Pharma analytics

Moving forward: rapid detection of bacteria and fungi in cell therapy products

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Abstract

Purpose: This study demonstrates the use of a rapid qPCR-based method for in-process testing for bacterial and/or fungal contamination in cell therapy-based production.

Methods: Bacteria and fungi species listed in USP chapter <71> were mixed with cell culture matrix prior to DNA extraction. Extracted samples were then tested using the Applied Biosystems™ SteriSEQ™ Rapid Sterility Testing Kit to detect bacteria or fungal DNA. Two of these species were also independently tested by a CRO using the same assay to confirm the reproducibility of the results. A separate experiment evaluated the impact of centrifugation on removal of free-floating DNA from test samples as a potential strategy to reduce detection from lysed microbial cells.

Results: All 6 USP<71> species were successfully detected with an LOD range of 10-99 CFU in a matrix containing 10^6 mammalian cells. An independent study also confirmed the LOD of 2 USP<71> species as <99 CFU. Finally, the centrifugation step resulted in a significant increase in the mean Ct values, which indicated a removal of >99% of the floating DNA. This suggests that the centrifugation step can help reduce the potential for detecting DNA from lysed microbes in the samples.

Introduction

In recent years, cell therapy products have shown tremendous potential in various medical fields. These products are often derived from complex processes involving the isolation of chimeric antigen receptor (CAR) T or Natural Killer (NK) cells, which renders the final product vulnerable to microbial contamination. Therefore, ensuring the sterility of the final cell therapy matrices is crucial to guarantee product quality and safety.

The study uses a qPCR-based rapid testing kit that leverages the Applied Biosystems™ SteriSEQ™ Rapid Sterility Testing Kit to detect both bacteria and fungi from complex cell product matrices in a single well. This study demonstrates the use of this method to detect the 6 species listed in USP<71>. Two of these species were also independently tested by a CRO to demonstrate reproducibility. In addition, the study also demonstrates how the use of a centrifugation step before sample prep helps to minimize the detection of DNA from lysed, non-viable microbes.

Materials and methods

USP<71> species testing

The species tested included *Aspergillus brasiliensis, Bacillus subtilis, Candida albicans, Clostridium sporogenes, Pseudomonas aeruginosa, and Staphylococcus aureus.* Organisms were spiked into either dilution buffer or a cell culture matrix with 10^6 Jurkat cells and cryopreservation media. Spiked samples were pelleted at 15,000 x *g* and extracted using either of two third-party sample prep kits, Kit 1 and Kit 2. Eluted samples were then tested using the SteriSEQ Rapid Sterility Testing Kit and the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System with Applied Biosystems™ AccuSEQ™ software.

Independent LOD study

Bacillus subtilis and *Staphylococcus aureus* were also sent for testing to a CRO (PPD® Laboratory Services) to evaluate the LOD of these organisms using the SteriSEQ assay workflow and to confirm earlier data. Each organism was spiked into a matrix containing 10^6 Jurkat cells and cryopreservation media at high (50-75), medium (25-50) and low (8-17) CFU titers. Spiked samples were pelleted at 15,000 x *g* and extracted using Sample Prep Kit 2. Eluted samples were then tested using the SteriSEQ™ Rapid Sterility Testing Kit and the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System with Applied Biosystems™ AccuSEQ™ software.

Pelleting study

A discriminatory positive control, included in the kit, was diluted in DNA dilution buffer and then added to a cell culture matrix prior to centrifuging at 15,000 x *g.* The cell culture matrix contained 10^6 Jurkat cells and cryopreservation media. Following the centrifugation step, the pellet was then processed further. Eluted samples were then tested using the qPCR kit and run using the QuantStudio™ 5 system with AccuSEQ™ software.

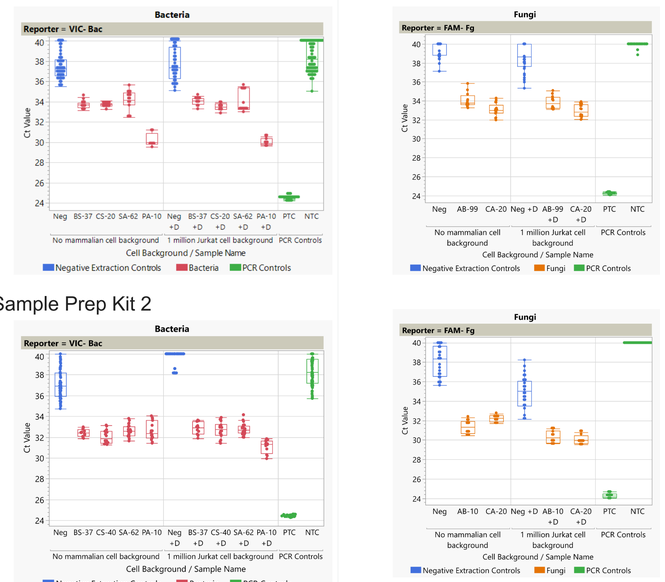
Results

Live bacteria and fungi detection

All 6 USP<71> species were detected at an LOD of 10-99 CFU (**Figure 1**). The workflow has shown compatibility with cell culture matrices containing 10^6 mammalian cells.

Figure 1. Ct values for bacteria and fungi samples spiked at LOD. AB: *Aspergillus brasiliensis,* CA: *Candida albicans*, BS: *Bacillus subtilis,* CS: *Clostridium sporogenes,* PA: *Pseudomonas aeruginosa,* SA: *Staphylococcus aureus.* Numbers following abbreviated species names refer to the titer (LOD in CFU) that was spiked in prior to sample preparation. Samples noted with "+D" included a matrix containing 10^6 Jurkat cells in cryopreservation media.

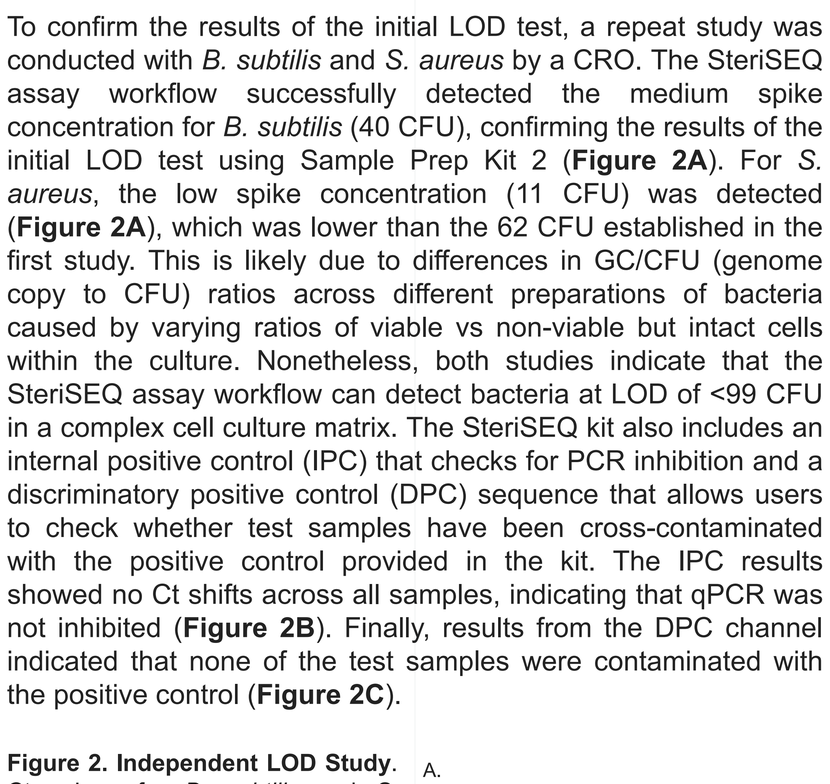
A. Sample Prep Kit 1



Independent LOD study

To confirm the results of the initial LOD test, a repeat study was conducted with *B. subtilis* and *S. aureus* by a CRO. The SteriSEQ assay workflow successfully detected the medium spike concentration for *B. subtilis* (40 CFU), confirming the results of the initial LOD test using Sample Prep Kit 2 (**Figure 2A**). For *S. aureus*, the low spike concentration (11 CFU) was detected (**Figure 2A**), which was lower than the 62 CFU established in the first study. This is likely due to differences in GC/CFU (genome copy to CFU) ratios across different preparations of bacteria caused by varying ratios of viable vs non-viable but intact cells within the culture. Nonetheless, both studies indicate that the SteriSEQ assay workflow can detect bacteria at LOD of <99 CFU in a complex cell culture matrix. The SteriSEQ kit also includes an internal positive control (IPC) that checks for PCR inhibition and a discriminatory positive control (DPC) sequence that allows users to check whether test samples have been cross-contaminated with the positive control provided in the kit. The IPC results showed no Ct shifts across all samples, indicating that qPCR was not inhibited (**Figure 2B**). Finally, results from the DPC channel indicated that none of the test samples were contaminated with the positive control (**Figure 2C**).

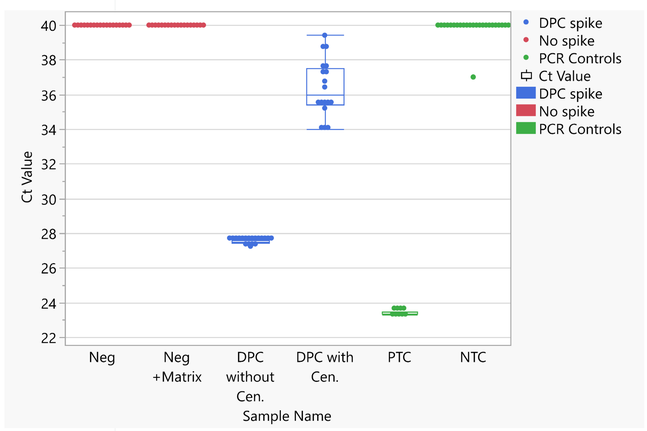
Figure 2. Independent LOD Study. Ct values for *B. subtilis* and *S. aureus* samples spiked in a matrix containing 10^6 Jurkat cells in cryopreservation medium and tested using the SteriSEQ assay. Numbers after bacteria species indicate spike titers in CFU. Graphs are shown for A) bacteria B) IPC and C) DPC targets. PEC: positive extraction control, S-PEC: sample positive extraction control, NEC: negative extraction control, S-NEC: sample negative extraction control.



Pelleting study

Centrifugation of test samples prior to sample prep can reduce some of the detection from free-floating genomic DNA from lysed microbial cells. When compared against samples that did not undergo centrifugation, samples which were centrifuged prior to extraction displayed an 8 Ct increase in mean Ct values. This indicates a removal of >99% of floating DNA. This data suggests that including a centrifugation step at the start of sample preparation helps enable the removal of a large proportion of floating DNA, reducing the potential for detection of DNA from lysed microbes in test samples.

Figure 3. Effect of centrifugation on detection of floating DNA. DPC without Cen.: positive control-spiked samples without centrifugation; DPC with Cen.: positive control-spiked samples that were centrifuged at 15,000 x *g* for 5 minutes prior to sample preparation. Negative samples refer to samples without positive control spike.



Conclusions

The qPCR assay used in this study detected both bacteria and fungi in a complex cell culture matrix within a single well at LOD ranging from 10-99 CFU. The data collectively demonstrates that the 6 species listed in USP<71> can be detected using two different sample preparation kits. A separate study done on 2 bacteria species also confirmed these results.

Furthermore, the centrifugation step was observed to remove over 99% of floating DNA. This finding suggests that incorporating a centrifugation step at the beginning of sample preparation can minimize the likelihood of detecting floating DNA from lysed microbes in test samples.

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