SARTORIUS

Application Note

June 21, 2021

Keywords or phrases:
Mycoplasma detection, Real time PCR, qPCR, EP 2.6.7, compliance, Rapid Microbiological Methods (RMM), Microsart® AMP Extraction, Microsart® ATMP Mycoplasma, CellSafe MycoQSearch™ qPCR

Benchmarking of Two Mycoplasma Real-time PCR Kits suitable for Release Testing According EP 2.6.7

Sartorius Microsart® ATMP Mycoplasma qPCR vs. CellSafe MycoQSearch™ qPCR

Lisa Hollstein , Dr. Alexandra Müller-Scholz
Lab Essentials Applications Development, Sartorius Stedim Biotech, Göttingen, Germany

Correspondence:
E-Mail: PCR@sartorius.com

Abstract

This benchmark study compares the capabilities of two different qPCR kits for the detection of Mycoplasma, namely, the Sartorius Microsart® ATMP Mycoplasma qPCR kit and the CellSafe MycoQSearch™ qPCR kit. The EP 2.6.7 required sensitivity of 10 CFU / mL of selective species is tested using three different Sartorius Microsart® Validation Standards (*Acholeplasma laidlawii, Mycoplasma orale, Mycoplasma pneumoniae*) and one CellSafe Standard (*Mycoplasma arginini*).

Despite some differences in the overall qPCR performance between the benchmarked products, mainly the different shipping conditions (Sartorius qPCR kit needs to be chilled [4]; CellSafe qPCR kit needs to be transported on dry ice [6]) and the number of pipetting steps are crucial.

The Sartorius qPCR kit shows a higher sensitivity (detected 16 / 16 positive) than the CellSafe qPCR kit (detected 12 / 16 positive).

Introduction

Among the world's smallest bacteria, Mycoplasmas are capable of independent reproduction. They belong to the class of Mollicutes and have a very slow and parasitic growth. The contamination of cell cultures remains a major problem. Several physiological and biochemical parameters are affected by the presence of Mycoplasma in cell cultures. The infection potentially causes changes in metabolism, growth, viability, macromolecule synthesis, morphology etc. and therefore a sensitive routine testing for contamination of cell cultures is essential [1].

The traditional growth-based method requires a cultivation time of at least 28 days before a contamination can be ruled out with certainty. In comparison, Nucleic Acid Amplification Techniques (NAT) can reduce the time to result to just hours. As an alternative to the culture method the NAT test system must be shown to detect 10 CFU / mL of Mycoplasma [2]. For this reason, the capability of two different Mycoplasma PCR kits to detect spikes of no higher than 10 CFU / mL in Dulbecco's Modified Eagle Medium (DMEM) plus 5 % Fetal Calf Serum (FCS) was tested in this study.

Material

Table 1. Name, REF, LOT and expiration date of the benchmarked material.

Name	REF	LOT	Expiration Date
Sartorius Stedim Biotech GmbH Minerva Biolabs GmbH Microsart® AMP [3] **Extraction**	SMB95-2003	9523S1080	2022-12-03
Sartorius Stedim Biotech GmbH Minerva Biolabs GmbH Microsart® ATMP Mycoplasma [4] **qPCR**	SMB95-1003	9513S20L1	2022-06-30
Qiagen GmbH DNeasy® Blood & Tissue [5] **Extraction**	69504	166030841	-
CellSafe Co., Ltd. MycoQSearch™ [6] **qPCR**	QDEP-100	QDEP012102	2022-01
Sartorius Stedim Biotech GmbH Minerva Biolabs GmbH Microsart® **Validation Standard** *Acholeplasma laidlawii* [7]	SMB95-2018	9521L1059	2021-05-31
Sartorius Stedim Biotech GmbH Minerva Biolabs GmbH Microsart® **Validation Standard** *Mycoplasma orale*[7]	SMB95-2012	9521O2069	2021-06-30
Sartorius Stedim Biotech GmbH Minerva Biolabs GmbH Microsart® **Validation Standard** *Mycoplasma pneumoniae*[7]	SMB95-2014	9521P1059	2021-05-31
CellSafe Co., Ltd. **Standard** *Mycoplasma arginini* [8]	M10 CFU-MA	MSA090120	2021-09

Instruments

PCR cycler: Bio-Rad CFX96 Deep Well™
Real-time System C1000 Touch™ Thermal Cycler
Software: Bio-Rad CFX Manager 3.1

PCR cycler: Agilent Technologies Stratagene MxPro 3005P
Software: MxPro

Both benchmarked qPCR analyses were performed using two different PCR cyclers.
First a Bio-Rad CFX96 cycler was used. The Sartorius qPCR showed evaluable Ct results, whereas the Ct values generated by the CellSafe qPCR kit were not to be evaluated. The CellSafe qPCR kit seems to be dependent on the addition of a ROX reference dye. However, the CellSafe qPCR manual [6] does not recommend adding the ROX reference dye using the Bio-Rad CFX96 cycler.

Switching to another Cycler (Agilent MxPro 3005P Cycler) resulted in evaluable Ct values for both benchmarked qPCR kits. These results are shown in the following.

Experimental Setup

In this Application Note the Sartorius Microsart® AMP Extraction kit and the Sartorius Microsart® ATMP Mycoplasma qPCR kit are benchmarked to the Qiagen DNeasy® Blood & Tissue Extraction kit and the CellSafe MycoQSearch™ qPCR kit.

The CellSafe MycoQSearch™ qPCR kit can be combined with different sample preparation procedures. In order to work complient to the EP 2.6.7 guideline, the Qiagen DNeasy® Blood & Tissue Extraction kit must be combined with the CellSafe qPCR.

Both product combinations are based on a manual DNA isolation and a subsequent DNA detection via qPCR.
As test material four different quantified colony forming unit (CFU) standards, Validation Standards, are rehydrated or diluted in DMEM and 5 % FCS, used as matrix. Four replicates of each standard are measured in the qPCR approaches. Three Validation Standards (*Acholeplasma laidlawii, Mycoplasma orale, Mycoplasma pneumoniae*) were supplied by Sartorius Stedim Biotech GmbH. Each vial contains a lyophilized pellet of 10 CFU. To achieve a concentration of 10 CFU / mL, 1 mL of matrix has to be added to each Validation Standard vial[7].

One Validation Standard (*Mycoplasma arginini*) was supplied by CellSafe Co., Ltd. and contains 100 CFU / mL. To receive a final concentration of 10 CFU / mL, 200 µL of the Validation Standard have to be mixed with 1800 µL of matrix.

Both DNA extraction methods are performed on the same day according to the manuals [3, 5, 7] of the manufacturer and are based on absorption and elution of DNA on silica columns. All reagents can be stored at room temperature and the DNA extraction can be performed within approximately one hour, valid for both kits.

Both PCR assays can be performed in less than three hours and are performed at the next day, following the manuals [4, 6] likewise. The Sartorius qPCR uses FAM labeled probes and ROX as internal inhibition control, whereas the CellSafe qPCR uses FAM labeled probes as well but HEX as internal control and ROX as a reference dye.

Further differences can be recognized regarding storage conditions and the number of pipetting steps.
The Sartorius qPCR kit includes lyophilized reagents and has to be stored at + 2 to + 8 °C. After opening and rehydration the kit must be stored at ≤ - 18 °C [4]. The CellSafe qPCR kit and the CellSafe Standard should always be stored at – 20 °C [6, 8] and therefore dry ice (solid carbon dioxide) is needed for shipment. Regarding the number of pipetting steps, it must be mentioned that the Sartorius qPCR kit includes a ready to use Mycoplasma Master mix [4]. To prepare a PCR reaction only two reagents (Master mix and sample/DNA extract) need to be mixed.

In contrast, the CellSafe qPCR kit provides a vial of Master mix, a vial of Primer and Probe Mix and a vial for ROX as reference dye [6]. Pipetting these three reagents, and in addition PCR grade water and the sample/DNA extract requires three more pipetting steps. Contaminations and handling mistakes are more likely than with the Sartorius product.

Results

The results of the mycoplasma detection via qPCR are shown in Table 2, as well as in Figures 1 and 2.

All qPCR Positive Controls (PC), No Template Controls (NTC) and Negative Extraction Controls (NEC) show expected results (Table 2).

Evaluating the results for the Validation Standard extractions, the Sartorius detection system shows 16 / 16 positive signals in the subsequent qPCR. It is confirmed that *A. laidlawii, M. orale, M. pneumoniae* and *M. arginini* with a concentration of 10 CFU / mL can be detected reliably.

Compared to the Sartorius results, the Qiagen extracts show 12 / 16 positive signals in the subsequent CellSafe qPCR. Two replicates of *M. orale* and one replicate of *M. pneumoniae* are not detected. In addition, one replicate of *A. laidlawii* shows a qPCR inhibition.

The amplification plots of the Sartorius qPCR show the typical amplification curve shape (Figure 1). In comparison, the amplification plots of the CellSafe show untypical curve shapes and relatively high Ct values (Figure 2) for the Mycoplasma detection.

Table 2. qPCR results of the FAM channel of controls and 10 CFU / mL Validation Standards of two different suppliers

	Sartorius / Sartorius **Microsart® AMP Extraction / Microsart® ATMP Mycoplasma qPCR**		**Qiagen / CellSafe** **Qiagen DNeasy® Blood & Tissue Extraction /CellSafe MycoQSearch™**	
Sample Material	**Ct Results**	**Summary**	**Ct Results**	**Summary**
Positive Control (PC)	27.3 25.1	2 / 2 positive	30.3 30.4	2 / 2 positive
No Template Control (NTC) (PCR grade Water)	No Ct No Ct	2 / 2 negative	No Ct No Ct	2 / 2 negative
Negative Extraction Control (NEC) (DMEM + 5 % FCS)	No Ct No Ct	2 / 2 negative	No Ct No Ct	2 / 2 negative
Sartorius Microsart® Validation Standard 10 CFU / mL *Acholeplasma laidlawii*	38.5 29.0 29.2 29.6	4 / 4 positive	No Ct 36.4 35.2 35.3	3 / 4 positive (1 sample inhibited)
Sartorius Microsart® Validation Standard 10 CFU / mL *Mycoplasma orale*	30.2 31.1 32.3 30.7	4 / 4 positive	38.3 38.8 No Ct No Ct	2 / 4 positive
Sartorius Microsart® Validation Standard 10 CFU / mL *Mycoplasma pneumoniae*	30.8 31.0 31.6 30.9	4 / 4 positive	38.5 38.5 40.1 39.0	4 / 4 positive
CellSafe Validation Standard 10 CFU / mL *Mycoplasma arginini*	32.8 31.8 32.3 31.1	4 / 4 positive	37.3 No Ct 37.5 37.0	3 / 4 positive
Summary		**16 / 16 positive**		**12 / 16 positive**

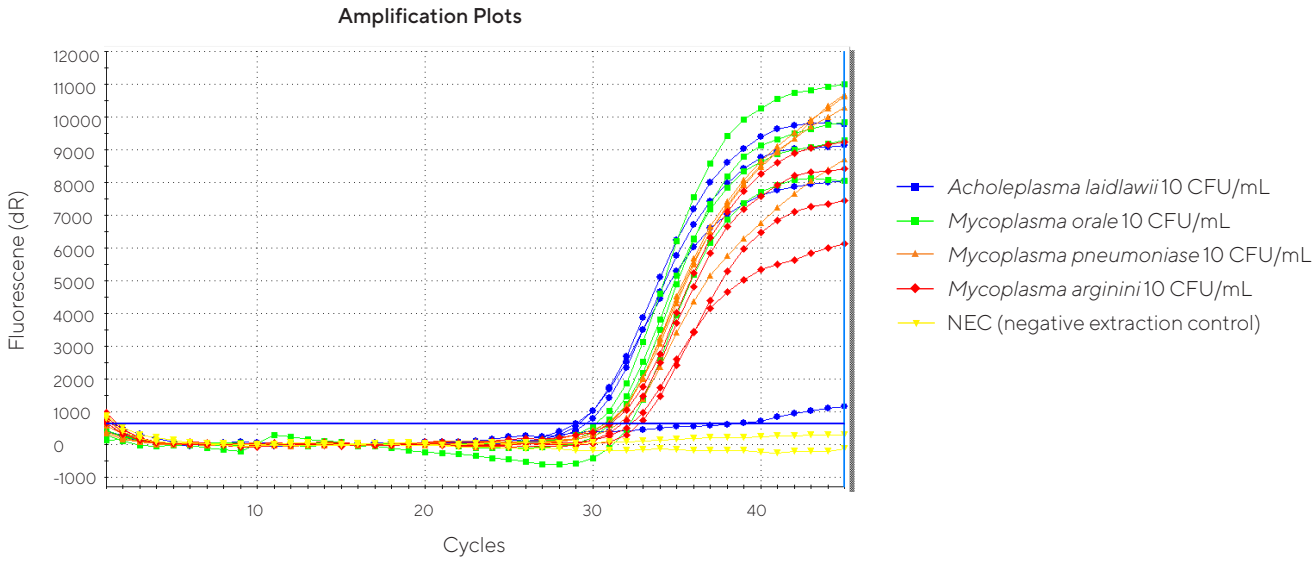

Figure 1. Amplification Plots of Validation Standards (10 CFU / mL) and NEC (0 CFU / mL) generated with Sartorius qPCR. Fluorescence (dR) signals in FAM™ channel; the blue amplification plot which shows a Ct value close to 40 can be regarded as an outlier value as we are detecting target concentrations close to the detection limit in this study.

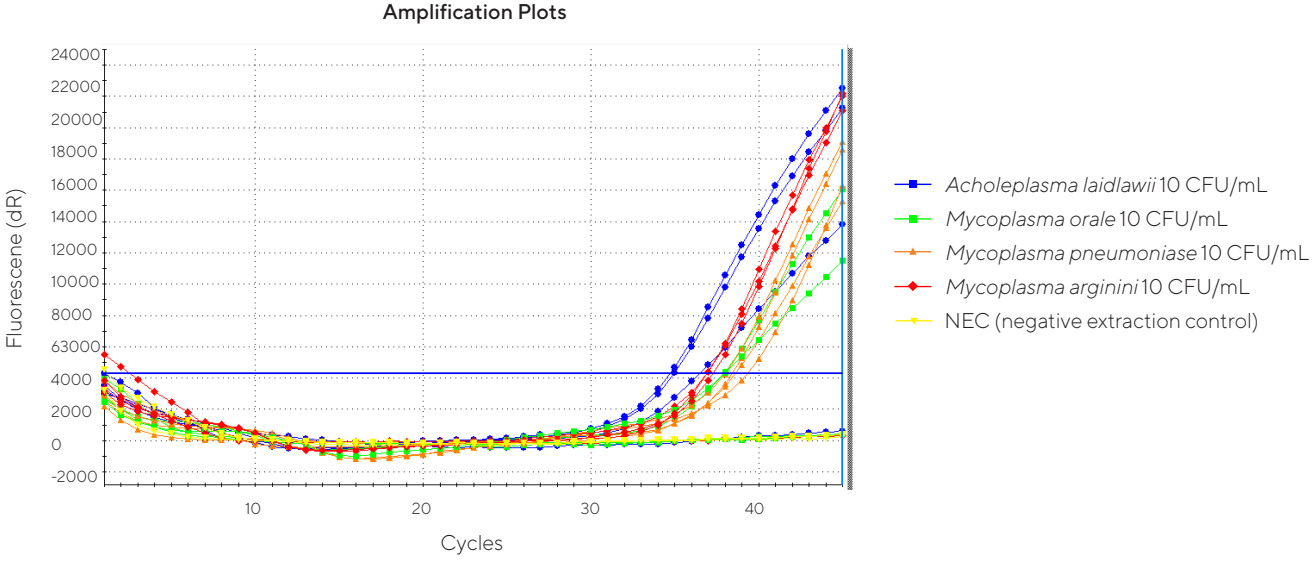

Figure 2. Amplification Plots of Validation Standards (10 CFU / mL) and NEC (0 CFU / mL) generated with CellSafe qPCR. Fluorescence (dR) signals in FAM™ channel.

Conclusion

In this Application Note two different extraction and qPCR products for regulated Mycoplasma testing (according to EP 2.6.7) are benchmarked.

In summary, the results (16 / 16 standards detected by the Sartorius qPCR and 12 / 16 standards detected by the CellSafe qPCR) show a higher sensitivity of the Sartorius qPCR.

References

1 Drexler HG, Uphoff CC. Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. Cytotechnology. 2002;39(2):75-90. doi:10.1023/A:1022913015916.

2 European Pharmacopoeia 7th edition, Strasbourg, FR; European Directorate for the Quality of Medicines; 2010, 2.6.7 Mycoplasmas

3 Instructions for use: Microsart® AMP Extraction, Sartorius, Prod. No. SMB95-2003, Ver. 08 | 2020

4 Instructions for use: Microsart® ATMP Mycoplasma, Sartorius, Prod. No. SMB95-1003, Ver. 09 | 2020

5 Instructions for use: DNeasy® Blood & Tissue Handbook, Qiagen, Prod. No. 69504, Ver. 07 | 2020

6 Instructions for use: MycoQSearch™, CellSafe, Prod. No. QDEP-100, Ver. 4.1 (EP2.6.7)

7 Instructions for use: Microsart® Validation Standard, Sartorius, Prod. No. SMB95-1012, SMB95-2014, SMB95-2018, Ver. 09 | 2020

8 Material Safety Data Sheet: CellSafe Co., Ltd. *Mycoplasma arginini* Prod. No. M10CFU- MA, Ver. 02 | 2018

Sales and Service Contacts

For further contacts, visit www.sartorius.com

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

Status: 06 | 2021