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Rapid Detection of Canine Parvovirus by Loop-Mediated Isothermal Amplification

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Loop-mediated isothermal amplification (LAMP) assay was developed for detecting the VP2 gene of canine parvovirus (CPV). The LAMP assay is a novel method of gene amplification that amplifies nucleic acid with high specificity, efficiency, and rapidity under isothermal conditions with a set of six specially designed primers that recognize six distinct sequences of the target. DNA was extracted from CPV strain (ATCC VR953) and 50 canine diarrheic samples suspected of suffering CPV infection by the DNAzol (MRC, INC). Reaction time and temperatures were optimized for 60 min at 63 °C, respectively. PCR was performed to identify 249 bp fragments situated on the VP2 gene. The sensitivity and specificity were compared for these two methods. A large amount of by-product, pyrophosphate ion, is produced yielding a white precipitate of magnesium pyrophosphate in the reaction mixture of LAMP. The presence or absence of this white precipitate allows easy detection of the amplification of CPV genomic DNA without gel electrophoresis. The detection limit using the LAMP method was up to 1 fg of DNA, when compared to 10 fg of DNA by PCR. The sensitivity of LAMP in comparison with that of PCR was 92% and the specificity was 100% (k=0.76). The advantage of the LAMP method is speed: only 1 hour was necessary for diagnosis of CPV infection. Therefore, this assay should be a useful aid to diagnosis of CPV infection in dogs, suggesting that this method may achieve clinical application.

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