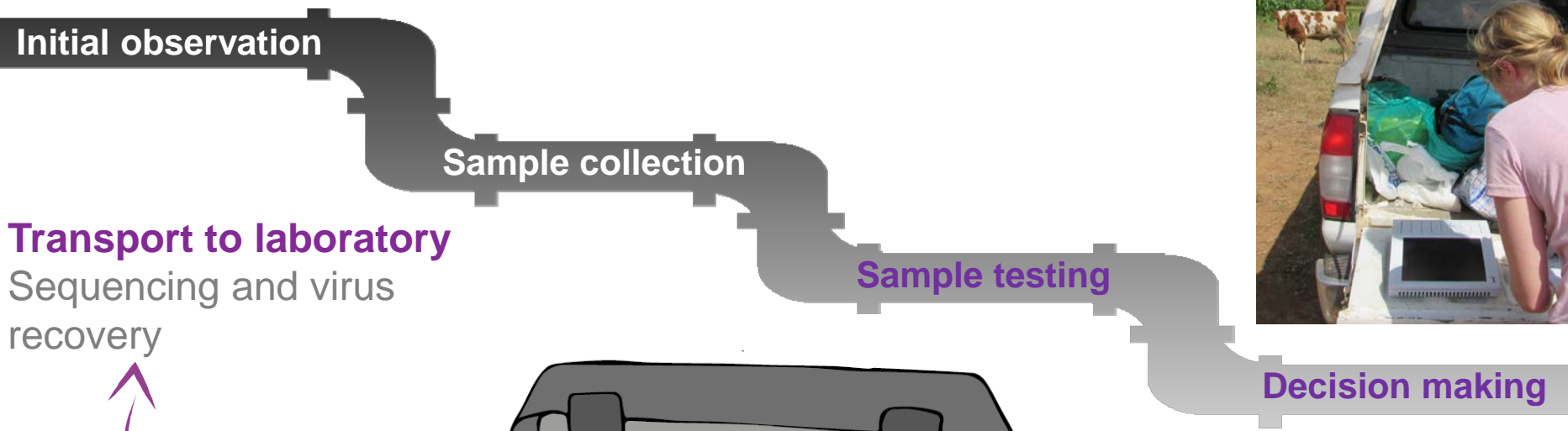
E. L. A. Howson<sup>1,2</sup> | B. Armonson<sup>1,2</sup> | N. A. Lyons<sup>1,3</sup> | E. Chepkwony<sup>4</sup> | C. J. Kasanga<sup>5</sup> | S. Kandus<sup>5</sup> | N. Ndujilo<sup>5</sup> | W. Yamazaki<sup>6</sup> | D. Gizaw<sup>7</sup> | S. Cleaveland<sup>2</sup> | T. Lembo<sup>7</sup> | R. Rauh<sup>8</sup> | W. M. Nelson<sup>9</sup> | B. A. Wood<sup>1</sup> | V. Mioulet<sup>1</sup> | D. P. King<sup>1</sup> | V. L. Fowler<sup>1</sup>

<sup>1</sup>The Pirbright Institute, Pirbright, Surrey, UK  
<sup>2</sup>Institute of Biomedical Animal Health and Comparative Medicine, College of Medical Veterinary & Life Sciences, University of Glasgow, Glasgow, UK  
<sup>3</sup>European Commission for the Control of Foot and Mouth Disease, Bulawayo, Zimbabwe

**Summary**

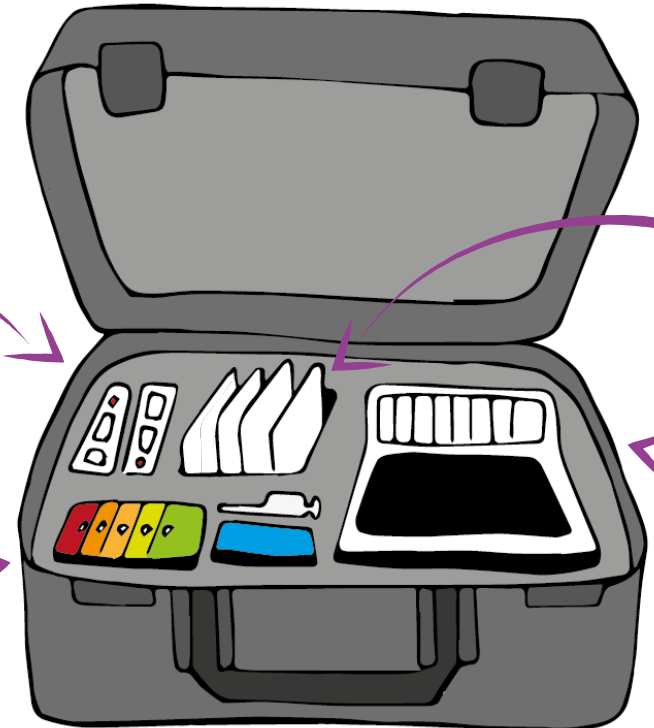
Effective control and monitoring of foot-and-mouth disease (FMD) relies upon rapid and accurate disease confirmation. Currently, clinical samples are usually tested to reference laboratories using standardized assays recommended by The World Organisation for Animal Health (OIE). However, the requirements for prompt and serotype-specific diagnosis during FMD outbreaks, and the need to establish robust

# The “on-farm” diagnostic pipeline



**Ag-LFD**  
Rapid confirmation of positive lesions and sample selection

**RNA extraction**  
For confirmation of negatives



- Lyophilised reagents**
- Pan-serotype specific
  - Serotype specific
  - Look-a-likes

**Molecular platform**  
Serotyping, sensitivity and other samples

# Challenges of implementation

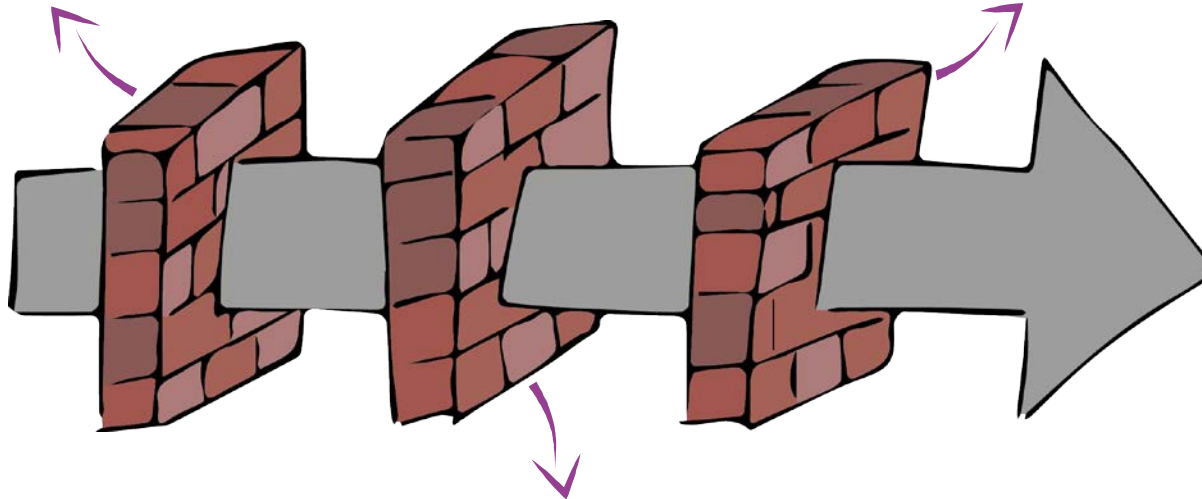
## Quality assurance

- OIE manual
- Outside of ISO 17025
- Acceptance of test data
- Secondary cases?

## Commercial sector

- Investment
- Viable markets (need and affordability)

R&D



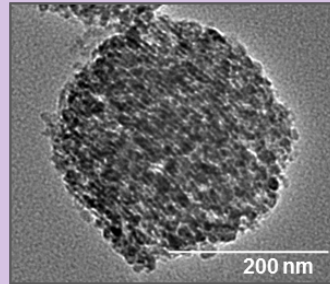
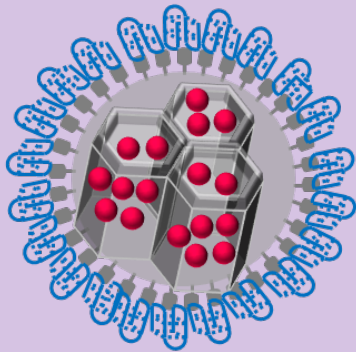
Routine  
use

## Diagnostic strategy

- What test / who runs it?
- Training requirements
- Stakeholder access to tests
- Reporting and storing test results

# Future directions and innovations

## Micro- and nano- technologies



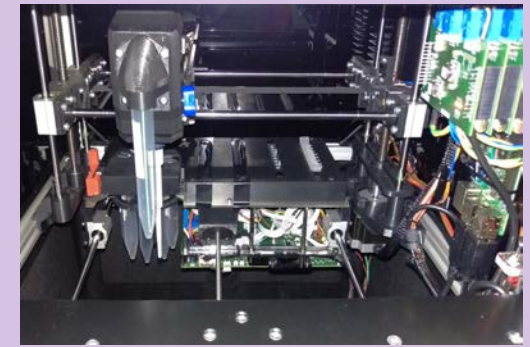
## Sequencing technologies



Biomeme

Disposable consumables

## Automation





# Acknowledgements



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Christopher Kasanga (SUA)  
Sengiumva Kandusi (SUA)



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Daniel Guizaw  
Livestock owners

