BIOMÉRIEUX

AUTOMATED 1-HOUR MYCOPLASMA TESTING IN BIOPRODUCTION

INTRODUCTION

Various pharmacopoeias, including the USP, specify a test for mycoplasma contamination must be performed as part of the release testing of a product manufactured in the presence of eukaryotic cells[1]. Current compendial methods require ≥28 days to generate results creating delays releasing product to downstream processes. Alternative conventional nucleic acid testing (NAT) methods are available; however, until recently these methods provided speed (~5 h) but not ease of use.

The BIOFIRE® FILMARRAY® 2.0 industry system utilizes a film array instrument and next generation PCR testing where all reagent components are contained in a closed pouch to detect the presence of >130 species of mycoplasma. The system provides sample to answer in ~1 hour with little technical training needed providing options for bringing mycoplasma testing directly to the production floor where critical testing is needed.

Testing Method	Refulation	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 – 10 mL
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

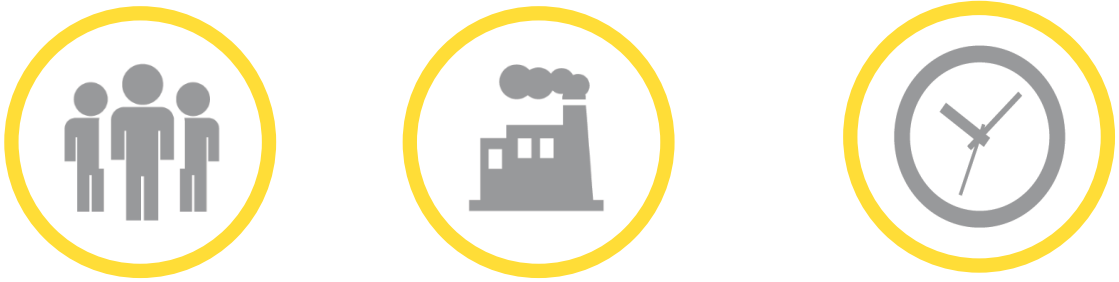
As presented at the 2021 PDA Pharmaceutical Microbiology Virtual Conference, the data summarized in this White Paper is from four bioproduction manufacturers that evaluated the BIOFIRE FILMARRAY 2.0 Industry system[2]. Samples were evaluated with up to 9 compendial mycoplasma species in the presence of high-density monoclonal antibody producing Chinese hamster ovary (CHO) cells. Studies were designed to assess product interference (false-positive rates), and detection (false-negative rates) including level of detection (LOD). Two distinct protocols were evaluated: a protocol using 10 mL of product sample that provides the sensitivity for release testing, and a direct-test protocol using 0.2 mL product sample that allows for at-line in-process control testing.

THE VALUE OF RAPID, EASY MYCOPLASMA TESTING

A faster, easier approach to mycoplasma testing can bring quantifiable value to bioproduction manufacturers. From simplifying training requirements, to reducing investigations linked to human error, to providing an early alert to contamination, an ultra rapid, ultra easy mycoplasma test can save time and money bringing value to any bioproduction organization.

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:

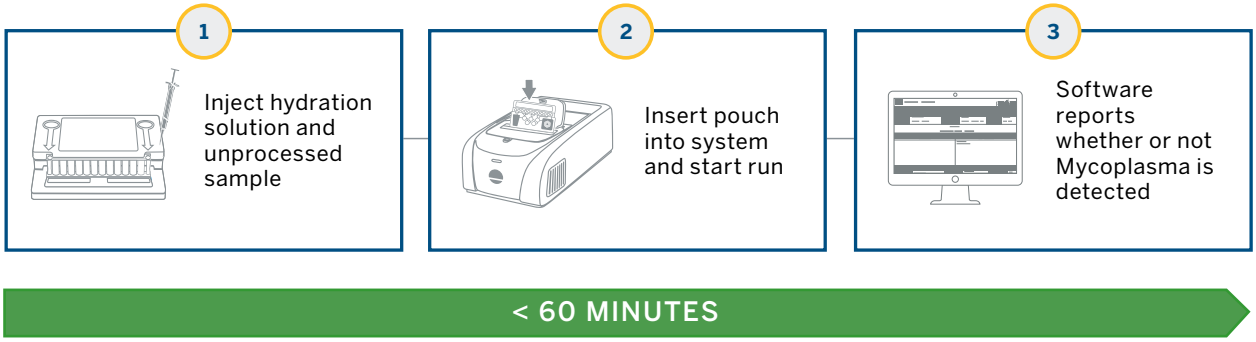
- Simplified training requirements
- Lower expertise required
- Objective results
- Less human error risk/improved Data Integrity
- Does not require PCR lab
- 1 hour time-to-result brings flexibility in testing planning
- Provides early alert to contamination and reduces costs of non-quality



ANYONE **ANYWHERE** **ANYTIME**

Because BIOFIRE FILMARRAY 2.0 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.

Bioproduction 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.



BIOFIRE FILMARRAY 2.0 INDUSTRY SYSTEM

The system utilizes a 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 of the necessary reagents for automated cell lysis, nucleic acid purification, reverse transcription, first and second stage nested PCR and analyst 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 PCR II controls. The instrument and 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 WxDxH). The system comes standard with 2 instruments; up to 8 instruments can be connected to a single PC.

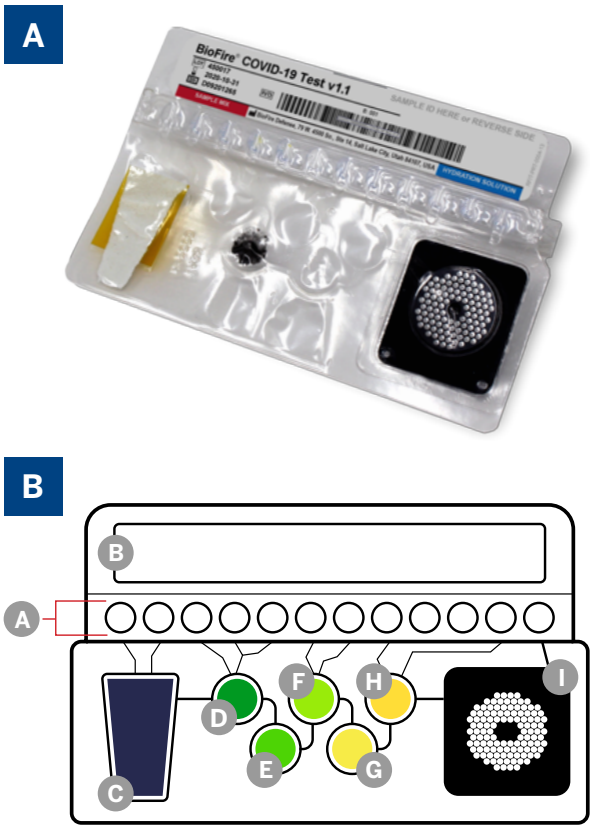


Figure 2: **A.** BIOFIRE Mycoplasma pouch. **B.** Pouch diagram: **(A)** Fitment with freeze-dried reagents; **(B)** Plungers-deliver reagents to blisters; **(C)** Sample lysis and bead collection; **(D)** Wash; **(E)** Magnetic bead collection blister; **(F)** Elution; **(G)** Multiplex Outer PCR blister;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

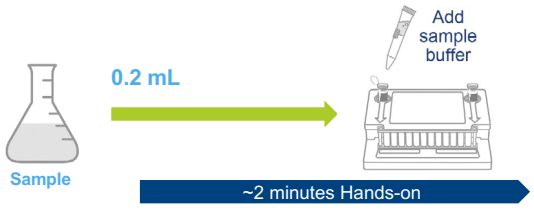
SAMPLE PROTOCOLS

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

These protocols were followed by evaluators except that Evaluator B deviated from the manufacturer recommendations with customized centrifugation times and forces. Following sample pre-processing, samples were then loaded onto a fully prepared and hydrated pouch and run on the FILMARRAY 2.0 Industry instrument.

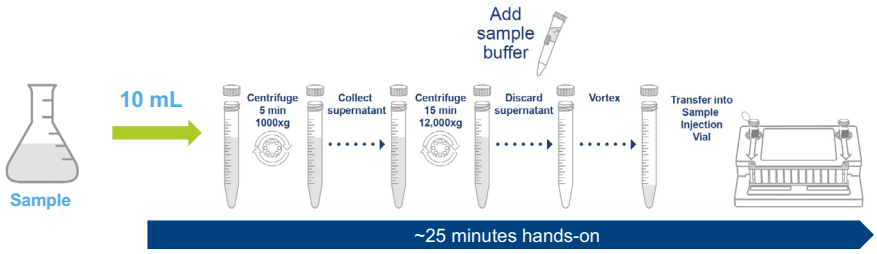
SUITABLE FOR IN-PROCESS CONTROLS

LOD ≤ 33 CFU/mL



SUITABLE FOR RELEASE TESTING

LOD ≤ 10 CFU/mL



EVALUATOR PRODUCTS & STUDY OUTLINE

Table 1 lists the different product matrices tested by each evaluator. All four evaluators tested high density monoclonal antibody producing CHO cells. Product samples were tested with and without inoculated mycoplasma. Evaluator C performed inoculation studies at different concentrations for 2 mycoplasma species to determine level of detection. The different species tested by each evaluator is shown in Table 2.

Table 1: Product matrices tested by each evaluator.

Evaluator	Sample Type
A	CHO bulk in-progress (10^7 cells/mL)
	ME medium (2 lots)[1]
	10% CHO bulk (10^6 cells/mL)+ME
B	Product 1: CHO bulk ($2x10^7$ cells/mL)
	Product 2: CHO bulk ($4x10^7$ cells/mL)[2]
C	CHO bulk at harvest (10^7 cells/mL)
D	CHO bulk 1 ($4x10^6$ cells/mL)
	CHO bulk 2 (unknown concentration)[3]

[1]Interference studies only (no mycoplasma inoculation).
[2]10 mL protocol tested only.
[3]0.2 mL protocol tested only.

Table 2: Mycoplasma species tested by each evaluator.

Test Species	A	B	C	D
Mycoplasma pneumoniae	✓	✓	✓	✓
Acholeplasma laidlawii	✓	✓		
Spiroplasma citri	✓			
Mycoplasma arginini		✓		
Mycoplasma fermentans		✓		✓
Mycoplasma hyorhinis		✓	✓	✓
Mycoplasma orale		✓	✓	✓
Mycoplasma gallisepticum			✓	✓
Mycoplasma salivarium		✓		✓

RESULTS AND DISCUSSION

In this evaluation study, 4 bioproduction manufacturers ran a total of 81 matrix interference tests and none of the evaluators reported false positive results when testing their product samples in the absence of mycoplasma (data not shown). Our internal data shows that only 1% bleach interferes with the assay which is to be expected. All other tested materials commonly found in bioproduction sample types did not show interference with detection of Mycoplasma using the BIOFIRE FILMARRAY system.

Tables 4 and 5 summarizes the data from the 4 bioproduction manufacturers testing the 0.2 mL in-process control, and 10 mL release testing protocols, respectfully. Internal interference studies were conducted on on a variety of substances by bioMerieux prior to product launch. Table 3 shows a list of materials tested which do not interfere with the BIOFIRE mycoplasma test. Only 1% bleach was found to interfere with the test (data not shown).

Table 3. Internal list of substances compatible with BIOFIRE.

Culture Media and Serum	Antimicrobials	Cell Lines	Disinfectants
DMEM (100%)	Gentamicin (50µg/mL)	CHO ($5x10^6$ cells/mL)	Ethanol 1%
Fetal Bovine Serum (1-100%)	Neomycin (50µg/mL)	SP2/0 ($4.7x10^5$ cells/mL)	Isopropanol 1%
Horse Serum (100%)	Polymyxin B (50µg/mL)	Sf9 ($1x10^6$ cells/mL)	Phenolic Acid (Low pH) 1%
Typical Soy Broth (100%)	Ciprofloxacin (10µg/mL)	HEK293 ($7.7x10^5$ cells/mL)	Phenolic Acid (High pH) 1%
Grace's Insect Medium (100%)	Penicillin/Streptomycin (100 U/mL)	COS-7 (10%)	
FRIIS Broth (100%)	Plasmocin (25 µg/mL)	Vero ($8.7x10^4$ cells/mL)	
Frey Medium (100%)	Amphotericin (2.5µg/mL)	Cryoprotectants	Microorganisms
Enzymes		DMSO 10%	E. coli ($2.2x10^7$ cfu/mL)
Trypsin EDTA (100%)		Glycerol 10%	S. cerevisiae ($1.43x10^8$ cfu/mL)
Collagenase (5 mg/mL)			
Thermolysin (50 µg/mL)			

Table 4. Inoculation results from 4 bioproduction manufacturers testing the 0.2 mL in-process control testing protocol.

Evaluator	CHO cell density per mL	Organism	Target concentration (CFU/mL) number replicates / number positive (%detected)	
			50	<10
A	1.0×10^7	*M. pneumoniae*	10/10 (100)	5/10 (50)
		A. laidlawii	10/10 (100)	3/10 (30)
		S. citri	10/10 (100)	4/10 (40)
B	2.1×10^7	*A. laidlawii*	3/3 (100)	3/3 (100)
		M. arginine	3/3 (100)	2/3 (67)
		M. fermentans	3/3 (100)	1/3 (33)
		M. hyorhinis	3/3 (100)	2/3 (67)
		M. orale	3/3 (100)	0/3 (0)
		M. pneumoniae	3/3 (100)	3/3 (100)
		M. salivarium	3/3 (100)	3/6 (50)
C	1.0×10^7	*M. pneumoniae*	Not tested	4/6 (67)
		M. orale	Not tested	3/3 (100)
		M. hyorhinis	Not tested	3/3 (100)
D	4.4×10^6	*M. pneumoniae*	Not tested	4/5 (80)
		M. orale	Not tested	5/5 (100)
		M. hyorhinis	Not tested	4/5 (80)
		M. gallisepticum	Not tested	5/5 (100)
	unknown	*M. fermentans*	Not tested	4/5 (80)
		M. salivarium	Not tested	5/5 (100)

The results in Table 4 from the 0.2 mL in-process control testing show:

- 51/51 (100%) CHO cell samples inoculated with a target concentration of 50 CFU/mL detected the inoculated mycoplasma species
- 63/96 (66%) samples tested at a target concentration of <10 CFU/mL (results as expected with manufacturer LOD for protocol at ~30 CFU/mL) detected the inoculated mycoplasma species
- Results show appropriate sensitivity at concentrations needed for fast in-process control testing

Table 5. Inoculation results from 4 bioproduction manufacturers testing the 10 mL release testing protocol

Evaluator	CHO cell density per mL	Organism	Concentration CFU/mL	Replicates	% Detection
A	1.0×10^7	*M. pneumoniae*	2.5	3	100
B	2.1×10^7	*A. laidlawii*	≤ 10	3	100
		M. arginine		4	100
		M. fermentans		3	100
		M. hyorhinis		4	100
		M. orale		3	100
		M. pneumoniae		1	100
		M. salivarium		4	100
	4.1×10^7	*A. laidlawii*	≤ 10	3	100
		M. arginine		3	100
		M. fermentans		3	100
		M. hyorhinis		3	100
		M. orale		3	100
		M. pneumoniae		3	100
		M. salivarium		3	100
C	1.0×10^7	*M. pneumoniae*	4.0	6	100
		M. orale	5.4	6	100
		M. hyorhinis	5.3	3	100
			0.6	3	100
		M. gallisepticum	8.0	3	100
			2.7	3	100
D	4.4×10^6	*M. pneumoniae*	≤ 10	5	100
		M. orale		5	100
		M. hyorhinis		5	100
		M. gallisepticum		5	100

- 90/90 (100%) CHO cell samples inoculated with a target concentration of ≤ 10 CFU/mL detected the inoculated mycoplasma species using the 10 mL release protocol
- Results show high sensitivity at concentrations needed for release testing
- Evaluator C reported LOD of 2.7 CFU/mL and <1 CFU/mL for M. gallisepticum and M. hyorhinis, respectfully
- No evaluator reported false-negative results

CONCLUSION

The results from these independent evaluators show that the BIOFIRE FILMARRAY Industry system is well-suited as both a release test or as an in-process control test for high-density CHO cell samples. The studies showed no product interference and high sensitivity providing reliable mycoplasma results in less than 1 hour. With a faster and easier approach to mycoplasma testing offered by BIOFIRE, bioproduction manufacturers can save time and reduce costs while ensuring quality.

ACKNOWLEDGEMENTS & REFERENCES

We graciously thank the 4 external bioproduction 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 Bioproduction Samples Using the BIOFIRE Mycoplasma Molecular Lab in a Pouch System, PDA Pharmaceutical Microbiology Virtual Conference, October 4-6, 2021

3. BIOFIRE FILMARRAY 2.0 Industry system manual

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