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Navigating Through the EP 2.6.7 Revision with Sartorius: Ensuring Reliable and Compliant Mycoplasma Detection

The European Pharmacopoeia (EP) chapter 2.6.7 is a cornerstone for ensuring reliable detection of mycoplasma contamination in pharmaceutical and biotechnological products. 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 provides harmonized regulatory guidance for mycoplasma testing, creating consistency and confidence across the industry.

Embracing the Revised EP 2.6.7 Framework

The latest revision of EP 2.6.7 introduces major updates that reflect modern molecular testing capabilities and evolving regulatory expectations. The chapter now provides clearer recognition and expanded guidance for nucleic acid amplification techniques (NAT), particularly PCR-based methods. It establishes explicit performance criteria for replacing traditional culture methods and embeds mycoplasma testing within a structured risk-based framework.

Nucleic acid-based testing now has a dedicated regulatory framework and is recognized as providing results equivalent to culture-based methods. This approach highlights the reduced testing time compared with traditional culture and underscores the ability of NAT to detect both culturable and non-culturable mycoplasma. This aligns with the USP's evolving guidance on rapid microbial methods, including the draft of USP <77>: Mycoplasma Nucleic Acid Amplification Tests.

At a technical level, the revised chapter defines concrete performance criteria for NAT comparability studies. PCR methods intended to replace culture testing must demonstrate detection sensitivities of ≤ 10 CFU/mL or ≤ 100 genomic copies/mL . The validation framework requires formal assessment of assay specificity against phylogenetically related non-mycoplasma bacteria, evaluation of robustness through controlled parameter variation, and defined acceptance criteria for GC/CFU ratios of reference preparations.

The revision also strengthens expectations for process control. External controls should be added prior to nucleic acid extraction, whenever possible, to monitor the entire analytical workflow, including extraction efficiency, amplification performance, and inhibition. In addition, the chapter advises testing both the cellular fraction and the supernatant whenever feasible, since mycoplasmas can attach to or exist within host cells.
Another key development is the integration of risk-based species selection. Validation panels must reflect mycoplasma species realistically associated with the biological origin of raw materials and production systems. The recommended panel of reference strains has been broadened (*M. salivarium* was added, and other *Spiroplasma* species besides *S. citri* can be used) and the WHO International Standard for mycoplasma DNA is explicitly incorporated to support calibration and inter-laboratory comparability.

Spotlight on CFU and GC Standards in the Revised EP 2.6.7

Together, these changes create a strong demand for well-characterized quantified standards that support both routine process control and risk-based validation. Appropriately designed CFU and GC standards can function as integrated tools for extraction control, inhibition monitoring, and species-relevant performance verification. This alignment between regulatory expectations and laboratory workflows enables robust assay performance while simplifying compliance with modern EP requirements. Sartorius supports laboratories in implementing these new CFU and GC standards through a portfolio of validated solutions optimized for PCR-based mycoplasma detection.

Cyclus® RT-qPCR Mycoplasma Detection Kit

Cyclus® RT-qPCR Mycoplasma Detection is designed to detect both mycoplasma DNA and RNA, providing coverage for more than 120 mollicute species, including all pharmacopeial reference strains. The assay meets the sensitivity criteria defined in the revised EP 2.6.7 (12.2), demonstrating a validated detection limit of ≤ 10 CFU/mL or ≤ 100 genomic copies/mL.

Validated in combination with Cyclus® Bead Extraction using viable CFU standards, the system delivers high robustness and reproducibility. Integrated internal controls monitor the full analytical workflow, ensuring system suitability and alignment with EP, USP, and JP expectations.

Cyclus® Bead Extraction

Cyclus® Bead Extraction enables efficient isolation of mycoplasma DNA and RNA using a magnetic bead-based workflow compatible with both manual and automated (KingFisher™ Flex System) processing. The system supports high-throughput sample preparation while maintaining consistent nucleic acid recovery, which is critical for reliable downstream PCR detection.

Rapid parallel processing reduces turnaround time and increases laboratory capacity without compromising precision. Straightforward protocols minimize operator induced variability and support reproducible extraction performance, making the kit well suited for routine and high-throughput testing environments aligned with the revised EP 2.6.7 (12.2) requirements.

Cyclus® 10 CFU Standards and Cyclus® 100 GC Standards

The revised EP 2.6.7 emphasizes the importance of accurate reference standards for validation and performance verification. Sartorius addresses this need by implementing digital PCR (dPCR) in the quality control of its mycoplasma standards, increasing quantification accuracy and reliability. The Cyclus® 10 CFU and 100 GC standards can be used for product specific validation and for routine testing as external positive control required due to the revision.

Cyclus® 10 CFU/mL Standards provide ready-to-use inactivated and precisely characterized CFU references that support validation of assays targeting the ≤ 10 CFU/mL sensitivity threshold. These standards enable safe and controlled performance verification without introducing viable mycoplasma into laboratory environments.

Cyclus® 100 GC Standards complement CFU standards by delivering highly accurate, ready-to-use genomic copy references aligned with the ≤ 100 genomic copies/mL requirement. Digital PCR (dPCR)-based characterization ensures traceable quantification and supports robust assay calibration.

By combining CFU and GC standards with validated PCR and extraction solutions, Sartorius provides the complete package for laboratories seeking to implement the revised EP 2.6.7 efficiently. These tools support compliant validation and release testing strategies while enhancing workflow efficiency and analytical reliability in modern biopharmaceutical production.

Empowering You to Meet the New EP 2.6.7 Requirements

As regulatory expectations evolve alongside advances in biopharmaceutical manufacturing, you are faced with the challenge of translating increasing technical requirements into reliable routine practice. The revised EP 2.6.7 represents a decisive shift toward modern, risk-based, and statistically robust mycoplasma testing — and successfully implementing these expectations requires more than compliant methods alone. You need practical tools, dependable CFU or GC standards, and expert guidance that fit seamlessly into your daily workflows. Sartorius partners with you throughout this transition by providing validated technologies, traceable CFU and GC standards, and application expertise that simplify adoption and strengthen confidence in your quality control processes. Together, we help you turn regulatory compliance into a driver of performance, efficiency, and long-term success in biopharmaceutical manufacturing.

For more information visit our webpage or contact **PCR@Sartorius.com**

Ordering Information

Mycoplasma Kit		
SMB95-6002	Cyclus® RT-qPCR Mycoplasma	25 reactions
Nucleic Acid Extraction Kit		
SMB95-6000	Cyclus® Bead Extraction	100 extractions
SMB95-6003	Cyclus® Bead Extraction Lysis Buffer	27.5 mL
Cyclus® 100 GC , 3 vials		
SMB95-3001	Cyclus® 100 GC *Mycoplasma arginini*	3 vials
SMB95-3002	Cyclus® 100 GC *Mycoplasma orale*	3 vials
SMB95-3003	Cyclus® 100 GC *Mycoplasma gallisepticum*	3 vials
SMB95-3004	Cyclus® 100 GC *Mycoplasma pneumoniae*	3 vials
SMB95-3005	Cyclus® 100 GC *Mycoplasma synoviae*	3 vials
SMB95-3006	Cyclus® 100 GC *Mycoplasma fermentans*	3 vials
SMB95-3007	Cyclus® 100 GC *Mycoplasma hyorhinis*	3 vials
SMB95-3008	Cyclus® 100 GC *Acholeplasma laidlawii*	3 vials
SMB95-3009	Cyclus® 100 GC *Spiroplasma citri*	3 vials
SMB95-3010	Cyclus® 100 GC *Mycoplasma salivarium*	3 vials

Cyclus® 10 CFU , 3 vials		
SMB95-3011	Cyclus® 10 CFU *Mycoplasma arginini*	3 vials
SMB95-3012	Cyclus® 10 CFU *Mycoplasma orale*	3 vials
SMB95-3013	Cyclus® 10 CFU *Mycoplasma gallisepticum*	3 vials
SMB95-3014	Cyclus® 10 CFU *Mycoplasma pneumoniae*	3 vials
SMB95-3015	Cyclus® 10 CFU *Mycoplasma synoviae*	3 vials
SMB95-3016	Cyclus® 10 CFU *Mycoplasma fermentans*	3 vials
SMB95-3017	Cyclus® 10 CFU *Mycoplasma hyorhinis*	3 vials
SMB95-3018	Cyclus® 10 CFU *Acholeplasma laidlawii*	3 vials
SMB95-3019	Cyclus® 10 CFU *Spiroplasma citri*	3 vials
SMB95-3020	Cyclus® 10 CFU *Mycoplasma salivarium*	3 vials
Cleaning Spray		
SMB95-5001	DNA Decontamination Reagent, spray bottle	250 mL
SMB95-5002	DNA Decontamination Reagent, refill canister	5 L
Cleaning Wipes		
SMB95-5003	DNA Decontamination Reagent, wipes	50 wipes

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