LATE-PCR DETECTION OF FOOT AND MOUTH DISEASE VIRUS

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INTRODUCTION

Foot and Mouth Disease (FMD) is a highly contagious disease of cloven-hoofed animals (cattle, sheep, pigs, goats). FMD is caused by the most economically important disease affecting farm livestock. Rapid and accuracy of diagnosis, essential to contain and eradicate disease.

Time to transport suspect material to central laboratory can delay and may preclude laboratory confirmation in event of an FMD outbreak. FMD is caused by a positive-strand RNA virus. Virus detection is complicated by the presence of 7 serotypes (A, C, O, Asia 1, SAT 1, 2 & 3) and variation within serotypes.

AIM

To develop a pan-FMDV assay, suitable for a field device.

METHODS

1) Assay design using LATE PCR technology [1–3]

- LATE PCR is an advanced form of asymmetric PCR
- Highly conserved sequences in the 3D (RNA polymerase) gene were used to design a targeting primer and probe
- RT-PCR was performed using the excess primer to initiate cDNA synthesis
- Phase I: exponential amplification of number of double-stranded amplicons
- Phase II: linear amplification of one strand to generate single-stranded amplicons
- Single-strands accumulate, 10-20 fold more abundant than double-strands
- Single-strand amplicons detected after the extension-step, or at end-point
- Low-Tm probe mismatch tolerant and hybridizes to sequence variants
- Assay includes an internal control target that is amplified by the same primers and hybridizes to a probe with a different fluoroprobe

2) Assay feasibility

- Initial optimisation performed using synthetic/mimic targets
- Test the assay concept
- Determine optimal reaction conditions
- Assay sensitivity
- Source reagents and compare

3) Assay verification using samples containing FMD viral RNA

- Laboratory work performed at the Institute for Animal Health
- Used samples of epithelial tissue submitted to World Reference Laboratory
- FMD epithelial suspensions (ELS) prepared and stored at 80°C
- Suspensions had been assayed for FMD by using VI and Ag-ELISA
- Samples used covered all 7 FMDV serotypes
- Suspect samples where no FMDV had been detected by VI / Ag-ELISA were classified as unknown
- Suspect samples from animals with 'look-like' clinical symptoms, but infected with non-FMDV, vesicular virus samples (VSV, PPV, VESV, MSV)-1 were tested to assess assay specificity
- ELS used as the starting material for RNA extraction
- In parallel experiments, RNA was tested by the LATE assay and by the reference laboratory, 1-step RT-PCR assay, both targeting 3D RNA polymerase region [4 – 6]

4) LATE assay tested on samples with predicted mismatches to primers

In parallel experiments, the LATE and reference RT-PCR assays were: (a) challenged with samples with predicted mismatches to both primers and (b) select samples re-tested to assess assay repeatability.

RESULTS

Concept & feasibility of LATE FMDV PCR assay (fig. 1)

- Synthetic target

a) Real-time detection
b) End-point melt analysis

c) End-point fluorescence proportional to initial number of targets

- Amplification of internal control in the absence of target

Verification of LATE FMDV RT-PCR assay (fig. 2)

Samples of all 7 serotypes

Non-FMDV

Vesicular virus samples (negative controls)

FMDV amplicons melt from probe at temperatures that vary with strain sequences (fig. 3)

End-point melt analysis

Internal control detected only in absence of FMDV virus (fig. 4)

Confirms PCR reaction worked when test is negative

FMDV Isolates

<table>
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<tr>
<th>Serotype</th>
<th>Samples Tested</th>
<th>Culture (pct standard)</th>
<th>Symmetric PCR</th>
<th>Positive Results by:</th>
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Control Isolates: Tests for false positives

Non-FMDV

4

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Control Isolates: Tests for false positives

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SUMMARY & CONCLUSION

- LATE PCR quantification can be done using either real-time or end-point detection (fig 1a & 2)
- Melt analysis confirms that strains with different sequence variations can be detected using a single mismatch tolerant probe (fig 3)
- An internal positive control included in the assay confirms PCR worked in absence of FMD virus (fig 1 & 4)
- LATE RT-PCR FMDV assay shows similar specificity and sensitivity as the world reference RT-PCR assay (3D region) (table 1 & 2) (4 – 6)
- LATE RT-PCR assay detected samples containing predicted mismatches to primers (table 2)

This assay will be evaluated on a portable sample preparation and PCR device developed by Smiths Detection

REFERENCES: