Mycoplasma Cell Culture Contamination Test

Fast, qPCR-based detection with a limit of detection of 6 copies per µl of sample
Detects all 11 Mycoplasma species causing >95% of contaminations, and more
Internally validated for specificity and robustness

Protect the Integrity of Your Research

High-quality research begins with healthy, contamination-free cell cultures. Mycoplasma contamination is a silent threat that can compromise cell culture integrity and alter cellular physiology, leading to distorted experimental outcomes[1,2]. To ensure your research remains reliable, reproducible, and acceptable to critical stakeholders—such as journals, institutional review boards, quality management teams, and funding agencies—the Mycoplasma testing method you choose must deliver both scientific rigor and credibility.

Microsynth's **qPCR-based Mycoplasma detection service** provides a fast, cost-effective, and highly reliable solution. Our carefully optimized assay design combines **maximum sensitivity** with **exceptional specificity**, ensuring accurate detection and full confidence in your results. Our **validated and continuously monitored process** provides the assurance demanded by modern research environments and regulatory expectations.

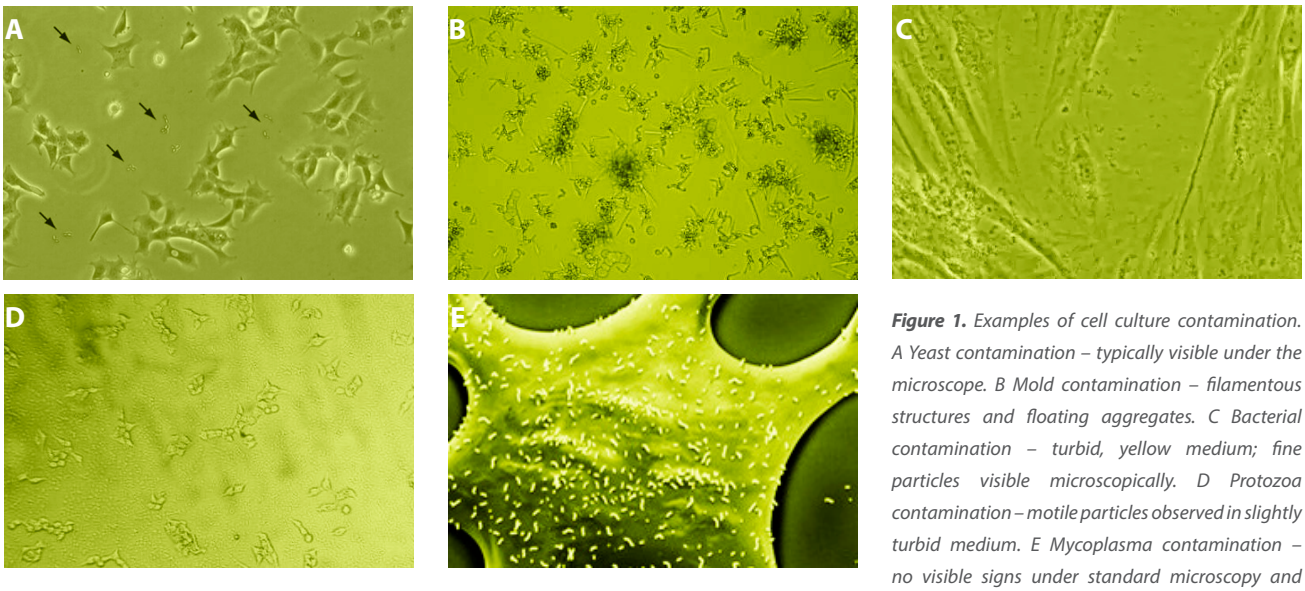


Figure 1. *Examples of cell culture contamination. A Yeast contamination – typically visible under the microscope. B Mold contamination – filamentous structures and floating aggregates. C Bacterial contamination – turbid, yellow medium; fine particles visible microscopically. D Protozoa contamination – motile particles observed in slightly turbid medium. E Mycoplasma contamination – no visible signs under standard microscopy and therefore easily overlooked.*

Detectable Species

Our qPCR assay has been validated using genomic DNA from **authentic Mycoplasma organisms**, ensuring that detection performance reflects real-world contamination scenarios. We have successfully detected all **11 Mycoplasma species** listed by Drexler & Uphoff (2002)[1] and by the **European Pharmacopoeia**[3], including:

Microsynth AG, Switzerland
Schützenstrasse 15 · P.O. Box · CH-9436 Balgach · Phone +41 71 722 83 33 · info@microsynth.ch · www.microsynth.com

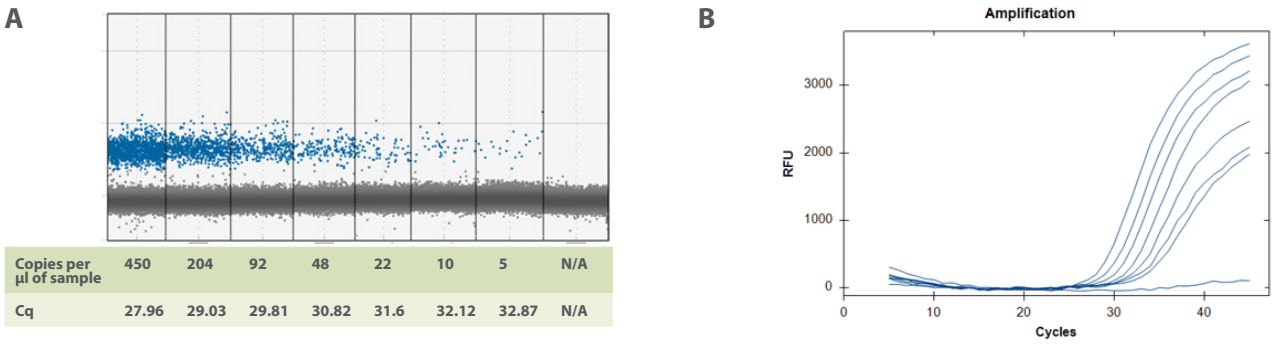
- *Mycoplasma hyorhinis*
- *Mycoplasma orale*
- *Mycoplasma arginini*
- *Mycoplasma fermentans*
- *Mycoplasma hominis*
- *Mycoplasma pneumoniae*
- *Mycoplasma salivarium*
- *Mycoplasma synoviae*
- *Mycoplasma gallisepticum*
- *Acholeplasma laidlawii*
- *Ureaplasma urealyticum*

These 11 species—out of more than 190 known Mycoplasma species—are responsible for **approximately 95%** of all reported cell culture contaminations worldwide. Additionally, internal in-silico sequence alignment analysis confirms that our assay detects a broader range of species from the genera Mycoplasma, Spiroplasma, and Acholeplasma, providing **comprehensive coverage** and minimizing the risk of undetected contamination.

Limit of Detection

Our assay achieves a **limit of detection as low as six Mycoplasma genome copies per microliter** of culture medium. This performance was independently confirmed by **digital PCR (dPCR)**, the most accurate technology available for absolute nucleic acid quantification[4].

For applications requiring compliance with **European Pharmacopoeia guidelines**[3], the standard **10 CFU/mL detection limit** (required for medicinal product testing) can be reached and validated through a dedicated **contract research project** tailored to your specific sample type and requirements.



Copies per µl of sample	450	204	92	48	22	10	5	N/A
Cq	27.96	29.03	29.81	30.82	31.6	32.12	32.87	N/A

Figure 2. *Sensitivity performance graph. A) Digital PCR measurement B) qPCR measurement*

Specificity and Robustness

Our qPCR assay demonstrates **high analytical specificity**, with **no cross-reactivity** to non-Mycoplasma bacterial DNA (e.g., E. coli) or eukaryotic DNA (e.g., human genomic DNA). This has been verified through internal validation experiments under controlled laboratory conditions.

This ensures that every positive signal truly indicates Mycoplasma contamination.

The assay also shows **strong robustness under realistic sample handling conditions**. Heat-inactivated samples stored at –20 °C for up to 3 weeks—or at room temperature for several days—showed **no detectable loss of sensitivity under internal test conditions**.

This stability minimizes the risk of false negatives caused by sample degradation and supports **flexible, reliable logistics** for sample transport and processing.

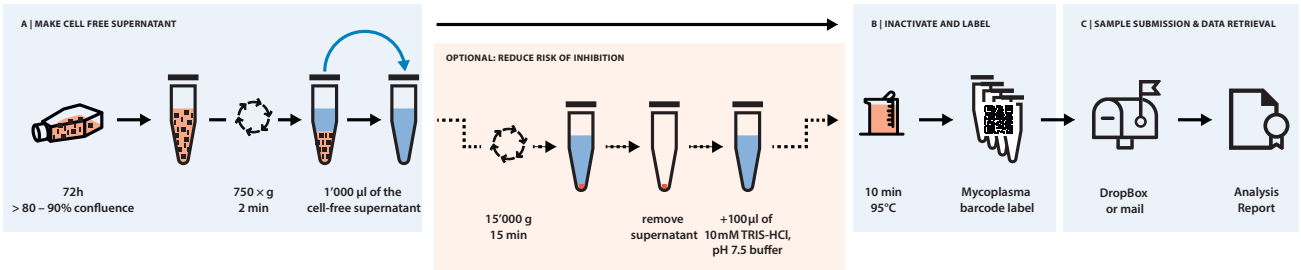


Figure 3. *Process and QC workflow.*

Comprehensive Quality Control

Each qPCR run includes **positive and negative controls** with defined acceptance criteria to verify performance and maintain process integrity. In addition, a **PCR amplification control** is included in every sample to monitor potential inhibition. Approximately **3% of samples** contain inhibitors that can affect amplification efficiency. When inhibition is detected, our reporting and **user guide** clearly outline the **mitigation strategy**, ensuring transparent communication and reliable interpretation. This comprehensive control strategy underscores our commitment to **data quality and analytical accountability.**

Value Proposition

Microsynth's Mycoplasma testing service delivers:

- **Validated and transparent methodology**
- **Scientifically rigorous documentation for stakeholder assurance**
- **Simple logistics and clear instructions**
- **Exceptional customer support from experienced molecular biologists**

Related Services

- **Cell Line Authentication (STR Profiling)**: Verify the genetic identity and integrity of your cell lines to complement Mycoplasma testing.
- **Custom Contract Research Projects:** Achieve a 10 CFU/mL detection limit (required for medicinal product testing) through a dedicated contract research project tailored to your specific sample type and requirements.
- **Species Identification:** Optional species-level identification of detected Mycoplasma.
- **Next Generation Sequencing**: Genomic and metagenomic analysis for contamination tracking and strain identification.
- **Custom qPCR Development**: Tailored assays for specific contamination or pathogen detection needs.

Data Insights: Most Common Contaminants

A retrospective analysis of our testing database identified the four most common contaminating Mycoplasma species (see also Drexler & Uphoff 2002[1] and Lincoln & Gabridge 1998[5]).

- *M. hyorhinis* – frequently introduced via porcine-derived reagents (e.g., trypsin, collagenase)
- *M. fermentans* – naturally associated with human microbiota
- *M. hominis* – naturally associated with human microbiota
- *M. arginini* – often introduced through bovine-derived reagents (e.g., fetal bovine serum)

Understanding the likely **source of contamination** supports targeted preventive strategies and enhances stakeholder confidence in contamination control.
On request, **species-level identification** is available through sequencing of positive samples, providing additional insight into contamination sources and routes.

References

[1] Drexler, H.G., & Uphoff, C.C. (2002). *Mycoplasma contamination of cell lines: Incidence, sources, effects, detection, elimination, prevention.* **Cytotechnology**, 39(2): 75–90.
[2] Nikfarjam L., Farzaneh P. (2012). *Prevention and detection of Mycoplasma contamination in cell culture.* **Cell Journal** 13 (4): 203–212.
[3] European Pharmacopoeia (Ph. Eur. 2.6.7): *Mycoplasmas (Testing for the presence of),* 11th Edition, 2023.
[4] Huggett, J.F. et al. (2015). *The digital MIQE guidelines: Minimum Information for Publication of Quantitative Digital PCR Experiments.* **Clinical Chemistry**, 61(9): 1333–1342.
[5] Lincoln, C.K. & Gabridge, M.G. (1998). *Cell culture contamination: sources, consequences, prevention.* **Methods in Cell Biology**, 57: 49–65.

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