BIOMÉRIEUX

EASY 1 HOUR MYCOPLASMA DETECTION IN CELL THERAPY SAMPLES

INTRODUCTION

While pharmacopoeias specify a test for mycoplasma contamination must be conducted prior to releasing product manufactured in the presence of eukaryotic cells (1), current compendial methods require ≥ 28 days to generate test results. Waiting 28 days or longer to release product isn't a feasible option for most autologous cell and gene therapy processes where patients are waiting to be infused and time is critical to effective treatment.

Conventional nucleic acid testing (NAT) methods offer a faster alternative but they often require specialized laboratories, elevated skill levels and significant training to interpret results. Because of these complexities, many cell and gene therapy companies outsource their mycoplasma testing to a third party laboratory. While often this solution provides results faster than compendial methods, the cost of outsourced labor can be high.

The BIOFIRE® Mycoplasma solution utilizes the FILMARRAY® 2.0 Industry instrument and a closed pouch PCR test to detect the presence of >130 species of mycoplasma. The system provides sample to answer in ~1 hour with little technical training required. This provides options for bringing mycoplasma testing in-house, thus saving time and outsourcing costs.

Testing Method	Regulation	Time to Result	Hands On Time	Expertise Needed	Contamination Risk	Reagent Storage	Testing Location	Sensitivity	Sample Size
BIOFIRE®	EP 9.0 <2.6.7> USP 39 <63> USP 39 <1223> JP 17 <G3>	<1 Hour	Minutes	Novice	Low	RT	Anywhere	≤10 CFU/mL*	0.2 mL-10mL
Other PCR Methods		5-7 hours	Hours	Expert	High	-20°C	PCR Lab		
Traditional Methods		6-28 days	Days	Expert	High	4°C	Specialized Lab		

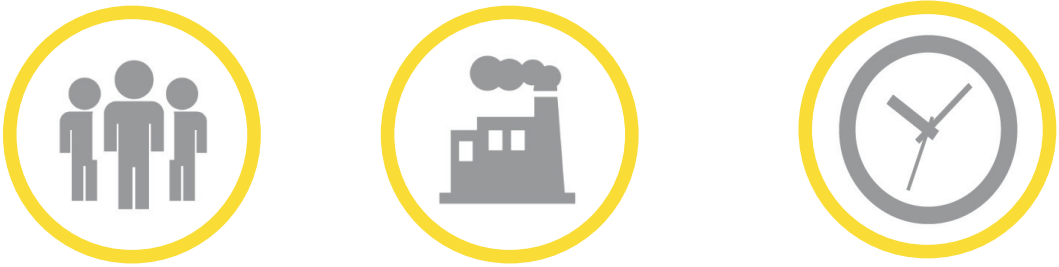
*10 mL protocol

The data summarized in this White Paper is from two cell therapy manufacturers that evaluated the BIOFIRE® FILMARRAY® 2.0 Industry System and presented their results at the 2022 Association for the Advancement of Blood and Biotherapies annual conference (2). Samples were evaluated with up to 3 compendial mycoplasma strains in the presence of high-density chimeric antigen receptor T (CAR T) cells or autologous cultured chondrocytes. Studies were designed to assess product interference (false-positive rates) and detection (false-negative rates). In addition, data from numerous internal studies that tested product compatibility & low level inoculation on commonly used media and raw materials is presented.

THE VALUE OF RAPID, SIMPLIFIED MYCOPLASMA TESTING

Because the BIOFIRE system is so easy to use, manufacturers who previously lacked the resources to perform mycoplasma testing in-house can now easily perform mycoplasma screening at-line with little specialized equipment or training saving on costly outsourced laboratory testing.

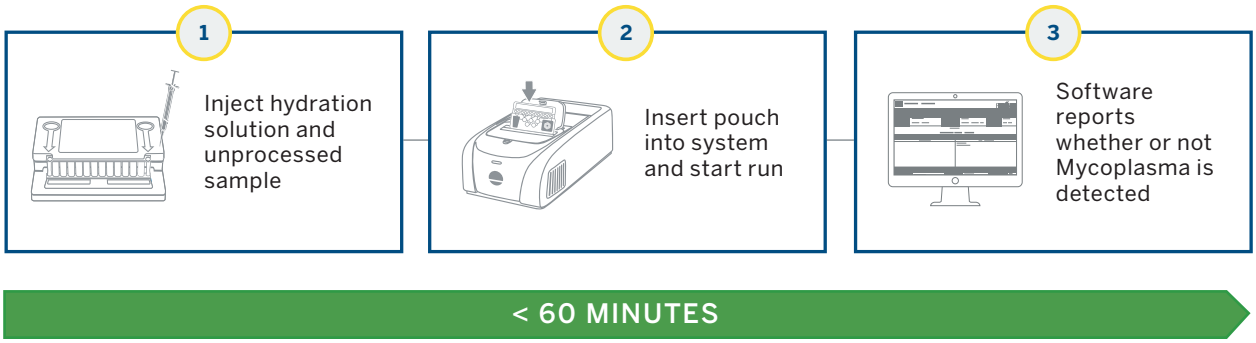
Cell and gene therapy manufacturers today waiting hours or weeks for results using complex testing methods can realize significant value with a more rapid, simplified approach to mycoplasma testing using BIOFIRE FILMARRAY 2.0 Industry.



ANYONE **ANYWHERE** **ANYTIME**

The BIOFIRE mycoplasma test can be performed by anyone, anywhere at anytime allowing manufacturers to take critical quality testing out of the lab and closer to manufacturing to realize the following benefits:

- 1-hour time to result for faster patient treatment
- Simplified training requirements
- Lower expertise required
- Objective results
- Less human error risk/improved Data Integrity
- Does not require molecular lab



BIOFIRE FILMARRAY 2.0 INDUSTRY SYSTEM

The BIOFIRE system utilizes the FILMARRAY 2.0 Industry instrument and next generation PCR testing in a closed pouch to detect the presence of mycoplasma (Figures 1 and 2). The disposable BIOFIRE Mycoplasma pouch contains all the necessary reagents for automated PCR and analyte detection in order to isolate, amplify and detect over 130 different mycoplasma species (Figure 2).

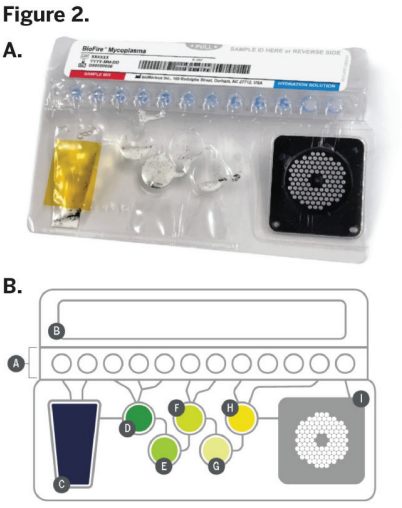
Several controls are integrated into the pouch to ensure the quality of the results including a total process control, reverse transcription control, and PCR I and II controls. The instrument & software process the pouch with results in less than an hour.

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

Figure 1.

Firgure 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.



Firgure 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

Two standard protocols have been designed to detect mycoplasma contamination (3). These include a 0.2 mL direct assay that can be used for in-process control testing with a validated LOD of ~≤ 30 CFU/ mL, and a 10 mL release test protocol that concentrates the sample using centrifugation and has a validated LOD of ≤ 10 CFU/mL (Figure 3).

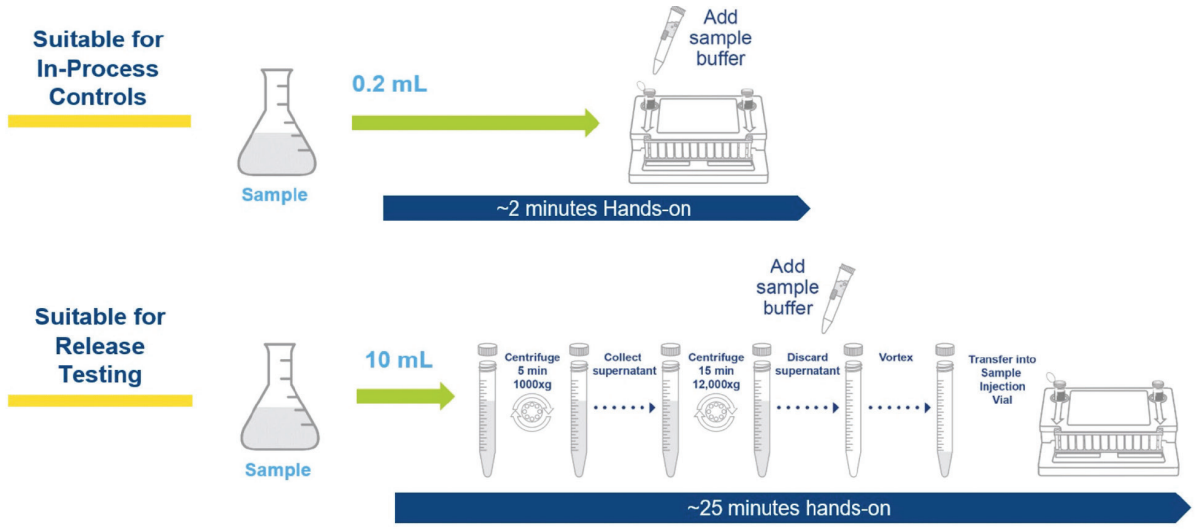


Figure 3. BIOFIRE FILMARRAY 2.0 Industry system sample protocols.

These protocols were customized by both evaluators during the study (figure 4). Evaluator A deviated from the manufacturer recommendations with customized sample volumes and evaluator B tested the 10 mL protocol bypassing the initial low-speed centrifugation due to the low concentration of cells in their product matrix. Following sample pre-processing, samples were then loaded onto a fully prepared and hydrated pouch and run on the FILMARRAY 2.0 Industry instrument.

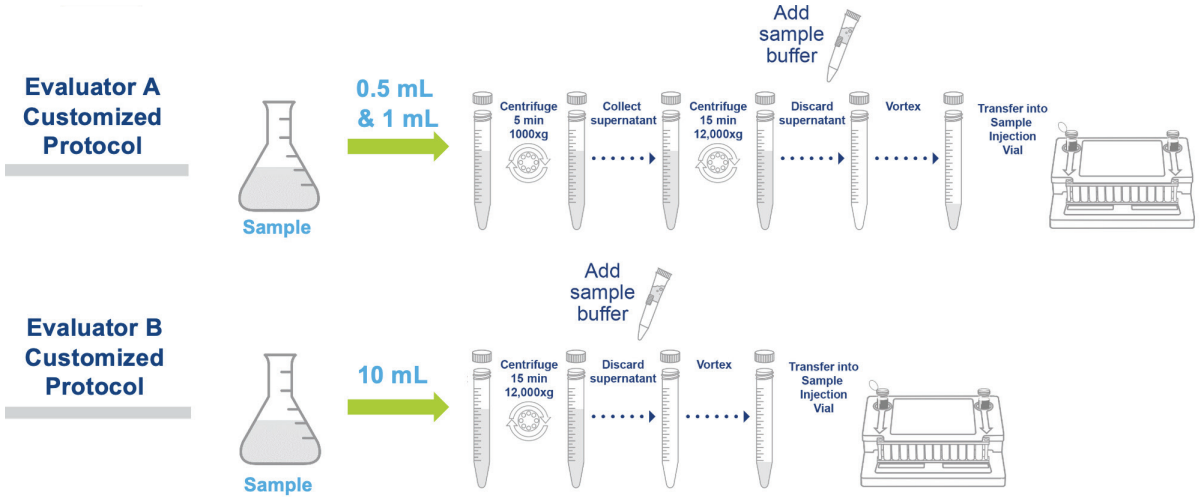


Figure 4. Customized protocols performed by evaluators A and B.

EVALUATOR PRODUCTS & STUDY OUTLINE

Table 1 lists the different product matrices tested by each evaluator. Product samples were tested with and without inoculated mycoplasma. Both evaluators performed inoculation studies at different concentrations to determine level of detection. The different species tested by each evaluator are shown in Table 2.

Table 1. Product matrices tested on the BIOFIRE FILMARRAY 2.0 Industry system.

Evaluator Designation	Sample Type
A	CAR T-cell ($3x10^6$ cells/mL)[1]
B	Autologous cellularized scaffold (2.5×10^4 cell/mL)[2]

[1]0.2 mL direct & 0.5 and 1 mL centrifugation protocols tested. [2]10 mL protocol tested with single high speed centrifugation.

Table 2. Mycoplasma species tested by each evaluator.

Test Species	A	B
Mycoplasma arginini		✓
Mycoplasma hyorhinis	✓	
Mycoplasma orale	✓	✓
Mycoplasma pneumoniae	✓	

RESULTS AND DISCUSSION

In this evaluation study, two cell therapy manufacturers ran a total of 30 matrix interference tests and neither evaluator reported false positive results when testing their product samples in the absence of mycoplasma (data not shown).

Table 3 summarizes the data from both cell therapy manufacturers testing the 0.2 mL in-process control and 10 mL release testing protocols.

Table 3. Inoculation results testing the 0.2 mL in-process control and 10 mL release testing protocols.

Evaluator	Cell Density per mL	BIOFIRE Testing Protocol	Organism	Concentration CFU/mL	Replicates	% Detection
A[1]	$3.0x10^6$	0.2 mL	*M. pneumoniae*	41.5	3	100
		10 mL	*M. hyorhinis*	34.2	5	100
				5.4	5	100
			M. orale	39.8	5	100
				8.0	5	100
			M. pneumoniae	41.5	5	100
				8.3	5	100
B[2]	$2.5x10^4$	10 mL	*M. arginini*	10	3	100
				1	3	100
			M. orale	10	3	100
				1	3	100

[1]Evaluator A used 0.5 mL product samples and followed a 2-step centrifugation protocol. [2]Evaluator B used a single high speed centrifugation.

- 3/3 (100%) CAR T-cell samples inoculated with a target concentration of ~40 CFU/mL detected the inoculated mycoplasma species using the direct 0.2 mL in-process control protocol.
- 42/42 (100%) of the cell therapy samples inoculated with 1-40 CFU/mL detected the inoculated mycoplasma species using a concentrating centrifugation protocol.
- Results show high sensitivity at concentrations needed for fast in-process control and release testing.
- Cell therapy evaluator A reported an LOD of < 10 CFU/mL for *M. hyorhinis*, *M. orale*, and *M. pneumoniae* while evaluator B reported an LOD of 1 CFU/mL for *M. arginini* and *M. orale*.
- No false-negative results were reported.

In addition to the matrices listed in Table 1, internal interference studies were performed as part of product development on a number of commonly used bioproduction materials (Table 4). Of all the matrices tested, only 1% bleach was found to interfere with the technology.

Table 4. List of materials tested that do not interfere with mycoplasma detection using the BIOFIRE FILMARRAY 2.0 Industry systrem.

Category	Material	Concentration
Cell Lines	CHO	5 x 10^5 cells/mL
	SP2/0	4.7 x 10^5 cells/mL
	Sf9	1 x 10^6 cells/mL
	HEK293	7.7 x 10^5 cells/mL
	COS-7	10% (v/v)
	Vero	8.7 x 10^4 cells/mL
Culture Media and Serum	DMEM	100% (v/v)
	Fetal Bovine Serum	1%; 10%; 100% (v/v)
	Horse Serum	100% (v/v)
	Tryptic Soy Broth	100% (v/v)
	Grace's Insect medium	100% (v/v)
	FRIIS Broth	100% (v/v)
	Frey Medium	100% (v/v)
Antimicrobials	Gentamicin	50 µg/mL
	Penicillin/Streptomycin	100 U/mL
	Neomycin	50 µg/mL
	Polymyxin B	50 µg/mL
	Ciprofloxacin	10 µg/mL
	Plasmocin	25 µg/mL
	Amphotericin	2.5 µg/mL
Cryoprotectants	DMSO	10% (v/v)
	Glycerol	10% (v/v)
Disinfectants*	Ethanol	1% (v/v)
	Isopropanol	1% (v/v)
	Phenolic Acid (low pH)	1% (v/v)
	Phenolic Acid (high pH)	1% (v/v)
Enzymes	Trypsin EDTA	100% (v/v)
	Collagenase	5 mg/mL
	Thermolysin	50 µg/mL
Microorganisms	*Escherichia coli*	2.2 x 10^7 CFU/mL
	Saccharomyces cerevisiae	1.43 x 10^8 CFU/mL

*Only 1% bleach was found to interfere.

Since launching the BIOFIRE FILMARRAY 2.0 Industry system, a number of internal studies have been performed on cell therapy product samples and found compatible with the following components:

- Jurkat and Tumor-Infiltrating Lymphocyte (TIL) cell lines
- mTeSRTM stem cell culture media
- AIM-V medium + GlutaMAXTM supplement + gentamicin + streptomycin + Plasma-Lyte A + CryoStor® CS-10 + 0.5% human serum albumin + human recombinant interleukin-2 proteins
- Human serum + HEPES + GlutaMax + Pluronic + sodium pyruvate + MEM Vitamin Solution + Proleukin®
- HyCloneTM medium + human serum albumin
- Stromal Cell Growth Medium (SCGM) + fetal bovine serum (FBS) + L-glutamine
- X-VIVOTM medium + GlutaMAX supplement + growth factors
- Dulbecco's Modified Eagle Medium (DMEM) containing FBS + HEPES + GlutaMAX + gentamicin + phenol red

CONCLUSION

The results of these studies by independent evaluators show that the BIOFIRE FILMARRAY 2.0 Industry system is well suited as both a release test or as an in-process control test for cell therapy samples that may contain high concentrations of mammalian cells. The results showed no product interference and high sensitivity providing reliable mycoplasma results in less than 1 hour.

In addition, numerous internal studies have shown compatibility for a wide-range of commonly used cell therapy components giving the ability to perform fast and simple screening for mycoplasma contamination.

A faster, easier approach to mycoplasma testing can bring significant benefits. From automated testing with a rapid result to reduced outsourcing costs, an ultra rapid, ultra easy mycoplasma test can save time and money. BIOFIRE's simple 3-step workflow, minimal hands-on time, 1-hour result and small footprint enable mycoplasma testing of a wide range of cell therapy products by anyone, anywhere at anytime.

ACKNOWLEDGEMENTS & REFERENCES

We graciously thank the external cell therapy manufacturers for evaluating the BIOFIRE Mycoplasma system.

1. USP <63> Mycoplasma Tests. United States Pharmacopoeia and National Formulary (USP43-NF38 - 6472). The United States Pharmacopeial Convention, Rockville, MD. DocID: GUID-05436D42-6984-45C8-9A43- 490147FE118A_1_en-US
2. Testing Cell Therapy Samples for Mycoplasma: Sample to Results in Less Than an Hour, AABB Annual Meeting, October 1-4, 2022
3. BIOFIRE FILMARRAY 2.0 Industry system manual

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