Mycoplasma testing
White paper

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

Mycoplasma contamination presents a significant challenge in cell culture and bioprocessing, particularly in the biopharmaceutical industry. This white paper explores the nature of mycoplasma, its impact on cell cultures, and advanced detection methods, with a focus on nucleic acid testing (NAT) techniques compliant with the World Health Organization and international pharmacopeias.

INTRODUCTION

Mycoplasmas are small, wall-less bacteria that frequently contaminate cell cultures, affecting 15-35% of continuous cell cultures and at least 1% of primary cell cultures. Their small size and flexible shape allow them to pass through standard bacterial filters, making them difficult to eliminate from bioprocessing systems.

Over 190 mycoplasma species exist, but only 20 distinct species of human, bovine, and porcine origin have been identified in cell culture. Eight species account for approximately 95% of all mycoplasma contamination. Due to their tiny size, mycoplasmas can easily pass through 0.1 µm sterilizing filters. Detecting mycoplasma in cell cultures is challenging because it doesn't affect turbidity and may be missed under a microscope.

Mycoplasma contamination primarily originates from personnel, equipment, and contaminated materials. Human-associated species like M. pneumoniae can be introduced by operators, affecting cell line development and upstream processing. Six species account for 90-95% of contaminations: M. orale, M. fermentans, and M. hominis (human); M. arginini and A. laidlawii (cattle); and M. hyorinis (swine). M. orale causes 20-40% of cell line infections, while other human, cattle, and swine-associated strains contribute to the remaining cases

METHODS OF DETECTION

Traditional mycoplasma detection methods, as outlined by regulatory agencies, involve:

- Off-line sample analysis
- Culturing on solid agar under aerobic and anaerobic conditions
- Liquid broth enrichment
- Cell line co-culture test with Hoechst staining and fluorescent microscopy

While reliable, these methods can take up to 28 days to complete and require significant manual handling.

Nucleic Acid Testing (NAT)

NAT, particularly qPCR-based methods, is emerging as a leading technique for mycoplasma detection.

- Faster results
- Potential for real-time manufacturing decisions
- Expedited lot release testing

The World Health Organization (WHO) recommends a limit of detection (LOD) corresponding to 10 CFU/mL of A. laidlawii, ensuring qPCR methods are at least as sensitive as compendial culture methods.

Advanced Detection: Digital PCR (dPCR)

Digital PCR (dPCR) is becoming the preferred NAT for mycoplasma detection, offering:

- Timely results
- Reliable and specific detection
- Ability to detect ribosomal RNA (rRNA) common to >110 mycoplasma species
- Compatibility with various sample types (culture media, final ATMPs, cell banks, virus harvests)
- Absolute quantification of contaminating species

OUR INNOVATION

We have developed a probe-based assay that enables the detection of mollicute species at single-copy resolution in a digital PCR platform.

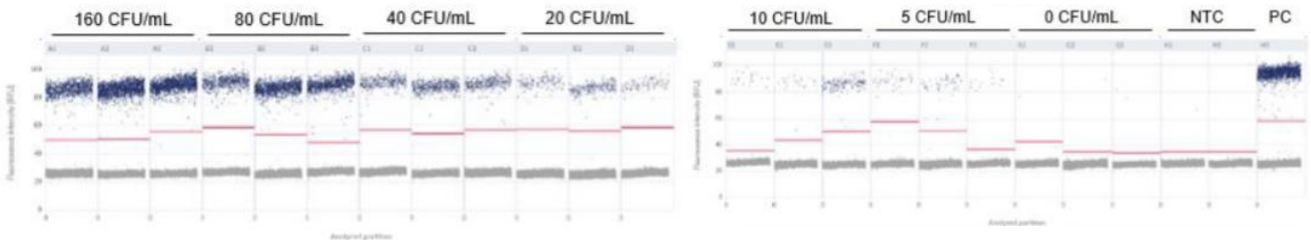
- Our assay detects ribosomal RNA (rRNA) common to >110 mycoplasma species. Performed in a duplex format: Mycoplasma assay (FAM), Internal Control (HEX).
- Results are delivered to the client in 48-72h after receipt of the sample.
- Our assay detects various mycoplasma species, including the WHO standard, down to 10 CFU/mL, as recommended by different pharmacopeias.

WORKFLOW EFFICIENCY

Our workflow is faster and superior to other existing methodologies

Types of samples compatible with our assays

- Culture media/ supernatant
- Cell pellet (as low as 10K cells)
- Cell banks (cryo-stocks)
- Virus harvest



Example of data output- 1-dimensional scatterplots of *M. orale* in serial dilutions detected in a contaminated sample (cell lysate). CFU=colony forming units

Our assay detects various mycoplasma species, including the WHO standard, down to 10 CFU/mL, as recommended by different pharmacopeias.

Species	Specifications	40 CFU/mL				10 CFU/mL				2.5 CFU/mL			
		Run 1	Run 2	Run 3	Run 4	Run 1	Run 2	Run 3	Run 4	Run 1	Run 2	Run 3	Run 4
WHO International Standard for Mycoplasma DNA[1]	Hit rate	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	1/6	3/6	2/6	2/6
	Mean value	6.14 copies/µL				2.37 copies/µL				0.13 copies/µL			
	SD	5.93 copies/µL				3.52 copies/µL				0.12 copies/µL			
	Out of 24	24				24				8			

1) Nübling CM, et al. World Health Organization International Standard To Harmonize Assays for Detection of Mycoplasma DNA. Appl Environ Microbiol. 2015;81(17):5694-5702

Scalable

Validated

Compliant

Sensitive

Our designed assays for mycoplasma detection are unique and qualify for various testing conditions.

We detect and provide absolute quantification of the mollicute species in various biological samples.

For free consultation about our testing services contact us at-

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