Validation of a Real-time RT PCR (qRT-PCR) Assay for the Detection of Foot-and-Mouth Disease Virus in Thailand Using a Portable Real-time PCR Machine

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Abstract

Background: Foot and mouth disease (FMD) is a highly contagious viral disease affecting cloven-hoofed animals. Molecular diagnostic techniques, especially qRT-PCR, show high sensitivity and specificity and save time compared to traditional diagnostic methods. However, in an FMD diagnostics, a qRT-PCR assay by the portable real-time PCR machine (Picogene®) needs to be validated and evaluated to ensure test performance.

Objectives: This study aims to validate a qRT-PCR assay by the portable real-time PCR machine (Picogene®) to ensure its accuracy under laboratory conditions.

Methods: FMDV serotype Asia 1 was used to evaluate the limit of detection (LOD) and PCR efficiency, while 10 infectious samples from cloven-hoof animals were used to evaluate analytical specificity (Asp). 45 Positive and 15 negative reference samples from viral fluids were used to determine the diagnostic sensitivity and specificity (DSe and DSp), and accuracy. All samples were extracted, and qRT-PCR was performed using primers and probes specific to the 3D region of FMD virus using a portable real-time PCR machine. Then, compared the DSe results with the qRT-PCR assay using a conventional real-time PCR machine.

Results: The qRT-PCR assay by the portable real-time PCR machine for FMD detection showed 100% of DSe, DSp, and accuracy. The LOD of the portable real-time PCR machine was 10^3 $TCID_{50}$/mL. For linearity results, the portable real-time PCR machine exhibited R^2 greater than 0.99 ($R^2 = 0.9947$), confirming that the test is consistent and reliable across different concentrations of FMD virus. Moreover, this experiment showed that PCR efficiency was 90%, confirming the suitability of the protocol, including primer/probe concentrations and PCR temperature. In part of Asp, the results presented 100% Asp, which showed no cross-reactivity to other viruses, including Bluetongue virus (BTV), Vesicular Stomatitis Virus (VSV), Bovine Viral Diarrhea Virus (BVDV), Bovine respiratory syncytial virus (BRSV), Infectious Bovine Rhinotracheitis/Bovine Herpesvirus (IBR/BHV), and Parapoxvirus. Lastly, the qRT-PCR assay by the portable PCR machine presented a Ct value (mean Ct = 24.28) greater than the conventional qRT-PCR machine (mean Ct = 20.83) ($p < 0.01$).

Conclusions: This study validated the qRT-PCR assay by the portable real-time PCR machine as an effective, reliable, and reproducible diagnostic tool for FMD virus detection, which is suitable for use in FMD virus diagnostics, contributing to improving FMD surveillance and control of FMD outbreaks in Thailand.

Keywords: Foot and mouth disease, qRT-PCR, Validation

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