MYCOPLASMA RELEASE TESTING OF CELL AND GENE THERAPY PRODUCT SAMPLES CONTAINING CELLS IN 1-HOUR USING A CLOSED-SYSTEM NEXT GEN PCR

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INTRODUCTION

Testing for mycoplasma contamination is a required release test for Cell & Gene Therapy (CGT) products as specified in the major pharmacopeias[(1-3)]. Current compendial methods require ≥ 28 days to generate results and are not suitable for short shelf-life products. Alternative nucleic acid test (NAT) methods are available and can reduce the time to result to hours instead of days.

The BIOFIRE® Mycoplasma test is a closed system sample-to-answer presence/absence NAT that is designed to detect over 130 mycoplasma species in less than an hour. It requires minimal hands-on time and contains everything needed to run a molecular test. A closed system NAT lowers the risk of contamination and answers the needs of CGT manufacturers for a fast & easy Mycoplasma release test.

Currently, a double centrifugation sample preparation protocol (Figure 3A) has been validated as a release test following current regulatory guidelines with a Limit Of Detection (LOD) of ≤ 10 CFU/mL[(4)]. Although, this protocol is compatible with a wide range of Bioproduction and CGT matrices[(5)], it excludes mammalian cells and therefore does not align with the new draft European pharmacopeia (EP) guideline[(6)].

PURPOSE

Present a new single-centrifugation protocol that allows the inclusion of mammalian cells thus aligning with the pending EP guideline, whilst providing the required level of detection appropriate as a Mycoplasma release test.

Present the compatibility studies of two CGT sample type using this new protocol.

MATERIALS AND METHOD

BIOFIRE® FILMARRAY® 2.0 Industry System

The BIOFIRE® FILMARRAY® 2.0 Industry system utilizes the FILMARRAY 2.0 instrument and next generation PCR testing in a closed pouch to detect the presence of over 130 different Mycoplasma species (Figures 1 and 2). The disposable BIOFIRE Mycoplasma pouch contains all the reagents for automated cell lysis, nucleic acid purification, reverse transcription, first and second stage nested PCR and analyte detection (Figure 2). Several controls are integrated into the pouch to ensure the quality of the results including a total process control, reverse transcription control, and PCR I and II controls. The instrument & software process the pouch with results in less than an hour.

The FILMARRAY 2.0 Industry software (21 CFR Part 11 compliance ready) performs all of the complex meta-analysis and provides presence/absence results as either "Mycoplasma Detected" or "Mycoplasma Not Detected".

Figure 1. FILMARRAY 2.0 Industry instrument performs the extraction, amplification and detection (25.4 x 39.3 x 16.5 cm/10 x 15.5 x 6.5 in WxDxH). The system comes standard with 2 instruments; up to 8 instruments can be connected to a single PC.

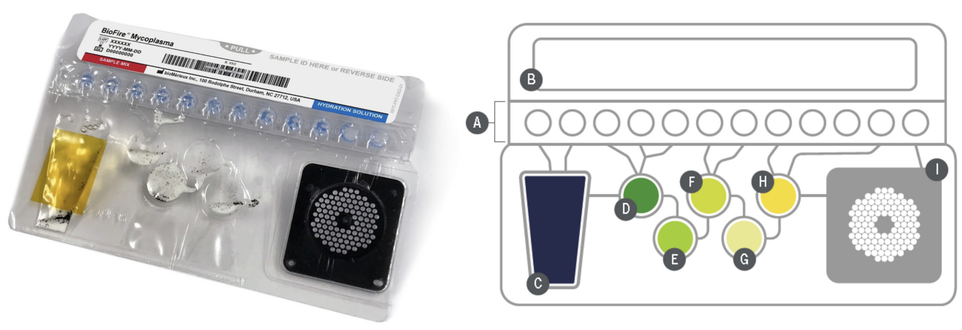

Figure 2. A. BIOFIRE Mycoplasma pouch. B. Pouch diagram A. Fitment with freeze-dried reagents B. Plungers-deliver reagents to blisters C. Sample lysis and bead collection D. Wash E. Magnetic bead collection blister F. Elution G. Multiplex Outer PCR blister H. Dilution blister I. Inner Nested PCR array.

Sample Protocols

In order to comply with upcoming EP requirements for inclusion of mammalian cells, we tested a 10 mL single centrifugation protocol that bypasses the initial low-speed centrifugation (Figure 3B).

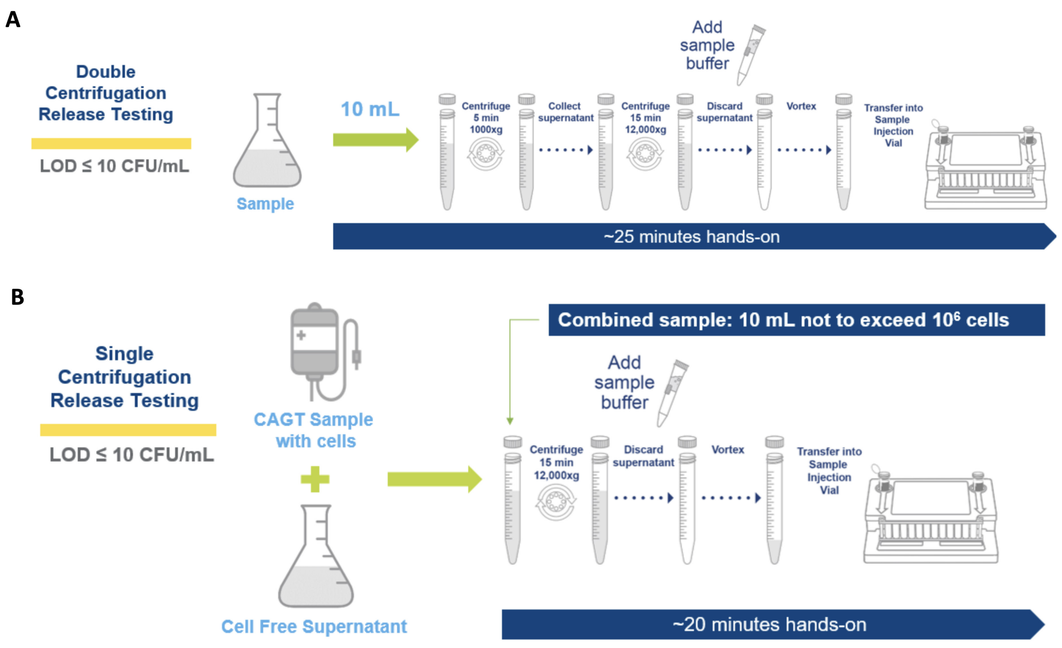

Figure 3: BIOFIRE FILMARRAY 2.0 Industry system release protocols for Mycoplasma testing. A. Double centrifugation and B. Single centrifugation.

Sample preparation & Mycoplasma inoculation

Jurkat cells (Clone E6-1) were grown in RPMI based media, harvested and stored in 10% Bovine Serum Albumin & 5% Cryostor® at -80°C.

Prior to inoculation, 10 mL of samples were normalized to 10^5 cells/mL using Phosphate Buffer Saline (PBS) or supernatant. Previous studies demonstrated that the sample to be tested was not to exceed a cell concentration greater than 10^5 cells/mL (data not shown).

Mycoplasma inoculation was carried out with viable titrated reference stocks from the American Type Culture Collection (ATCC) and displaying a ratio of GC/CFU (Genome Copy / colony forming unit) below 10 (except *M. synoviae* GC/CFU = 60). Based on the supplier Certificate of Analysis (CoA), stocks were aliquoted and diluted with PBS; a precise volume was pipetted to inoculate the sample at the target of ≤ 10 CFU/mL.

Inoculated samples were processed following the 10 mL single centrifugation protocol (Figure 3B), loaded onto a fully prepared and hydrated pouch and run on the FILMARRAY 2.0 Industry instrument.

Study outline

The new 10 mL single centrifugation performance was evaluated on Jurkat cells targeting a LOD ≤ 10 CFU/mL. Confirmation of LOD required a detection rate of at least 95% from a minimum of 24 tests (≥23/24 Detected) using individually inoculated samples from at least three independent stock dilution events[(2)]. The study outline for the evaluation was as follows:

- 24 replicates were tested for each of the 10 Mycoplasma strains,
- 2 operators carried out 4 replicates each, on 3 different days,
- 8 different FILMARRAY instruments were used.

In addition, internal studies were performed on CGT samples aimed at assessing their compatibility with the BIOFIRE Mycoplasma solution using the new 10 mL single centrifugation protocol. Multiple Mycoplasma strains were included (see Table 2). The compatibility of a sample was determined by non-interference with BIOFIRE internal controls (uninoculated product) & Mycoplasma detection (inoculated product). These studies were performed in triplicate with a minimum of 2 pouch lots.

RESULTS AND DISCUSSION

10 mL single centrifugation performance

Table 1 : Results of Mycoplasma detection in 10^5 Jurkat cells/mL using the 10 mL single centrifugation protocol at a target of 10 CFU/mL

Mycoplasma strain	ATCC-titrated stock reference	Number of positive replicates	Detection rate
Acholeplasma laidlawii	23206-TTR™	24/24	100%
Mycoplasma arginini	23838-TTR™	23/24	95%
Mycoplasma fermentans	19989-TTR™	24/24	100%
Mycoplasma gallisepticum	19610-TTR™	24/24	100%
Mycoplasma hominis	27545-TTR™	23/24	95%
Mycoplasma hyorhinis	17981-TTR™	23/24	95%
Mycoplasma pneumoniae	15531-TTR™	24/24	100%
Mycoplasma orale	23714-TTR™	24/24	100%
Mycoplasma salivarium	23064-TTR™	24/24	100%
Mycoplasma synoviae	25204-TTR™	24/24	100%

Appropriate sensitivity (10 CFU/mL) was reached for all Mycoplasma strains tested with a detection rate ≥ 95% in the presence of 10^5 Jurkat cells/mL (Table 1) as per Pharmacopeia requirements[(2)].

These results show that the 10 mL single centrifugation protocol is an acceptable approach that allows the inclusion of mammalian cells and thus complies with the upcoming EP regulatory Requirements[(6)].

Internal studies on CGT samples

Three internal studies were performed on CAR-T cells and on Tumor cells using the single centrifugation protocol. Samples were normalization to 10^5 cells/mL using supernatant prior to testing.

All samples demonstrated compatibility with no internal control failure occurring. No false positive results were observed, and Mycoplasma detection was achieved when inoculated at 10 CFU/mL, for all samples (Table 2).

Table 2: Compatibility studies in CGT product samples using the 10 mL single centrifugation protocol

Sample type	Sample compatibility without spiking	Inoculation study	
		Organisms	Mycoplasma detection in product
CAR-T cells (1)	Pass 3/3	*A. laidlawii*	Mycoplasma detected 3/3
	Pass 3/3	*M. fermentans*	Mycoplasma detected 3/3
	Pass 3/3	*M. gallisepticum*	Mycoplasma detected 3/3
	Pass 3/3	*M. synoviae*	Mycoplasma detected 3/3
CAR-T cells (2)	Pass 3/3	*M. pneumoniae*	Mycoplasma detected 3/3
Tumor cells	Pass 3/3	*M. pneumoniae*	Mycoplasma detected 3/3

CONCLUSION

The new 10 mL single centrifugation protocol including 10^5 Jurkat cells/mL was successfully evaluated on the BIOFIRE FILMARRAY 2.0 Industry system and will comply with upcoming regulatory requirements[(6)]. Sensitivity of ≤ 10 CFU/mL in presence of mammalian cells was demonstrated across 10 Mycoplasma strains including Mycoplasma referenced in the major pharmacopeia[(1-3)].

Three CGT product samples normalized to 10^5 cells/mL were tested using the new single centrifugation protocol and were confirmed compatible. There was no product interference and Mycoplasma was detected at LOD for all three samples.

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