Appendix 65

LINEAR-AFTER-THE-EXPONENTIAL (LATE) PCR: NEW DETECTION TECHNOLOGIES FOR PAN-FMDV AND SEROTYPE-SPECIFIC ASSAYS IN THE FIELD

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INTRODUCTION

Detection and serotype identification of Foot-and-mouth Disease virus (FMDV) RNA is complicated by high sequence variation between strains. Linear-After-The-Exponential (LATE)-PCR efficiently generates single-stranded DNA that is freely available to hybridize with fluorescently labelled, mismatch-tolerant probes over a wide temperature range, enabling the detection of numerous sequence variants. Two assays are being developed; a pan-FMDV assay intended for detection of all strain variants and a second to distinguish between FMDV serotypes.

MATERIAL AND METHODS

Limiting and excess primers were designed for the relatively conserved sequences in the FMDV 3D (RNA polymerase) gene or in the 1D (VP1 capsid protein) to 2B genes using LATE-PCR design criteria. RNA targets are pre-incubated with primers, and then amplified using a one-step RT-PCR reaction. Fluorescent signals from the probes are measured over a wide range of temperatures following RT-PCR. Control RNA sequences are co-amplified and detected with a separate probe to guard against false negatives.

RESULTS

Initial experiments using synthetic DNA templates demonstrated that the fluorescent intensity of the probe at end point was proportional to the initial concentration of the templates over a wide concentration range, 10 copies to 1 million copies. Subsequent testing showed that viral RNA from each of the 7 FMDV serotypes could be amplified and then detected with a single mismatch-tolerant probe in the pan-FMDV assay. All samples with unrelated viruses remained negative for FMDV signals. All non-FMDV samples and other control samples without virus showed amplification of the internal RNA control. Tests with the serotype-specific assay are ongoing. A probe for the Asia 1 serotype generated a strong fluorescent signal with synthetic targets for Asia 1 variants, but no signal with an O serotype target with the most similar sequence.

DISCUSSION AND CONCLUSIONS

LATE-PCR with a single mismatch-tolerant probe can be used for pan-detection of FMDV. A second assay with specific probes should provide serotype characterization of the virus. These quantitative end-point assays can be used in laboratory thermal cyclers or in field instruments, such as the BioSeeq^{TM}-Vet Portable Diagnostic Laboratory, for rapid detection of FMDV in samples from suspect animals.