A Rapid Alternative to Culture Based Mycoplasma Detection

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ABSTRACT

Per regulatory requirements, cell-culture based therapies must be free of mycoplasma. Manufacturers have traditionally outsourced testing to labs that specialize in the 28-day culture-based test method. For manufacturers of gene and cell therapy products, as well as other low-dose and short shelf-life therapeutics, it is not feasible to wait 28 days for test results. Thus, the need for rapid mycoplasma test results has also increased. Real-time PCR based assays provide a viable alternative to the culture based method and provide results in hours while meeting the required sensitivity. Following validation, regulatory filing and review, users across multiple therapeutic modalities have received regulatory acceptance to use the MycoSEQ assay for lot release testing.

INTRODUCTION

Mycoplasma contamination represents a serious and costly problem for biomedical research laboratories and facilities involved in development and manufacture of cell-derived biological and pharmaceutical products. Undetected mycoplasma contamination in pharmaceutical products has serious consequences for patient safety and product quality. Testing guidelines to ensure mycoplasma-free, cell-based biotherapeutics are provided by multiple international guidelines and regulatory agencies (e.g., United States Pharmacopoeia (USP), European Pharmacopoeia (EP), Japanese Pharmacopoeia (JP), Section 21 of the Code of Federal Regulations (CFR), International Conference on Harmonization (ICH) and Food and Drug Administration (FDA).

Traditionally, this testing involved the culture of viable mycoplasmas in broth, agar plates and indicator cells. While this is an efficient method of detection, it is costly, time consuming (28 days) and requires specialized training to interpret the results. The amount of on-test time for these culture-based assays does not allow for timely decision making during routine in-process testing. Additionally, the emergence of single- or low-dose therapeutics with short shelf lives, such as gene and cell therapy, has made the 28-day culture test impractical and driven the need for an accurate, sensitive and rapid mycoplasma detection assay.

Chapters on mycoplasma testing in both The United States Pharmacopoeia (USP<63>) and the European Pharmacopoeia (EP 2.6.7) allow for the use of properly validated nucleic acid amplification (NAT) methods as an alternative to the 28-day culture based test. Following validation, regulatory filing and review, our customers have received regulatory acceptance to use the Applied Biosystems MycoSEQ Mycoplasma Detection assay for lot release testing applications across multiple therapeutic modalities (Table 1).

Here we describe the MycoSEQ assay, an accurate and sensitive real-time PCR mycoplasma detection assay that provides results in under 5 hours. Additionally, we present 2 case studies from users who have validated and received regulatory approval to use the assay for product lot release.

Table 1. Number of companies using MycoSEQ for product lot release

Product category	Number of companies who have approved products	Number of companies who have products in the process of approval	Regulatory agency for approval
Cell therapy	7	5	EMA/FDA/Local Agency
Tissue therapy	2		EMA/FDA/Local Agency
Mammalian cell-culture derived products (fed-batch)	2	3	FDA
Mammalian cell-culture derived products (perfusion)		1	FDA/EMA
Vaccines	1	2	EMA/Local Agency

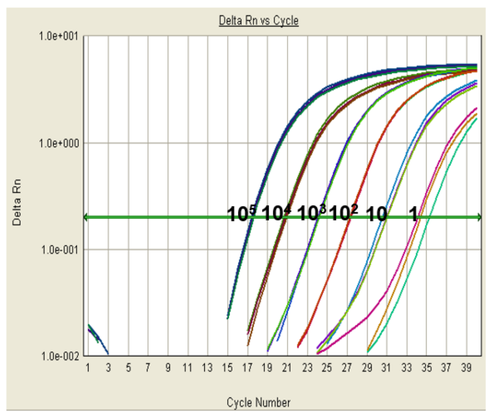
The MycoSEQ Assay

The MycoSEQ Mycoplasma detection system is an integrated sample preparation and qPCR assay, that includes automation systems for sample preparation, the real-time PCR instrument and fully integrated software package that includes a module to help meet 21 CFR Part 11 compliance (Figure 1). The assay provides quantitative detection of more than 90 Mycoplasma species in under 5 hours with consistent and comprehensive detection down to 1 genome copy (Figure 2).

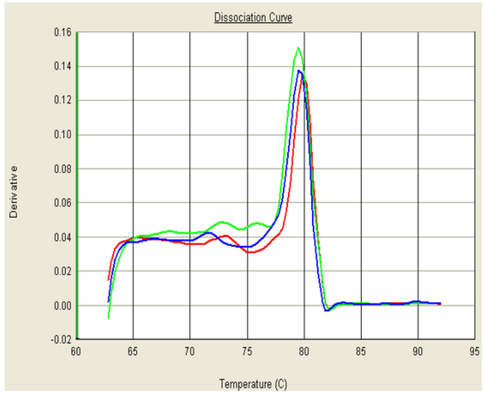
Figure 1. The MycoSEQ Mycoplasma Detection Assay

Figure 2. Assay sensitivity down to 1 genome copy.

Analysis of a 10-fold dilution Series of Purified *M. arginini* DNA:



Melt Analysis at 1 GC/Rxn



Case Study #1 - Mycoplasma lot release testing for a cell therapy product

Background

- Customer produces an autologous cell therapy product with a high number of samples.
- Regulatory expectations are that testing is required at various stages in the production process for therapeutic biological products intended for human use

The scenario

- The product has a short shelf life with the expectation of same day release. Standard testing methods take at least 28 days to complete.
- The main objective was to mitigate risks of Mycoplasma contamination
- A risk-based approach to mitigate validation issues during implementation was adopted and is consistent with the objectives of ICH Guideline Q90

Derived Value

- EMA approval was received for the cell therapy product in 2013 and FDA approval in 2016
- Mycoplasma test results are now available for same day release of autologous cell products.
- qPCR can be used to rapidly identify potential contamination and significantly reducing the risk to other processes in the facility.

Key to Success

The customer discussed mycoplasma testing validation plans with FDA before submitting the BLA, thus facilitating its use in the application. Table 2 summarizes the validation protocol and results. The validation study found the new method's specificity and limit of detection (LOD) to be equivalent to or better than the traditional culture method. The rapid PCR method detected mycoplasma in samples spiked with 10 CFU/mL, which the culture method did not detect.

Table 2. Summary of validation protocol

Parameter	Samples	Acceptance Criteria	Results
Specificity	Unspiked	No mycoplasma detected	6/6 negative
	Mycoplasma DNA	Detection in spiked samples	6/6 positive replicates for six species
Detection limit	Mycoplasma DNA	Detection in spiked samples	6/6 positive replicates for six species
	Mycoplasma <10 CFU/mL	Detection in spiked samples	6/6 positive replicates for six species
Repeatability	Unspiked	All replicates negative	24/24 negative
	Mycoplasma DNA	All replicates positive	24/24 positive replicates for six species
Ruggedness	Analyst to analyst	Δ(Average C_t) < 3	Δ(Average C_t) = 0.1
	Instrument to instrument	Δ(Average C_t) < 2	Δ(Average C_t) = 0.1
	Reagent lot to reagent lot	Δ(Average C_t) < 3	Δ(Average C_t) = 0.0
	Laboratory to laboratory	Δ(Average C_t) < 4	Δ(Average *A. laidlawii* C_t) = 3.1 Δ(Average *M. arginini* C_t) = 0.2 Δ(Average *M. fermentans* C_t) = 1.2 Δ(Average *M. hyorhinis* C_t) = 3.4 Δ(Average *M. orale* C_t) = 1.4 Δ(Average *M. pneumoniae* C_t) = 3.1
Equivalence	*Mycoplasma orale* 7 CFU/mL	NAT positive ≥ PTC positive	NAT 6/6 and PTC 0/6 positive
	MACI SUMMIT clinical trial samples	NAT results = PTC positive	NAT 78/78 and PTC 78/78 negative

Case Study #2 – In-house alternative to outsourced culture-based testing

Background

- Customer produces a human recombinant factor product in a perfusion process
- Current culture based method was outsourced

The scenario

- A large number of samples made the outsourced testing cost prohibitive
- The main objective was to find an in-house alternative to outsourcing
- Determined a commercially available assay provided a faster timeline to validation.

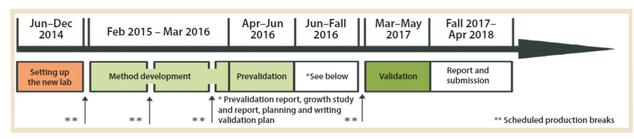
Derived Value

- qPCR can be used to rapidly identify potential contamination
- MycoSEQ implemented as replacement for culture based test and Mycoplasma testing is no longer outsourced
- After validation and filing, regulatory approvals are in-process

Key to Success

Due to the scope of the project, early communication with all relevant departments was critical to success. Fully implementation took about four years from the point of investigating the PCR method as an alternative to the traditional method to regulatory submission (Figure 3). Thermo Fisher Scientific technical support and assistance throughout all phases of the project was also critical.

Figure 3. Project phase timeline



CONCLUSIONS

The MycoSEQ assay was designed specifically for lot-release testing and meets or exceeds the guidance in EP 2.6.7 for an NAT method. The rapid time to results allow for same day release of cell therapy products. After validation, the assay has been accepted by regulatory agencies for lot release testing across multiple therapeutic modalities.