

SARTORIUS

Simplifying Progress

Matrix specific Validation Proposal

Microsart® ATMP Extraction
Microsart® ATMP Bacteria & Fungi

Microsart® ATMP Sterile Release



Proposal according to European Pharmacopoeia 2.6.7

The matrix specific validation proposal gives an overview of how to validate that a matrix (final product, cell culture, etc.) can be tested for bacterial or fungal contamination (<100 CFU/mL) using **either** the Microsart® ATMP Extraction kit in combination with the Microsart® ATMP Bacteria and Microsart® ATMP Fungi kit **or** the Microsart® ATMP Sterile Release kit.

The kits are not suitable yet to replace classical sterility testing according to EP 2.6.1 and USP <71>.

EP 5.1.6|USP <1223>|EP 2.6.27| TR33 were used as guidance for validation for alternative methods and **compliant with USP <1071>**.

- The following 4 bacteria and 2 fungi species are mandatory to be tested according to EP/USP
 - *Bacillus subtilis*
 - *Clostridium sporogenes*
 - *Pseudomonas aeruginosa*
 - *Staphylococcus aureus*
 - *Aspergillus brasiliensis*
 - *Candida albicans*
- **24 independent replicates of matrix spiked with 99 CFU/mL (Colony Forming Unit)** of each species need to be tested, including DNA extraction and qPCR analysis.
 - Split the 24 replicates to at least **3 independent experiments on different days**. Each experiment contains:
 - 8 replicates of spiked matrix per species
 - 2 **Negative Extraction Controls (NEC)**; matrix without spiked microorganisms)
 - 2 **PCR No Template Controls (NTC)**; PCR grade water as template)
 - 2 **PCR Positive Controls (PC)**

Acceptance Criteria

Matrix spiked with...	Exp. 1*	Exp. 2*	Exp. 3*	Sum	PCR Acceptance Criteria
99 CFU / mL <i>Bacillus subtilis</i>	8	8	8	24	<ul style="list-style-type: none"> all PCR controls (NEC, NTC, PC) must meet expectations. 23 out of 24 replicates of each species must be positive.
99 CFU / mL <i>Clostridium sporogenes</i>	8	8	8	24	
99 CFU / mL <i>Pseudomonas aeruginosa</i>	8	8	8	24	
99 CFU / mL <i>Staphylococcus aureus</i>	8	8	8	24	
99 CFU / mL <i>Aspergillus brasiliensis</i>	8	8	8	24	
99 CFU / mL <i>Candida albicans</i>	8	8	8	24	

*optionally: perform experiment on different days or different lab technicians could run the experiments to challenge robustness

Microsart® Validation Standards

- Microsart® Validation Standards contain lyophilized non-viable bacteria and fungi suitable for matrix validation
 - One vial has a concentration of **99 CFU/mL** after rehydration with 1 mL matrix.
- **1000 µL of the 99 CFU/mL matrix suspension are required per extraction**
 - **One vial** is sufficient for **one extraction**
 - One Microsart® Validation Standard contains **6 vials**



Summary of required Material

Process step	Quantity for 4 bacterial and 2 fungal species	Material
DNA extractions	<p>Number of extractions = 6 mandatory species x (3 experiments x (8 spiked matrix replicates + 2 NEC)) = $6 \times (3 \times (8 + 2)) = 180$ extractions</p> <p>Number of Microsart® ATMP Extraction kits = 180 extractions / 50 extractions per kit = 3.6 kits → 4 Kits</p>	<p>Microsart® ATMP Extraction</p> <p>SMB95-2001</p>
Bacteria PCR	<p>Number of PCRs = 4 bacterial species x (3 experiments x (8 spiked matrix replicates + 2 NEC + 2 NTC + 2 PC)) = $4 \times (3 \times (8 + 2 + 2 + 2)) = 168$ PCRs</p> <p>Number of Microsart® ATMP Bacteria kits = 168 PCRs/100 reactions per kit = 1.7 → 2 Kits</p>	<p>Microsart® ATMP Bacteria</p> <p>SMB95-1008</p>
Fungi PCR	<p>Number of PCRs = 2 fungal species x (3 experiments x (8 spiked matrix replicates + 2 NEC + 2 NTC + 2 PC)) = $2 \times (3 \times (8 + 2 + 2 + 2)) = 84$ PCRs</p> <p>Number of Microsart® ATMP Fungi kits = 84 PCRs/100 reactions per kit = 0.8 → 1 Kit</p>	<p>Microsart® ATMP Fungi</p> <p>SMB95-1012</p>
Non-viable bacteria and fungi spikes	<p>24 spiked matrix replicates per species are required</p> <p>Number of Microsart® Validation Standard kits = 24 spiked matrix replicates/1 extractions per vial / 6 vials per kit = $24/1/6 = 4$ kits → 4 Kits per bacterial or fungal species</p>	<p>Microsart® Validation Standard</p> <p>SMB95-2005 - 2010, SMB95-2037 - 2043</p>





Summary of required Material

Quantity	Order no.	Product name
4	SMB95-2001	Microsart® ATMP Extraction
3	SMB95-1008	Microsart® ATMP Bacteria
1	SMB95-1012	Microsart® ATMP Fungi
4	SMB95-2005	Microsart® Validation Standard <i>Bacillus subtilis</i>
4	SMB95-2006	Microsart® Validation Standard <i>Pseudomonas aeruginosa</i>
4	SMB95-2007	Microsart® Validation Standard <i>Kocuria rhizophila</i> <i>Micrococcus luteus</i>
4	SMB95-2008	Microsart® Validation Standard <i>Clostridium sporogenes</i>
4	SMB95-2009	Microsart® Validation Standard <i>Bacteroides vulgatus</i>
4	SMB95-2010	Microsart® Validation Standard <i>Staphylococcus aureus</i>
4	SMB95-2037	Microsart® Validation Standard <i>Candida albicans</i>
4	SMB95-2038	Microsart® Validation Standard <i>Aspergillus brasiliensis</i>
4	SMB95-2039	Microsart® Validation Standard <i>Aspergillus formigatus</i>
4	SMB95-2040	Microsart® Validation Standard <i>Penecillium chrysogenum</i>
4	SMB95-2041	Microsart® Validation Standard <i>Candida glabrata</i>
4	SMB95-2042	Microsart® Validation Standard <i>Candida krusei</i>
4	SMB95-2043	Microsart® Validation Standard <i>Candida tropicalis</i>

Species printed in bold are mandatory

Thank you.