MHSRS-25-13920

Detection of Biothreat Agents Using a Multiplexed PCR Test for the BIOFIRE® SPOTFIRE® Instrument

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ABSTRACT

BioFire Defense has provided PCR-based testing capabilities to military personnel and first-responders for the detection of biothreat pathogens, including the Joint Biological Agent Identification and Diagnostic System (JBAIDS), RAZOR® Mk II, and Next Generation Diagnostic System (NGDS) FILMARRAY® 2.0 platforms. These portable systems have provided timely and reliable answers to support critical decision making and countermeasures related to biological warfare agents. The ability to reliably detect biothreat pathogens with the state-of-the-art BIOFIRE® SPOTFIRE® System represents the next generation of biothreat detection capabilities provided by BioFire Defense.

The BioThreat Panel was developed for use on the FILMARRAY 2.0 instrument for detection of bacterial and viral pathogens, and toxin-encoding genes, directly from environmental samples. The BioThreat Panel targets: *Bacillus anthracis, Brucella melitensis, Burkholderia mallei/pseudomallei, Coxiella burnetii, Francisella tularensis, Rickettsia prowazekii, Yersinia pestis*, Eastern equine encephalitis virus, *Marburg Marburgvirus, Orthopoxvirus* spp., variola virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus, *Zaire ebolavirus*, and toxin-encoding genes from *Clostridium botulinum* (botulinum toxin) and *Ricinus communis* (Ricin toxin).

The study presented demonstrates that the BioThreat Panel produces similar sensitivity on the next generation, state-of-the-art SPOTFIRE instrument to FILMARRAY 2.0 performance.

Figure 1. The BIOFIRE SPOTFIRE Instrument

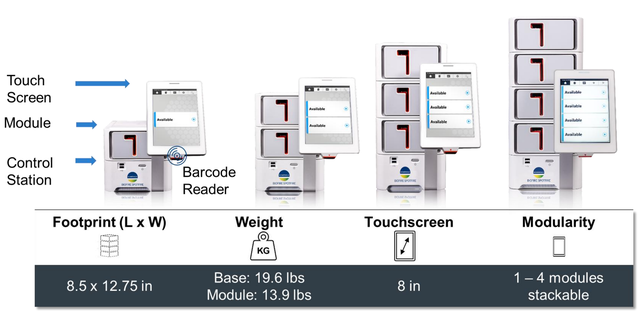

Figure 2. The BIOFIRE Pouch

BACKGROUND

The SPOTFIRE platform (Figure 1), is a rapid multiplex diagnostic system that can identify many viral, bacterial, fungi, or protozoan pathogens simultaneously. The SPOTFIRE platform is compatible with the existing BIOFIRE Panel consumable format. Following modifications to the instrument instructions governing a pouch run and modifications to the software required to analyze pouch runs, existing BIOFIRE Panels can be compatible with the SPOTFIRE system, achieving run times of ~60 minutes without any changes to reagent chemistry.

The SPOTFIRE System incorporates automated sample extraction (Figure 2A), nucleic acid purification (Figure 2B and 2C), reverse-transcription and nested multiplex first-stage PCR (Figure 2D and 2E) and individual second-stage PCR reactions in a PCR array (Figure 2G) to evaluate multiple targets in a single run (Figure 2). With minimal hands-on time, a comprehensive result is returned.

STUDY DESIGN

OVERALL DESIGN: The study was conducted in two phases, limit of detection (LoD) estimation and LoD confirmation. Testing was performed in phosphate-buffered saline on the SPOTFIRE and FILMARRAY 2.0 instruments in parallel.

ANALYTE SELECTION: Live/inactive organisms or genomic DNA were desired. Four analytes: *Eastern equine encephalitis* virus, Variola virus, *Clostridium botulinum,* and *Coxiella burnetii*, did not have material available at a Biosafety Level 2 or below. Synthetic templates were used in place of organism or genomic DNA for these target analytes.

ESTIMATED LOD:
The lowest concentration that provides 100% analyte detection when testing four pouches.

CONFIRMED LOD:
The lowest concentration that provides ≥ 95% analyte detection when testing 20 pouches.

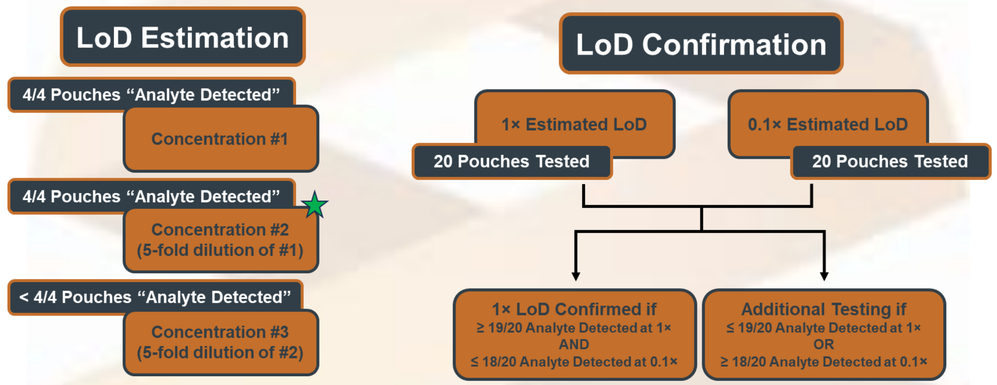

Table 1. Analytes Tested for Each Target on the BioThreat Panel[1]

	Target Analyte	Analyte Strain[1]	Vendor ID[2]	Type of Analyte	Targeted Assays	Analyte Interpretation
Bacteria	*Bacillus anthracis*	Ames	AGD 1331	Inactivated	Chromosome Element, pXO1, pXO2	*Bacillus anthracis*
		Sterne 34F2	NR-1400	Live	Chromosome Element, pXO1	Bacillus spp.
	Brucella melitensis	16M	AGD 0074	Inactivated (Formalin)	BRT2, BRT4	*Brucella melitensis*
						Brucella spp.
	Burkholderia pseudomallei	MSHR 146	AGD 1595	Inactivated (Formalin)	Burk2, Burk8	*Burkholderia mallei/pseudomallei*
	Coxiella burnetii	*NA*	CHEM-GBK-0485	Synthetic Nucleic Acid	CBT1	*Coxiella burnetii*
	Francisella tularensis	SCHU S4	NR-15753	Inactivated (Formalin)	FTT2, FTT3	*Francisella tularensis*
	Rickettsia prowazekii	Brienl	AGD 0079	Inactivated (Formalin)	RIK2	*Rickettsia prowazekii*
	Yersinia pestis	CO92	NR-2717	Genomic DNA	YPT1, YPT3	*Yersinia pestis*
Viruses	*Zaire ebolavirus*	Mayinga	NR-31807	Inactivated (Gamma-irradiated)	EB2	Ebola Zaire
	Eastern equine encephalitis virus	*NA*	CHEM-RNA-0039	Synthetic Nucleic Acid - RNA	EEE01	EEE Virus
	Marburg marburgvirus	German Voege	NR-31816	Inactivated (Gamma-irradiated)	Marb2, Marb3	Marburg virus
		Ravn	NR-31819	Inactivated (Gamma-irradiated)	Marb2, Marb3	
	Orthopoxvirus spp.	Modified vaccinia ankara (MVA) virus	NR-1	Attenuated Live	OPX2, Var3	Orthopox genus virus
	Variola virus	*NA*	CHEM-GBK-0752	Synthetic Nucleic Acid	OPX2	Variola virus
		NA	Var1_BTNIVD_g1QC	Synthetic Nucleic Acid	Var1a, Var1b	
		NA	Var3_BTNIVD_g1QC	Synthetic Nucleic Acid	Var3	
	Venezuelan equine encephalitis virus	Trinidad	AGD 0108	Inactivated (Formalin)	VEE-MP2, VEE-RC3	VEE virus
	Western equine encephalitis virus	CBA87 Alpha 025	AGD 0110	Inactivated (Gamma-irradiated)	WEE01	WEE virus
Toxin	*Clostridium botulinum* (Botulinum toxin)	*NA*	CHEM-RNA-0031	Synthetic Nucleic Acid	BoNTA	*Clostridium botulinum*
	Ricinus communis (Ricin toxin)	Castor Bean gDNA	NR-44091	Genomic DNA	RCN2	*Ricinus communis*

[1]Analyte strain listed for organism isolates; *NA* listed for synthetic nucleic acid analytes
[2]Vendor ID or BioFire Defense part number, for synthetic nucleic acid templates

RESULTS

- Similar (within 5-fold) or improved sensitivity on SPOTFIRE for all analytes, except *Yersinia pestis* (Table 2).
- Decrease in sensitivity towards *Yersinia pestis* on SPOTFIRE expected due to changes in the SPOTFIRE software calling scheme.

Table 2. Confirmed LoD Values on SPOTFIRE and FILMARRAY 2.0

	Target Analyte	Analyte Strain[1]	Confirmed LoD (copies/mL)	
			SPOTFIRE	FILMARRAY
Bacteria	*Bacillus anthracis*	Ames	8.0E+03	8.0E+03
		Sterne 34F2	4.0E+03	4.0E+03
	Brucella melitensis	16M	1.0E+02	1.0E+02
	Burkholderia pseudomallei	MSHR 146	4.8E+02	4.8E+02
	Coxiella burnetii	*NA*	3.0E+04	3.0E+04
	Francisella tularensis	SCHU S4	2.4E+02	2.4E+02
	Rickettsia prowazekii	Brienl	8.7E+03	1.7E+03
	Yersinia pestis	CO92	2.2E+03	4.4E+01
Viruses	*Zaire ebolavirus*	Mayinga	3.7E+04	1.8E+05
	Eastern equine encephalitis virus	*NA*	7.4E+03	5.9E+05
	Marburg marburgvirus	German Voege	1.4E+04	1.4E+04
		Ravn	3.2E+04	6.5E+03
	Orthopoxvirus spp.	MVA poxvirus	3.0E+03	6.0E+02
	Variola virus	*NA*	8.0E+03	4.0E+03
	Venezuelan equine encephalitis virus	Trinidad	2.4E+03	1.2E+04
	Western equine encephalitis virus	CBA87 Alpha 025	4.8E+02	2.4E+03
Toxin	*Clostridium botulinum* (Botulinum toxin)	*NA*	3.1E+05	3.1E+06
	Ricinus communis (Ricin toxin)	Castor Bean gDNA	4.0E+02	8.0E+02

[1]Analyte strain listed for organism isolates; *NA* listed for synthetic nucleic acid analytes Confirmed LoD (copies/mL) are listed for each analyte on each instrument. Cells shaded in blue indicate reduced sensitivity on SPOTFIRE and cells shaded in orange indicate increased sensitivity on SPOTFIRE

TAKEAWAY

- BIOFIRE systems have been widely adopted by the US Department of Defense and others because they provide:
  - Broad capability (tests for more than 100 common and 30 rare infectious disease pathogens)
  - Ease-of-use
  - Speed
  - Low logistics burden (no cold chain required)
- BIOFIRE SPOTFIRE is the next generation of BIOFIRE system
  - FDA-cleared Panels for detection of respiratory pathogens are available for SPOTFIRE
  - Additional Panels in development to provide same capabilities as earlier BIOFIRE platforms, such as the NGDS Warrior Panel
- **The SPOTFIRE BioThreat Panel will be available December 2025**

NOTE: The BioThreat Panel is not for diagnostic use

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Presented at the Military Health System Research Symposium (MHSRS)
August 4 – 7, 2025