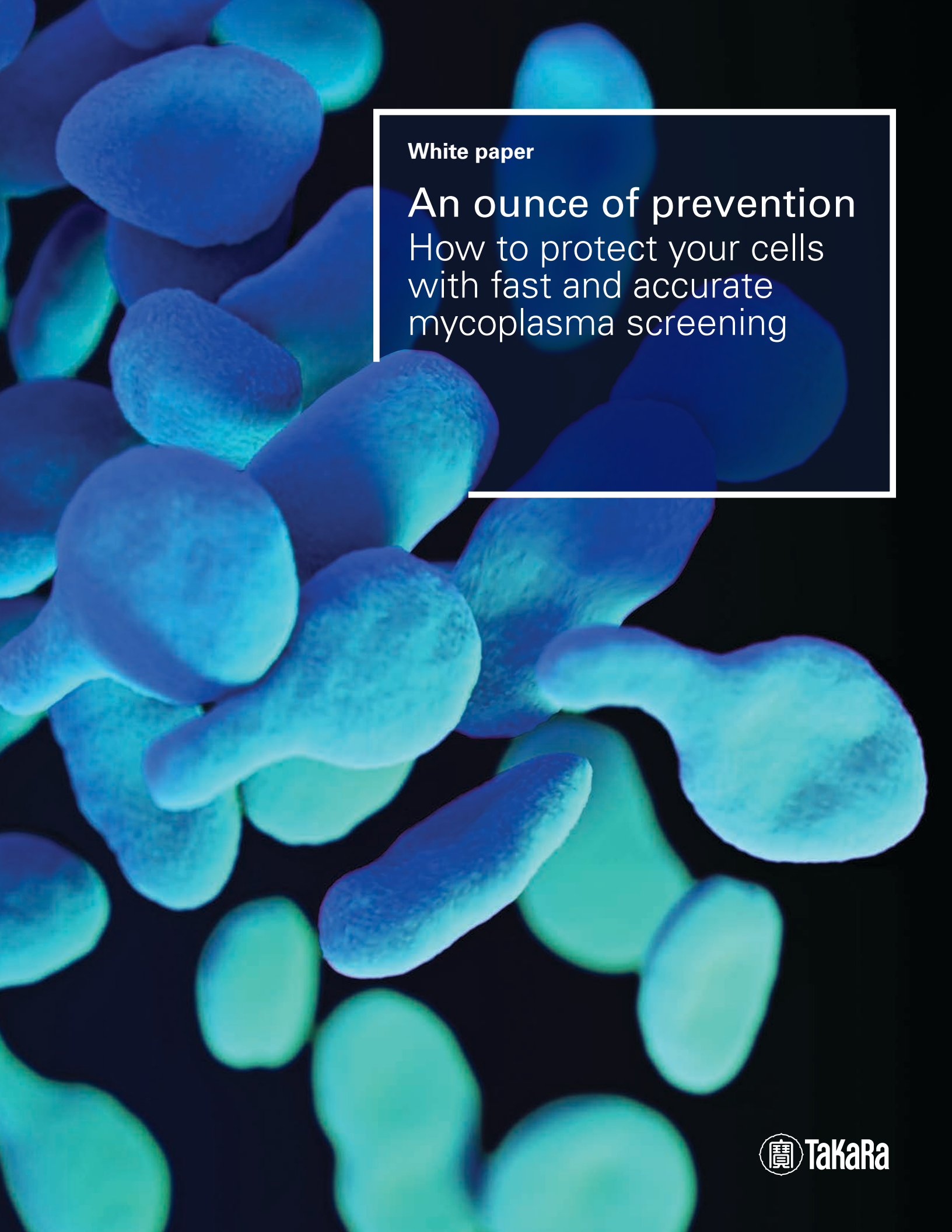


White paper

# An ounce of prevention

## How to protect your cells with fast and accurate mycoplasma screening

TaKaRa

For cell culture-based research, rigorously ensuring the health and quality of each cell line is essential for the success of downstream experiments. For over a decade, mycoplasma—a widespread and notoriously undetectable cell culture contaminant—has gained recognition as one of the greatest challenges for scientists performing cell culture.

## Introduction

The term "mycoplasma" is colloquially used to describe any bacteria of the class Mollicutes that infect humans, several animal species, and cultured cells in laboratories. Mycoplasma infection in cell lines is remarkably common, with an estimated 62% of cell cultures infected worldwide (Kazemiha et al. 2016). While over 100 species of mycoplasma exist, only eight species (*M. arginini, M. fermentans, M. orale, M. hyorhinis, M. hominis, M. salivarium, M. pirum and Acholeplasma laidlawii*) account for >95% of cell line contaminations (Nikfarjam and Farzaneh 2012). Surprisingly, this high infection rate is largely due to the highly advantageous adaptations mycoplasma have evolved and not to low-sterility laboratory practices. Due to their minute size (300–800 nm diameter) and absence of a cell wall, mycoplasma is capable of easily passing through standard 0.2 µm media filter membranes and spreading by touch or aerosol from relatively distant contaminated surfaces. As robust facultative anaerobes, mycoplasma can survive a broad range of temperatures, oxygen levels, and other harsh conditions, including exposure to liquid nitrogen commonly used for cell cryopreservation. Mycoplasma infections are unusually problematic to identify. Infected cultures do not produce any clear visual indicator such as color or turbidity change in culture media (Young, Sung, and Masters 2010), enabling mycoplasma to thrive in cell cultures for extended time periods while escaping detection.

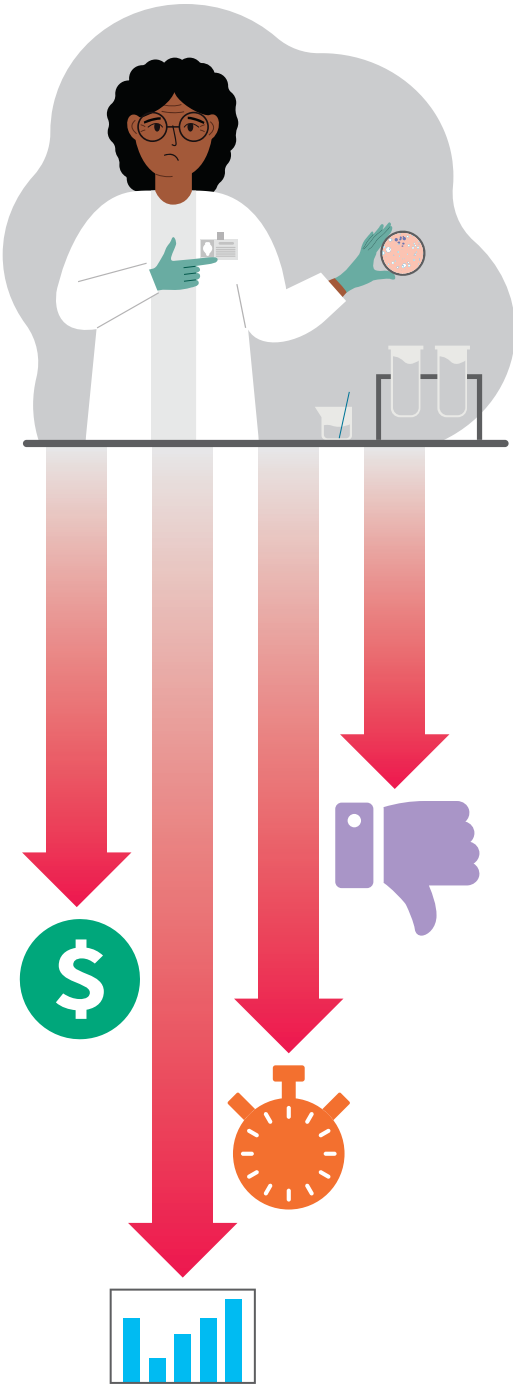
In addition to being resilient and elusive, mycoplasma is also relatively ubiquitous. Cell culture contaminations can originate from many sources, including cells from collaborators or commercial suppliers, improperly sterilized laboratory equipment, airborne particles from nearby surfaces or lab staff. As mycoplasma naturally infects many plant and animal species, serums and supplements derived from infected animals can also harbor mycoplasma contaminants.

**Mycoplasma contaminations can arise from a variety of sources**

## Impact of mycoplasma infection

Mycoplasma infection can severely impact laboratories, cores, and companies alike. Cells can be adversely affected by mycoplasma contamination in many ways, including altered proliferation, survival, morphology, gene expression, and functional characteristics. As the number and severity of symptoms manifest inconsistently, data produced from mycoplasma-infected cells are considered unreliable. Research predicated upon those results must be discarded or—if already published—retracted. The potential disruptions mycoplasma can cause are wide-reaching. In the biopharmaceutical and biotechnology industries, contaminations can lead to significant financial losses and production delays, including costly decontamination efforts and potential product recalls, as well as compromised research data and regulatory setbacks. Mycoplasma infection compromises the quality and safety of cell-generated biological products and undermines the validity of research studies or drug screens conducted with infected cells (Armstrong, Mariano, and Lundin 2010; Baronti et al. 2013). In addition to wasting current batches of cells or products, future productions must be paused until affected cell culture facilities undergo thorough (and expensive) decontamination. Biohazard decontamination can cost between $1,500 and $5,000, with potentially higher costs for larger facilities and more severe contaminations (Carlson 2024). For laboratories and companies dealing with mycoplasma contamination, an ounce of prevention is worth more than a pound of precious data, labor, time, and money.

Options to limit mycoplasma infections in cell culture are scant and often inadequate. Post-infection treatments with antibiotics are rarely successful, as its lack of cell wall renders mycoplasma resistant to most antibiotics (Lanao, Chakraborty, and Pearson-Shaver 2023). Treating cells is recommended solely as an attempt to salvage irreplaceable samples, and cells undergoing treatment must be maintained in strict quarantine to avoid spreading infection. In most cases, it is highly recommended to promptly dispose of infected cultures before they can infect other cells. Timely identification and disposal of infected cells is crucial to limit spread. Preventative measures like sterile technique, UV irradiation, and quarantining can reduce the risk of infection, but mycoplasma is robust and pervasive—one mycoplasma cell can grow to 1 x $10^6$ colony forming units per ml within three to five days in an infected cell culture (Drexler and Uphoff 2002).



**Mycoplasma infection of cell cultures can severely impact laboratories, cores, and companies.** Any product generated from infected cultures must be discarded, potentially resulting in extensive losses of profits, data, time, labor, and more.

As a result, the most successful method to deal with mycoplasma is to regularly screen cell lines to identify and isolate potential infections as quickly as possible.

## Frequency and methods of mycoplasma testing

Mycoplasma testing of cell lines should be performed every 6 months at a minimum, with additional testing recommended preceding large-scale experiments or upon receipt of new cell lines (Cell Culture Services - Penn Genetics 2024). Cell banks are highly susceptible to mycoplasma spread—one study showed up to 87% of cell lines in different cell banks were infected with mycoplasma (Kazemiha et al. 2016). As such, cells should always be purchased from trusted facilities with mycoplasma-free certification, and newly received cell lines should immediately undergo mycoplasma testing, remaining isolated from other cell lines until deemed negative for infection.

Considering the high impact of infection, mycoplasma screening methods must be rapid enough for effective decision-making, sensitive enough to detect low levels of contamination, and robust enough that results are conclusive. Currently, three standard methods are primarily used to test for mycoplasma: culture testing, DNA staining, and PCR-based detection (CDC 2024).

### Comparing methods of mycoplasma detection

**Culture testing**
Bacterial culture media is inoculated with cell culture samples and incubated on a mycoplasma agar plate for four to five weeks. Samples that produce a colony indicate a positive result. This method is simple and definitive, but has an incredibly long turnaround time, which reduces the relevance dependability of a negative mycoplasma test result, wastes valuable culture resources during the accompanying cell quarantine period, and precludes timely decision-making to limit infection spread. The long incubation period also leads to high direct costs for culture-based detection, ranging from $400 to $2,000 per test.

**Cost**
$400–$2,000/test

**Timeline**
4–5 weeks

**Reliability**
100%

**Slower than PCR**

**DNA staining**
Confluent cells are fixed and stained with Hoechst or DAPI to mark nuclei. Upon examination with a fluorescence microscope, filamentous staining indicates a positive result. This method is fast and convenient but lacks the sensitivity to definitively detect infections in low-contamination cultures. Relying on subjective morphological assessment of polymorphic mycoplasma, DNA staining analysis is used mainly for initial screening or in tandem with other detection methods. While costs for DNA staining detection appear relatively low, averaging $200 to $1,000 per analysis, initial equipment investment and ongoing training fees can be significant.

**Cost**
$200–$1,000/test

**Timeline**
<1 day

**Reliability**
50%

**Less consistent than PCR**

**PCR-based detection**
Mycoplasma-specific gene targets are selectively amplified from cell culture samples and subsequently run on a gel. The presence of a band of amplified bacterial DNA indicates a positive result. This method is both definitive and fast, taking less than a day to generate results. PCR-based mycoplasma tests usually cost around $20 to $30 per sample to run, making it the most affordable option of the three conventional methods. Still, the necessity of running gel electrophoresis post-PCR can increase sample contamination risk and reduces quantification sensitivity.

**Cost**
$20–$30/test

**Timeline**
<1 day

**Reliability**
98%

Of these three conventional options, PCR-based detection has historically proven to be the most reliable, fast, and sensitive method for mycoplasma detection (Uphoff and Drexler 2011). However, recent years have seen the emergence of an additional option for mycoplasma detection—real-time PCR/qPCR. Studies have shown that qPCR-based mycoplasma tests demonstrate additional detection sensitivity, specificity, and accuracy compared to conventional PCR (Kazemiha et al. 2016). Without the need for gel electrophoresis, qPCR also offers a more streamlined and cost-effective workflow, making it an ideal option for repeated routine screening. For facilities seeking simple and rigorous preventative mycoplasma screening, consider the TaKaRa Mycoplasma qPCR Detection Kit today.

## References


Armstrong, S. E., Mariano, J. A. & Lundin, D. J. The scope of mycoplasma contamination within the biopharmaceutical industry. Biologicals 38, 211–213 (2010).

Baronti, C., Pastorino, B., Charrel, R. & de Lamballerie, X. Mycoplasma removal: Simple curative methods for viral supernatants. J. Virol. Methods 187, 234–237 (2013).

Carlson, J. Average cost of biohazard cleanup. HomeGuide (2024). at <https://homeguide.com/costs biohazard-cleanup-cost#:~:text=Biohazard cleanup costs %2425 to,level and type of contaminants.>

CDC. Laboratory Testing for Mycoplasma pneumoniae | M. pneumoniae | CDC. at <https://www.cdc.gov/mycoplasma/php/laboratories/index.html>

Cell Culture Services - Penn Genetics. at <https://genetics.med.upenn.edu/cores/cell-center-services/services/cell-culture-services/>

Drexler, H. G. & Uphoff, C. C. Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. Cytotechnology 39, 75–90 (2002).

Kazemiha, V. M. et al. Real-time PCR assay is superior to other methods for the detection of mycoplasma contamination in the cell lines of the National Cell Bank of Iran. Cytotechnology 68, 1063–80 (2016).

Lanao, A. E., Chakraborty, R. K. & Pearson-Shaver, A. L. Mycoplasma Infections. (StatPearls Publishing, 2023). at <https://www.ncbi.nlm.nih.gov/books/NBK536927/>

Nikfarjam, L. & Farzaneh, P. Prevention and Detection of Mycoplasma Contamination in CellCulture. Cell J. 13, 203 (2012).

Uphoff, C. C. & Drexler, H. G. Detecting mycoplasma contamination in cell cultures by polymerase chain reaction. Methods Mol. Biol. 731, 93–103 (2011).

Young, L., Sung, J. & Masters, J. R. Detection of Mycoplasma in cell cultures. Nat. Protoc. 2010 55 5, 929–934 (2010).





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