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A reverse transcription loop-mediated isothermal amplification (LAMP) assay for the detection of feline Coronavirus.

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Abstract

Loop-mediated isothermal amplification (LAMP) is a molecular method that amplifies DNA under isothermal conditions. It relies on the use of 4 different primers recognizing 6 regions of the template sequence and on the use of a DNA polymerase with strand displacement activity (Notomi et al., 2000). The addition of two *loop primers* allows the reaction time to be of one hour only (Nagamine et al., 2002). The aim of this study was to develop a reverse transcription LAMP assay for an easy and inexpensive detection of feline Coronavirus (FCoV). Six primers binding the conserved 3'UTR region of the FCoV were designed with the Primer Explorer software. Thirty-two samples of RNA (11 feces, 8 effusions, 9 blood samples and 4 tissues) on which a reverse transcription polymerase chain reaction (RT-PCR) for the 3'UTR region was performed were used. The reaction was carried out in 25µL reaction volume and the mixture was incubated in a thermocycler at 63°C for 1 hour followed by 10 minutes at 80°C. LAMP products were visualized under UV after electrophoresis migration on a 1.5% agarose gel stained with ethidium bromide, where they produce a ladder-like pattern if positive. Results where compared with those obtained on standard PCR. Sensitivity and specificity were respectively 60% and 100% on feces, 40% and 100% on effusions, 25% and 100% on blood, and 100% and 100% on tissues. The overall sensitivity and specificity of this method were of 57.1% and 100%, thus limiting a clinical application of this method, except for tissues.

References

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