

Analytical sensitivity of MycoQSearch™ Mycoplasma Detection Kit for the WHO International Standard for mycoplasma DNA and other mycoplasma 10 CFU standards

Introduction

The MycoQSearch™ Plus Mycoplasma Detection Kit utilizes a hydrolysis probe-based qPCR assay designed for the rapid and accurate detection of mycoplasma contamination in cell culture and biopharmaceutical manufacturing processes.

The assay enables broad-spectrum detection of more than 200 mycoplasma species, including *Acholeplasma*, *Spiroplasma*, and *Ureaplasma*. The test procedure has been validated for sensitivity, specificity, and robustness in accordance with European Pharmacopoeia 2.6.7, providing reliable and reproducible results.

Here, we report the performance parameters of the qPCR-based assay for the detection of the WHO International Standard for mycoplasma DNA and other mycoplasma 10 CFU standards.

European Pharmacopoeia 2.6.7 Revision Clarifies Sensitivity Requirements for NAT Assays

Following the extensive revision of European Pharmacopoeia 2.6.7 (12.2), the sensitivity requirements for the use of nucleic acid amplification techniques (NAT) as an alternative method have been further clarified.

Specifically, the genome copy (GC) to colony-forming unit (CFU) ratio of reference preparations should be less than 10. As an alternative to the culture method, the NAT-based test system must be demonstrated to detect either 10 CFU/mL or less than 100 GC/mL, with both criteria explicitly defined.

In addition, the WHO International Standard (WHO IS) for mycoplasma DNA (8293/13), intended for nucleic acid amplification technique-based assays designed for generic mycoplasma detection, can be used to compare different PCR kits or different strain preparations. It serves to harmonize and standardize NAT assays for mycoplasma detection.



Figure 1. Overall workflow for utilizing the MycoQSearch™ Mycoplasma qPCR Detection Kit. The kit is compatible with most PCR systems and extraction methods.

MycoQSearch™ Detects 1 IU per PCR of the WHO International Standard for Mycoplasma DNA

The WHO International Standard (WHO IS) consists of *Mycoplasma fermentans* with a potency of 200,000 international units (IU) per mL. The standard was reconstituted with 0.5 mL of sterile nuclease-free water, followed by serial dilution in PBS and spiked into CHO cell suspensions at a concentration of 1×10^6 cells/mL.

Genomic DNA was manually extracted using the MycoQSearch™ Mycoplasma gDNA Extraction Kit (MGE-50, CellSafe), and amplification plots were generated using the MycoQSearch™ Plus Mycoplasma qPCR Detection Kit (QDPEP-100, CellSafe).

The assay demonstrated high sensitivity, achieving a detection limit of 1 IU per PCR (Fig. 2).

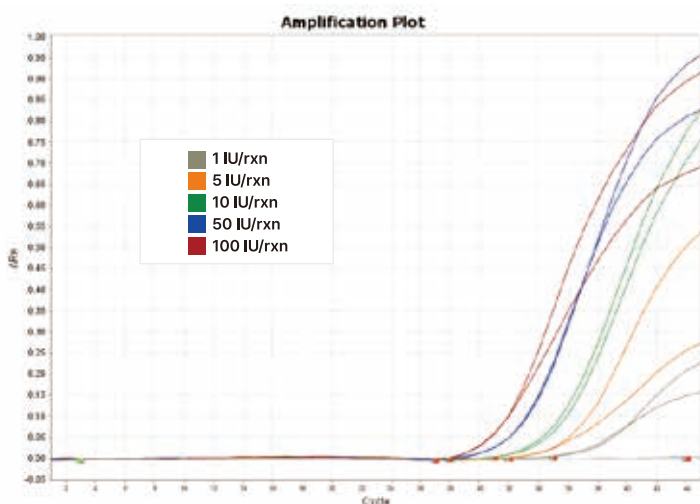


Figure 2. Serial dilutions of the WHO International Standard for mycoplasma DNA were extracted using the MycoQSearch™ Mycoplasma gDNA Extraction Kit and analyzed with the MycoQSearch™ Plus Mycoplasma qPCR Detection Kit (duplicate). Detection was achieved at 1 IU per PCR.

MycoQSearch™ Detects 10 CFU/mL with a GC:CFU Ratio Below 10

Inactivated mycoplasma species with GC:CFU ratios below 10 were prepared and spiked into HEK293 cell suspensions at 1×10^6 cells/mL, resulting in a final concentration of 10 CFU/mL. Genomic DNA was extracted from the spiked samples using the MycoQSearch™ Mycoplasma gDNA Extraction Kit (MGE-50, CellSafe) and subsequently analyzed with the MycoQSearch™ Plus Mycoplasma qPCR Detection Kit (QDPEP-100, CellSafe). A detection limit of 10 CFU/mL was confirmed for all Mollicutes species listed in Table 1.

| Species | Equivalent ATCC strain | GC:CFU | LOD (CFU/mL) |
|---------------------------------|------------------------|--------|--------------|
| <i>Acholeplasma laidlawii</i> | ATCC 23206 | 8.7 | 10 |
| <i>Mycoplasma arginini</i> | ATCC 23838 | 6.7 | 10 |
| <i>Mycoplasma fermentans</i> | ATCC 19989 | 6.3 | 10 |
| <i>Mycoplasma hyorhinis</i> | ATCC 17981 | 6.6 | 10 |
| <i>Mycoplasma orale</i> | ATCC 23714 | 9.0 | 10 |
| <i>Mycoplasma pneumoniae</i> | ATCC 15531 | 7.6 | 10 |
| <i>Mycoplasma salivarium</i> | ATCC 23064 | 7.2 | 10 |
| <i>Mycoplasma gallisepticum</i> | ATCC 19610 | 6.1 | 10 |
| <i>Mycoplasma pirum</i> | ATCC 25960 | 9.6 | 10 |
| <i>Mycoplasma hominis</i> | ATCC 23114 | 9.4 | 10 |
| <i>Mycoplasma synoviae</i> | ATCC 25204 | 6.6 | 10 |
| <i>Spiroplasma citri</i> | ATCC 27556 | 6.1 | 10 |

Table 1. Detection limit of inactivated mycoplasma spiked into HEK293 cell suspension.

Summary

The MycoQSearch™ Plus Mycoplasma qPCR Detection Kit ensures high sensitivity through rigorous quality control. Both mycoplasma reference strains and genomic DNA are utilized to determine sensitivity and ensure consistent kit performance.

A detection limit was demonstrated as follows: 1 IU of the WHO International Standard per PCR, 10 copies of mycoplasma DNA per PCR, and 10 CFU/mL for common mycoplasma species.

Contact CellSafe

Want to learn more? Connect with CellSafe and explore the latest regulatory-compliant Mycoplasma testing solution, MycoQSearch™.



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