Rapid, Sensitive and Effective Diagnostic Tools for Infectious Diseases Surveillance

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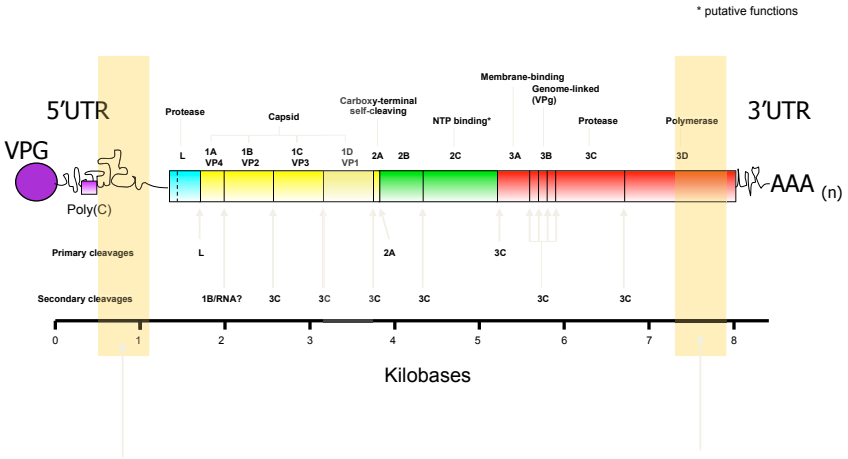
Phakalane, Gaborone, Botswana

FMDV: conserved genome regions

Assay targets:



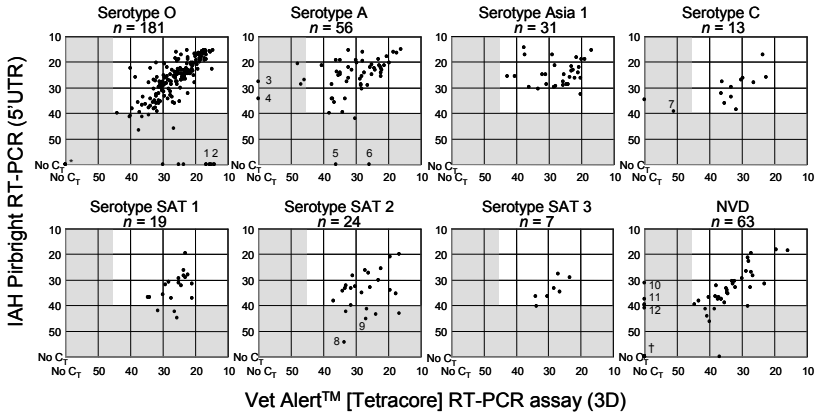
conserved **IRES** and **3D** regions
targets for pan-serotype reactive assays

Automated real-time RT-PCR

- 2 pan-serotype assays in routine use
  - 5'UTR and 3D
- Automated extraction equipment can be used to prepare nucleic acid
  - Roche MagNA PURE LC
  - Qiagen BioRobot Universal

•Reid et al., (2003) J Virol Methods 107:129-39.
Evaluation of automated RT-PCR to accelerate the laboratory diagnosis of FMDV

Real-Time RT-PCR comparison



RNA extracted from 394 suspensions of epithelial tissue obtained from suspect FMD cases

Validation of diagnostic rRT-PCR

- Comparison with “established” diagnostic approaches
- Evaluation of different sample matrices
- Comparison of different genome targets
- Inter-laboratory “ring-tests”

New technologies / new opportunities

Suspect case of disease

LOCAL CLINICAL OBSERVATION

LABORATORY DIAGNOSIS (Local or NRL)

1. Lateral-flow devices for FMDV antigen
2. Mobile PCR
3. Isothermal assays

- Faster (more rapid detection)
- Ability to support diagnosis based on clinical signs

Lateral-flow devices - FMDV Antigen detection

- Developed by IAH in collaboration with international partners
- Quick and simple to perform
- Recognises all 7 FMDV serotypes
- Also useful in the Lab
- LFD marketed by Svanova

Validation data for pan-reactive LFD

- Sensitivity data generated for archived clinical samples

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

Ferris et al – J. Virol. Methods

Rapid detection of FMDV in the field: Portable PCR platforms

Enigma-FL™ (EnigmaDiagnostics)

- Non-specialist user
  - Nucleic acid extraction
  - PCR set-up
  - Analysis
- Battery operated
- Platform for other (livestock) diseases

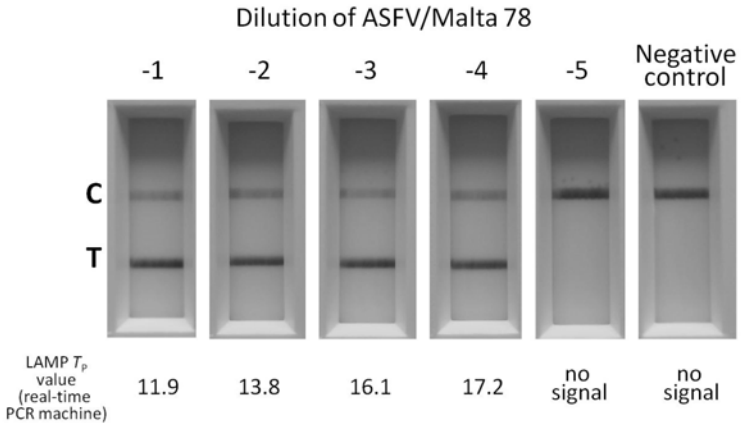
Alternative amplification technologies

- Nucleic acid amplification at a single temperature
- No need for fragile precision instrumentation
- More suitable for use in the field

- **LAMP** – Loop-mediated isothermal amplification
- Very rapid
- Similar performance to rRT-PCR
- Assays developed for many livestock pathogens
- Portable formats being developed/evaluated

Simple detection of LAMP products

- LFD Forsite diagnostics
- Disposable test?



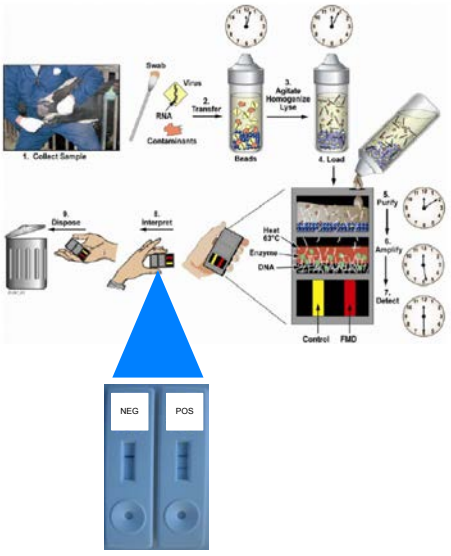
James et al., 2010

What might a disposable mol. test look like?

Integrated assay format:

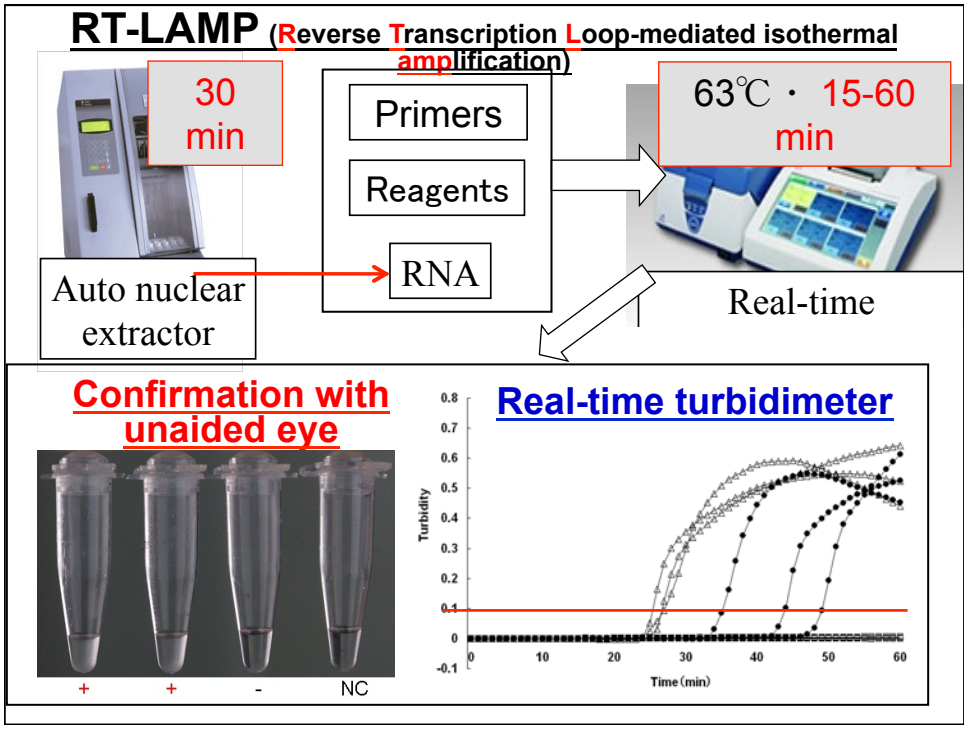
- Simple sample-prep
- Isothermal amplification
  LAMP?
- Simple detection of products

Concept picture from LLNL/IAH collaboration



Case study: rRT-PCR vs RT-LAMP

- 179 probang samples were used in this study
- Samples for testing assays were randomly selected
- RNA was prepared by an automated BioRobot machine
- All samples were tested for FMDV genome (targeting the 3D region) by rRT-PCR and RT-LAMP
- Detection: real-time *thermocycler* and *turbidimeter*
- Sensitivity: based on the end-point analysis



Results: rRT-PCR vs RT-LAMP

- All rRT-PCR positive samples were also positive for RT-LAMP
- Some RT-PCR negative samples (Ct values 32 <X≤36) were positive by RT-LAMP
- Both assays were specific for FMDV

FMDV detection by rRT-PCR vs RT-LAMP

Location	Spp	Number Tested	rRT-PCR Positive	RT-LAMP positive
Tanzania - Katavi	Buffalo Cattle	31 30	15 3	18 5
Malawi - Lengwe	Buffalo Cattle	30 30	4 0	9 3
Mozambique - Morromeu	Buffalo Cattle	29 29	7 2	14 5
Total		**179**	**31 (17.3%)**	**54 (30.2%)**

Note: The Ct values ranged from 26.20 – 40.25
Ct values ≤ 32 were regarded as FMDV genome positive

Discussion and conclusion

- FMDV genome was detected in both rRT-PCR and RT-LAMP
- Both methods were specific to FMDV
- RT-**LAMP** indicated superior performance to rRT-PCR, and is more suitable for use in the field
- RT-LAMP has high potential for specific detection and surveillance of infectious diseases

Recommendation(s)

- Serotype-specific RT-LAMP methods should be developed and deployed for field use in Africa
- Sensitive and pathogen-specific pshould be used for rapid diagnosis and surveillance of FMD and other infectious diseases

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wellcome trust

UNIVERSITY OF ZAMBIA

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