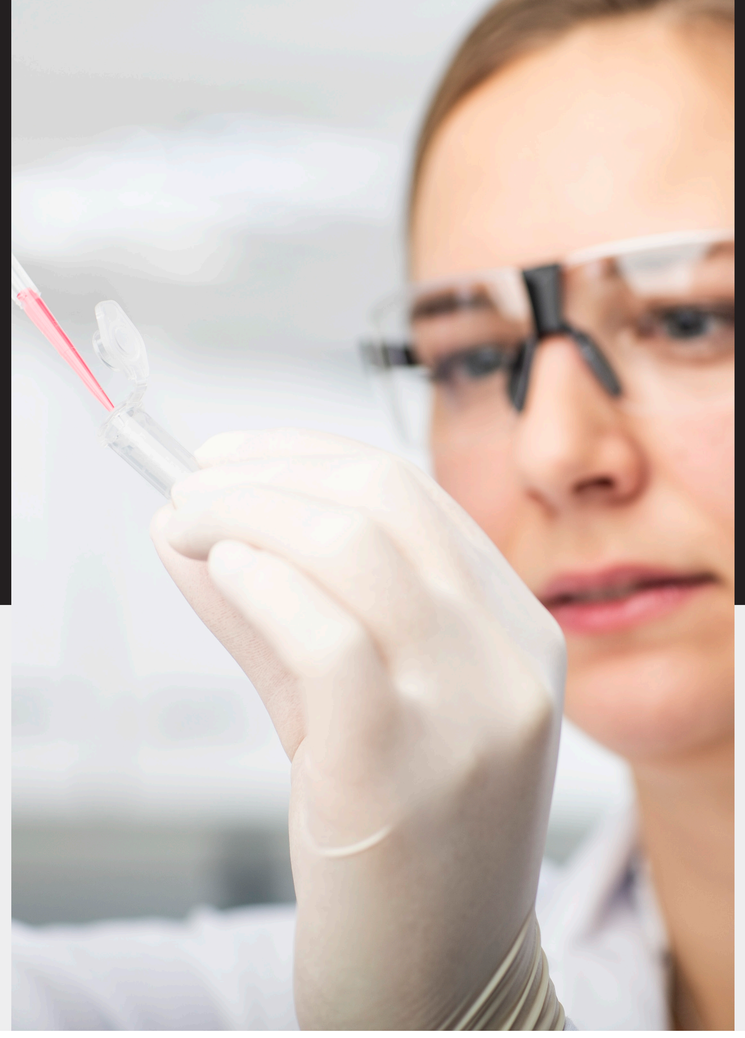

Rapid detection of Mycoplasma and other cell wall-less bacterial species via quantitative PCR

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ABSTRACT

Detecting mycoplasma, acholeplasma, or spiroplasma (mollicutes) in cell cultures is crucial for the pharmaceutical industry, particularly for Advanced Therapy Medicinal Products (ATMP). Traditional culturing methods are time-consuming and may miss viable mycoplasma, risking false-negative results. The Microsart® ATMP Mycoplasma (Sartorius) offers rapid detection, adhering to European Pharmacopoeia standards.[1] Quantification of genomic copy numbers is vital and achievable with the Microsart® Calibration Reagent. Implementation of these solutions on the qTOWERiris 96 and 384 ensures high specificity and timely Mycoplasma testing for cell-based therapeutics.

INTRODUCTION

Mycoplasmas, part of the bacterial class mollicutes, are small, wall-less bacteria thriving as commensals or infectious agents across diverse hosts. They easily contaminate cell cultures, impacting cell growth and therapeutic protein expression. Despite their microscopic size and variable shape, they resist antibiotics and form biofilms, posing challenges in detection and eradication. In the realm of Advanced Therapy Medicinal Products (ATMP), mycoplasma contamination presents significant risks, particularly in autologous cell therapy, threatening product safety and patient outcomes. Traditional culturing methods involve incubating samples for up to 28 days, which can miss viable but non-culturable (VBNC) mycoplasma, leading to false-negative results and impacting production efficiency. In contrast, qPCR methods like the Microsart® ATMP Mycoplasma (Sartorius) offer rapid and accurate detection, providing an alternative for timely mycoplasma detection in pharmaceutical industries.

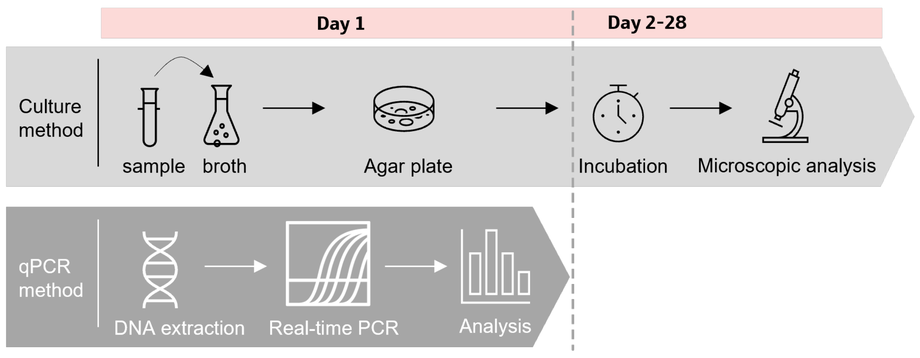

Figure 1 | Schematic of mycoplasma detection methods.

MATERIAL & METHODS

Chemicals

- Microsart® Calibration Reagent (Sartorius)
- Microsart® ATMP Mycoplasma (Sartorius)

Instrumentation

- Real-time PCR thermocycler qTOWERiris 96 and qTOWERiris 384 (Analytik Jena) including color module 1 (455 nm / 515 nm), used for FAM™ dye and color module 4 (580 nm / 620 nm), used for ROX™ dye

- Dilution series of the Microsart® Calibration Reagent was prepared according to manufacturer's instructions to create a calibration curve
- qPCR reaction was prepared following the manufacturer's instructions and the temperature-time protocol defined in Table 1
- An internal control was used as amplification control in the experiment

Table 1 | Temperature and time protocol.

Step	Cycle	Profile	Temperature	Holding time	Ramp rates
1	1	Initial denaturation	95 °C	3 min	8 °C/s
2	45	Denaturation	95 °C	30 sec	8 °C/s
		Annealing	55 °C	30 sec	5.5 °C/s
		Elongation*	60 °C	45 sec	8 °C/s

*Data acquisition with color module 1 and 4 for qTOWERiris series, gain 5.0.

RESULTS

The standard dilutions yielded excellent amplification results and formed a standard curve with corresponding R^2 and PCR efficiency values (see Figure 2; Table 2). The samples showed high homogeneity with a standard deviation below 0.2 (Table 3). The concentration of the positive control was determined with 4175 genome copies for qTOWERiris 96. The validity of the assay was confirmed with an internal amplification control (Figure 3). The experiment repeated with qTOWERiris 384 showed comparable results, with optimal PCR efficiency and coefficient of determination; amplification data for qTOWERiris 384 are available in the corresponding Application Note.

Table 2 | Results of the standard curve.

	FAM ■
R^2	0.99789
Slope	-3.33
Offset	33.66
PCR efficiency	1

Table 3 | Mean Ct, standard deviation, and concentration of all samples with threshold 3.224.

Target	Mean Ct	Standard deviation	Concentration [genome copies per reaction]
Standard 1	13.92	0.02	1,000,000
Standard 2	16.60	0.01	100,000
Standard 3	20.22	0.08	10,000
Standard 4	24.06	0.00	1000
Standard 5	27.05	0.18	100
Standard 6	30.19	0.00	10
Positive control	21.60	0.09	4175.15
No target control (NTC)	No Ct	-	-

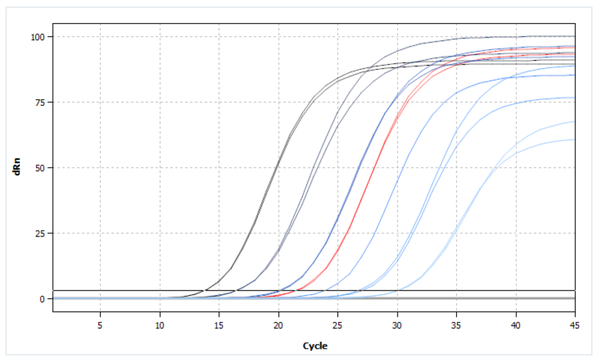

Figure 2 | Amplification curves of the target for FAM™, with an automatic threshold of 3.224.

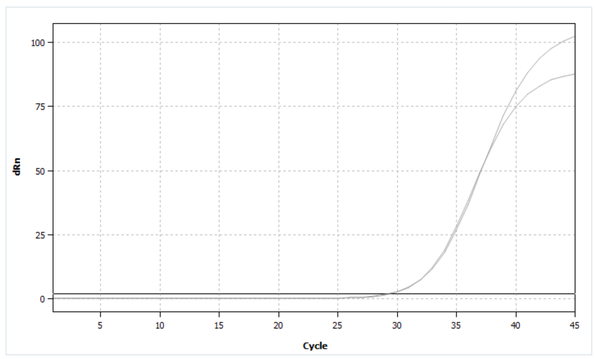

Figure 3 | Amplification curve for the NTC for ROX™ as internal control with an automatic threshold of 3.224.

CONCLUSION

The detection of mollicutes contamination, crucial for those working with cell cultures, especially in Advanced Therapy Medicinal Products (ATMP), is facilitated by assays like the Microsart® ATMP Mycoplasma from Sartorius Stedim Biotech GmbH. Combined with the Microsart® Calibration Reagent, it allows for quantification, demonstrating excellent performance with the qTOWERiris and enabling high-throughput applications with the qTOWERiris 384. The combined use of qTOWERiris 96 and 384, offered by Analytik Jena GmbH+Co. KG, along with Microsart® ATMP Mycoplasma, represents a robust solution for Mollicutes detection, meeting European Pharmacopoeia standards for quality assurance.

Reference: [1] European Pharmacopoeia – Chapter 2.6.27 Microbiological Examination of cell-based Preparations revised: https://www.gmp-compliance.org/gmp-news/revision-of-chapter-2-6-7-of-the-european-pharmacopoeia-published-for-comment

For more information check out the related AppNote.

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