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Pharma analytics

Advancing cell therapy manufacturing: rapid sterility testing to help ensure the safety and quality of cell therapy products

Introduction

In recent years, cell therapy products have shown tremendous potential in regenerative medicine and the treatment of various diseases. These products are often derived through complex processes involving the isolation of human cells, particularly the expansion and manipulation of chimeric antigen receptor (CAR) T cells, which renders them vulnerable to microbial contamination. Therefore, ensuring the sterility of cell therapy products is crucial to guaranteeing product quality and safety.

The traditional sterility testing method, as outlined by USP chapter <71>, is a compendial method that relies on microbial growth–based detection. This method requires a 14-day culture, which can create difficulties in promptly releasing cell therapy products that may have a limited shelf life or require immediate administration to patients.

USP chapter <1071> outlines alternative, rapid methods such as quantitative polymerase chain reaction (qPCR) for sterility testing. By targeting conserved regions of microbial genomes, qPCR can identify a wide range of microorganisms with high specificity, enabling rapid detection of microorganisms within hours rather than days, allowing for early detection of microbial contamination in cell therapy products. USP chapter <1071> also provides comprehensive guidance on using qPCR-based methods for sterility testing and microbiological control, emphasizing the importance of validation, specificity, sensitivity, and regulatory compliance.

Studies have previously shown that PCR assays can be utilized to detect microbial contamination in cell therapy products. In particular, Tokuno et al. [1] demonstrated the sensitivity of a bacterial 16S ribosomal DNA (rDNA) PCR assay to be 10–100 CFU/mL. The authors concluded that this PCR assay is a useful alternative for rapid sterility testing for stem cell products used in regenerative medicine, as compared to a rapid growth-based method. Additionally, Kleinschmidt et al. [2] developed a qualitative real-time PCR method for microbiological quality control testing in mammalian cell culture production. The real-time PCR assays were tested on the genomic DNA of *Escherichia coli* and *Candida albicans*, and the sensitivity was established as 10^2–10^3 CFU/mL in a sample containing approximately 10^6 mammalian cells/mL.

The Applied Biosystems™ SteriSEQ™ Rapid Sterility Testing Kit is a probe-based qPCR assay designed using Applied Biosystems™ TaqMan™ chemistry. It is designed to swiftly detect bacteria and fungi in complex cell therapy product matrices and deliver actionable results within 5 hours. The kit can be used for in-process testing, along with the traditional growth-based method, to help gain confidence in a cell therapy bioprocessing workflow. With the SteriSEQ kit, it is possible to address the challenges of timely release of cell therapy products that may have a limited shelf life or require immediate administration to patients. Here we illustrate how the SteriSEQ kit can be used to detect the 6 species listed in USP chapter <71>, as well as additional relevant bacterial and fungal species.

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Detection of live bacteria and fungi

Methods

Limit-of-detection (LOD) experiments for the 6 species listed in USP chapter <71> were conducted using Thermo Scientific™ Quanti-Cult™ Plus QC Organisms. These species are *Aspergillus brasiliensis* (Cat. No. R4711100), *Bacillus subtilis* (Cat. No. R4711221), *Candida albicans* (Cat. No. R4711503), *Clostridium sporogenes* (Cat. No. R4711700), *Pseudomonas aeruginosa* (Cat. No. R4715210), and *Staphylococcus aureus* (Cat. No. R4717016). Microorganisms were reconstituted according to instructions in the Quanti-Cult Plus QC Organisms user guide before being spiked into either SteriSEQ DNA Dilution Buffer (included with the SteriSEQ kit) or cell culture matrix at titers ranging from 10 CFU to 99 CFU. The cell culture matrix tested contained 10^6 Jurkat cells and cryopreservation medium (50% CryoStor™ CS10 Cryopreservation Medium (Biolife Solutions), 40% Plasma-Lyte™ A solution (Baxter), and 10% (v/v) 25% human serum albumin (Octapharma)). Spiked samples were pelleted at 15,000 x *g* for 5 min, and supernatants were removed prior to extraction using third-party kits. The sample elution volume was 80 µL. Eluted samples were then tested using the SteriSEQ kit and run using the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System with Applied Biosystems™ AccuSEQ™ software (Figure 1). Samples of the Quanti-Cult Plus QC Organisms from the lots used in LOD experiments were also sent to an external vendor to confirm titer and viability. LOD was defined as the concentration that produced a mean difference in C_t of more than 3 when compared against negative extraction controls.

Figure 1. Default qPCR cycling conditions on AccuSEQ software version 3.2.1.

Results

All 6 species were detected with an LOD range of 10 to 99 CFU/mL (Table 1, Figure 2A–D).

The SteriSEQ kit is compatible with cell culture matrices containing 10^6 mammalian cells.

Table 1. LOD (in CFU/mL) of 6 USP chapter <71> species.*

Species	Classification	MolYsis™ Complete5 Kit (Molzym)	DNeasy™ PowerSoil™ Pro Kit (Qiagen)
A. brasiliensis	Fungus	99	10
C. albicans	Fungus	20	20
B. subtilis	Bacterium	37	37
C. sporogenes	Bacterium	20	40
P. aeruginosa	Bacterium	10**	10
S. aureus	Bacterium	62	62

* The LOD values indicated reflect spiked samples without matrix, as well as with cell culture matrix containing 10^6 Jurkat cells.

** Used a modified protocol for *Pseudomonas aeruginosa* LOD for the MolYsis Complete5 Kit.

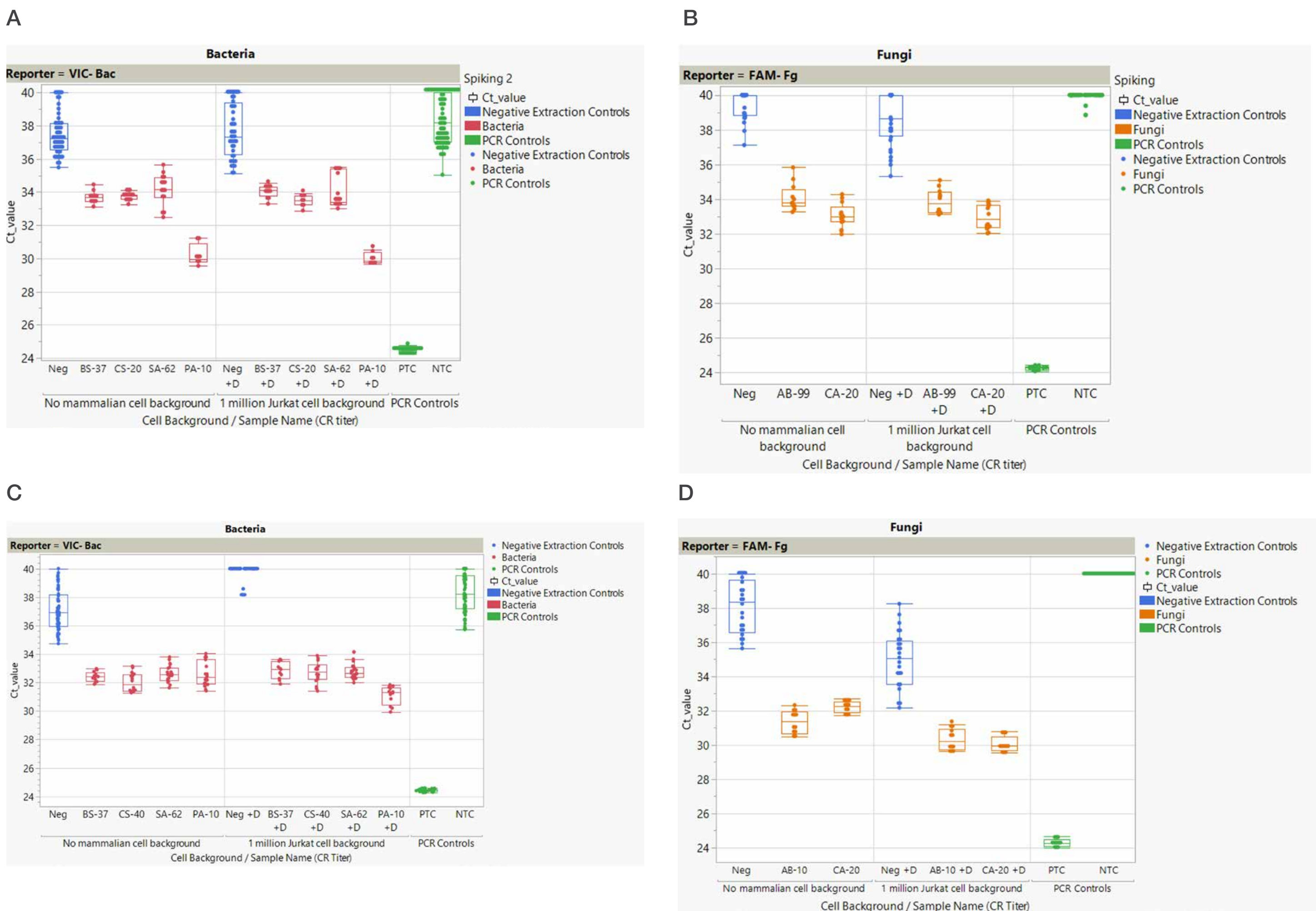

Figure 2. C_t values for bacterial and fungal samples spiked at LOD prior to extraction with the MolYsis Complete5 Kit (A, B) and the DNeasy PowerSoil Pro Kit (C, D). AB: *A. brasiliensis*; CA: *C. albicans*; BS: *B. subtilis*; CS: *C. sporogenes*; PA: *P. aeruginosa*; SA: *S. aureus*. Numbers after species name abbreviations refer to the titer (in CFU) that was spiked in prior to sample prep. Samples with "+D" included a matrix containing 10^6 Jurkat cells in cryopreservation medium.

LOD of additional bacterial and fungal species

Methods

Genomic DNA was extracted from live organisms of 5 bacterial species: *Afipia felis* (ATCC 53690), *Lactobacillus acidophilus* (ATCC 4356), *Lactobacillus delbrueckii* (ATCC 9649), *Niallia circulans* (ATCC 4513), and *Ralstonia pickettii* (ATCC 27511). Mass was quantified using the Invitrogen™ Qubit™ dsDNA High Sensitivity Assay Kit. Genomic DNA for the remaining 3 species was purchased from ATCC: *Burkholderia cepacia* (ATCC 25416DQ), *Komagataella pastoris* (ATCC 28485D-5), and *Cryptococcus neoformans* (ATCC 208821D-2). Apart from *B. cepacia*, for which quantified DNA was available from ATCC, copy numbers for all other organisms were determined using theoretical conversion from mass to genome copies for dsDNA based on the respective DNA sequences. Genomic DNA was tested at concentrations ranging from 5 to 50 genome copies per qPCR reaction using the QuantStudio 5 Real-Time PCR System with AccuSEQ software. LOD was defined as the concentration for which 95% of data points produced C_t values under 35.5 for bacteria and 36 for fungi.

Results

The LOD of all 8 species was between 5 to 50 genome copies when tested using the SteriSEQ kit (Table 2, Figure 3A, B).

Table 2. LOD of bacterial and fungal species.

Species	Classification	LOD (genome copies/reaction)
L. acidophilus	Bacterium (gram-positive)	10
L. delbrueckii	Bacterium (gram-positive)	5
N. circulans	Bacterium (gram-positive)	5
A. felis	Bacterium (gram-negative)	50
B. cepacia	Bacterium (gram-negative)	10
R. pickettii	Bacterium (gram-negative)	10
C. neoformans	Fungus (yeast)	5
K. pastoris	Fungus (yeast)	5

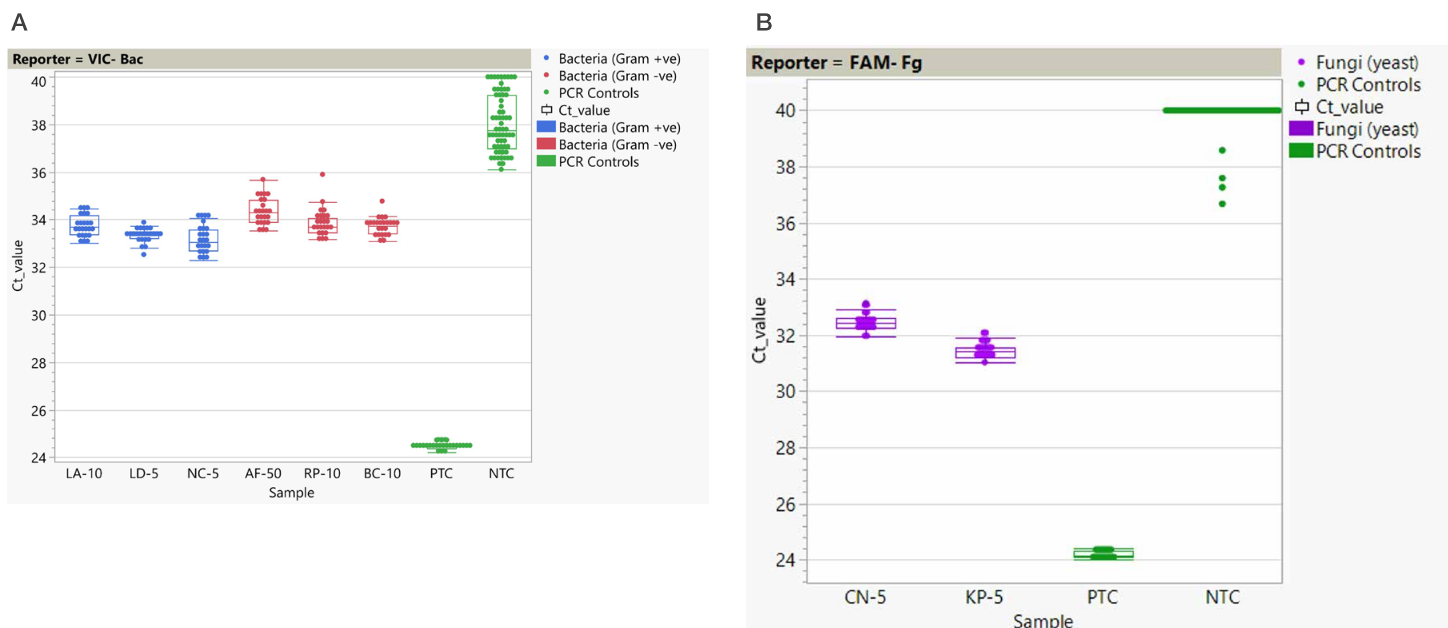

Figure 3. C_t values for bacterial (A) and fungal (B) genomic DNA at LOD. LA: *L. acidophilus*; LD: *L. delbrueckii*; NC: *N. circulans*; AF: *A. felis*; BC: *B. cepacia*; RP: *R. pickettii*; CN: *C. neoformans*; KP: *K. pastoris*. Numbers after species name abbreviations refer to the LOD in genome copies/reaction.

Pelleting study

Background

qPCR-based testing often enables a turnaround time of less than 1 day, which makes it well suited as a rapid sterility testing method. However, it is unable to differentiate between live and dead microbial cells, since DNA from both will be amplified during qPCR. One way to circumvent this issue is to introduce a centrifugation step at the start of sample preparation, to pellet live, intact microbial cells, leaving genomic DNA released from dead cells in the supernatant, which can be discarded. This study demonstrates the effect of centrifugation on removal of released DNA in the sample.

Methods

The SteriSEQ discriminatory positive control (DPC) was diluted in DNA dilution buffer, and 6,514 copies were added to a cell culture matrix before centrifugation at 15,000 x *g* for 5 minutes. A separate control set was also performed, with addition of DPC to the matrix without centrifugation. The cell culture matrix contained 10^6 Jurkat cells and cryopreservation medium (50% CryoStor CS10 Cryopreservation Medium, 40% Plasma-Lyte A solution, and 10% (v/v) 25% human serum albumin). After centrifugation all of the supernatant was removed, leaving behind a cell pellet that was processed using the DNeasy PowerSoil Pro Kit (Qiagen) according to the manufacturer's instructions. The sample elution volume was 80 µL. Eluted samples were then tested using the SteriSEQ kit and run using the QuantStudio 5 system with AccuSEQ software.

Results

Centrifugation resulted in a significant (8.7) increase in mean C_t value, indicating >99% removal of released DNA (Figure 4). This suggests that a centrifugation step at the start of sample preparation enables removal of a large proportion of released DNA, reducing the potential for detection of DNA from lysed microbes in test samples.

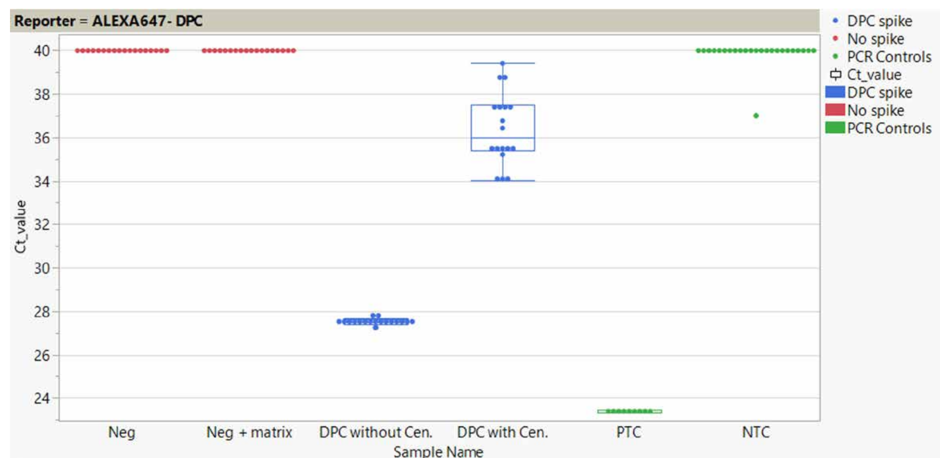

Figure 4. Effect of centrifugation on detection of released DNA. DPC without Cen.: positive control–spiked samples without centrifugation; DPC with Cen.: positive control–spiked samples that were centrifuged at 15,000 x *g* for 5 min. Negative samples refer to samples without a positive control spike.

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Conclusions

The SteriSEQ Rapid Sterility Testing Kit provides a qPCR assay designed to swiftly detect bacteria and fungi in complex cell therapy product matrices within a single well. The data presented here collectively demonstrate that 6 species listed in USP chapter <71> were detected within an LOD range of 10–99 CFU/mL using two different sample prep kits. Additionally, centrifugation was found to significantly increase the mean C_t by 8.7, indicating the removal of over 99% of released DNA. This finding suggests that incorporating a centrifugation step at the beginning of sample preparation can minimize the likelihood of detecting DNA released from lysed microbes in test samples.

References

1. Tokuno O, Hayakawa A, Yanai T et al. (2015) Sterility testing of stem cell products by broad-range bacterial 16S ribosomal DNA polymerase chain reaction. *Lab Med* 46(1):34–41. **doi:10.1309/LMKT4P9FFI2BBSIU**
2. Kleinschmidt K, Wilkens E, Glaeser SP et al. (2017) Development of a qualitative real-time PCR for microbiological quality control testing in mammalian cell culture production. *J Appl Microbiol* 122(4):997–1008. **doi:10.1111/jam.13387**

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