WHITEPAPER

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GenieColor Mycoplasma Detection Kit: A Visual Alternative to qPCR

Product: GenieColor Mycoplasma Detection Kit (MORV0013) | **Author:** Miren Ruiz de Eguilaz PhD

Introduction

Mycoplasma are a unique class of bacteria that lack a cell wall and possess very small genomes. Over time, several Mycoplasma species have lost their original host specificity (e.g., bovine, porcine, and human cells) and are now capable of colonizing a wide variety of cell cultures (1). In modern laboratory settings, infections are most commonly introduced through cross-contamination. Once present, Mycoplasma can thrive in cell culture media, attach firmly to cell membranes, or even invade the cytoplasm of eukaryotic cells (2). Their high pathogenicity, persistence, and remarkable ability to bypass standard sterilization and filtration techniques make them a significant and ongoing challenge for cell culture scientists..

While qPCR provides exceptional sensitivity and specificity for detecting Mycoplasma nucleic acids, it also demands specialized instrumentation, rigorous optimization, and complex workflows that can be both time-consuming and resource-intensive.

However, the GenieColor Mycoplasma Detection Kit (MORV0013) is a rapid, one-step colorimetric assay designed to detect mycoplasma contamination with high accuracy and minimal equipment requirements. By leveraging LAMP-based amplification and visual color change (**Figure 1**), GenieColor provides a fast and user-friendly alternative to conventional methods.

This technical note evaluates GenieColor's detection limits and accuracy relative to qPCR.

Figure 1. GenieColor Mycoplasma Detection Kit results interpretation, If Mycoplasma DNA is present, reagent colour changes from pink to yellow.

Validation Data

Purpose

This study aims to benchmark the GenieColor Mycoplasma Detection Kit (against qPCR, the gold standard for mycoplasma detection. The focus is on comparing sensitivity, accuracy, and practical usability across a range of sample types, concentrations and conditions.

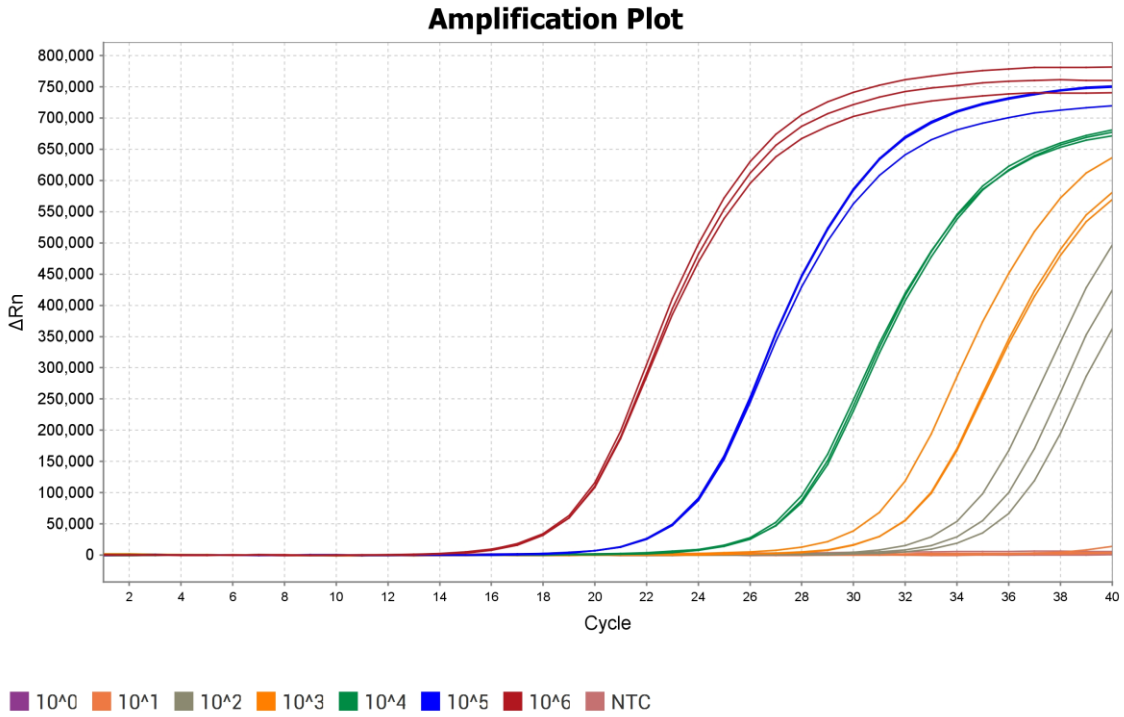
Methodology

- Plasmid Gradient Testing: Plasmid samples with varying copy numbers tested using GenieColor and qPCR.
- Cell Sample Gradient Testing: Mycoplasma-contaminated cell samples diluted at different ratios.
- Multi-Cell Line Testing: Diverse cell lines spiked with mycoplasma to compare detection outcomes.

Key Findings

Specificity

qPCR detects down to 10^2 copies/reaction (20 µL), while GenieColor reliably detects 10^4 copies/reaction. For cell samples, GenieColor matched qPCR at 1:1,000 dilutions but showed decreased detection at 1:10,000 (**Figure 2**).



Validation Data

Copies/rxn	Ct
10^6	18.75
10^5	23.11
10^4	27.10
10^3	31.45
10^2	34.82
10^1	–
10^0	–
NTC	–

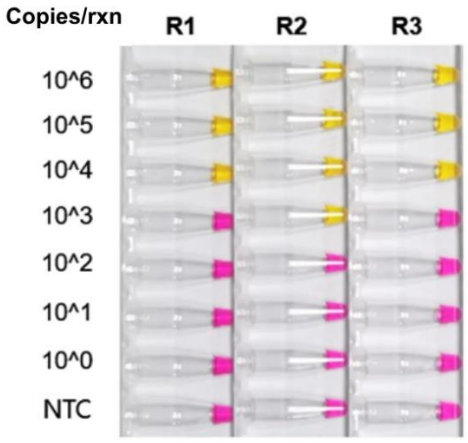
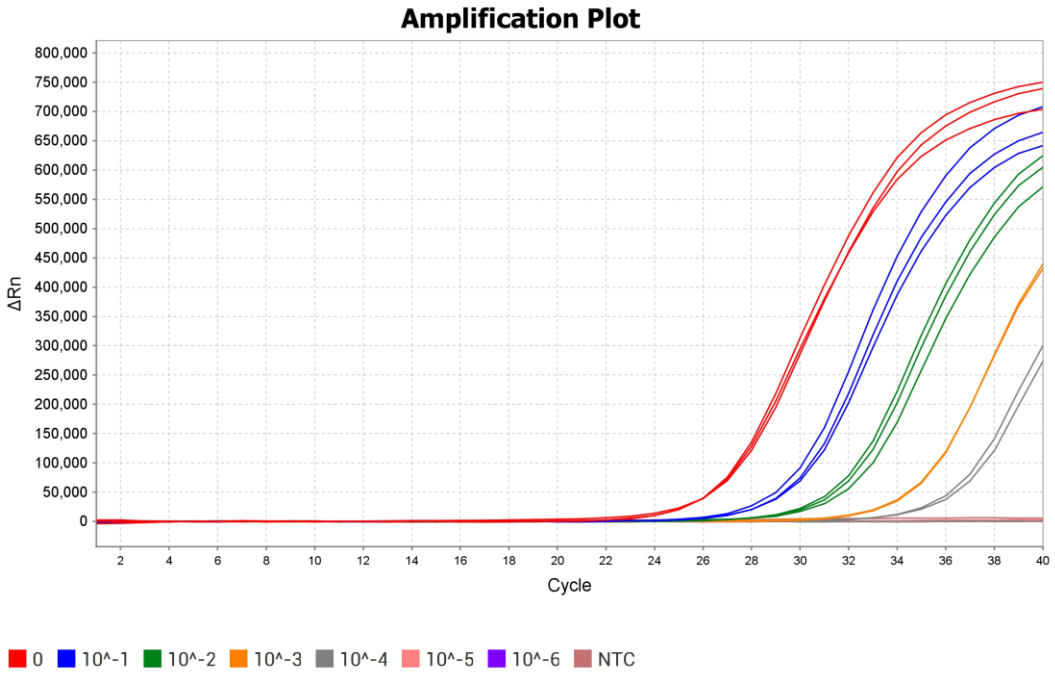


Figure 2: qPCR vs GenieColor results when testing with different plasmid concentrations. Samples were run in triplicates (n=3).

Sensitivity in cell cultures

The results indicate that GenieColor can reliably detect mycoplasma contamination in cell samples diluted up to 1,000-fold. However, at higher dilutions (greater than 10^-4), mycoplasma was not detected by either method. (**Figure 3**).



Validation Data

Dilution ratio	Ct
0	26.44
10^-1	29.35
10^-2	31.59
10^-3	35.62
10^-4	36.39(2/3)
10^-5	–
10^-6	–
NTC	–

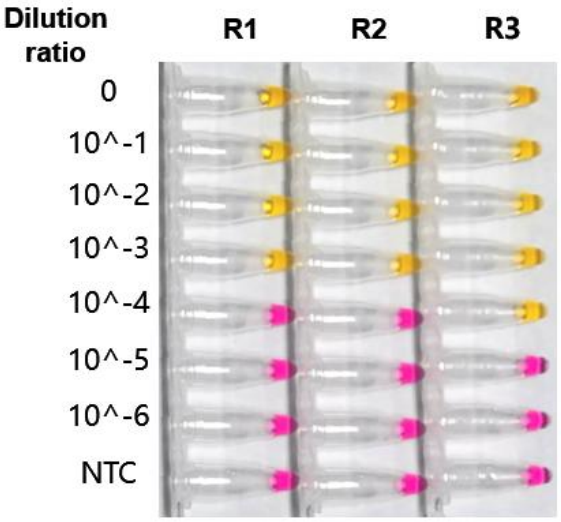
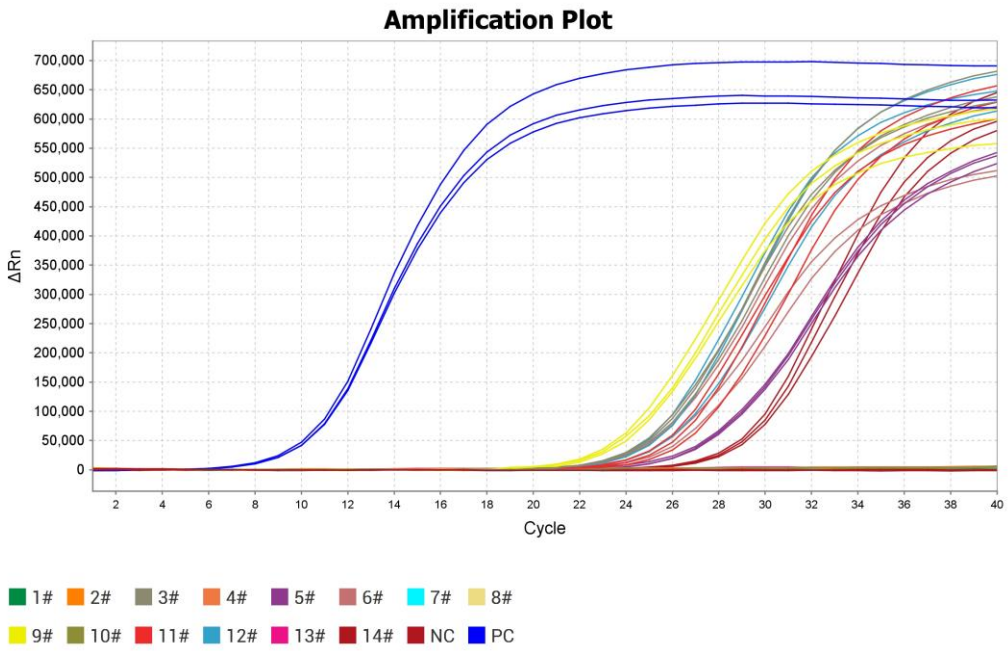


Figure 3: qPCR vs GenieColor results with different concentrations of mycoplasma contaminated cell samples. Samples were run in triplicates for GenieColor.

Compatible with multiple cell lines

Tests on multiple cell samples demonstrated that Mycoplasma detection using MORV0013 was consistent with qPCR results across various cell lines **(Figure 4).**



Validation Data

Sample	NC	PC	1#	2#	3#	4#	5#	6#
qPCR-Ct	–	8.83	–	–	23.49	–	25.87	24.25

Sample	7#	8#	9#	10#	11#	12#	13#	14#
qPCR-Ct	–	–	22.3	–	24.7	23.86	–	27.63

Figure 4: qPCR vs MOR0013 results with different 14 different cell lines. (NC= Negative Control, PC= Positive Control).

Conclusion

The GenieColor Mycoplasma Detection Kit provides robust and reliable performance for practical mycoplasma monitoring. Its speed and ease of use make it an ideal solution for routine screening and quality control, offering specificity and sensitivity comparable to those of qPCR.

References

1. Siegl, D., Kruchem, M., Jansky, S., Eichler, E., Thies, D., Hartwig, U., ... & Bockamp, E. (2023). A PCR protocol to establish standards for routine mycoplasma testing that by design detects over ninety percent of all known mycoplasma species. *Iscience*, *26*(5).
2. Uphoff, C. C., & Drexler, H. G. (2014). Detection of Mycoplasma contamination in cell cultures. Current protocols in molecular biology, 106(1), 28-4.

Validation Data

SKU Code	Product	Size
MORV0013-25	GenieColor Mycoplasma Detection Kit	25 Tests
MORV0013-50	GenieColor Mycoplasma Detection Kit	50 Tests

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