

# Titered Mycoplasma Reference Strains Panel

## Prevalence of Mycoplasma Contamination

Mycoplasmas are frequent contaminants of cell cultures and bioprocessing fluids. Numerous studies have been published that establish mycoplasma contamination of continuous cell cultures in the range of 15-35%, with primary cell cultures exhibiting a minimum 1% contamination rate<sup>1,2</sup>.

There are over 190 species of mycoplasma, but only 20 distinct species of human, bovine and porcine origin have been identified in cell culture<sup>3,4</sup>. Of those twenty, eight species account for approximately 95% of all mycoplasma contamination in cell culture, including *M. arginini* (bovine), *M. fermentans* (human), *M. hominis* (human), *M. hyorhinis* (porcine), *M. orale* (human), *M. pirum* (human), *M. salivarium* (human), and *Acholeplasma laidlawii* (bovine).

## Importance of Routine Testing

Mycoplasma can affect the phenotypic and functional characteristics of cells *in vitro*, including morphology, protein expression, and virus production. Implementation of early detection methods that are rapid and sensitive is an important step towards preventing the deleterious and costly effects of mycoplasma on research and development projects.

## Assay Development & Validation

ATCC mycoplasma reference standards with low genome copy (GC) to colony forming unit (CFU) ratios are ideal for use in the development and validation of PCR-based methods of detection. The Titered Mycoplasma Reference Strains Panel (ATCC® MP-7™) represents a unique collection of species that are:

- Commonly associated with cell culture contamination
- Essential for comparing the sensitivity of nucleic acid-based testing against conventional methods
- Useful for evaluating the entire isolation and detection process

The panel is composed of 10 species of mollicutes isolated from both clinical and environmental sources. Each titered sample provides 0.5 mL mycoplasma in suspension that has been:

- Evaluated for genome copy number\*
- Quantified by colony forming units (CFU)\*\*
- Rigorously characterized and authenticated by ATCC ISO 9001:2008 certified laboratories
- Optimized to yield high-viability upon thaw

Items from the ATCC Titered Mycoplasma Reference Strains Panel can be ordered individually with bulk discounts available. Genomic DNA from each strain is also available upon request.

**TABLE 1.** ATCC Genuine Cultures® included in the Titered Mycoplasma Reference Strains Panel (ATCC® MP-7™)

ATCC® No.	Organism	Designation	Source
27545-TTR™	<i>Mycoplasma hominis</i>	—	Human - blood
15531-TTR™	<i>Mycoplasma pneumoniae</i>	FH strain of Eaton Agent	Human - atypical pneumonia
23206-TTR™	<i>Acholeplasma laidlawii</i>	PG8	Sewage
23064-TTR™	<i>Mycoplasma salivarium</i>	—	Saliva
25204-TTR™	<i>Mycoplasma synoviae</i>	WVU 1853	Hock joint of chicken
19989-TTR™	<i>Mycoplasma fermentans</i>	PG18	Ulcerative balanitis
23838-TTR™	<i>Mycoplasma arginini</i>	G230	Mouse brain experimentally infected with scrapies
19610-TTR™	<i>Mycoplasma gallisepticum</i>	—	Suspension of tracheal and air sac tissues of chickens with chronic respiratory disease
17981-TTR™	<i>Mycoplasma hyorhinis</i>	BTS-7	Nasal cavity of pig
23714-TTR™	<i>Mycoplasma orale</i>	CH 19299	Human - oropharynx of child

\*The genome copy number calculation is determined using genome-size reported for the strain or the species and the concentration of genomic DNA determined by PicoGreen® from three separate extractions; the values provided for each distribution lot are an average of these three results. Using an alternative method of gDNA quantification may yield different results.

\*\*CFU are quantified by absorbance or plate counts, depending on the ability of each strain to be cultured on solid media. Use of media or culture conditions other than those recommended by ATCC may yield different results.

## Sources of Mycoplasma Contamination

Despite having limited metabolic pathways and no cell wall, mycoplasmas are resilient. Mycoplasmas are dispersed by aerosol droplets or particles generated while pipetting and handling media and different cell types simultaneously. Possible sources of mycoplasma contamination are<sup>1</sup>:

- Infected incoming cells (cross-contamination)
- Cell culture media, sera or trypsin
- Laboratory personnel

The primary source of mycoplasma contamination is cross contamination from infected cell cultures. Laboratory personnel are also a key source of contamination, as human mycoplasmas (*M. orale*, *M. fermentans* and *M. salivarium*) are major species detected in cell cultures. Laboratory equipment, benches and flow hoods have also been identified as sources of contamination, along with cell culture reagents such as media and sera.

## Effect of Mycoplasma in Cell Cultures

Mycoplasma contamination can cause a wide variety of adverse effects on the function and activities of cells in culture, leading to misinterpretation of results and compromising the validity of data generated for research and/or development projects. Consequences of mycoplasma contamination include<sup>1,4,6</sup>

- Inhibition of cell metabolism
- Induction of chromosomal abnormalities
- Disruption of DNA and RNA synthesis
- Changes in virus and antibody production
- Interference with growth rate of cells
- Depletion of arginine
- Change in pH
- Activation of B cells
- Altered gene expression in cells

## Antibiotics and Mycoplasma


Use of standard antibiotics does not protect cell cultures against mycoplasma contamination. Penicillin has no effect on mycoplasma since mycoplasma lack a cell wall. Streptomycin inhibits about half the mycoplasma strains but is ineffective against many others. Gentamycin is generally ineffective at the concentrations routinely used in cell culture. In fact, mycoplasmas are generally resistant to most antibiotic mixtures commonly used in cell culture<sup>1</sup>.

## References

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