

The background of the slide features two overlapping circular images. The upper circle shows a green fluorescence micrograph with numerous bright green spots, likely representing Mycoplasma cells. The lower circle shows a phase-contrast micrograph of Mycoplasma cells, which appear as purple, pleomorphic structures. The text 'Mycoplasma Testing - An Overview' is overlaid in yellow on the green fluorescence image.

# Mycoplasma Testing – An Overview

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# Background on *Mycoplasma*

- ✓ *Mycoplasmas, Ureaplasmas, Acholeplasmas, Anaeroplasmas, Entomoplasmas* and *Thermoplasmas* lack a rigid cell wall. They are classified in the class *Mollicutes*.
- ✓ The above species belong to the family *Mycoplasmataceae*
- ✓ *Mollicutes* means soft skin, referring to the lack of a rigid bacterial cell wall.
- ✓ Average genome size in *Mycoplasma* is 600-1700 Kb while other bacteria have genomes ranging in size from 4000-6000 kb.
- ✓ Smallest free-living bacteria (0.2 - 0.8  $\mu\text{m}$ )
- ✓ Similarities in the ribosomal RNA sequences suggest that *Mycoplasma* may have evolved from a *Bacillus-Lactobacillus-Sterptococcus* phylogenic origin.
- ✓ The smaller genome size probably resulted from selective pressure for energy economy.

# Background on *Mycoplasma* – Continued

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- ✓ The smaller genome allows for faster multiplication and the ability to grow in environments with limited nutritional elements.
- ✓ *Mycoplasmas* and *Ureaplasmas* have been recovered from humans, animals, birds, insects, and plants.
- ✓ There are some free-living species that were isolated from water and soil as well.
- ✓ There have been many studies implicating *Mycoplasma* in autoimmune conditions via molecular mimicry.
- ✓ *Mycoplasmas* are nicknamed the “crabgrass” of cell culture because their infections are persistent, tedious to detect and hard to eradicate.

# Background on *Mycoplasma* – Continued

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- ✓ *Mycoplasmas* were first discovered as the etiological agent of contagious bovine pleuropneumoniae in 1898 and classified in the mid 1950's.
- ✓ Statistics prove that 30-60% of cell cultures are contaminated with *Mycoplasmas*, resulting in alterations of growth rates, morphology and cell viability.
- ✓ *Mycoplasma* contamination can alter experimental outcomes significantly.
- ✓ Almost **100** *Mollicute* species have been classified, among which, about 20 *Mycoplasmas* and *Acholeplasma* species have been isolated as cell culture contaminants.
- ✓ Most common are *M. arginini*, *M. fermentans*, *M. orale*, *M. hyorhinae*, *M. hominis*, *M. salivarium*, *M. pirum* and *A. laidlawii*.

# Background on *Mycoplasma* – Continued

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**Differentiation of Species: Different species use different nutritional elements as a source of carbon.**

- ✓ *M. pneumoniae* - glucose
- ✓ *M. hominis* - arginine
- ✓ *U. urealyticum* - urea
- ✓ *M. genitalium* - difficult to culture

# Background on *Mycoplasma* – Continued

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## Clinical Aspects & Laboratory Diagnosis - *M. pneumoniae* (as an example)

- ✓ Microscopy: Difficult to stain
- ✓ Can help eliminate other organisms Culture (definitive diagnosis)
- ✓ Sputum (usually scant) or throat washings
- ✓ special transport medium needed
- ✓ Must suspect *M. pneumoniae*
- ✓ May take 2-3 weeks
- ✓ ELISA for antibodies, Complement fixation, cold agglutinin

# Background on *Mycoplasma* – Continued

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*M. hominis*, *M. genitalium* and *U. urealyticum*:

Clinical syndromes

- ✓ *M. hominis* - pyleonephritis, pelvic inflammatory disease and postpartum fever
- ✓ *M. genitalium* - nongonococcal urethritis
- ✓ *U. urealyticum* - nongonococcal urethritis
- ✓ Epidemiology: Colonization at birth - usually cleared  
Colonization with *M. hominis* - 15% Colonization with *U. urealyticum* - 45% -  
75% Colonization with *M. genitalium* - ??

# Background on *Mycoplasma* – Continued

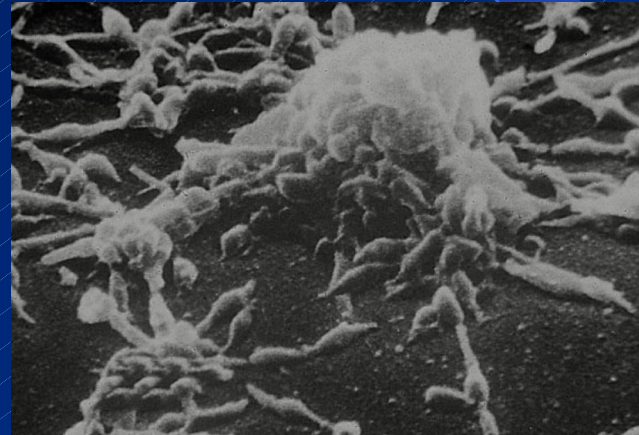
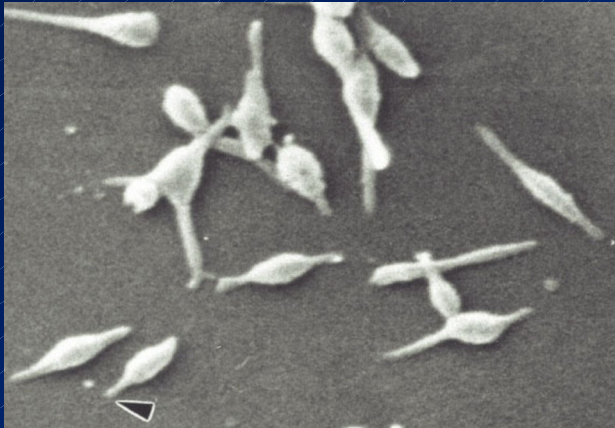
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*Mycoplasma* colonies on *Mycoplasma* Agar  
Fried Egg Colony Morphology  
Growth is by Binary Fission

# Microscopic Images of *Mycoplasma*

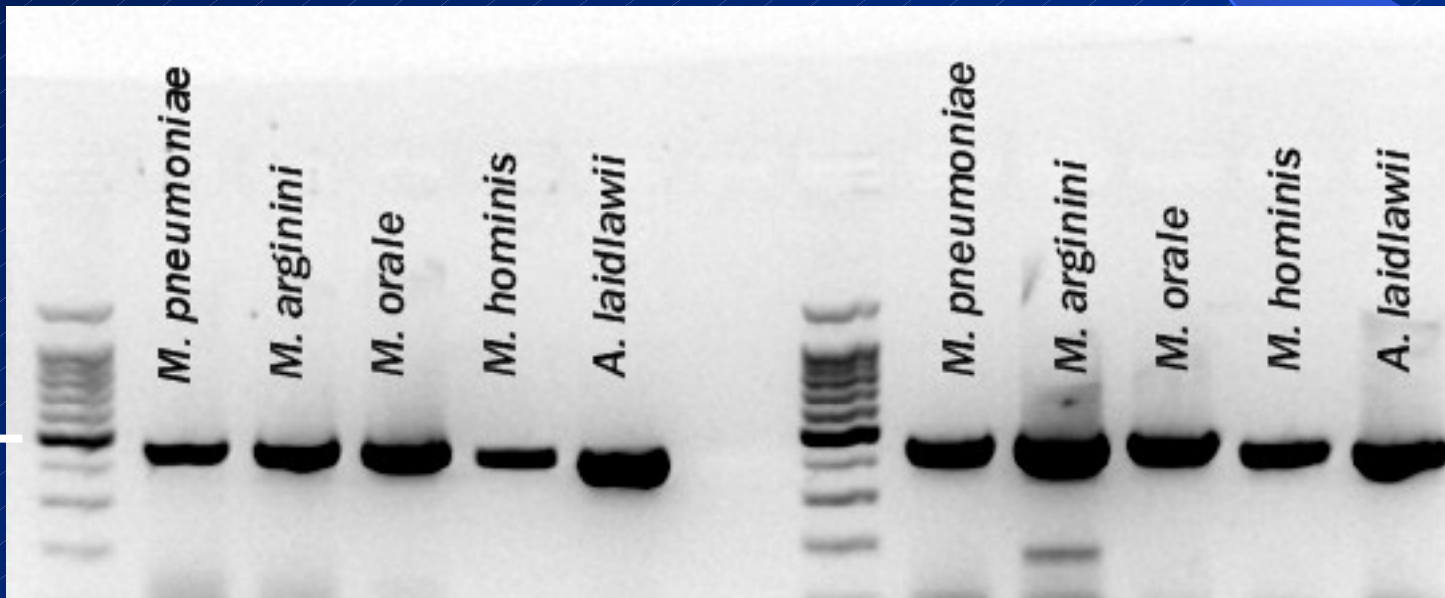
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# Published Primer Sets Assay - Internal

CLON-Set A

CLON-Set B

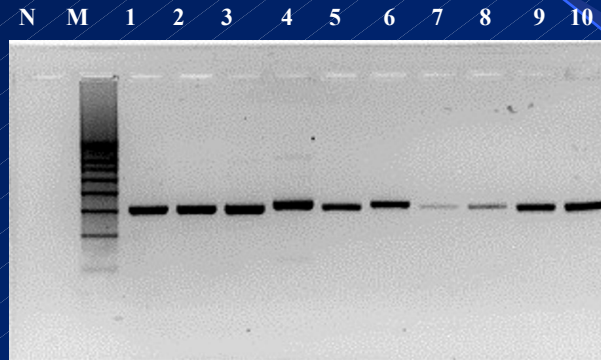


## Commercial Kit Assay

**N:** Negative control

**M:** Size Marker

1. *M. genitalium*
2. *M. pneumoniae*
3. *M. fermentans*
4. *M. hominis*
5. *M. penetrans*
6. *M. suis*
7. 10 copies of *M. hominis*
8. 100 copies of *M. hominis*
9. 1000 copies of *M. hominis*
10. 10,000 copies of *M. hominis*



## Clinical Importance of *Mollicutes*

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- ✓ *Mollicutes* are obligate saprophytes associated with a number of opportunistic infections in humans mainly pneumoniae (known as walking pneumoniae) and genitourinary infections.
- ✓ They are also implicated in autoimmune diseases and arthritis.
- ✓ Although they are associated with the host cell wall, they will grow in nutrient-rich, cell-free media.

## Regulatory Sources and Process Monitoring for *Mollicute* Contamination

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Detection of *Mollicutes* using conventional culture methods remains the gold standard and is described in the following documents:

- ✓ Code of Federal Regulations 21 CFR Ch. I (4-4-01 Edition) §610.30 Subpart D-*Mycoplasma*
- ✓ European Pharmacopeia (2.6.7 '*Mycoplasmas*')
- ✓ Center for Biologicals Evaluation and Research, FDA, *Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals*.
- ✓ There are active discussions taking place in Europe and the USA regarding adopting novel, rapid methods to detect *Mycoplasma* contamination. Some of these technologies are PCR coupled with agarose gel electrophoresis and hybridization using rRNA as a target.

## Procedural Notes on *Mycoplasma* Testing

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- ✓ **Three antibiotic-free passages of cell lines tested for *Mycoplasma* are recommended in order to achieve the highest test sensitivity.**
- ✓ **Cell-based assay in Vero cells followed by Hoechst staining requires 3-6 days to obtain results.**
- ✓ **Microbiology media growth assay requires three subculture steps from broth to fresh agar plates and takes 28 days to complete.**
- ✓ **Large sample volume required for CFR and Points to Consider testing (a minimum of 26 mls of test article).**

## Advantages of PCR Method for *Mycoplasma* Detection

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- ✓ **Rapid:** takes 6-8 hours to complete the test if results are negative.
- ✓ **Sensitive:** assay can detect as little as 10 copies of *Mycoplasma* genomic DNA material.
- ✓ **Specific:** primers are designed to detect target sequences unique to *Mycoplasma* species. Individual sets of primers for detection of individual species are available and widely used.
- ✓ **Requires a very small sample volume** (200 – 400  $\mu$ l, or  $10^6$  cells)
- ✓ **Inexpensive:** cost is 30-50% lower than conventional methods.

## Limitations of *Mycoplasma* Detection by PCR

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- ✓ Assay detects non-viable *Mycoplasma* raising a false alarm in some cases.
- ✓ False negatives occur if cells had been treated with antibiotics.
- ✓ Assay, by nature, is highly sensitive and the risk of contamination does exist.
- ✓ Primer sets may not detect certain species that were not accounted for when primers were designed.
- ✓ Assay not accepted by FDA for clinical trial submissions with very few exceptions.

# Conclusions and Summary

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- ✓ Polymerase Chain Reaction remains one of the most robust Tests for *Mycoplasma* Testing.
- ✓ The existing PCR assays detect the most common *Mollicute* species.
- ✓ PCR and other amplification assays such as PCR-based ELISA And rRNA hybridization protection assay (Gen-Probe) are Good options for testing for *Mycoplasma*.
- ✓ PCR can detect *Mollicutes* that are hard to isolate in culture.