

SARTORIUS

Simplifying Progress

Matrix specific Validation Proposal

Microsart® AMP Extraction
Microsart® ATMP Mycoplasma



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 Mycoplasma contamination (10 CFU/mL) using the Microsart® ATMP Mycoplasma and the Microsart® AMP Extraction.

- At least **4 different Mycoplasma species** need to be tested
 - For matrices of human origin:
 - *A. laidlawii*
 - *M. fermentans*
 - *M. orale*
 - *M. pneumoniae*
 - Matrices of other origin require different Mycoplasma species (“Choice of Mycoplasma Species” slide)
- **24 independent replicates of matrix spiked with 10 CFU/mL** (Colony Forming Unit) of each species need to be tested, including DNA extraction and PCR 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 Mycoplasma)
 - 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
10 CFU / mL <i>A. laidlawii</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.
10 CFU / mL <i>M. fermentans</i>	8	8	8	24	
10 CFU / mL <i>M. orale</i>	8	8	8	24	
10 CFU / mL <i>M. pneumoniae</i>	8	8	8	24	

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

Choice of Mycoplasma Species

The following species are recommended in the present validation proposal in accordance with EP 2.6.7:

- *Mycoplasma fermentans*
- *Mycoplasma orale*
- *Mycoplasma pneumonia*
- *Acholeplasma laidlawii*

M. arginini, *M. synoviae*, *S. citri*, *M. gallisepticum*, *M. hyorhinis* might be considered when the manufacturing process does use ruminant, insect, plant, avian and porcine materials.

It is important to check the origin of all components of the culture media and process supplements. It is not necessary, but usually accepted by the regulatory authorities, to restrict validation to such species of the highest importance according to risk assessment.

According to EP 2.6.7, the listed species need to be tested if material is of the following origin:




- **Human**
M. orale, *M. fermentans*, *M. pneumoniae*
- **Bovine**
M. arginini
- **Avian**
M. synoviae, *M. gallisepticum*
- **Porcine**
M. hyorhinis
- **Plant and insects**
S. citri
- **Please always include**
A. laidlawii

Microsart® Validation Standards

- Microsart® Validation Standards contain lyophilized non-viable Mycoplasma suitable for matrix validation
 - One vial has a concentration of 10 CFU/mL after rehydration with 1 mL matrix.
- Only 200 µL of the 10 CFU/mL matrix suspension are required per extraction
 - One vial is sufficient for $(1000 \mu\text{L} / 200 \mu\text{L} =) 5$ extractions (**4 extractions are recommended**)



Summary of required Material

Process step	Quantity for 4 Mycoplasma species	Material
DNA extractions	<p>Number of extractions = 4 Mycoplasma species x (3 experiments x (8 spiked matrix replicates + 2 NEC)) = 4 x (3 x (8 + 2)) = 120 extractions</p> <p>Number of Microsart® AMP Extraction kits = 120 extractions / 50 extractions per kit = 2.4 kits → 3 Kits</p>	<p>Microsart® AMP Extraction</p> <p>SMB95-2003</p> 
Mycoplasma PCR	<p>Number of PCRs = 4 Mycoplasma species x (3 experiments x (8 spiked matrix replicates + 2 NEC + 2 NTC + 2 PC)) = 4 x (3 x (8 + 2 + 2 + 2)) = 168 PCRs</p> <p>We offer different sizes of the Microsart® ATMP Mycoplasma Kits (25x and 100x PCRs).</p> <p>For 168 PCRs → 3 small kits (25x) and 1 large kit (100x)</p>	<p>Microsart® ATMP Mycoplasma</p> <p>SMB95-1003 / SMB95-1004</p> 
Non-viable Mycoplasma spikes	<p>24 spiked matrix replicates per Mycoplasma species are required (e.g. <i>A. laidlawii</i>, <i>M. fermentans</i>, <i>M. orale</i>, <i>M. pneumoniae</i>)</p> <p>Number of Microsart® Validation Standard kits = 24 spiked matrix replicates / 4 extractions per vial / 3 vials per kit = 24/4/3 = 2 kits → 2 Kits per Mycoplasma species</p>	<p>Microsart® Validation Standard</p> <p>SMB95-2012 / 2014 / 2016 / 2018</p> 

Summary of required Material



Quantity	Order no.	Product name
3	SMB95-2003	Microsart® AMP Extraction
3	SMB95-1003	Microsart® ATMP Mycoplasma (25x)
1	SMB95-1004	Microsart® ATMP Mycoplasma (100x)
2	SMB95-2011	Microsart® Validation Standard <i>Mycoplasma arginini</i>
2	SMB95-2012	Microsart® Validation Standard <i>Mycoplasma orale</i>
2	SMB95-2013	Microsart® Validation Standard <i>Mycoplasma gallisepticum</i>
2	SMB95-2014	Microsart® Validation Standard <i>Mycoplasma pneumoniae</i>
2	SMB95-2015	Microsart® Validation Standard <i>Mycoplasma synoviae</i>
2	SMB95-2016	Microsart® Validation Standard <i>Mycoplasma fermentans</i>
2	SMB95-2017	Microsart® Validation Standard <i>Mycoplasma hyorhinis</i>
2	SMB95-2018	Microsart® Validation Standard <i>Acholeplasma laidlawii</i>
2	SMB95-2019	Microsart® Validation Standard <i>Spiroplasma citri</i>
2	SMB95-2020	Microsart® Validation Standard <i>Mycoplasma salivarium</i>

Choose at least four species.

Thank you.