

February 20, 2020

**Keywords or phrases:**

Rapid sterility testing of short shelf-life therapeutics;  
Equivalency of Real-Time PCR- and growth based  
detection

# Equivalency of PCR-Based Rapid Sterility Testing and the Compendial Culture Method According to Ph. Eur. 2.6.1., JP 4.06 and USP <71>

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## Abstract

In this study, we compared the microbial detection capability of the Microsart® ATMP Bacteria and Microsart® ATMP Fungi Real-time PCR kits with the compendial sterility test. We spiked samples, using 6 different bacterial (*Bacillus subtilis*, *Staphylococcus aureus*, *Clostridium sporogenes*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and *Pseudomonas protegens*) and 2 fungal (*Candida albicans* and *Aspergillus brasiliensis*) species at concentration levels between 2.5 CFU/mL and 198 CFU/mL, and compared our results to the growth-based method performed in parallel at an external contract lab, according to Ph. Eur. 2.6.1, JP 4.06 and USP <71><sup>2,3,4</sup>. Our results show full equivalency of Microsart® ATMP Bacteria and Microsart® ATMP Fungi with the compendial method. Moreover, the Microsart® ATMP Fungi detected *Candida albicans* with higher sensitivity.

## Introduction

Sterility testing is a critical component of the release testing for any cell therapy product since microbial contamination of cell therapy products can potentially kill recipients. The current compendial sterility test for most bacteria takes 14 days and 28 days for mycoplasma testing before contamination can be ruled out with certainty<sup>1,2,3</sup>. However, time-to-result is an important attribute of testing for short shelf-life cellular therapeutics, especially for autologous cell therapies intended to for terminally ill patients.

As a result, growth-independent rapid assays are in increasing demand. To fulfill this demand, we developed and comprehensively validated a highly sensitive and broad range microbial detection system, consisting of an efficient Microsart® ATMP Extraction DNA isolation protocol, and followed by a real-time PCR assay using the Microsart® ATMP Bacteria/Fungi/Mycoplasma kit.

We designed this validation study to evaluate the bacterial and fungal detection capability of this system, according to the requirements of the European Pharmacopeia chapter 5.1.6<sup>1</sup>. *In silico* sequence alignment analysis demonstrated that the Microsart® ATMP Bacteria kit can detect > 94% of Gram-positive and Gram-negative bacteria. Also, *in silico* analysis for Microsart® ATMP Fungi demonstrated an additional coverage of > 37% of all fungi species. Due to the higher variability in fungal genera, the fungal coverage is seemingly low; however, all USP/EP relevant species are covered, as well as further typical contaminants. In addition, PCR assays even allow the detection of bacterial and fungal contaminations that are not covered by growth tests.

In this study, we show that both PCR assays are comparable in detection of microbial contaminants to the compendial culture method. For comparison, spiked samples were tested in parallel at an external contract lab, according to Ph. Eur. 2.6.1, JP 4.06 and USP <71><sup>2,3,4</sup>.

## Methods

EZ-CFU™ standards (quantified reference cultures; Microbiologics) for eight microbial species (Table 1) were rehydrated in 2 mL of rehydration buffer, according to the instructions for use<sup>5</sup>. The bacteria suspensions were diluted in DMEM + 5 % FBS and the fungal suspensions in DMEM to generate concentrations of 2x LOD<sub>95</sub>, LOD<sub>95</sub> and ½ LOD<sub>95</sub>. Samples were split into aliquots. LOD<sub>95</sub> values of the different bacterial and fungal species were determined during their respective PCR kit validation and are listed in Table 1.

An aliquot of each concentration was used for sterility testing (direct inoculation) at Labor LS. Each sample was cultivated in thioglycolate medium and soya-bean casein medium for 14 days, according to the recommendation of the guidelines. In parallel, aliquots were extracted in duplicates and analyzed using a CFX96 Real-time PCR instrument according to the instructions for use of the Microsart® ATMP Extraction kit, the Microsart® ATMP Bacteria, or the Microsart® ATMP Fungi detection kit,<sup>6,7,8</sup>. The test setup is described in Table 2.

## Results

Our results are summarized in Table 3. For the Microsart® ATMP Bacteria and Microsart® ATMP Fungi results are given in Ct values (cycle threshold values). Ct values < 40 are positive.

Species	Strain	Atmosphere	LOD <sub>95</sub>
<i>Staphylococcus aureus</i>	ATCC 6538	aerobic	25 CFU/ml
<i>Clostridium sporogenes</i>	ATCC 19404	anaerobic	50 CFU/ml
<i>Pseudomonas aeruginosa</i>	ATCC 9027	aerobic	5 CFU/ml
<i>Streptococcus pyogenes</i>	ATCC 19615	aerobic	99 CFU/ml
<i>Pseudomonas protegens</i>	ATCC 17386	aerobic	10 CFU/ml
<i>Candida albicans</i>	ATCC 10231	aerobic	50 CFU/ml
<i>Aspergillus brasiliensis</i>	ATCC 16404	aerobic	50 CFU/ml

Table 1: Bacterial and fungal strains used in this study, respective incubation conditions, and LOD<sub>95</sub>

## Comparison with culture method with defined starting material quantity at external contract lab

1	Spiked DMEM + 5 % FBS with <i>B. subtilis</i> , <i>S. aureus</i> , <i>C. sporogenes</i> , <i>P. aeruginosa</i> , <i>S. pyogenes</i> or <i>P. protegens</i> at 2x LOD <sub>95</sub> , LOD <sub>95</sub> and ½ LOD <sub>95</sub> . One aliquot without spike was processed as NC.	
	A 1 mL aliquot of each sample was used per each cultivation media (thioglycolate medium and soya-bean casein medium) at the external contract lab L+S AG.	DNA was extracted from 1 mL starting material with Microsart® ATMP Extraction. Real-time PCR was performed according to Microsart® ATMP Bacteria.
2	Spiked DMEM with <i>C. albicans</i> or <i>A. brasiliensis</i> at 2x LOD <sub>95</sub> , LOD <sub>95</sub> and ½ LOD <sub>95</sub> . One aliquot without spike was processed as NC.	
	A 1 mL aliquot of each sample was used per each cultivation media (thioglycolate medium and soya-bean casein medium) at the external contract lab L+S AG	DNA was extracted from 1 mL starting material with Microsart® ATMP Extraction. Real-time PCR was performed according to Microsart® ATMP Fungi.

Table 2: Test setup for comparison between Microsart® ATMP Bacteria and Microsart® ATMP Fungi with compendial sterility test

Species	Microsart® ATMP Bacteria			Compendial culture method (External Lab)		
	Results available within 3 hours			Results available after 14 days		
	2 x LOD <sub>95</sub>	LOD <sub>95</sub>	½ x LOD <sub>95</sub>	2 x LOD <sub>95</sub>	LOD <sub>95</sub>	½ x LOD <sub>95</sub>
<i>Bacillus subtilis</i>	33.16	34.23	35.47	Positive	Positive	Positive
	33.23	34.32	34.38			
<i>Staphylococcus aureus</i>	35.42	35.77	36.56	Positive	Positive	Positive
	34.13	35.67	39.90			
<i>Clostridium sporogenes</i>	34.20	34.87	35.45	Positive	Positive	Positive
	34.10	33.43	35.61			
<i>Pseudomonas aeruginosa</i>	36.40	36.74	<b>37.22</b>	Positive	Positive	<b>Negative</b>
	36.22	37.96	<b>No Cq</b>			
<i>Streptococcus pyogenes</i>	34.89	35.53	36.55	Positive	Positive	Positive
	35.09	35.93	35.88			
<i>Pseudomonas protegens</i>	34.14	34.38	36.52	Positive	Positive	Positive
	33.28	34.51	35.61			
Species	Microsart® ATMP Fungi			Compendial culture method (External Lab)		
	2 x LOD <sub>95</sub>	LOD <sub>95</sub>	½ x LOD <sub>95</sub>	2 x LOD <sub>95</sub>	LOD <sub>95</sub>	½ x LOD <sub>95</sub>
<i>Candida albicans</i>	32.25	32.27	<b>33.96</b>	Positive	Positive	<b>Negative</b>
	31.94	32.12	<b>32.96</b>			
<i>Aspergillus brasiliensis</i>	34.38	37.06	34.94	Positive	Positive	Positive
	32.40	33.17	34.20			

Table 3: Results of bacterial and fungal detection using Microsart® ATMP Bacteria and Microsart® ATMP Fungi in comparison to the compendial culture method according to Ph. Eur. 2.6.1, JP 4.06 and USP <71>

## Discussion

In this study, we compared the bacterial and fungal detection performance of the Microsart® ATMP Bacteria and Microsart® ATMP Fungi detection kits and the compendial method. We simultaneously tested spiked samples using rapid real-time PCR based detection and the compendial testing (performed at an external contract lab).

Our results, summarized in Table 3, show that the Microsart® ATMP Bacteria and Microsart® ATMP Fungi detection kits give equivalent results to the growth-based, compendial method. Only one sample containing *P. aeruginosa* at a concentration of 2.5 CFU/mL gave negative results using both methods. During validation of the Microsart® ATMP Bacteria kit, we determined an LOD<sub>95</sub> of 5 CFU/ml for *P. aeruginosa*. However, 2.5 CFU/ml are still detectable with a probability of 83% and we detected one out of two. Of note, the lowest tested concentration of *C. albicans* was detected only with the Microsart® ATMP Fungi kit, while the traditional growth-based method failed to detect the contaminant.

The Microsart® ATMP Bacteria and Fungi assays enable simultaneous testing for bacteria and fungi within the same PCR, and give results in 3 hours instead of weeks. A rapid detection of such contaminants in short shelf-life cellular therapeutics, especially autologous cell therapies, is urgently needed prior to administration to terminally ill patients.

Our results emphasize that, in addition to the classic sterility testing, rapid real-time PCR-based detection of microbial contaminations contribute to a risk reduction, as they facilitate the availability of results prior treatment and, therefore, contribute to patient safety.

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