FASTFACTS

Balancing precision and efficiency in cell therapy assays: low volume sampling for mycoplasma detection

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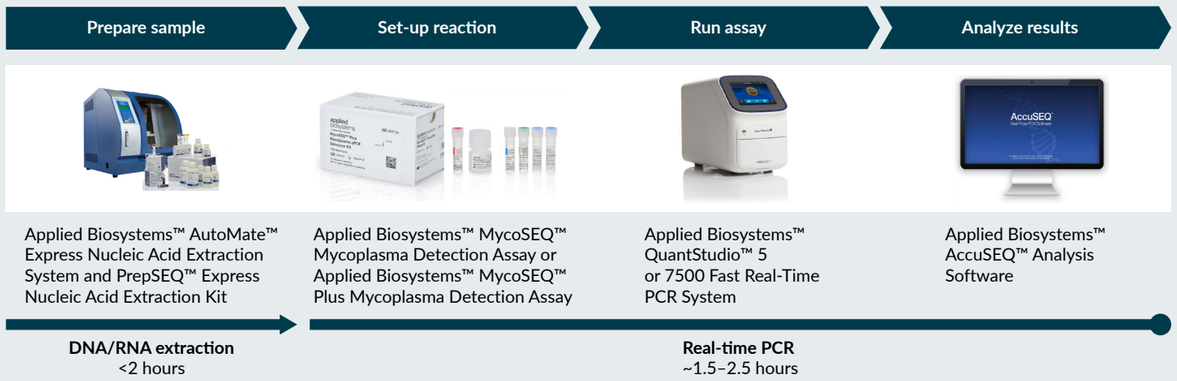
Rapid, sensitive, and specific mycoplasma detection, which can be achieved through PCR-based techniques, is essential for ensuring the quality and safety of cell therapy products. This poster presents the Applied Biosystems™ MycoSEQ™ Plus workflow for accurate and rapid detection of mycoplasma DNA in cell cultures, including cell-based therapies. This case study demonstrates the kit's sensitivity with low sample volumes across different sample matrices.

Figure 1. MycoSEQ assay workflow



With the increasing demand for cell therapies and their potential to revolutionize patient care, ensuring the safety and quality of these novel products is paramount. Mycoplasma, a contaminant of cell cultures, poses significant risks to both the efficacy and safety of cell therapies, and thus it is critical to employ robust and efficient mycoplasma detection techniques.

The MycoSEQTM Plus Kit is a qPCR assay that leverages Applied Biosystems™ TaqManTM chemistries, and utilizes a multiplexed pool of primers and probes for amplifying and detecting multiple target species, including mycoplasma.

The MycoSEQ assay workflow, shown in **Figure 1**, involves four key steps: sample preparation with Applied Biosystems™ AutoMate Express and PrepSEQ Express kits; reaction setup with MycoSEQ Plus assay; PCR reaction using the Applied Biosystems™ QuantStudio 5 or 7500 Fast Real-Time PCR system; and results analysis with GMP-compliant Applied Biosystems™ AccuSEQ™ software.

TESTING METHOD SENSITIVITY

This study tested a method designed for situations where a high level of mycoplasma detection sensitivity is required, but only a small amount of testing material is available.

Firstly, the mycoplasma species were spiked into 1.5 or 2 mL of spent T cell media containing 1×10^6 cells at 10 genome copies (GC)/mL. Samples were then processed using the PrepSEQ AutoMate Express workflow followed by ethanol precipitation, and the MycoSEQ Plus kit. The results were then analyzed using AccuSEQ software.

As illustrated in **Figure 2**, all data points were below the cycle threshold (Ct) cutoff value of 38, which indicates that the sample is positive for mycoplasma detection and demonstrates the method's ability to detect 10 GC/mL using 1.5–2 mL of sample material.

EVALUATING DIFFERENT SAMPLE MATRICES

In another experiment, genomic DNA from various mycoplasma species was spiked into 1.5 or 2 mL of mock sample before automated extraction and precipitation. Mock sample types included fresh media, spent media, and a cryopreservation medium, all containing 1×10^6 T cells. As shown in **Figure 3**, this method detected 10 GC/mL for all tested species and sample types, as indicated by the box plots being below the 38 Ct cutoff value.

Figure 2. Detection of mycoplasma species into spent T cell media. Here, the Ct values of each replicate are plotted, with red dots representing data points from the 1.5 mL sample matrix and blue dots from the 2 mL sample matrix.

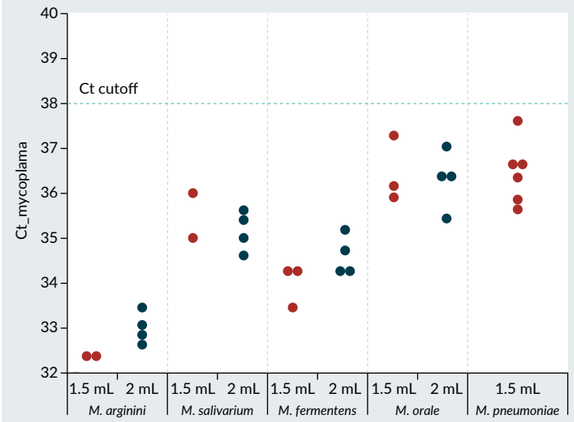
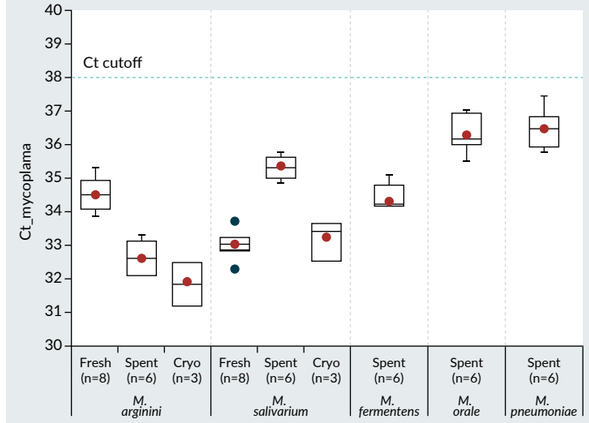


Figure 3. Detection of mycoplasma species into spent T cell media. Here, the Ct values of each replicate are plotted, with red dots representing data points from the 1.5 mL sample matrix and blue dots from the 2 mL sample matrix.



SUMMARY

In summary, rapid and accurate detection of mycoplasma contamination can be achieved with the qPCR-based MycoSEQ Plus assay workflow. Based on the case studies described above, the MycoSEQ system can detect 10 GC/mL using sample volumes as low as 1.5–2 mL, helping to ensure the safety and efficacy of cell therapy products.

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