

DETECTION OF ALL SEROTYPES OF FOOT AND MOUTH DISEASE VIRUS USING LATE-PCR

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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

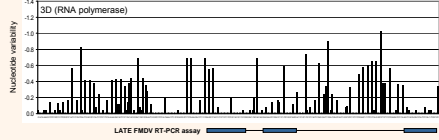
AIM

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

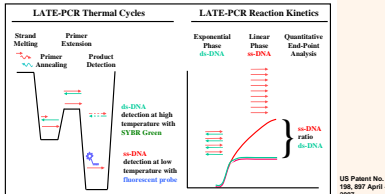
METHODS

1) 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



2) Assay feasibility

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

3) Assay verification using clinical samples containing FMD viral RNA

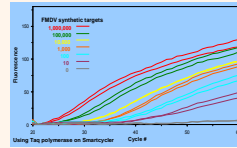
- Laboratory work performed at the Institute for Animal Health
- Used samples of epithelial tissue submitted to World Reference Laboratory
- FMDV epithelial suspensions (ES) prepared and stored at -80°C
- Suspensions had been assayed for FMDV by using VI and Ag-ELISA
- Samples used covered all 7 FMDV 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]

RESULTS

1) Concept & feasibility of LATE FMDV PCR assay

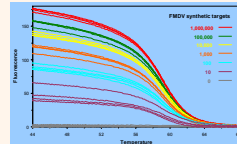
Synthetic (DNA) target (fig. 1)

a) Real-time detection

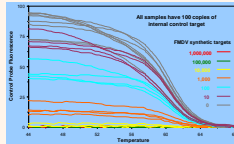


- End-point fluorescence is proportional to initial number of targets
- assay detection to 10 genome equivalents

b) End-point melt analysis



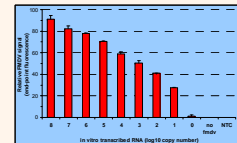
c) Amplification of internal control in the absence of target



2) Verification using samples containing FMDV RNA

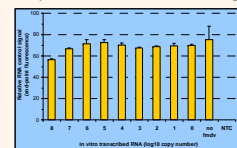
Absolute sensitivity (fig. 2)

a) End-point fluorescence is proportional to RNA standard

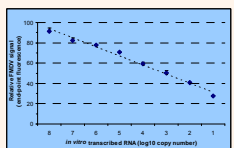


- FMDV signal is proportional to RNA copy number in the presence of sample preparation (RNA) control
- assay detection to 10 genome copy number

b) RNA control shows consistent signal in samples with and without FMDV RNA targets

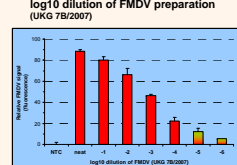


c) RNA standard curve



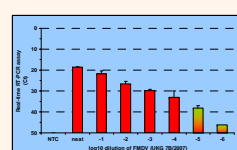
Analytical sensitivity (fig. 3)

a) End-point fluorescence is proportional to log10 dilution of FMDV preparation (UKG 7B/2007)

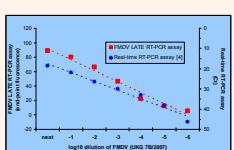


- LATE FMDV 2-plex RT-PCR assay sensitivity compared to real-time RT-PCR assay used at the World Reference Laboratory
- Limit of detection of both assays was to same dilution of FMDV isolate (UKG 7B/2007)

b) Real-time RT-PCR assay [4]



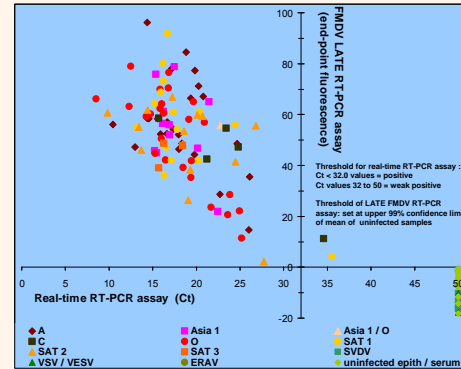
c) Comparative analytical sensitivity



²Supported by Smiths Detection, Inc

RESULTS

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



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

FMDV Isolates Serotype	Strains Tested	Positive Results by:		
		Culture (gold standard)	Real-time PCR ^a	LATE-PCR
A	24	24	24	24
C	5	5	4	5
O	25	25	25	25
Asia 1	12	12	12	12
SAT 1	15	15	14	15
SAT 2	14	14	14	14
SAT 3	3	3	3	3
TOTAL	98	98	96†	98
Control Isolates: Tests for false positives				
Non-FMDV [‡]	11	NA	0	0
uninfected epithelium / serum	17	0	0	0

& - Callaghan 3D 1-step RT-PCR assay [3 - 6]; † - Two samples had Ct values >32, and classified as weak positives; ‡ - Suspect samples from animals with 'look-alike' clinical symptoms, but infected with non-FMD vesicular virus (VSV, VESV, SVDV, ERAV); NA - not applicable

SUMMARY & CONCLUSIONS

- LATE-PCR quantification 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

³Supported by Defra UK (project SE 1121 / SE 1124)



Bio-Seq@-Vet Portable Diagnostic Laboratory

Automated Sample Preparation and PCR Instrument

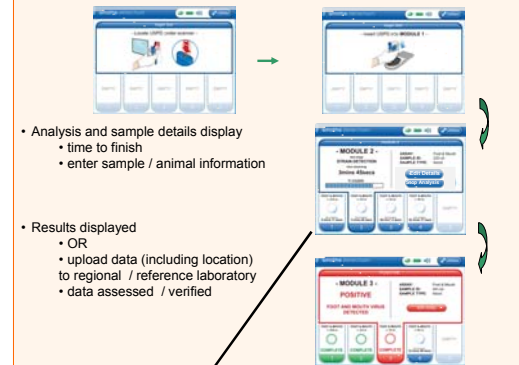


- Purifies DNA or RNA from a wide range of sample types (blood, faecal/nasal swabs, vesicular tissue and milk)
- Completely sealed unit containing bio hazardous material can be immersed for decontamination after use
- Simple, completely automated sample preparation and PCR driven by the Bio-Seq@ instrument
- All enzymes and additives supplied as temperature stable beads
- Communicates with Bio-Seq@ to download assay formats and conditions for latest assays

Overview of workflow



- Insert reagent pack → collect sample → put sample in → close & mix / macerate
- Scan barcode and insert sample preparation unit into module to start process



- Analysis and sample details display
 - time to finish
 - enter sample / animal information
- Results displayed
 - OR
 - upload data (including location) to regional / reference laboratory
 - data assessed / verified



References:
 [1] Rice, J.E., Sanchez, J.A., Pierce, K.E., Reis, A. H., Osborne A and Wangh, L.J. (2007). Multiplex/multiplex linear after-the-exponential-PCR assays combined with PrimeSafe and Dilute-N-Go sequencing. Nature Protocols, 2 (10), 2429 - 38.
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 [3] Pierce, K.E. and Wangh, L.J. (2007). LATE-PCR and allied technologies: Real-time detection strategies for rapid, reliable diagnosis from single cells. In: Single Cell Diagnostics, A.R. Thornton (ed.), Humana Press, pages 65-85.
 [4] Callaghan, J.D., Brown, F., Osorio FA, Sur, J.H., Kramer, E., Long, W.G., Lutbroth, J., Ellis, S.J., Shoulars, K.S., Gaffney, K.L., Rock, D.L., Nelson WM. (2002). Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. J Am Vet Med Assoc, 220 (11), 1636-42.
 [5] Reid SM, Olsen SS, Ferris NP, Hutchings GH, Alexandersen S. (2003). Evaluation of automated RT-PCR to accelerate the laboratory diagnosis of foot-and-mouth disease virus. J Virol Methods 107 (2): 129-39.
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