Technical Note | Compatibility and Performance Assessment of the GenieColor Mycoplasma Detection Kit Across Diverse Laboratory Conditions

1. Introduction

The GenieColor Mycoplasma Detection kit (MORV0013) is a rapid and user-friendly solution designed to detect mycoplasma contamination in cell cultures with high sensitivity and specificity. Leveraging isothermal amplification technology, the kit enables easy and visual detection of mycoplasma by changing the reaction solution color from purple-red to yellow in the presence of contamination, making it ideal for routine lab use. This one-tube system requires only a single 1 µL sample of cell culture supernatant and operates at 65°C for one hour, providing clear results without the risk of false positives from aerosol contamination.

To confirm the GenieColor Mycoplasma Detection kit (MORV0013) broad applicability and robustness across various laboratory setups, this report evaluates its compatibility with multiple conditions, including reaction instruments, temperature ranges, prolonged reaction times, different cell types, and culture media with serum. By testing these variables, we ensure that the kit delivers consistent and reliable results under diverse experimental settings, supporting its effectiveness for a wide range of cell culture applications.

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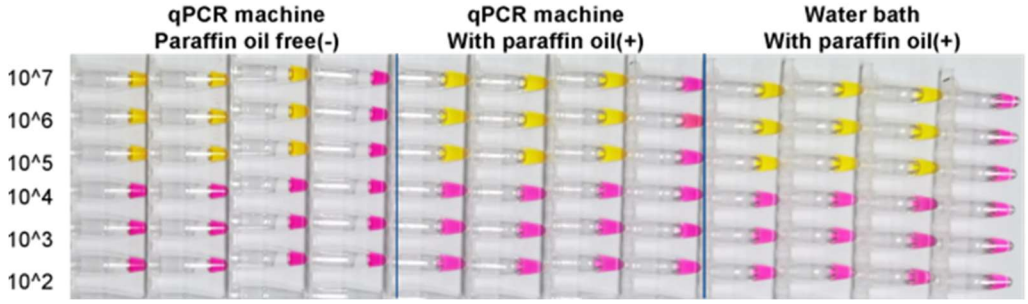
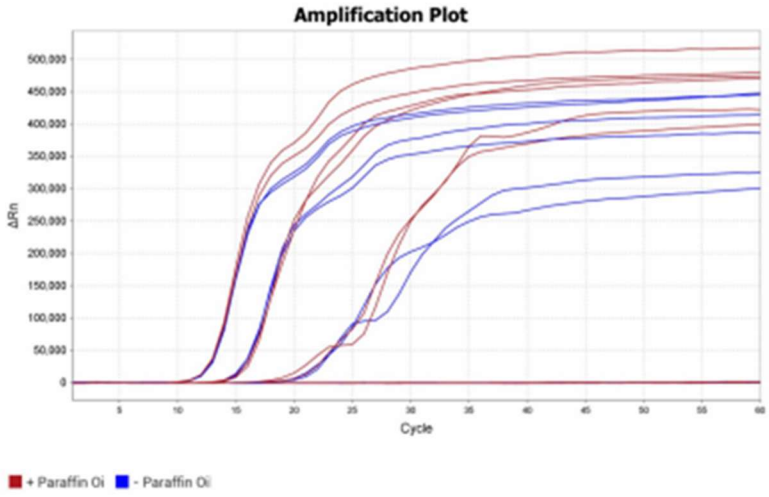
2. Compatibility Tests

2.1 Compatibility of Reaction Instrument

- **Purpose**: Assess the compatibility of MORV0013 with different reaction instruments, specifically a qPCR machine with and without paraffin oil, and a water bath setup.
- **Method**:
  Fluorescent dye was added to MORV0013, and various concentrations of Mycoplasma hyorhinis plasmid were used as templates. Reactions were conducted in a qPCR machine with and without paraffin oil at 65°C for a 60-minute cycle, with 1 minute per cycle. Additionally, the water bath was set to 65°C, and paraffin oil was added to MORV0013 before the reaction to compare sensitivity and specificity across setups.

- **Results**:



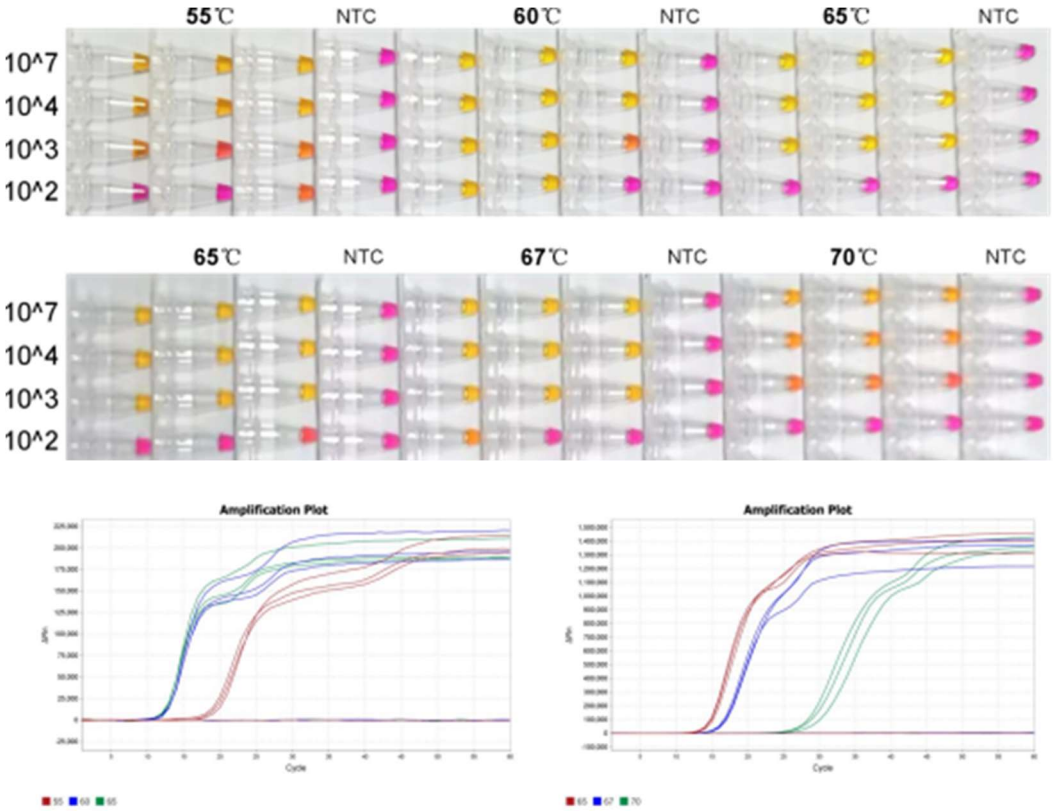


The presence of paraffin oil did not impact the amplification performance of MORV0013. The kit functioned effectively in both qPCR machines and the water bath setup, maintaining consistent amplification speed, sensitivity, and specificity.

2.2 Compatibility of Reaction Temperature

- **Purpose**: Determine the optimal temperature range for MORV0013 and assess performance across a temperature spectrum.
- **Method**:
  The qPCR instrument was set at 55°C, 60°C, 65°C, 67°C, and 70°C, with varying concentrations of Mycoplasma hyorhinis plasmid as the template. The sensitivity and specificity of MORV0013 were measured to identify temperature compatibility within the specified range.

- **Results**:

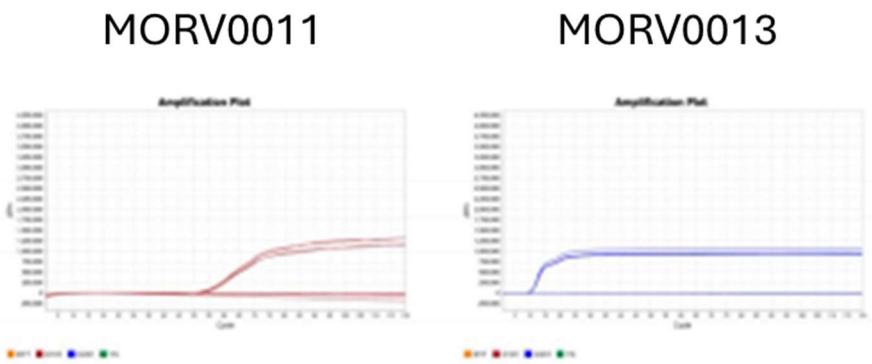


MORV0013 performed reliably between 60°C and 67°C, demonstrating a broad temperature tolerance suitable for instruments with varying precision levels. The sensitivity and specificity remained consistent across this range, confirming that MORV0013 can detect mycoplasma effectively under diverse thermal conditions.

2.3 Compatibility of Long-Time Reaction

- **Purpose**: Test the stability and accuracy of GenieColor Mycoplasma Detection Kit (product code MORV0013) over an extended reaction period versus MycoGenie Rapid Mycoplasma Detection Kit (MORV0011).
- **Method**:
  Using positive controls from MORV0013, four replicates of negative and positive controls were prepared and reacted at 65°C for 2 hours. The results were assessed by observing any color changes in both positive and negative controls to evaluate the impact of prolonged reaction times.

- **Results**:

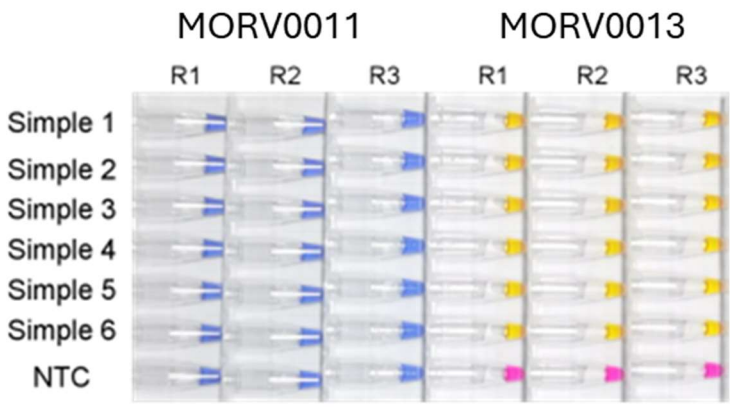


After the 2-hour reaction, negative controls for MORV0011 and MORV0013, demonstrating that MORV0013 maintains specificity and stability during extended reactions.

2.4 Compatibility with Different Cell Samples

- **Purpose**: Comparing GenieColor Mycoplasma Detection Kit (product code MORV0013) effectiveness versus MycoGenie Rapid Mycoplasma Detection Kit (MORV0011) with various cell sample types, including different culture methods and sources.
- **Method**:
  In 19 µL of MORV0013, 1 µL of cell samples from different culture methods (suspension and adherent) and sources (human and mouse) were added. The reaction was performed at 65°C for 60 minutes, allowing assessment of MORV0013's compatibility with a diverse range of cell types.

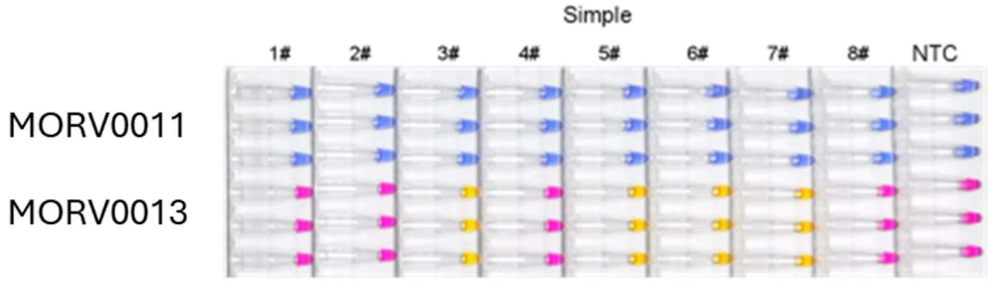
- **Results**:



Samples 1-3: VERO Cells

Samples 3-6: U373 Cells

MORV0013 was found to be positive for mycoplasma from all six cell culture fluids. MORV0011 showed no detectable mycoplasma in any of the six samples.



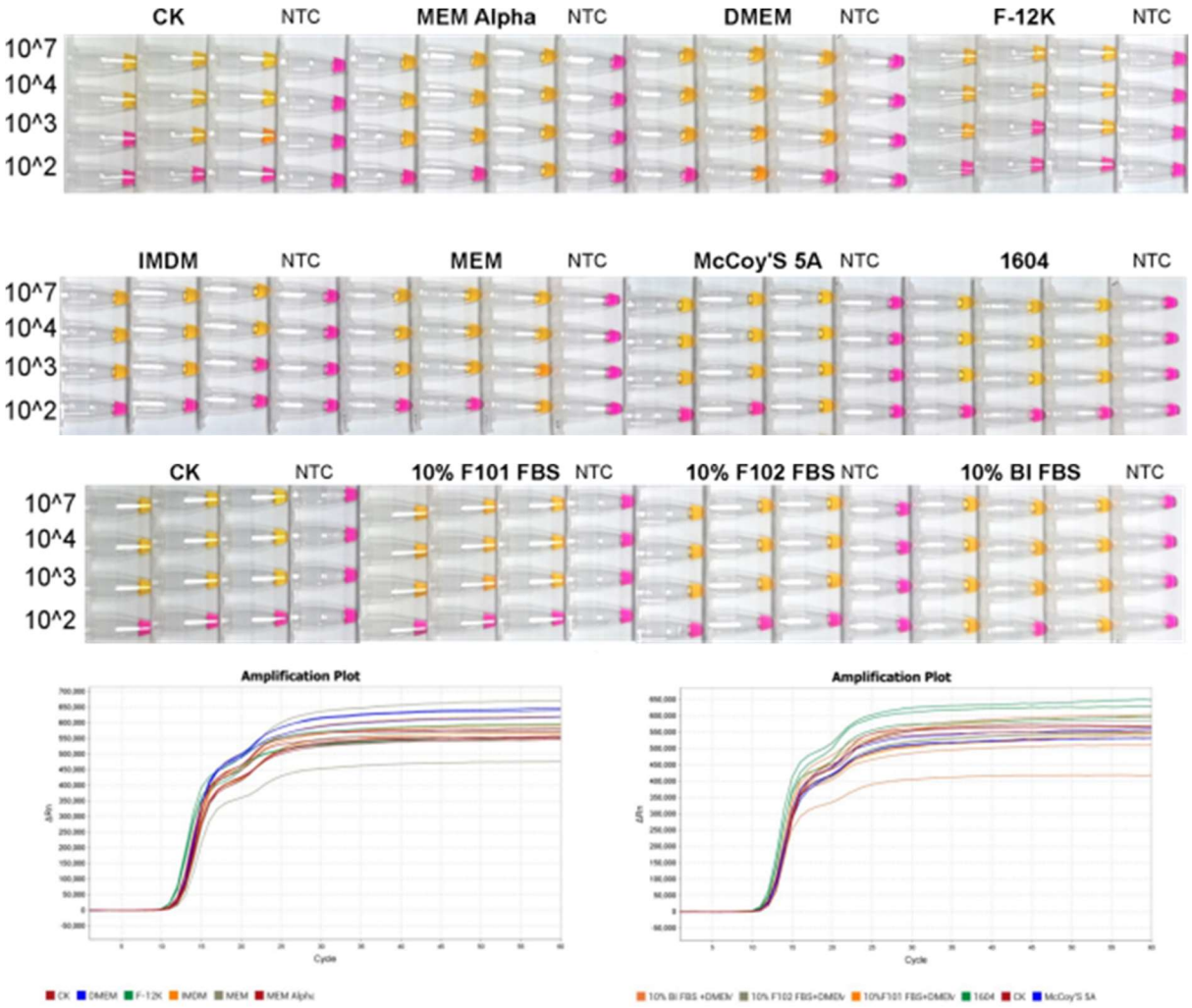
1#Jurkat	2# THP1	3# U373	4# A549	5# MDA231	6# H9c2	7# SW480	8# OKT3

MORV0013 tested positive for mycoplasma in samples 3, 5, 6, and 7, with the remaining samples testing negative. MORV0011 showed no detection in all 8 samples.

2.5 Compatibility with Different Media and Serum Types

- **Purpose**: Assess the influence of different culture media and serum types on MORV0013's detection ability.
- **Method**:
  Using a mycoplasma plasmid as the template, 1 µL of different types of media and serum were added to MORV0013. Reactions were conducted at 65°C for 60 minutes to observe any effects of media and serum on amplification speed, sensitivity, and specificity.

- **Results**:



MORV0013 demonstrated compatibility with all tested media and serum types, with no significant impact on amplification speed (ΔCt≤1), sensitivity, or specificity. This confirms that the kit can reliably detect mycoplasma in the presence of various media and serum environments without performance loss.

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3. Test Analysis & Conclusion

1. **Instrument and Temperature Compatibility**:
   MORV0013 showed stable performance across different reaction instruments, regardless of the presence of paraffin oil. It also performed consistently across a broad temperature range of 60°C to 67°C, making it adaptable for instruments with limited temperature precision.

2. **Sample, Media, and Serum Compatibility**:
   MORV0013 demonstrated excellent compatibility with diverse cell types, culture media, and serum, with no impact on amplification speed (ΔCt≤1), sensitivity, or specificity. Its performance was equivalent to that of Supplier A, confirming its suitability across varied cell culture applications.