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A rapid and sensitive loop-mediated isothermal amplification procedure (LAMP) for *Mycoplasma hyopneumoniae* detection based on the *p36* gene

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ABSTRACT. The aim of this study was to establish a method for sensitive and rapid diagnosis of *Mycoplasma hyopneumoniae* in clinical specimens. To this effect, we employed three sets of primers specifically designed for amplification of nucleic acids under isothermal conditions. After optimization of reaction conditions, *M. hyopneumoniae* could be successfully detected at 63°C in 45 min through use of the loop-

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mediated isothermal amplification (LAMP) assay. A positive reaction was identified visually as white precipitate and confirmed by gel electrophoresis. The detection limit for this assay was 10 copies/μL, as observed by electrophoretic analysis. The accuracy of the LAMP reaction was confirmed by restriction endonuclease digestion as well as by direct sequencing of the amplified product. This method can specifically detect *M. hyopneumoniae*; other species with high homology and other bacterial and virus strains gave negative results. To test the utility of this procedure, the LAMP assay was applied to 40 clinical samples collected from swine lung tissues experimentally challenged with *M. hyopneumoniae* isolates, and compared to the results from a real-time polymerase chain reaction (PCR) assay. A concordance of 100% was observed between the two assays. In conclusion, the results from our study demonstrated that the LAMP assay provided a rapid reaction and was inexpensive to perform, with no need of complex instruments or systems such as Geneamp PCR. The LAMP assay may therefore be applied in routine diagnosis in the clinical laboratory and for in-field detection of *M. hyopneumoniae* infection.

Key words: *p36* gene; Loop-mediated isothermal amplification; *Mycoplasma hyopneumoniae*

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