Development of a SYBR Green I based real-time RT-PCR assay for detection and quantification of bovine coronavirus

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Abstract

A novel two-step, SYBR Green I based real-time RT-PCR assay was developed for detection and quantification of BCoV using ABI PRISM 7500 sequence detection system. The assay was carried out using two sets of primers designed to amplify highly conserved sequences of the nucleocapsid gene of BCoV and the internal control, bovine glyceraldehyde-3-phosphate dehydrogenase, RNA. Specific identification of both targets was elucidated by melt curve analysis, in which the BCoV amplified product generated a melt peak at 78.35 ± 0.26 °C and the internal control RNA at 82.54 ± 0.32 °C. The assay was highly specific since all negative controls and other viruses of clinical and structural relevance failed to develop any positive results. The detection limit of the reaction was 10(3) plasmid copies and 1.17 × 10(-3) TCID(50) of the tissue culture propagated virus. Standard deviation and coefficient of variation was low for both intra-assay and inter-assay variability. The assay performance on field samples was evaluated on 103 (68 fecal and 35 nasal) swab specimens and compared with the conventional RT-PCR assay. The results of both assays matched for the diagnosis of 65 fecal and 33 nasal samples. However, three fecal and two nasal samples tested negative in gel-based assay were positive for the real-time RT-PCR. The robustness and a high-throughput performance of the developed assay make it a powerful tool in diagnostic applications and in BCoV research.

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