Development of a Loop-mediated Isothermal Amplification Assay for Screening *Mycoplasma* Contamination to Reduce Contamination Problems in Cell Culture

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Abstract

Background: *Mycoplasma* contamination is a major issue in cell culture, leading to abnormal cell growth and often necessitating the disposal of contaminated cultures, resulting in significant loss of time and resources. It also poses a risk of contaminating vaccine products derived from cultured cells. Common contaminating species in cell culture include *Mycoplasma orale*, *Mycoplasma arginini*, *Mycoplasma hyorhinis*, *Mycoplasma fermentans*, and *Acholeplasma laidlawii*. Standard detection methods such as culture and PCR provide high accuracy but are time-consuming or require costly equipment, while imported commercial test kits, although convenient, are expensive.

Objectives: To develop a Loop-mediated Isothermal Amplification (LAMP) assay for rapid screening of *Mycoplasma* contamination in cell culture and to evaluate its diagnostic performance.

Methods: A LAMP assay was developed for detecting *Mycoplasma* species commonly found in contaminated cell cultures including *Mycoplasma orale*, *Mycoplasma arginini*, *Mycoplasma hyorhinis*, *Mycoplasma fermentans*, and *Acholeplasma laidlawii*. The assay performance was evaluated in terms of analytical sensitivity, specificity, diagnostic sensitivity, diagnostic specificity, and overall accuracy. The limit of detection (LOD) was determined using serial dilutions of target DNA. Positive reactions were interpreted by turbidity.

Results: The developed assay demonstrated high specificity for common *Mycoplasma* species found in cell culture, with a limit of detection (LOD) of 10^3 copies/µL, diagnostic sensitivity of 97.14%, diagnostic specificity of 100%, and overall accuracy of 99.36%. In addition, the assay provided rapid results, was simple to perform, did not require sophisticated instruments, and was four times more cost-effective than imported commercial kits.

Conclusion: The developed LAMP assay is a reliable, rapid, cost-effective, and user-friendly method for screening *Mycoplasma* contamination in cell culture systems. This assay has strong potential as an effective surveillance tool to reduce contamination problems in cell culture processes and improve biosafety in vaccine and biological product production by enabling rapid early detection and prompt management of contaminated cell cultures. However, the high amplification efficiency of LAMP increases the risk of carryover contamination and false-positive results if strict laboratory precautions are not followed. In addition, visual interpretation based on turbidity may be subjective, particularly in samples with low target concentrations or weak positive reactions. Therefore, further validation and standardized interpretation criteria are recommended.

Keywords: *Mycoplasma* contamination, Cell culture, LAMP assay, Screening test

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