

Detection of Foot-and-Mouth Disease Virus on an Automated Sample Preparation and PCR Point-of-Care Instrument

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INTRODUCTION

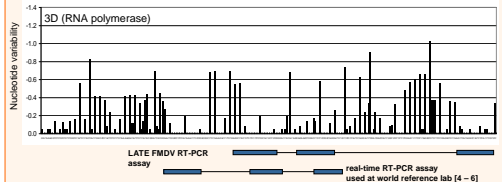
- Foot and mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals including cattle, sheep, pigs and goats
- FMD is considered the most economically important disease affecting farm livestock
- Speed and accuracy of diagnosis are essential to contain and eradicate disease
- Time to transport suspect clinical material to central laboratory can delay and may preclude laboratory confirmation in event of an FMD outbreak
- FMD is caused by a positive-stranded RNA virus
- Assay should be capable of detecting the presence of all 7 serotypes (A, C, O, Asia 1, SAT 1, 2 & 3) and variation within serotypes

AIM

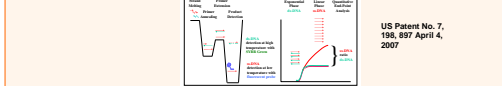
To develop an FMDV RT-PCR assay which detects all 7 serotypes and is suitable for a field device

METHODS

- Assay design using LATE-PCR technology
 - LATE-PCR is an advanced form of asymmetric PCR
 - Highly conserved sequences in the FMDV 3D (RNA polymerase) gene were used to design a limiting and excess primer & probe



- Primers were pre-incubated with RNA targets prior to reverse transcription
- Phase I: exponential amplification of limited number of double-stranded amplicons
- Phase II: linear amplification of one strand to generate single-strand amplicons
- Single-strands accumulate, 10 to 20 fold more abundant than double-strands
- Single-strand amplicons detected after the extension-step, or at end-point
- Low-Tm probe is mis-match tolerant and hybridizes to sequence variants
- Assay includes an internal control target that hybridizes to a probe with a different fluorophore



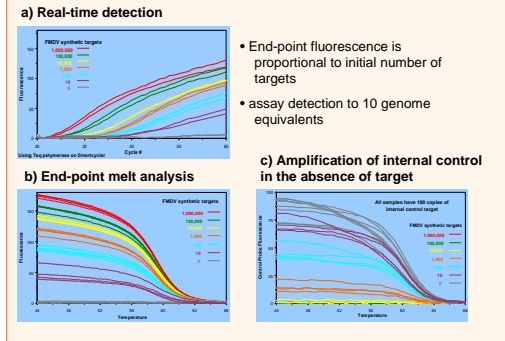
- Assay feasibility
 - Initial optimisation performed using synthetic DNA/RNA targets
 - Optimize reaction conditions and assay sensitivity
 - Source reagents and compare

- Assay verification using clinical samples containing FMD viral RNA
 - Laboratory work performed at the Institute for Animal Health
 - Used samples of epithelial tissue submitted to World Reference Laboratory
 - FMDV epithelial suspensions (ES) prepared and stored at -80°C
 - Suspensions had been assayed for FMDV by using VI and Ag-ELISA
 - Samples used covered all 7 FMDV serotypes
 - Suspect samples from animals with 'look-a-like' clinical symptoms, but infected with non-FMD vesicular virus (vesicular stomatitis virus (VSV), vesicular exanthema of swine virus (VESV), swine vesicular disease virus (SVDV), equine rhinitis A virus (ERAV)) were tested to assess assay specificity
 - ES used as the starting material for RNA extraction
 - In parallel experiments, RNA was tested by the LATE assay, and by the real-time RT-PCR assay used at the World Reference Laboratory, both targeting 3D RNA polymerase region [4-6]

- Assay performance on Bio-Seq-Vet compared to laboratory instruments
 - Parallel samples prepared and thermocycled on portable Bio-Seq Vet and compared with laboratory SmartCycler II and Stratagene Mx3005 instruments

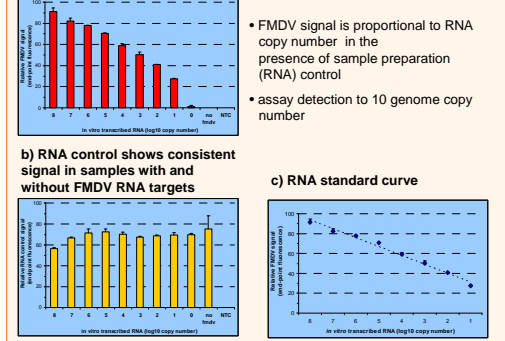
RESULTS

- Concept & feasibility of LATE FMDV PCR assay

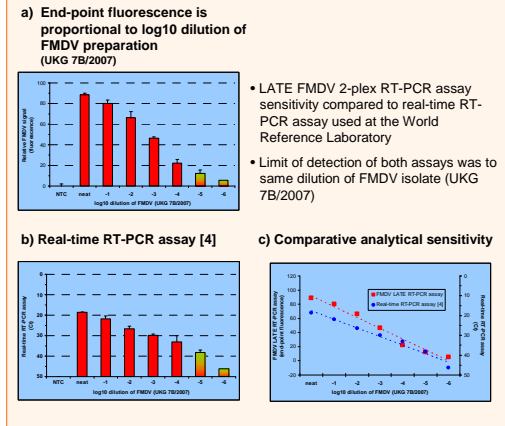


- Verification using samples containing FMDV RNA

Absolute sensitivity (fig. 2)

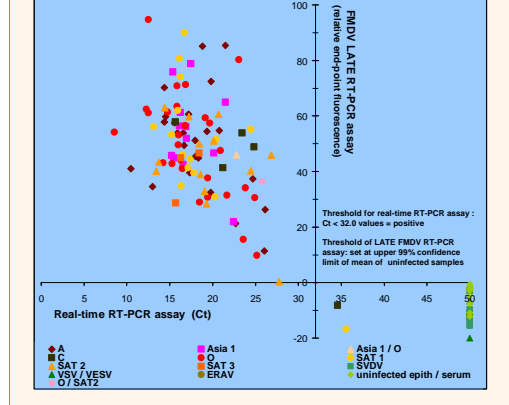


Analytical sensitivity (fig. 3)



RESULTS

- Comparison of LATE FMDV RT-PCR assay with real-time RT-PCR assay used at the World Reference Laboratory (fig. 4)



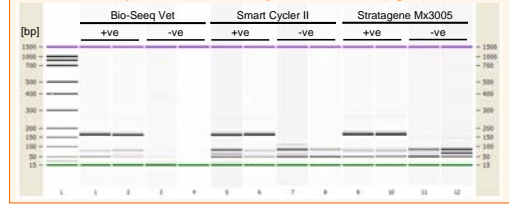
- LATE FMDV RT-PCR assay shows equivalent specificity to real-time RT-PCR assay routinely used at the World Reference Laboratory & (table 1)

FMDV Serotype	# of isolates tested	Positive Results by:		
		Culture (gold standard)	Real-time PCR &	LATE-PCR
A	30	30	30	30
C	5	5	4	4
O	36	36	36	36
Asia 1	12	12	12	12
SAT 1	16	16	15	15
SAT 2	14	14	14	14
SAT 3	3	3	3	3
Asia 1 / O	1	1	1	1
O / SAT 2	1	1	1	1
TOTAL	118	118	116	116

NON-FMDV negative control isolates²: Tests for false positives

Sample	Culture (gold standard)	Real-time PCR &	LATE-PCR
SVDV	8	NA	0
VSV / VESV	2	NA	0
ERAV	2	NA	0
Uninfected epithelium / serum	16	NA	0
TOTAL	28	NA	0

- Equivalent performance of LATE FMDV RT-PCR assay on point of care Bio-Seq-Vet instrument compared to laboratory instruments (fig. 5)



Bio-Seq-Vet Portable Diagnostic Laboratory

Automated Sample Preparation and PCR Instrument

Overview of work flow

Insert reagent pack **Collect sample** **Sample-in** **Cap-on** **Place on module** **Press start** **Result-out to regional / reference laboratory**

- Purifies DNA or RNA from a wide range of sample types (blood, faecal/nasal swabs, vesicular tissue and milk)
- Completely sealed unit containing bio hazardous material can be immersed for decontamination after use
- Simple, completely automated sample preparation and PCR driven by the Bio-Seq-Vet instrument
- All enzymes and additives supplied as temperature stable beads
- Communicates with Bio-Seq-Vet to download assay formats and conditions for latest assays
- Five independent modules
- Touch screen, step-by-step instructions
- Sample and location details logged
- Time to finish for each sample
- Result displayed QR data up-linked to regional / reference laboratory

SUMMARY & CONCLUSIONS

- LATE-PCR comparative detection can be done using either real-time or end-point detection (fig. 1, 2 & 3)
- An encapsulated RNA control will confirm sample preparation and RT-PCR process worked in absence of FMD virus (fig. 2b.)
- LATE RT-PCR FMDV assay shows equivalent sensitivity (fig. 2 & 3) and specificity (fig. 4 and table 1) as the 3D RT-PCR assay used at the World Reference Laboratory [4-6], and greater sensitivity than VI / ELISA
- This assay is currently being evaluated on the Bio-Seq-Vet Portable Diagnostic Laboratory (fig. 5)

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