

New detection technologies for pan-FMDV and serotype-specific assays in the field.

Kenneth E. Pierce^{1*}, Rohit Mistry², Suki Bharya², Scott M. Reid³, Katja Ebert³, Donald P. King³, and Lawrence J. Wangh¹

¹ Department of Biology, Brandeis University, Waltham, MA 02454, USA

² Smiths Detection, Watford, WD23 2BW, UK

³ Institute for Animal Health, Pirbright, GU24 0NF, UK

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 labeled, 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 BioSeeqTM-Vet Portable Diagnostic Laboratory, for rapid detection of FMDV in samples from suspect animals.