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

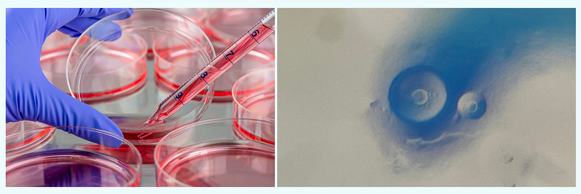
Surattana Boonsai*, Warisa Ketphan, Thammarath Sujit, Tapanut Songkasupa

National Institute of Animal Health, Kasetklang, Ladyao, Chatuchak, Bangkok, 10900 Thailand
*Corresponding author: Surattana Boonsai E-mail: surattanaboonsai@gmail.com

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 *M. orale*, *M. arginini*, *M. hyorhinis*, *M. fermentans*, *M. hominis*, 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. This study aimed to develop a Loop-mediated Isothermal Amplification (LAMP) assay for rapid screening of Mycoplasma contamination in cell culture and to evaluate its diagnostic performance.

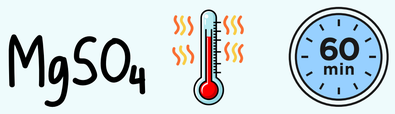
METHODOLOGY



1 Common cell culture *Mycoplasma* species (6 species)

2 Genomic DNA extraction

3 *16S rRNA* alignment and primer design by Primer Explorer V4



4 LAMP optimization ($MgSO_4$ conc., incubation temperature and period)

5 Analytical validation
- Limit of detection (LOD)
- Specificity
- Repeatability
- Reproducibility

6 Diagnostic validation
- n = 312 samples
- 180 cell cultures
- 90 culture media
- 42 laboratory reagents

compared with routine PCR

RESULTS AND DISCUSSION

- Successfully detected six common cell culture-associated *Mycoplasma* species
- Optimal LAMP conditions: 65°C for 60 min with 4 mM $MgSO_4$
- Visual interpretation with a total turnaround time of approximately 90 min **(Figure 1)**
- Requires only basic laboratory equipment (micropipettes, heating block, microcentrifuge, and reaction tubes) **(Figure 1)**

Cell culture supernatant
↓
DNA extraction
(~30 min)
↓
LAMP reaction
(65°C, 60 min)
↓
Visual interpretation
(~1 min)
↓
TOTAL TURNAROUND TIME ≈ 90 MIN

Figure 1. Workflow and basic laboratory equipment required for routine LAMP screening.

RESULTS AND DISCUSSION (CONTINUE)

- Limit of detection (LOD): 10^3 copies/µL (**Figure 2)**
- No cross-reactivity with non-target bacteria or yeast **(Table 1)**
- Diagnostic sensitivity, specificity, and accuracy compared with PCR were 97.14%, 100%, and 99.36%, respectively **(Table 2)**
- High agreement with routine PCR was observed (Cohen's κ = 0.98)
- Approximately 4-fold lower cost than commercial kits.
- Suitable for rapid and routine screening of *Mycoplasma* contamination in cell culture laboratories.

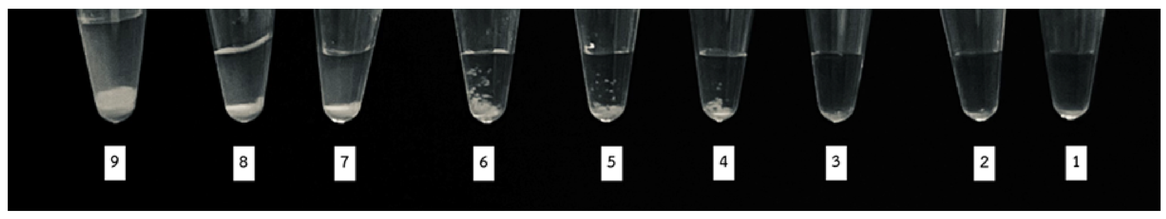


Figure 2 Limit of detection (LOD) of the developed LAMP assay.
Tube 1 = negative control; tubes 2–9 represent 10^1–10^8 copies/µL.
The assay detected as low as 10^3 copies/µL.

Table 1. Analytical validation

Parameter	Result
LOD	10^3 copies/µL
Analytical specificity	No cross-reactivity with non-target bacteria and yeast
Repeatability	100% agreement
Reproducibility	100% agreement

Table 2. Diagnostic validation (n = 312)

Parameter	Result
Diagnostic sensitivity	**97.14%**
Diagnostic specificity	**100%**
Accuracy	**99.36%**
Cohen's κ	**0.98**

CONCLUSION

- Rapid detection of *Mycoplasma* contamination within approximately 90 minutes.
- Requires only basic laboratory equipment.
- Demonstrated high agreement with routine PCR (Cohen's κ = 0.98).
- Comparable performance to commercial kits at approximately four-fold lower cost.
- Suitable for routine *Mycoplasma* screening in cell culture laboratories.

REFERENCE

[1] Drexler, G. and Uphoff, C. 2002. *Mycoplasma* contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. Cytotechnology. 39 (2): 75–90.
[2] Taylor, M., Gihoon C., Lawrence, D., Marc, S. and Robert, M.2021. LAMP Diagnostics at the Point-of-Care: Emerging Trends and Perspectives for the Developer Community. Expert Rev. Mol. Diagn. 21: 1, 43-61.

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