Rapid detection of porcine encephalomyocarditis virus (EMCV) by isothermal reverse transcription recombinase polymerase amplification assays

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Abstract

Reverse transcription recombinase polymerase amplification assays combined with the fluorescence detection platform (qRT-RPA) and lateral flow biosensor (LFB RT-RPA) were developed and validated for rapid detection of porcine EMCV. The primers and probes were designed based on the highly conserved region of 3D gene of porcine EMCV. The optimal reaction condition of qRT-RPA and LFB RT-RPA assay was at 42 °C for 20 min. The assays were highly specific to EMCV and no cross-reactions were observed with other 7 porcine viruses. With the 10-fold sieral diluted EMCV genomic RNA as template, the limit of detection of the qRT-RPA assay was 1.0×10^{2} copies and the limit of detection of LFB RT-RPA was 1.0×10^{1} copies. A total of 92 samples collected from EMCV inoculated mice (24), control mice (8) and pigs showing reproductive failures (60) were examined using developed RT-RPA and a described qRT-PCR, and the diagnostic agreement between qRT-RPA (23/92), LFB RT-RPA (25/92) and qRT-PCR (23/92) was 100% and 97.83, respectively. The performances of the developed RT-RPA assays were comparable to a qRT-PCR, while the RPA assays needed less time and easy to perform to obtain the positive results and LFB RT-RPA was higher sensitive than the qRT-PCR. The developed EMCV RT-RPA assays are rapid, reliable and easy to perform, which provide an attractive and promising tool for effective detection of EMCV in low-resource settings.

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