Simplifying Progress

Navigating Through the New EP 2.6.7. (12.2.) and USP<77>: Ensuring Rapid and Compliant Mycoplasma Detection with Sartorius

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Introduction

The European Pharmacopoeia (EP) chapter 2.6.7. is a cornerstone for ensuring reliable detection of mycoplasma contamination in pharmaceutical and biotechnological products and has just recently been complemented by the new USP<77>. Mycoplasmas present a significant safety risk in cell-based manufacturing because they are not detected by routine sterility tests and can directly impact product quality and patient safety. To address this risk, the EP/USP provides harmonized regulatory guidance for mycoplasma testing, creating consistency and confidence across the industry.

Embracing the Revised EP 2.6.7 Framework and USP<77>

The latest revision of EP 2.6.7. and USP<77> reflects modern molecular testing capabilities and evolving regulatory expectations. The chapter provides clearer recognition and expanded guidance for nucleic acid amplification techniques (NAT), particularly PCR-based methods, and establishes explicit criteria for replacing traditional culture methods within a risk-based framework.

Key changes include:

- Formal recognition of NAT methods as **equivalent** to culture-based testing.
- Demonstration of detection **sensitivities** of ≤ 10 CFU/mL **OR** ≤ 100 genomic copies/mL for PCR methods replacing culture assays.
- Enhanced validation requirements, including assessment of specificity, robustness, and defined acceptance criteria for **GC/CFU ratios** below 10.
- Stronger expectations for process control monitoring extraction, amplification, and inhibition through **external positive control, EPC,** and **internal amplification control, IAC.**
- Recommendation to test both cellular fractions and supernatants whenever feasible.
- Product-specific suitability: Demonstration that sample matrix doesn´t inhibit NAT assay.
- Integration of risk-based **species selection**, including an expanded reference strain panel (e.g. *M. salivarium*)

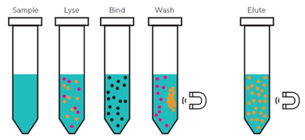
These updates strengthen the regulatory framework for reliable and standardized mycoplasma testing in pharmaceutical and biotechnological manufacturing.

Sartorius Way of Compliant Mycoplasma Detection

Nucleic Acid Extraction



Cyclus® Bead Extraction



- Magnetic Bead-based nucleic acid extraction kit | RNA & DNA
- Magnetic beads allow efficient binding of minimal DNA & RNA amounts, removal of PCR inhibitors
- Suitable for manual or **automated** extraction workflows (KingFisher Flex™)

RT-qPCR Mycoplasma Detection



Cyclus® RT-qPCR Mycoplasma Kit

- Enhanced sensitivity due to Reverse Transcription
- Incorporated Internal Amplification Control (IAC) with duplex TaqMan™ detection
- Ultra pure, lyophilized reagents - store at 2°to 8°C
- 18-month shelf-life
- Compatible with multiple PCR devices able to detect FAM™ & HEX™/VIC® or JOE™

For further information or support contact:
PCR@sartorius.com

Spotlight on CFU and GC Standards in revised EP 2.6.7. and USP<77>

Representative lot-specific GC/CFU ratio of EP/USP named Mycoplasma species

Species	GC/CFU ratio
A. laidlawii	3.4
M. arginini	2.1
M. fermentans	4.9
M. gallisepticum	1.4
M. hyorhinis	1.4
M. orale	2.4
M. pneumoniae	2.8
M. salivarium	2.8
M. synoviae	2.6
S. citri	5.7



- Non-infectious, inactivated 10 CFU standards (GC/CFU≤10)
- 100 GC Standards
- And/or EP 2.6.7. (12.2.) mandatory low-concentration controls in routine testing as EPC
- Certified, dPCR quantified, compliant, ready-to-use
- Lyophilized; stable at 2°C to 8°C; 18-month shelf-life
- Available for all relevant USP/EP species

dPCR Quantification of 100 GC *M. fermentans* and *M. orale**

M. fermentans 100 GC Lot 104-202-2505
M. orale 100 GC Lot 104-590-2505

Sample Preparation: 100 GC standards in 100 µL nuclease-free molecular biology grade water.

NAT Platform: QIAGEN QIAcuity One digital PCR

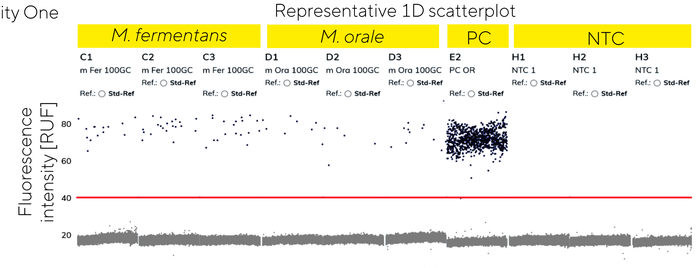
Nanoplate Type: 26K 24-well
Reaction volume: 40 µL
Sample volume: 20 µL
 # well replicates per sample: 3
 # nanoplates: 2

Results obtained from average of 6 nanoplate well replicates:

Sample	dPCR Conc. (cp/µL)	Conc. per Sample (cp/µL)	Total Copyies per Vial*
M. fermentans 100GC	1.145	2.290	114.5
M. orale 100GC	0.439	0.877	87.7

**M. fermentans contains 2 and M. orale 1 copies of 16S rRNA gene*

Representative 1D scatterplot



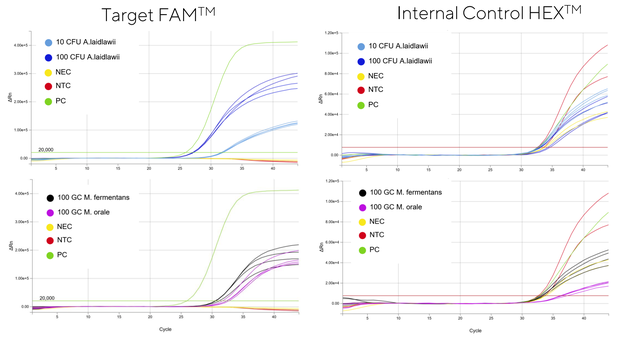
* Exemplary data used with kind permission of Minerva Biolabs GmbH

Sensitivity | Limit of Detection 95

Overview of hit rates by run and concentration in LoD analysis (3 individual runs, 8 replicates each, n=24)

Species	Sample concentration in CFU/ml					LOD95 in CFU/ml
	1000	100	20	10	5	
A. laidlawii	24/24	24/24	24/24	24/24	21/24	10
M. arginini	24/24	24/24	24/24	23/24	21/24	10
M. fermentans	24/24	24/24	24/24	24/24	23/24	5
M. gallisepticum	24/24	24/24	23/24	24/24	21/24	10
M. hyorhinis	24/24	24/24	24/24	24/24	23/24	5
M. orale	24/24	24/24	24/24	24/24	22/24	10
M. pneumoniae	24/24	24/24	24/24	24/24	21/24	10
M. salivarium	24/24	24/24	24/24	23/24	19/24	10
M. synoviae	24/24	24/24	24/24	23/24	18/24	10
S. citri	24/24	24/24	24/24	24/24	24/24	5

Robustness | 10 Mio HEK293T cells/mL + 5% FBS + 10% DMSO



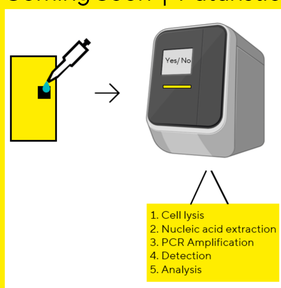
- All CFU and GC spiked samples were consistently detected in FAM™
- Robust assay suitability for cell-culture derived samples with high cell background

Conclusion

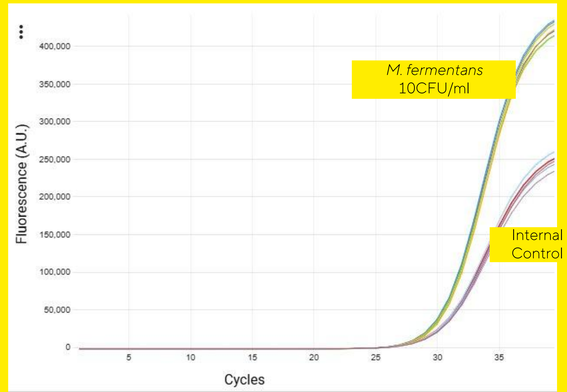
All previous and NEW regulatory requirements are met with the Cyclus® solution

- Sensitivity: 10CFU/ml AND 100GC for all named species
- dPCR-quantified Standards with GC/CFU ratio below 10
- EPC & IAC to control all steps
- Robustness despite high cell density

Coming Soon | Futuristic PCR-Automation



- **Plug-and-Play** | No user expertise required
- No manual sample preparation
- Sample Volume **1 ml of direct matrix**
- Completely **automated** assay
- Included cell lysis & nucleic acid extraction
- Pre-loaded sterile reagents
- Robust design | ≥ 10 Mio cells /mL
- Built-in controls
- Mycoplasma release testing in **compliance** to revised EP 2.6.7. and USP<77>



Amplification plot of a representative run of LoD testing for *M. fermentans*