

Mycoplasma Detection – Protect Your Continuous Cell Lines

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About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA and an R&D and Services center in Gaithersburg, MD
- World's premiere biological materials resource and standards development organization
 - 5,000 cell lines
 - 80,000 microbes
 - Genomic & synthetic nucleic acids
 - Media/Reagents
- ATCC collaborates with and supports the scientific community with industry-standard and innovative biological solutions
 - Growing portfolio of products and services
 - Sales and distribution in 150 countries, 12 International distributors
- Talented team of 450+ employees; over one-third with advanced degrees



An innovative global partner for authentic biomaterials, standards, and services

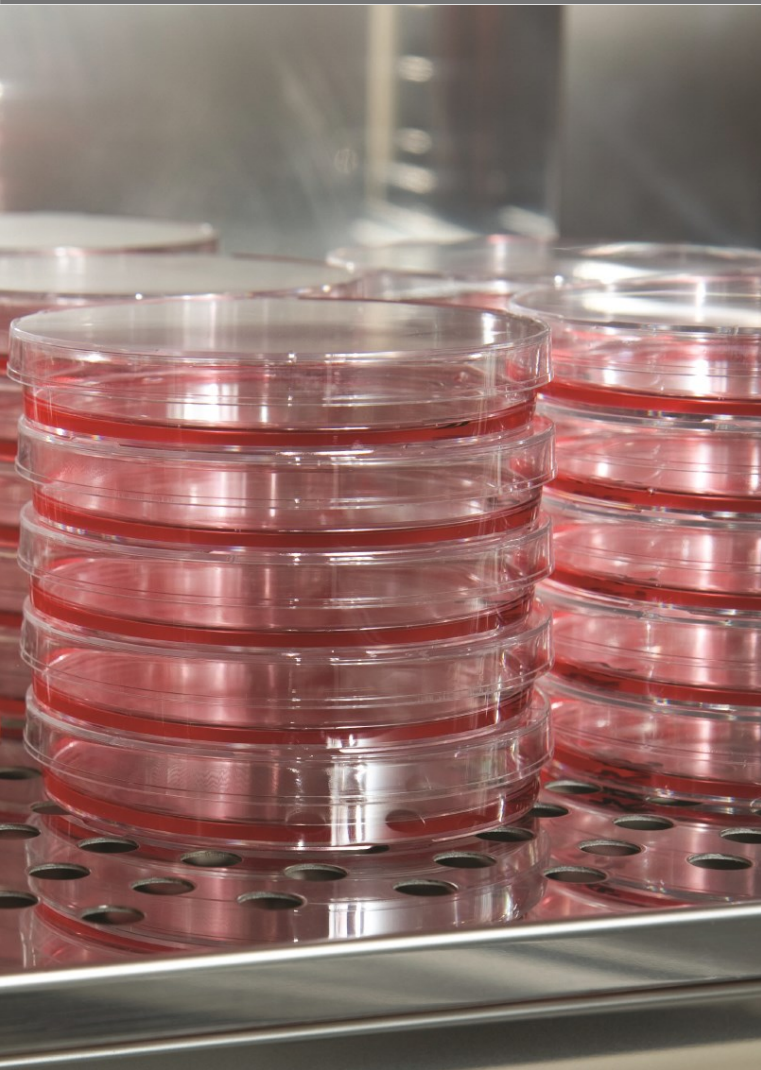


Outline



- Background on mycoplasma
- Mycoplasma contamination
- Sources of contamination
- Mycoplasma detection methods
- Eradicating mycoplasma contamination
- How to protect your cell cultures

Mycoplasma



- Bacteria
- Class Mollicutes
- Each species lacks a cell wall, but has a simple plasma membrane
- Small size (0.15-0.3 μm)
- Small genome
- Requires the presence of cholesterol, amino acids, fatty acids, vitamins, and other catabolites to survive

Mycoplasma



Mycoplasma is estimated to affect 15-35% of continuous cell cultures

Over 190 mycoplasma species known, but only eight are responsible for ~95% of cell culture contamination events

- *Mycoplasma arginini*
- *Mycoplasma fermentans*
- *Mycoplasma hominis*
- *Mycoplasma hyorhinis*
- *Mycoplasma orale*
- *Mycoplasma pirum*
- *Mycoplasma salivarium*
- *Acholeplasma laidlawii*

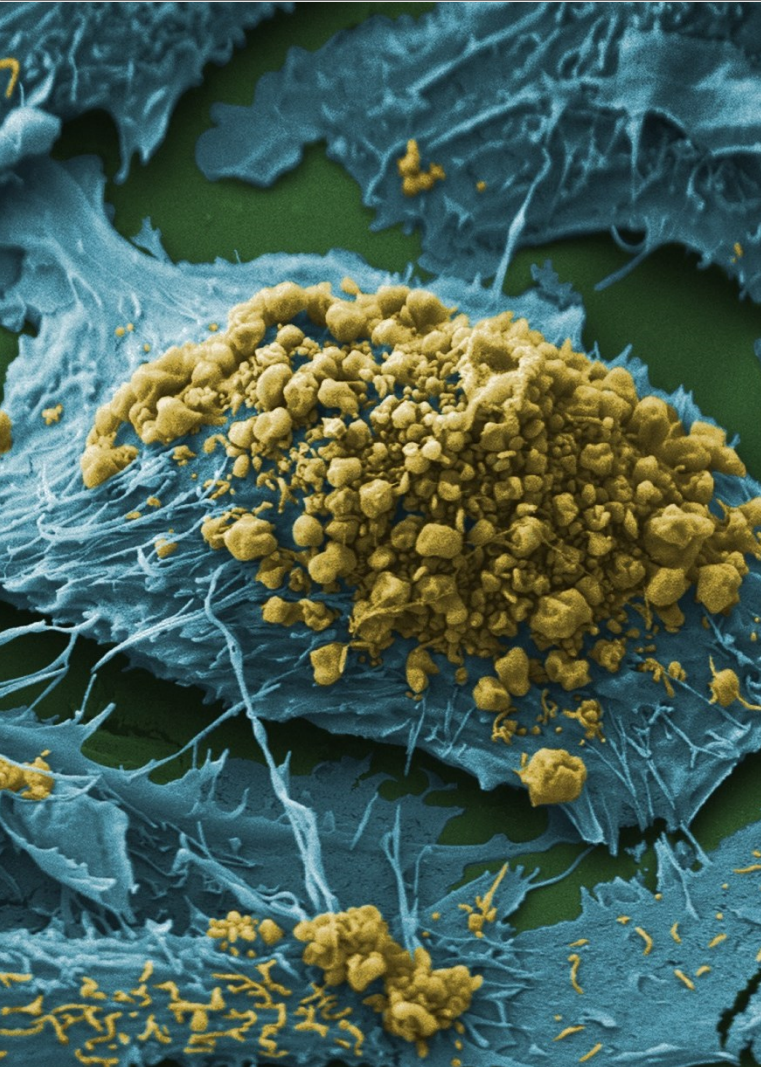
Mycoplasma contamination



Not easily detected

- Does not cause media turbidity
- Does not alter the pH of the media
- Few metabolic byproducts
- Cannot be detected by microscopy

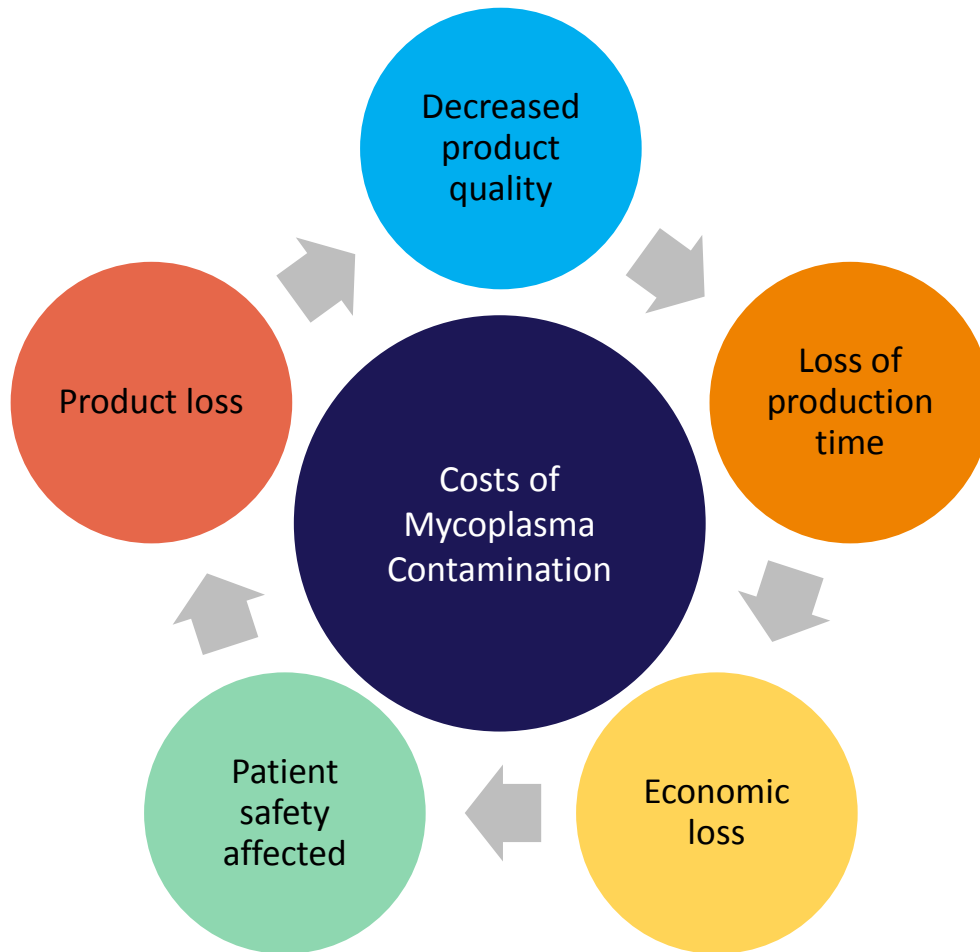
Mycoplasma contamination



Results in a number of deleterious effects

- Chromosomal aberrations
- Disruption of nucleic acid synthesis
- Changes in membrane antigenicity
- Inhibition of cell proliferation and metabolism
- Decreased transfection rates
- Changes in gene expression profiles
- Affects virus production
- Cell death

Cell-derived biopharmaceuticals



Mycoplasma contamination of cell lines used in the production of biopharmaceuticals poses a major safety and economic risk

Sources of contamination



Personnel and equipment

- Poor culturing practices
- Dust and aerosols

Cross contamination

- Aerosol dispersion of contaminated cell cultures
- Broken or faulty laminar flow

Culture reagents

- Sera, media, reagents

Common methods for mycoplasma detection



- Direct agar culture
- Indirect Hoechst DNA staining
- PCR-based testing

Direct agar culture

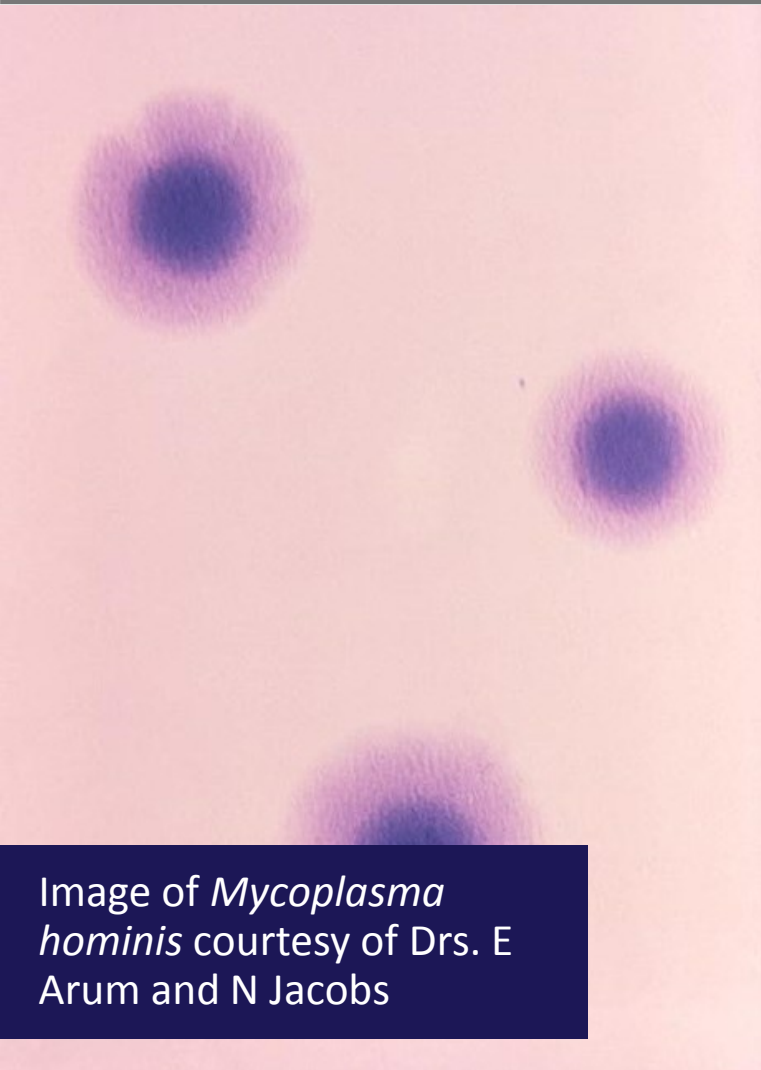


Image of *Mycoplasma hominis* courtesy of Drs. E Arum and N Jacobs

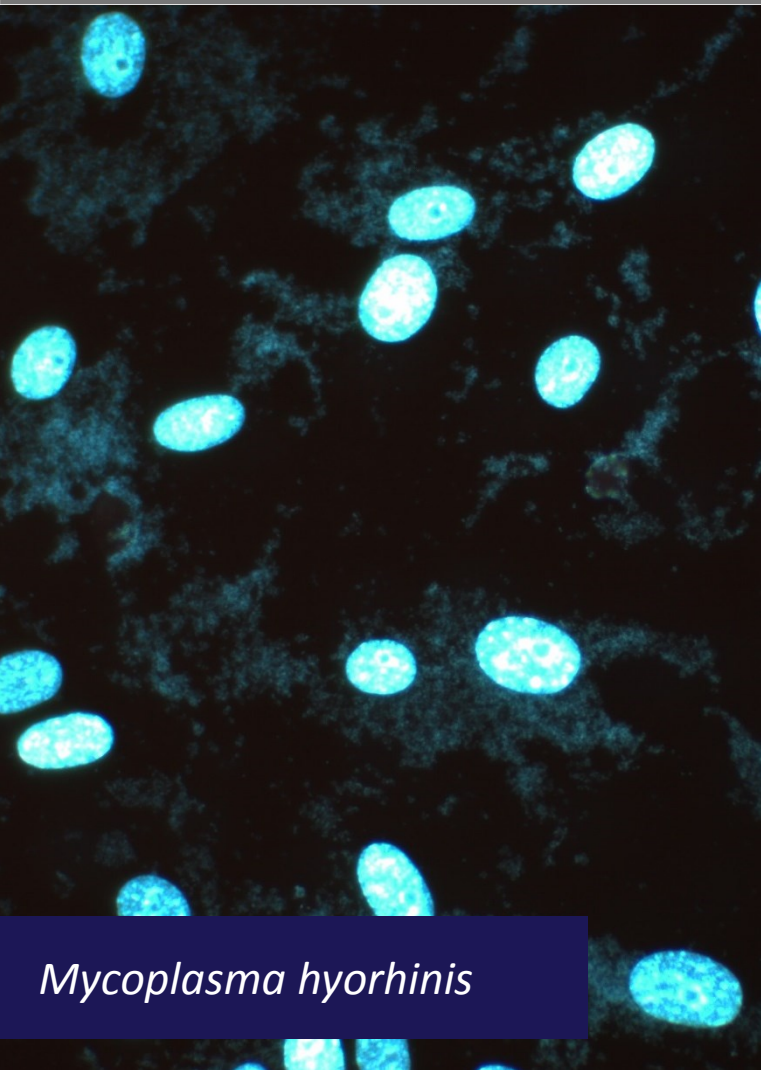
Advantages

- Considered the “gold standard” for testing
- Easy to perform
- Detects viable cells

Disadvantages

- Time-intensive
- Laborious
- Not all mycoplasma are culturable *in vitro*
- May require expert interpretation
- Requires selective media

Indirect Hoechst DNA staining



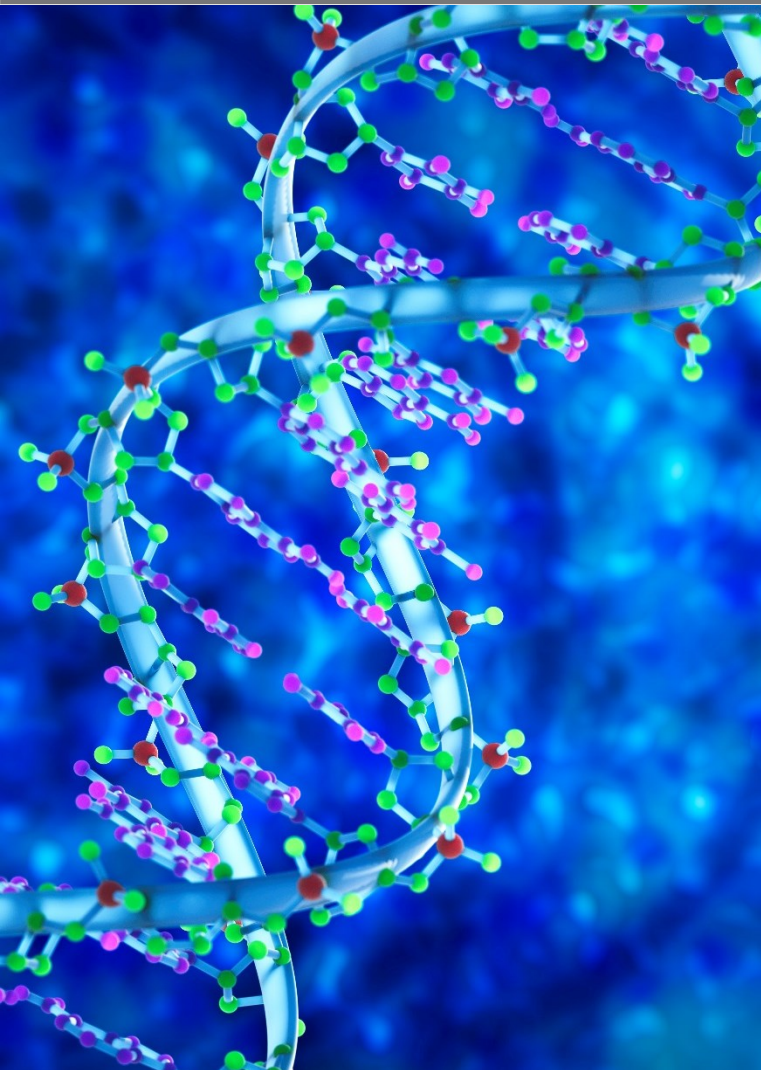
Advantages

- Easy to perform
- Rapid analysis
- Cost-effective

Disadvantages

- Interpreting results can be challenging
- Stains all nucleic acids, so you cannot differentiate between
 - Eukaryotes vs. prokaryotes
 - Mycoplasma vs. other bacteria

PCR-based methods



Advantages

- Easy to perform
- Reproducible
- High sensitivity and specificity
- Efficient
- Cost-effective

Disadvantages

- Cannot distinguish viable and non-viable cells
- Requires primers that are broad enough to amplify different mycoplasma, but specific enough to not amplify other bacterial contaminants
- Requires optimization and validation

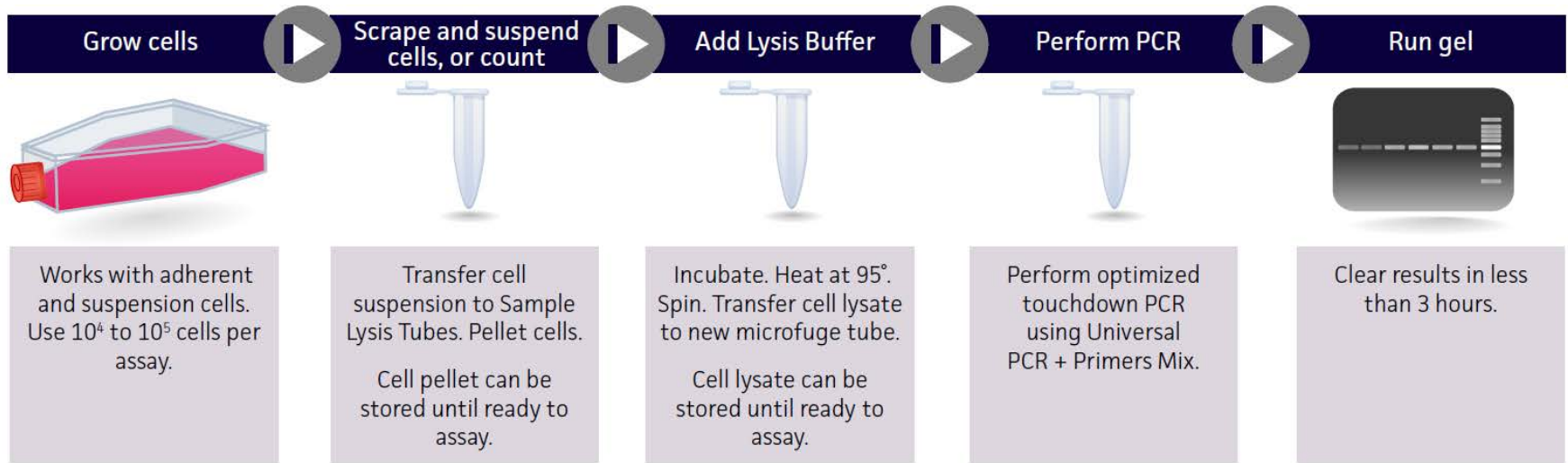
ATCC products and services



- Universal Mycoplasma Detection Kit
- Titered Mycoplasma Reference Strains Panel
- Quantitative Mycoplasma DNA Certified Reference Materials
- Mycoplasma Testing Service

Universal Mycoplasma Detection Kit

ATCC® 30-1012K™



- Detects over 60 species of *Mycoplasma*, *Acholeplasma*, *Spiroplasma*, and *Ureaplasma*
- All components for the PCR reaction are provided and optimized for amplification



Universal Mycoplasma Detection Kit

ATCC® 30-1012K™

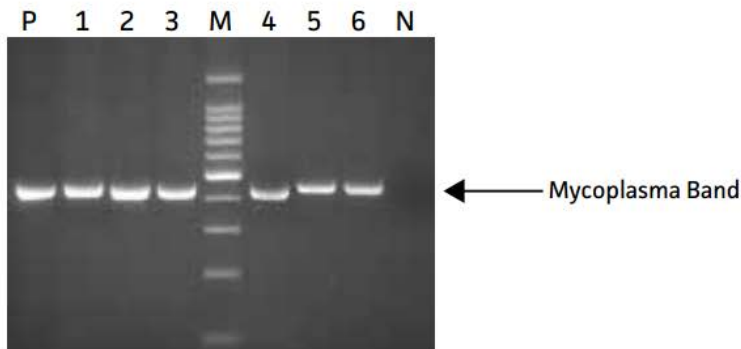
Summary of Results for BHK Cells Infected with *M. orale*

	Dilution					
	1	2	3	4	5	6
Number of Cells/mL	500,000	50,000	5,000	500	50	5
CFU/mL	2,208	154	13	2	0	0
ATCC	+++	+++	++	++	+	+
Supplier 1	++	+	+	+	+/-	+/-
Supplier 2*	+++	++	++	-	-	-
Supplier 3*	+	++	++	++	-	-

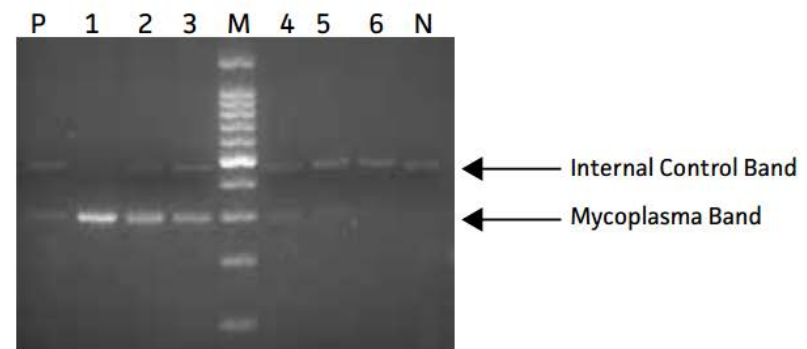
* Positive and negative scores were determined by following the Suppliers' instructions. Supplier 2 provides a metabolic assay for the detection of mycoplasma; Supplier 3 provides a PCR-ELISA test kit.

PCR gels were run for samples analyzed by Universal Mycoplasma Detection and the kit from Supplier 1. Suppliers 2 and 3 are kits that require an instrument for assay read-out. A distinct band was easily visualized in all samples when using the Universal Mycoplasma Detection Kit. Using the PCR kit from Supplier 1 gave less intense bands at all concentrations tested; the most dilute samples yielded ambiguous results. Instrumentation-based systems provided by Suppliers 2 and 3 were much less sensitive.

ATCC Universal Mycoplasma Detection



Supplier 1



P=Positive control N=Negative control M= 100 bp Ladder Lanes labeled 1 through 6 correspond to the dilution series

Titered Mycoplasma Reference Strains Panel

ATCC® MP-7™

- A panel of 10 titered strains representing common cell culture contaminants
- Low genome copy (GC) to colony forming unit (CFU) ratio
- Optimized to yield high-viability upon thaw
- Useful for assay development
- Ideal for comparing culture- and molecular-based detection assays
 - Dabrazhynetskaya *et al.*, 2013

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 hyorinis</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.

Quantitative Mycoplasma DNA CRM

- Genomic DNA quantitated for genome copy number per microliter
- Produced under an ISO Guide 34:2009 process to confirm identity, well-defined characteristics, and chain of custody
- Can be used as controls in inclusivity & exclusivity testing, and establishing limits of detection

ATCC® No.	Item Description	Designation
qCRM-15531D	<i>Mycoplasma pneumoniae</i>	FH strain of Eaton Agent [NCTC 10119]
qCRM-17981D	<i>Mycoplasma hyorhinis</i>	BTS-7 [ATCC 23234, PG 42, NCTC 10130]
qCRM-19610D	<i>Mycoplasma gallisepticum</i>	[NCTC 10115, PG 31, X95]
qCRM-19989D	<i>Mycoplasma fermentans</i>	PG18 [G, NCTC 10117]
qCRM-23064D	<i>Mycoplasma salivarium</i>	[H110, NCTC 10113, PG 20]
qCRM-23206D	<i>Acholeplasma laidlawii</i>	PG8 [NCTC 10116, PG8, A]
qCRM-23714D	<i>Mycoplasma orale</i>	CH 19299 [NCTC 10112]
qCRM-23838D	<i>Mycoplasma arginine</i>	G230 [NCTC 10129]
qCRM-25204D	<i>Mycoplasma synoviae</i>	WVU 1853 [NCTC 10124]
qCRM-27545D	<i>Mycoplasma hominis</i>	[LBD-4]



Mycoplasma testing service

Direct and indirect culture (bundled service)

- Direct culture – Uses both broth and agar
- Indirect culture – Hoechst DNA stain

PCR-based testing

- Detection using the ATCC Universal Mycoplasma Detection Kit



Check out this service at
www.atcc.org/services

Eradicating mycoplasma contamination



Cell culture

- Destroy contaminated cell cultures
- Antibiotic therapy
- Acquire fresh cells



Media

- Destroy contaminated media
- Use media guaranteed to be mycoplasma-free
- Sterilize media via filtration or UV irradiation



Laboratory

- Disinfect all laboratory surfaces and equipment – Biosafety cabinets, incubator, water bath, laboratory bench

How to protect your cell lines



- Use proper aseptic techniques and practices
- Quarantine new cell lines of any origin
- Routinely test cultures for contamination
- Use antibiotics responsibly
- Discard or treat contaminated cells
- Employ good cell banking practices

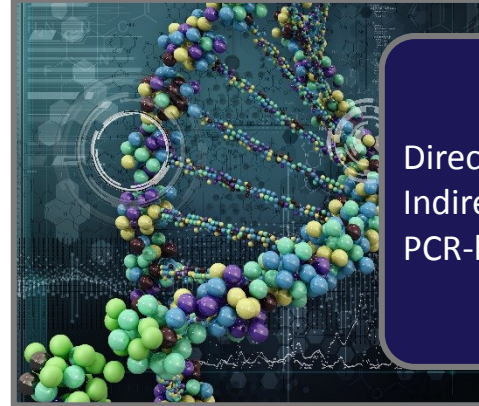
Summary points

Sources



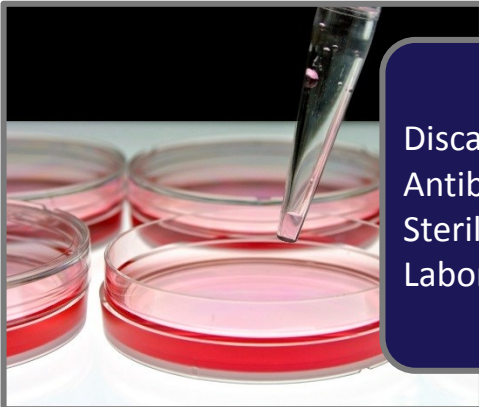
- Personnel
- Equipment
- Cross-contamination
- Media
- Reagents

Detection



- Direct agar culture
- Indirect DNA staining
- PCR-based testing

Eradication



- Discard cells and media
- Antibiotic therapy
- Sterilize media
- Laboratory disinfection

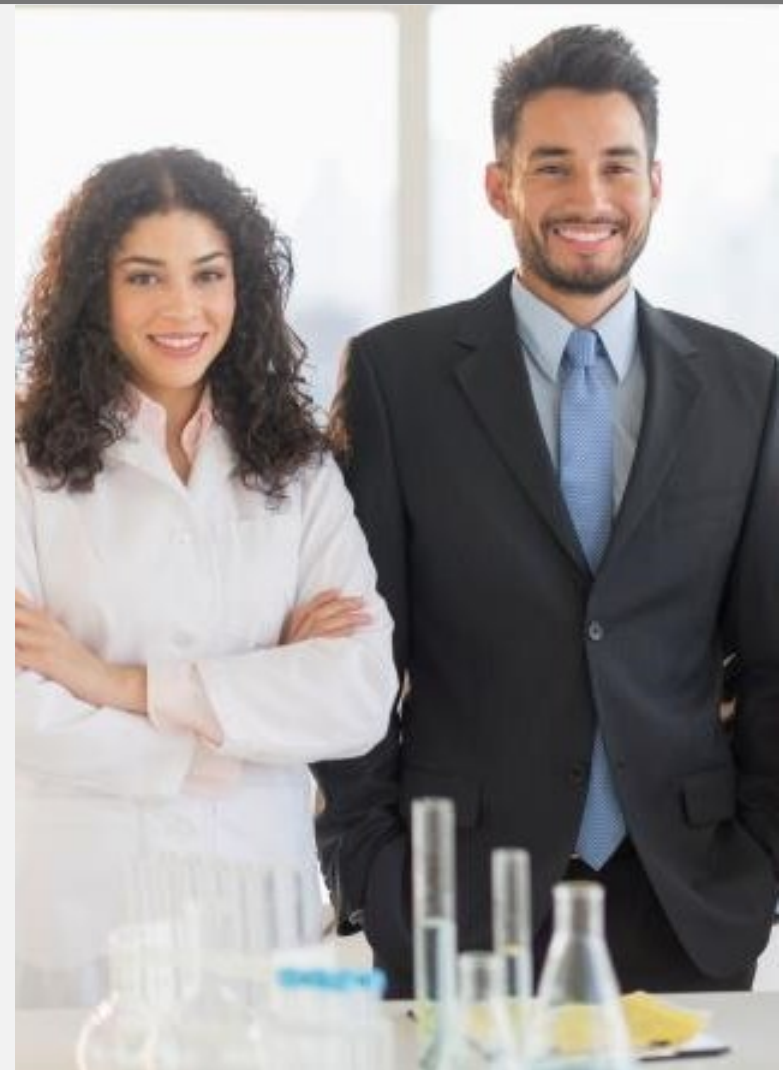
Prevention



- Aseptic technique
- Quarantine
- Routine testing
- Antibiotic stewardship
- Eradicate contamination
- Cell banking

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Check out our mycoplasma quality control
resources at www.atcc.org/MycoplasmaCRMs

