A Rapid Alternative to Culture Based Mycoplasma Detection

Darren J. Bauer and Michael Sherriff, Thermo Fisher Scientific, 35 Wiggins Avenue, Bedford, MA 01730 USA

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 medical 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) and the European Pharmacopoeia (EP) act 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>
<thead>
<tr>
<th>Table 1. Number of companies using MycoSEQ for product lot release</th>
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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.
- PCR 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.
- 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**

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

**Background**
- 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.

**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.