RAPIDIA FIELD
Validation of AMPlite

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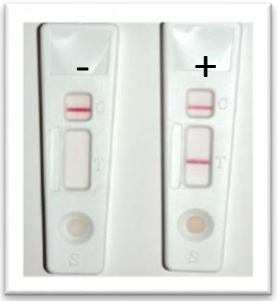
Detection platforms and technologies

- Largely driven by advances in human “personal” medicine



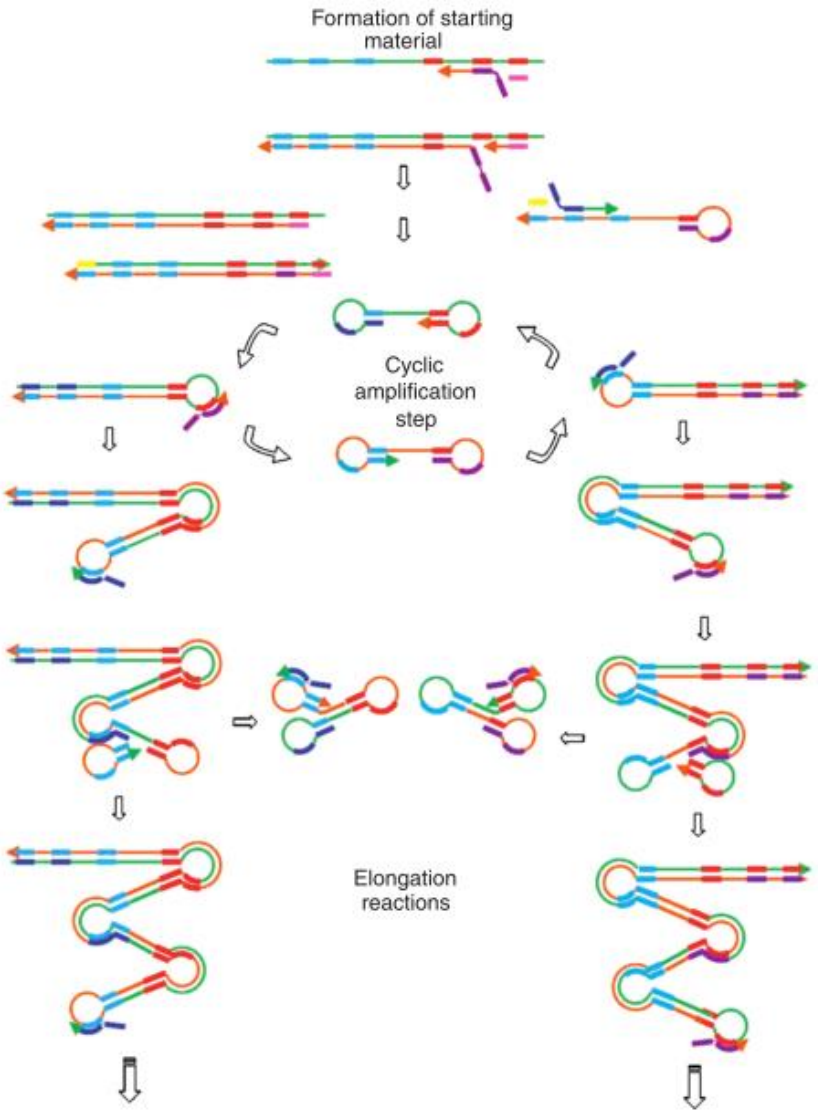
- <u>Immunoassays</u>
  - Lateral-flow devices

- <u>Molecular tests</u>
  - Mobile rRT-PCR
  - Local laboratory rRT-PCR
  - **Isothermal tests**

Loop-Mediated Isothermal Amplification:

The process

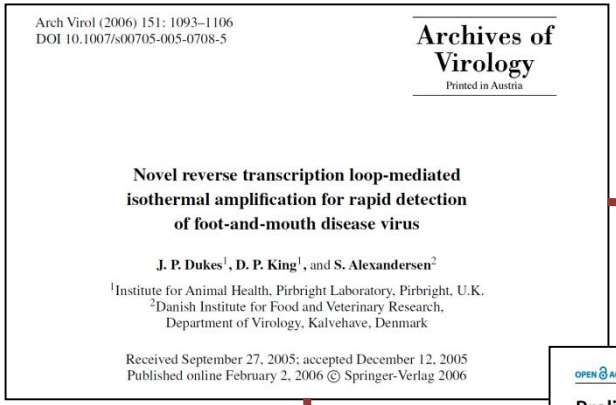
- **Strand-displacing** polymerases
- **Isothermal** amplification
- **Four-six** primers
- Formation of **loop structures**
- **Rapid** amplification
- **Autocycling**
- Double-stranded, multi-sized amplicons
- Reverse transcription (RT-LAMP)



Low cost, simple equipment

The Problem:

Field deployment of RT-LAMP



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Archives of Virology
Printed in Austria

Novel reverse transcription loop-mediated isothermal amplification for rapid detection of foot-and-mouth disease virus

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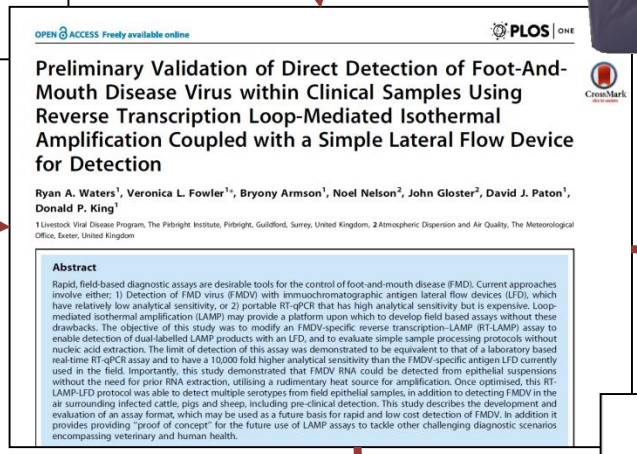

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Preliminary Validation of Direct Detection of Foot-And-Mouth Disease Virus within Clinical Samples Using Reverse Transcription Loop-Mediated Isothermal Amplification Coupled with a Simple Lateral Flow Device for Detection

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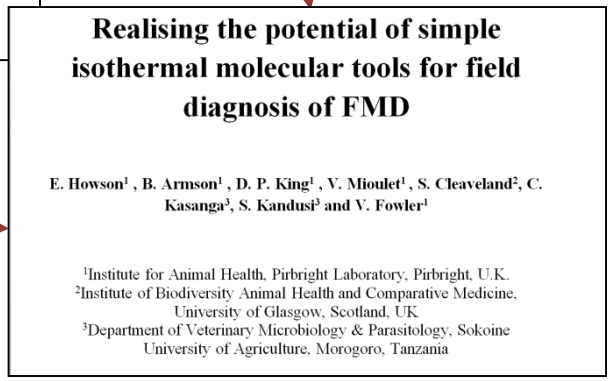
Abstract

Rapid, field-based diagnostic assays are desirable tools for the control of foot-and-mouth disease (FMD). Current approaches involve either; 1) Detection of FMD virus (FMDV) with immuochromatographic antigen lateral flow devices (LFD), which have relatively low analytical sensitivity, or 2) portable RT-qPCR that has high analytical sensitivity but is expensive. Loop-mediated isothermal amplification (LAMP) may provide a platform upon which to develop field based assays without these drawbacks. The objective of this study was to modify an FMDV-specific reverse transcription–LAMP (RT-LAMP) assay to enable detection of dual-labelled LAMP products with an LFD, and to evaluate simple sample processing protocols without nucleic acid extraction. The limit of detection of this assay was demonstrated to be equivalent to that of a laboratory based real-time RT-qPCR assay and to have a 10,000 fold higher analytical sensitivity than the FMDV-specific antigen LFD currently used in the field. Importantly, this study demonstrated that FMDV RNA could be detected from epithelial suspensions without the need for prior RNA extraction, utilising a rudimentary heat source for amplification. Once optimised, this RT-LAMP-LFD protocol was able to detect multiple serotypes from field epithelial samples, in addition to detecting FMDV in the air surrounding infected cattle, pigs and sheep, including pre-clinical detection. This study describes the development and evaluation of an assay format, which may be used as a future basis for rapid and low cost detection of FMDV. In addition it provides providing "proof of concept" for the future use of LAMP assays to tackle other challenging diagnostic scenarios encompassing veterinary and human health.



Realising the potential of simple isothermal molecular tools for field diagnosis of FMD

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Requirements for *in situ* diagnostics:

1. Three elements of a molecular test: (sample preparation, amplification, detection)
2. "Ready-to-use" kit
3. Reagents compatible with field deployment

Lyophilised singleplex RT-LAMP:

Analytical sensitivity comparable to rRT-PCR

- Laboratory concordance (rRT-PCR/Wet/Dry)
- Dilution series of RNA standard
- Comparison with the gold-standard Callahan rRT-PCR

Copy number of RNA standard →

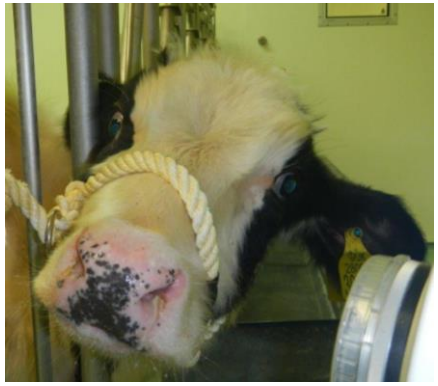
RT-LAMP (RNA standard)*		10^6	10^5	10^4	10^3	10^2	10^1	10^0	Negative
rRT-PCR		+	+	+	+	+	+	+/-	-
Wet reagents	rRT-LAMP	+	+	+	+	+	+	-	-
	RT-LAMP-LFD								
Lyophilised reagents	rRT-LAMP	+	+	+	+	+	+	+/-	-
	RT-LAMP-LFD								

Test ---
Control ---

*RNA standard supplied by Graham Freimanis

Expansion of sample preparation:

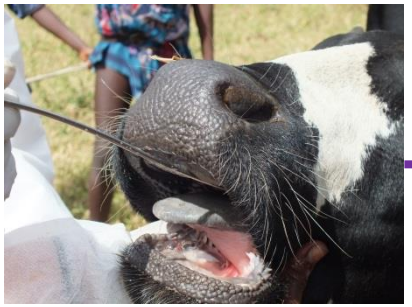
From the laboratory to the field



From Jose Gonzales

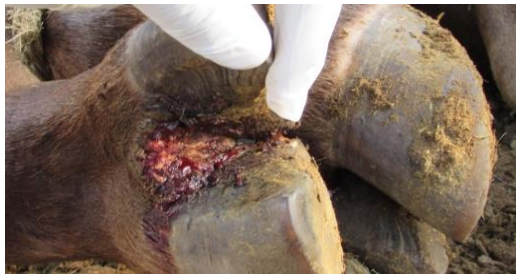
Cattle samples from experimental infection		Serum samples				OP* fluid samples			
		0dpc	1dpc	2dpc	3dpc	0dpc	1dpc	2dpc	3dpc
rRT-PCR		-	+	+	+	-	+	+	+
rRT-LAMP	Extracted RNA	-	+	+	+	-	+	+	+
	Neat	-	IN	IN	IN	NS	NS	NS	+
	1 in 5 dilution	-	+	+	+	NS	+	+	+
	1 in 10 dilution	-	+	+	+	-	+	+	+

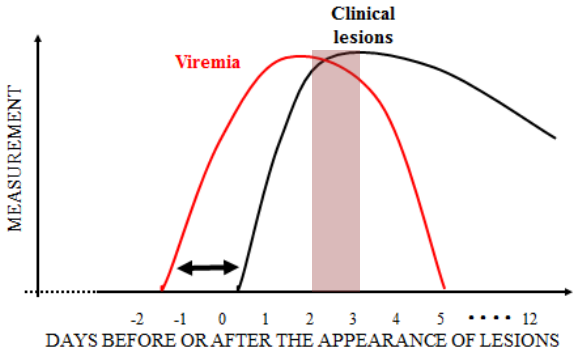
*oesophageal-pharyngeal (probang)
IN: Inhibitory
NS: Non-specific amplification



Detection of FMDV in clinical animals:

2 days after the appearance of clinical signs





King *et al.*, 2012 (data from Alexandersen *et al.* 2003)

Animal ear tag

		7803	7804	7805	7806	Negative**
Foot Epithelium	Mobile rRT-PCR*	+	+	+	+	-
	rRT-LAMP	+	+	+	+	-
	RT-LAMP-LFD					
Serum	rRT-LAMP	+	+	+	+	-
	RT-LAMP-LFD					
OP fluid	rRT-LAMP	+	+	+	+	-
	RT-LAMP-LFD					

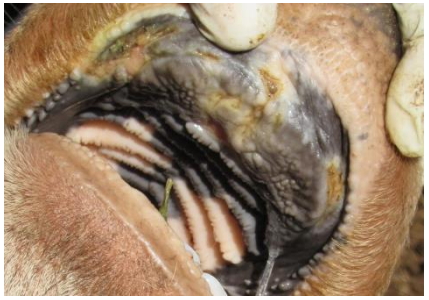
Test
Control

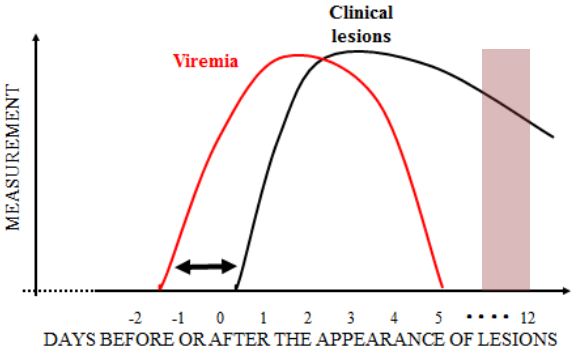
Epithelial samples positive by rRT-PCR were also positive by Ag-LFD

*mobile rRT-PCR is ~one log less sensitive than the OIE laboratory gold standard
**Negative water control

Detection of FMDV in late infection:

10 days after the appearance of clinical signs





King *et al.*, 2012 (data from Alexandersen *et al.* 2003)

Animal ear tag

		7807	7808	7809	7810	7812	Negative
Mouth Epithelium	Mobile rRT-PCR*	+	+	+	+	+	-
	rRT-LAMP	+	+	+	+	+	-
	RT-LAMP-LFD						
Serum	rRT-LAMP	-	-	-	-	-	-
	RT-LAMP-LFD						
OP fluid	rRT-LAMP	+	-	+	+	-	-
	RT-LAMP-LFD						

Test ---
Control ---

Epithelial samples were all negative on Ag-LFD

*mobile rRT-PCR is ~one log less sensitive than the OIE laboratory gold standard

Clinically normal cattle:

ca. 1 month after the appearance of clinical signs

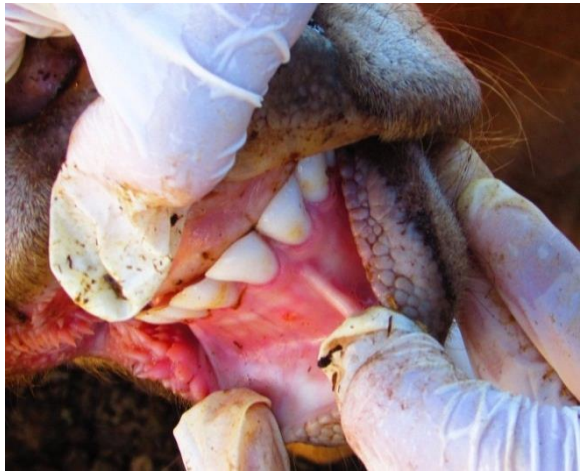
Animal ear tag

		7732	7733	7734	7735	7737	7739	7741	7742	7645	7648	7649	7650
Serum	**rRT-LAMP**	-	-	-	-	-	-	-	-	-	-	-	-
	RT-LAMP-LFD												
OP fluid	**rRT-LAMP**	-	+	+	-	-	+	-	-	-	-	-	-
	RT-LAMP-LFD												

Test --- Control ---

previously displayed clinical symptoms

no clinical symptoms



The Solution

RT-LAMP-LFD Closed system

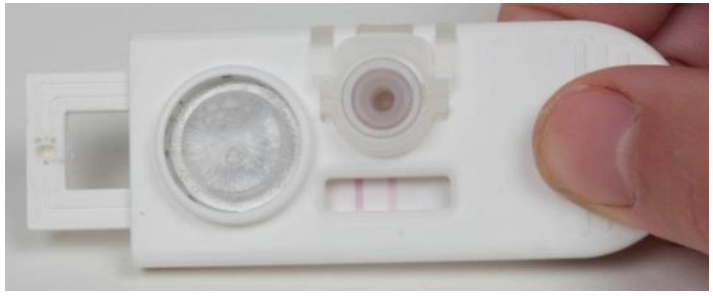
To develop and perform initial validation of closed RT-LAMP device (AMPlite) for detection of FMDV at the penside

What is the AMPlite?

- **Closed system** to minimise **technical input** required for assay and to **minimise cross-contamination**.
- **Inexpensive** heating block with **disposable** consumables.

OptiGene

Animal & Plant Health Agency



- Waters et al (2014) singleplex FMDV RT-LAMP assay was modified to incorporate a isothermal mastermix produced by Optigene.
- Analytical sensitivity was assessed using wet and lyophilised reagents, with and without internal control primers and control.
- Performance of assay and device was compared against rRT-PCR and real time RT-LAMP/ RT-LAMP-LFD.

life technologies

ThermoFisher SCIENTIFIC

AMPlite: Summary and impact

- Test confidence:

Analytical sensitivity for AMPlite is one log less than rRT-PCR using wet reagents
Integrated internal control line develops to confirm test negative

Multiplex RT-LAMP (RNA standard)		10^0	10^1	10^2	10^3	10^4	10^5	10^6
Wet	RT-LAMP	-	+	+	+	+	+	+
	Amplite	-	-	+	+	+	+	+
	rRT-PCR	-/+	+	+	+	+	+	+
Lyophilised	RT-LAMP	-	+	+	+	+	+	+
	Amplite	-	+/-	+	+	+	+	+
	rRT-PCR	+/-	+	+	+	+	+	+

- Test simplicity:

AMPlite LAMP can be performed directly on diluted clinical samples and; visualised at end point molecular LFD's with minimum user interference.
Epithelium suspensions can be made *in situ* using Svanova Ag extraction vials.

- Test rapidity: sample processing to test completion within 30 minutes.

AMPlite: Summary and impact

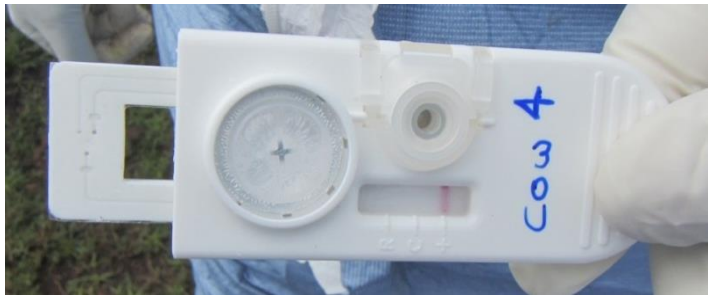
- Test robustness:

The robustness of the AMPlite was tested in Kenya in December 2014 with power provided via a vehicle auxiliary.

Epithelial and serum samples were collected and assayed on the AMPlite and compared to real time RT-LAMP and RT-LAMP-LFD run on Genie II.

Complete concordance was observed between AMPlite and real time RT-LAMP and RT-LAMP-LFD run on Genie II. More samples will be assayed in Nepal in 2015.

Cow	Sample	(a) RT-LAMP	(b) RT-LAMP-LFD	(c) AMPlite
4	Epithelium	+	+	+
4	Serum	-	-	-
9	Epithelium	+	+	+



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