



MYCOPLASMA QUALITY CONTROL

PREVALENCE AND EFFECT OF MYCOPLASMA CONTAMINATION

Mycoplasma contamination affects roughly 15-35% of continuous cell cultures, resulting in a number of deleterious effects, including:

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

These adverse effects can lead to the misinterpretation of results and compromise the validity of data generated for research and/or development projects.

IMPORTANCE OF ROUTINE TESTING

Because mycoplasma can affect the phenotypic and functional characteristics of cells *in vitro*, the 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.

The most frequently used detection methods include direct culture, Hoechst DNA staining, and PCR-based testing. While direct culture can take 6-12 weeks to complete and some DNA stains are difficult to interpret due to heavy cell lysis, PCR-based testing has been shown to be a rapid and reliable alternative when validated as a comparable method of detection.

Mycoplasma (-)

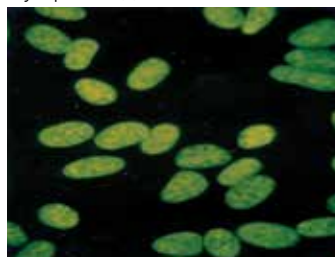


Direct culture method

Mycoplasma (+)

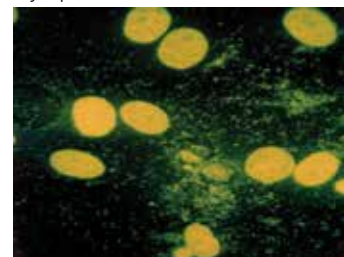


Mycoplasma (-)



Hoechst DNA staining method

Mycoplasma (+)



ASSAY DEVELOPMENT AND VALIDATION

Developing and implementing a novel PCR-based mycoplasma detection system can be challenging and time consuming with regard to sample preparation and validation of the system to ensure equivalency or superiority to conventional test methods. To meet this need, ATCC has developed titered mycoplasma reference standards for comparing PCR and culture-based detection methods, and quantitative mycoplasma DNA certified reference materials for use as external controls in inclusivity/exclusivity testing and establishing limits of detection. These products are rigorously authenticated and characterized by ATCC ISO 9001:2008 certified laboratories, and represent a unique collection of species that are commonly associated with 95% of all mycoplasma contamination in cell culture.

ATCC Titered Mycoplasma Reference Strains Panel (ATCC® MP-7™)

A panel of 10 titered mycoplasma reference strains commonly associated with cell culture contamination. Each strain is prepared with a low genome copy to colony forming unit ratio, which is ideal for use in the development and validation of PCR-based methods of detection.

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

ATCC Quantitative Mycoplasma DNA Certified Reference Materials

These certified reference materials are derived from the strains represented in the ATCC Titered Mycoplasma Reference Strains Panel (ATCC® MP-7™). Each preparation is produced under an ISO Guide 34:2009 accredited process to confirm identity, well-define characteristics, and an established chain of custody. These tools are ideal for use in establishing limits of detection, inclusivity/exclusivity testing, and validating or comparing test methods.

| ATCC® No. | Organism | Designation | Specification Range |
|-------------|---------------------------------|--------------------------|--|
| qCRM-23206D | <i>Acholeplasma laidlawii</i> | PG8 | 1x10 ⁶ – 1x10 ⁷ genome copies/μL |
| qCRM-23838D | <i>Mycoplasma arginini</i> | G230 | 1x10 ⁶ – 1x10 ⁷ genome copies/μL |
| qCRM-19989D | <i>Mycoplasma fermentans</i> | PG18 | 1x10 ⁶ – 1x10 ⁷ genome copies/μL |
| qCRM-19610D | <i>Mycoplasma gallisepticum</i> | -- | 1x10 ⁶ – 1x10 ⁷ genome copies/μL |
| qCRM-27545D | <i>Mycoplasma hominis</i> | -- | 1x10 ⁶ – 1x10 ⁷ genome copies/μL |
| qCRM-17981D | <i>Mycoplasma hyorhinis</i> | BTS-7 | 1x10 ⁶ – 1x10 ⁷ genome copies/μL |
| qCRM-23714D | <i>Mycoplasma orale</i> | CH 19299 | 1x10 ⁶ – 1x10 ⁷ genome copies/μL |
| qCRM-15531D | <i>Mycoplasma pneumoniae</i> | FH strain of Eaton Agent | 1x10 ⁶ – 1x10 ⁷ genome copies/μL |
| qCRM-23064D | <i>Mycoplasma salivarium</i> | -- | 1x10 ⁶ – 1x10 ⁷ genome copies/μL |
| qCRM-25204D | <i>Mycoplasma synoviae</i> | WVU 1853 | 1x10 ⁶ – 1x10 ⁷ genome copies/μL |



UNIVERSAL MYCOPLASMA DETECTION KIT (ATCC® 30-1012K)

A PCR-based test for the detection of over 60 mycoplasma species, including the eight species most likely to contaminate cell cultures. All components required for the PCR reaction are provided and have been optimized for amplification.

Browse our collection of titered mycoplasma reference strains and quantitative nucleic acids at www.atcc.org/MycoplasmaCRMs.

PHONE

800.638.6597
703.365.2700

EMAIL

tech@atcc.org

WEB

www.atcc.org



10801 University Blvd.
Manassas, VA 20110

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