PCR-Based Mycoplasma Testing: A Customer's Journey to Reliable Cell Therapy QC Release

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Advanced Therapy Medicinal Products (ATMPs) offer groundbreaking treatments for severe and life-threatening conditions. However, ensuring their safety before patient administration is a significant challenge, particularly due to stringent regulatory requirements for mycoplasma testing. Traditional growth-based methods can take up to 28 days—a duration that ATMP manufacturers cannot afford given the critical dosing windows.

In this article, we present BioRestorative Therapies' firsthand experience implementing real-time PCR-based mycoplasma testing for QC release, highlighting the impact of faster, more reliable detection methods.

The Challenge: Efficient Testing for High-Density Cells

BioRestorative Therapies is a regenerative medicine company developing BRTX-100, a bone marrow-derived stem cell therapy currently in Phase 2 clinical trials for the treatment of chronic lumbar disc degeneration. As part of the trial, each patient's autologous bone marrow-derived cell therapy required individual mycoplasma screening before QC release.

Outsourcing mycoplasma testing was slow—taking up to two weeks—and sometimes produced inconclusive results, delaying patient treatment. Bringing testing in-house required validating a method that could handle a high-density cell product (26 million mesenchymal stem cells per milliliter). This was necessary because the high nucleic acid load of the cells risked interfering with PCR detection by competing with the target DNA.

To overcome these obstacles, the company needed a robust, efficient mycoplasma testing method that met strict QC standards.

"Our QC department was concerned about bringing live mycoplasma species into the lab for validation, and we needed a method that was both safe and reliable," explained Michael Joyce, Director of Research & Development at BioRestorative Therapies. "The ability to use inactivated validation standards instead of live colony-forming units was a game-changer for us."

A

B

Figure 1: Microsart® AMP Extraction kit (A) and Microsart® ATMP Mycoplasma kit (B).

The Solution: Optimized PCR-Based Workflow

To address these challenges, BioRestorative Therapies partnered with Sartorius to implement the Microsart® AMP Extraction kit and Microsart® ATMP Mycoplasma kit (Figure 1). The kit's workflow was designed to streamline DNA extraction and PCR-based detection, significantly reducing testing time. The process involves extracting DNA from the sample using a column-based method, followed by PCR amplification that includes built-in internal and positive controls to ensure accurate detection (Figure 2).

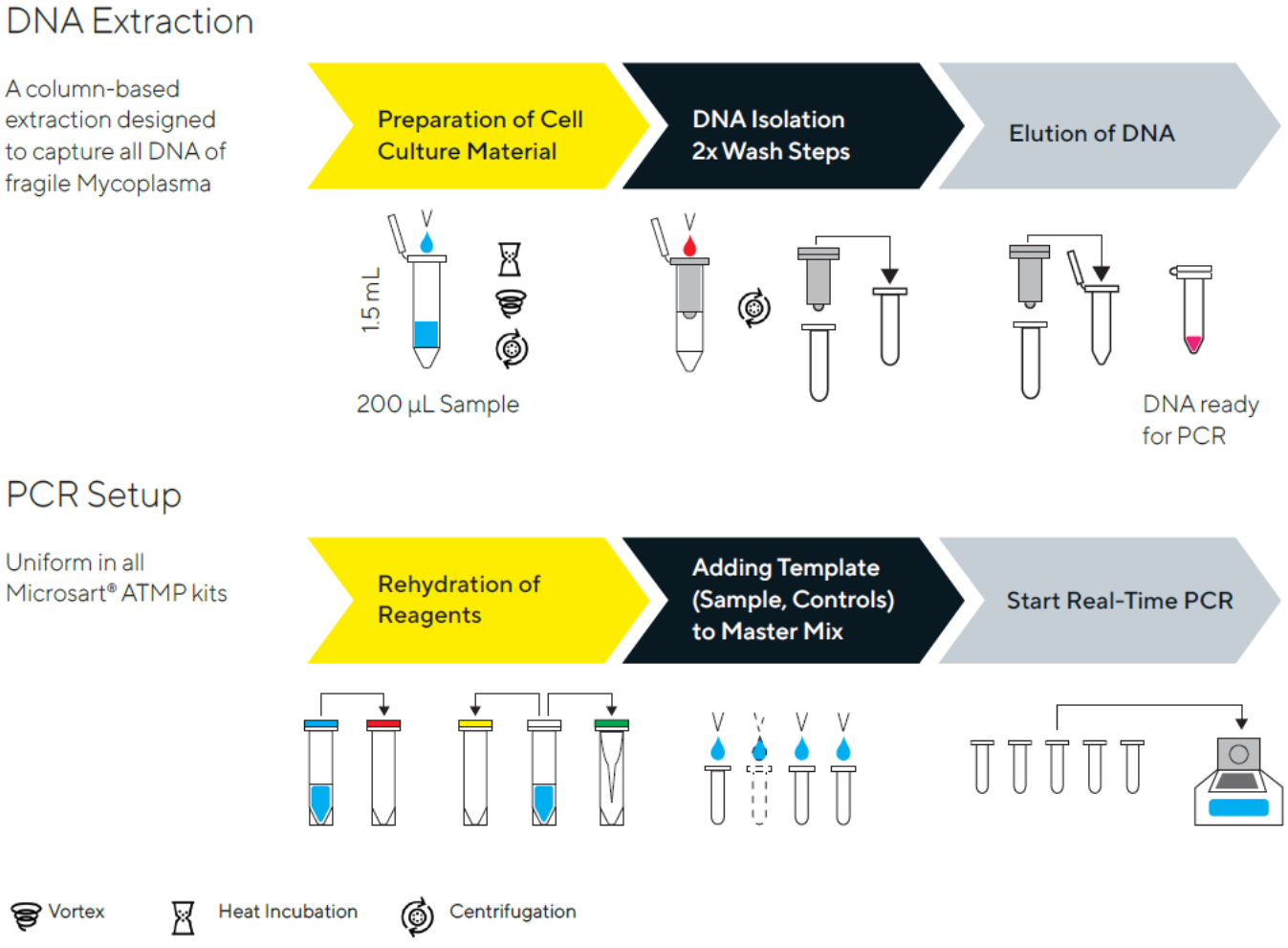

Figure 2: Standard Microsart® AMP Extraction Workflow and Microsart® ATMP Mycoplasma Workflow.

The Sartorius team helped address the challenges around high nucleic acid load. By integrating additional enzymatic treatments and optimizing centrifugation and washing steps to improve DNA purity, they reliably detected the four mycoplasma species, which are relevant for their matrix validation (Figure 3).

"We received incredible support throughout our validation process," Joyce noted. "They helped troubleshoot issues, adjust protocols, and ensure that we were consistently detecting 10 CFU or greater of mycoplasma DNA in our samples."

Impact: Faster Turnaround, Reliable QC Results

Bringing mycoplasma testing in-house with PCR-based detection transformed BioRestorative Therapies' QC workflow, cutting testing time from weeks to just a few hours. This validation paved the way for FDA approval to use the assay in ongoing phase-II clinical trials, highlighting the effectiveness of the protocol adjustments. For ATMP manufacturers facing similar challenges, this validation study demonstrates how real-time PCR can streamline QC release, ensuring timely, accurate, and compliant testing.

"Time is money, but more importantly, time is patients," Joyce emphasized. "PCR-based testing allowed us to confidently release our product faster without compromising safety. That reliability is invaluable."

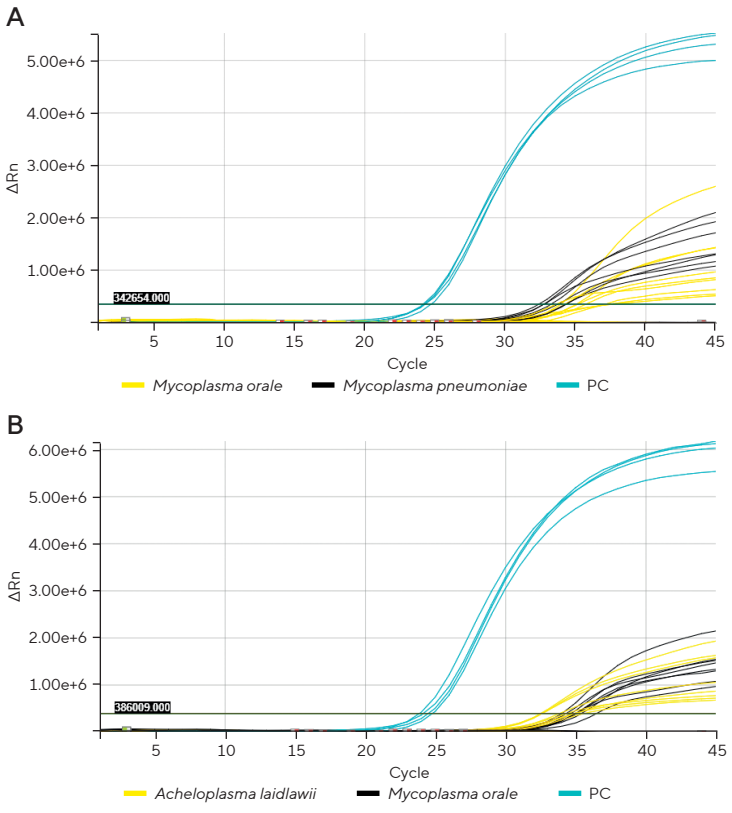

Figure 3: Amplification curves after extracting 10 CFU of *Mycoplasma orale, Mycoplasma pneumoniae* (A), *Mycoplasma fermentans,* and *Acheloplasma laidalawii* (B) by using the Microsart® AMP extraction kit (optimized protocol) with subsequent real-time PCR analysis (Microsart® ATMP Mycoplasma kit). Positive Control (PC).

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