BioFire Defense, LLC
David Rabiger
Associate Director of Regulatory and Clinical Affairs
79 W 4500 S, Suite 14
Salt Lake City, Utah 84107

March 22, 2023

Re: K213362
Trade/Device Name: BioFire Global Fever Special Pathogens Panel; BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel
Regulation Number: 21 CFR 866.4000
Regulation Name: Device To Detect And Identify Biothreat Microbial Agents In Human Clinical Specimens.
Regulatory Class: Class II
Product Code: QVR, QMV, PMN
Dated: October 8, 2021
Received: October 12, 2021

Dear David Rabiger:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's

requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Noel J. Gerald -S

Noel J. Gerald, Ph.D.
Branch Chief
Bacterial Respiratory and Medical Countermeasures Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*

K213362

Device Name

BioFire Global Fever Special Pathogens Panel

Indications for Use *(Describe)*

The BioFire® Global Fever Special Pathogens Panel is a qualitative, multiplexed, nucleic acid-based test intended for use with BioFire® FilmArray® 2.0 and BioFire® FilmArray® Torch Systems. The BioFire Global Fever Special Pathogens Panel is for the simultaneous qualitative detection and identification of multiple bacterial, viral, and protozoan nucleic acids directly from EDTA whole blood collected from individuals with signs and/or symptoms of acute febrile illness or recent acute febrile illness and known or suspected exposure to the target pathogens described below.

Pathogens identified:

Chikungunya virus
Dengue virus (serotypes 1, 2, 3 and 4)
Leishmania spp. that cause visceral leishmaniasis (e.g., *L. donovani* and *L. infantum*)
Leptospira spp.
Plasmodium spp. (including species differentiation of *Plasmodium falciparum* and *Plasmodium vivax/ovale*)
West Nile virus

Pathogens presumptively identified:

Bacillus anthracis
Crimean-Congo hemorrhagic fever virus
Ebolavirus spp.
Francisella tularensis
Lassa virus
Marburgvirus
Yellow fever virus
Yersinia pestis

Pathogens for which the panel provides presumptive identification results require additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be necessary.

Positive results do not rule out co-infections with pathogens not included on the BioFire Global Fever Special Pathogens Panel. Not all pathogens that cause acute febrile illness are detected by this test, and negative results do not preclude infection with the pathogens targeted by the device and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Evaluation for more common causes of acute febrile illness (e.g., infections of the upper and lower respiratory tract or gastroenteritis, as well as non-infectious causes) should be considered prior to evaluation with this panel. In the United States, patient travel history, exposure risk, and consultation of the CDC Yellow Book should be considered prior to use of the BioFire Global Fever Special Pathogens Panel as some pathogens are more common in certain geographical locations. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities.

The BioFire Global Fever Special Pathogens Panel is indicated for use in laboratories having appropriate biosafety equipment, personal protective equipment (PPE), containment facilities and personnel trained in the safe handling of diagnostic clinical specimens potentially containing any of the pathogens detected by this panel.

The BioFire Global Fever Special Pathogens Panel is indicated for use in laboratories that follow public health guidelines that address appropriate biosafety conditions, interpretation of test results, and coordination of findings with public health authorities.

For In Vitro Diagnostic Use.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

I. Submitter

BioFire Defense, LLC
79 West 4500 South, Suite 14
Salt Lake City, UT 84107
Phone: (801) 262-3592
Fax: (801) 447-6907

Contact Person: David Rabiger
Date Prepared: 2022-Oct-07

II. Device

Name of Device: BioFire® Global Fever Special Pathogens Panel
Common or Usual Name: Same
Product Code: QMV
Regulation: 21 CFR 866.3966
Classification Name: Device to detect and identify selected microbial agents that cause acute febrile illness
Regulatory Class: Class II (Special Controls)
Panel: Microbiology – 83

Additional Product Code: QVR
Regulation: 21 CFR 866.4000
Classification Name: A multiplex nucleic acid detection system for biothreat agents
Regulatory Class: Class II (Special Controls)
Panel: Microbiology – 83

III. Predicate Devices

BioFire® Global Fever Panel (BioFire Defense, LLC) [DEN200043]
This predicate has not been subject to a design-related recall.

FilmArray® NGDS Warrior Panel (BioFire Defense, LLC) [K170883]
This predicate has not been subject to a design-related recall.

IV. Device Description

The BioFire® Global Fever Special Pathogens Panel is a multiplexed nucleic acid-based test designed to be used with BioFire® FilmArray® Systems (BioFire® FilmArray® 2.0 or BioFire® FilmArray® Torch). The BioFire Global Fever Special Pathogens Panel pouch contains freeze-dried reagents to perform nucleic acid purification and nested, multiplexed polymerase chain reaction (PCR) with DNA melt analysis. The BioFire Global Fever Special Pathogens Panel conducts tests for the identification of bacterial, viral, and protozoan pathogens from whole blood specimens collected in EDTA tubes (Table 1**)**. Results from the BioFire Global Fever Special Pathogens Panel are available in about 1 hour.

Table 1. Pathogens Detected by the BioFire Global Fever Special Pathogens Panel

Type	Disease	Pathogen
Bacterial	Anthrax	*Bacillus anthracis* [1]
	Leptospirosis	*Leptospira* spp.
	Plague	*Yersinia pestis* [1]
	Tularemia	*Francisella tularensis* [1]
Viral	Crimean-Congo hemorrhagic fever	Crimean-Congo hemorrhagic fever virus [1]
	Chikungunya fever	Chikungunya virus
	Dengue fever	Dengue virus (serotypes 1, 2, 3 and 4)
	Lassa fever	Lassa virus [1]
	Ebola virus disease	*Ebolavirus* spp. (Bundibugyo, Reston, Sudan, Taï Forest, Zaire) [1]
	Marburg virus disease	*Marburgvirus* [1]
	West Nile fever	West Nile virus
	Yellow fever	Yellow fever virus [2]
Protozoan	Malaria	*Plasmodium* spp.
		Plasmodium falciparum
		Plasmodium vivax/ovale
	Visceral Leishmaniasis	*Leishmania* spp. including *L. donovani* and *L. infantum*

[1] Select agents are subject to additional requirements. Definitive identification requires confirmatory testing.
[2] Definitive identification requires confirmatory testing.

A test is initiated by loading Hydration Solution into one port of the pouch and a whole blood specimen mixed with the provided Sample Buffer into the other port of the pouch. The pouch contains all the reagents required for specimen testing and analysis in a freeze-dried format; the addition of Hydration Solution and the Sample Buffer rehydrates the reagents. After the pouch is prepared, the BioFire® FilmArray® Software guides the user through the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample identification, selecting the appropriate protocol, and initiating the run on the BioFire FilmArray system.

The BioFire FilmArray instruments contain a coordinated system of inflatable bladders and seal points, which act on the pouch to control the movement of liquid between the pouch blisters. When a bladder is inflated over a reagent blister, it forces liquid from the blister into connecting

channels. Alternatively, when a seal is placed over a connecting channel it acts as a valve to open or close a channel. In addition, electronically controlled pneumatic pistons are positioned over multiple plungers to deliver the rehydrated reagents into the blisters at the appropriate times. Two Peltier devices control heating and cooling of the pouch to drive the PCR reactions and subsequent melt.

Nucleic acid extraction occurs within the BioFire FilmArray pouch using mechanical and chemical lysis followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, a nested multiplexed PCR is executed in two stages. During the first stage, a single, large volume, multiplexed reverse transcription PCR (rt-PCR) reaction is performed. The products from first stage PCR are then diluted and combined with a fresh, primer-free master mix and a fluorescent double stranded DNA binding dye (LC Green® Plus, BioFire Defense, LLC). The solution is then distributed to each well of the array. Array wells contain sets of primers designed specifically to amplify sequences internal to the PCR products generated during the first stage PCR reaction. The second stage PCR and melt, or nested singleplex PCR, is performed in each well of the array. At the end of the second stage PCR, the array is interrogated by melt curve analysis for the detection of signature amplicons denoting the presence of specific targets. A digital camera placed in front of the array captures fluorescent images of the PCR2 reactions and software interprets the data.

Materials provided in each BioFire Global Fever Special Pathogens Panel Kit:

- Individually packaged BioFire Global Fever Special Pathogens Panel Pouches
- Single-use (1.0 mL) BioFire® FilmArray® Sample Buffer Tubes
- Single-use pre-filled (1.5 mL) BioFire® FilmArray® Hydration Injection Vials
- Individually packaged BioFire® FilmArray® Sample Injection Vials
- Individually packaged Transfer Pipettes
- *BioFire Global Fever Special Pathogens Panel – Quick Guide*
- Instructions available online at: *www.biofiredefense.com*
    - *BioFire Global Fever Special Pathogens Panel – Instructions for Use*

Materials required but not provided:

- BioFire FilmArray System including:
    - BioFire® FilmArray® 2.0 or BioFire® FilmArray® Torch Instrument System including accompanying platform-specific core software
    - BioFire® FilmArray® Pouch Loading Station
    - BioFire® Global Fever Special Pathogens Pouch Module Software is required to run the BioFire Global Fever Special Pathogens Panel and is available by request at *www.biofiredefense.com* if not already installed on the instrument system.
- 10% bleach solution or a similar disinfectant

V. Intended Use

The BioFire® Global Fever Special Pathogens Panel is a qualitative, multiplexed, nucleic acid-based test intended for use with BioFire® FilmArray® 2.0 and BioFire® FilmArray® Torch Systems. The BioFire Global Fever Special Pathogens Panel is for the simultaneous qualitative detection and identification of multiple bacterial, viral, and protozoan nucleic acids directly from EDTA whole blood collected from individuals with signs and/or symptoms of acute febrile illness or recent acute febrile illness and known or suspected exposure to the target pathogens described below.

Pathogens identified:

Chikungunya virus
Dengue virus (serotypes 1, 2, 3 and 4)
Leishmania spp. that cause visceral leishmaniasis (e.g., *L. donovani* and *L. infantum*)
Leptospira spp.
Plasmodium spp. (including species differentiation of *Plasmodium falciparum* and *Plasmodium vivax/ovale*)
West Nile virus

Pathogens presumptively identified:

Bacillus anthracis
Crimean-Congo hemorrhagic fever virus
Ebolavirus spp.
Francisella tularensis
Lassa virus
Marburgvirus
Yellow fever virus
Yersinia pestis

Pathogens for which the panel provides presumptive identification results require additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be necessary.

Positive results do not rule out co-infections with pathogens not included on the BioFire Global Fever Special Pathogens Panel. Not all pathogens that cause acute febrile illness are detected by this test, and negative results do not preclude infection with the pathogens targeted by the device and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Evaluation for more common causes of acute febrile illness (e.g., infections of the upper and lower respiratory tract or gastroenteritis, as well as non-infectious causes) should be considered prior to evaluation with this panel. In the United States, patient travel history, exposure risk, and consultation of the CDC Yellow Book should be considered prior to use of the BioFire Global Fever Special Pathogens Panel as some pathogens are more common in certain geographical locations. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities.

The BioFire Global Fever Special Pathogens Panel is indicated for use in laboratories having appropriate biosafety equipment, personal protective equipment (PPE), containment facilities and personnel trained in the safe handling of diagnostic clinical specimens potentially containing any of the pathogens detected by this panel.

The BioFire Global Fever Special Pathogens Panel is indicated for use in laboratories that follow public health guidelines that address appropriate biosafety conditions, interpretation of test results, and coordination of findings with public health authorities.

For In Vitro Diagnostic Use.

VI. Substantial Equivalence

The BioFire Global Fever Special Pathogens Panel is substantially equivalent to the BioFire Global Fever Panel (previously known as the FilmArray Global Fever Panel) and the FilmArray NGDS Warrior Panel. The BioFire Global Fever Panel was granted De Novo classification on November 20, 2020 [DEN200043] and was categorized as a Class II device. The FilmArray NGDS Warrior Panel was cleared on June 22, 2017 and was also categorized as a Class II device [K170883].

In addition, the BioFire Global Fever Special Pathogens Panel is substantially equivalent to the FilmArray NGDS Warrior Panel in that they both have the ability to detect select agents in whole blood specimens. While there are differences between these panels regarding pouch chemistry, pouch protocols, and pouch module software, both panels were developed using the same FilmArray technology and design principles.

Table 2 outlines the similarities and differences between the BioFire Global Fever Special Pathogens Panel and the predicate devices.

Table 2. Comparison of the BioFire Global Fever Special Pathogens Panel and Predicate Devices

Element	Subject Device: BioFire Global Fever Special Pathogens Panel	Predicate: BioFire Global Fever Panel [DEN200043]	Predicate: FilmArray NGDS Warrior Panel [K170883]
Specimen Type	Whole Blood (collected in EDTA tube)	Same as BioFire Global Fever Special Pathogens Panel	Whole Blood (collected in EDTA tube), positive blood culture, or sputum
Intended Use Setting	Individuals with signs and/or symptoms of acute febrile illness or recent acute febrile illness and known or suspected exposure to pathogens on the panel.	Same as BioFire Global Fever Special Pathogens Panel	Individuals with signs and symptoms of infection from biothreat agents and/or individuals who are at risk for exposure or may have been exposed to pathogens tested by the panel.

Element	Subject Device: BioFire Global Fever Special Pathogens Panel	Predicate: BioFire Global Fever Panel [DEN200043]	Predicate: FilmArray NGDS Warrior Panel [K170883]
Pathogens Detected	Identification: Chikungunya virus, Dengue virus (serotypes 1, 2, 3 and 4), *Leishmania* spp., *Leptospira* spp., *Plasmodium* spp. (including species differentiation of *Plasmodium falciparum* and *Plasmodium vivax/ovale*), West Nile virus Presumptive Identification: *Bacillus anthracis,* Crimean-Congo hemorrhagic fever virus, *Ebolavirus* spp., *Francisella tularensis*, Lassa virus, *Marburgvirus*, Yellow fever virus, *Yersinia pestis*	Identification: Chikungunya virus, Dengue virus (serotypes 1,2,3, and 4), *Leptospira* spp., *Plasmodium* spp. (including species differentiation of *Plasmodium falciparum* and *Plasmodium vivax/ovale*)	Presumptive Identification in whole blood EDTA: *Bacillus anthracis*, *Coxiella burnetii*, *Ebolavirus* spp., *Francisella tularensis*, *Marburgvirus, Yersinia Pestis* Presumptive Identification in positive blood culture: *Bacillus anthracis, Yersinia pestis* Presumptive Identification in sputum: *Francisella tularensis, Yersinia pestis*
Analyte	RNA/DNA	Same as BioFire Global Fever Special Pathogens Panel	Same as BioFire Global Fever Special Pathogens Panel
Technological Principles	Highly multiplexed nested nucleic acid amplification test with melt analysis	Same as BioFire Global Fever Special Pathogens Panel	Same as BioFire Global Fever Special Pathogens Panel
Instrumentation[1]	BioFire FilmArray 2.0 and BioFire FilmArray Torch	BioFire FilmArray 2.0	BioFire FilmArray 2.0
Time to result	About 1 hour	Same as BioFire Global Fever Special Pathogens Panel	Same as BioFire Global Fever Special Pathogens Panel
Reagent Storage	Room temperature	Same as BioFire Global Fever Special Pathogens Panel	Same as BioFire Global Fever Special Pathogens Panel
Test Interpretation	Automated test interpretation and report generation. User cannot access raw data.	Same as BioFire Global Fever Special Pathogens Panel	Same as BioFire Global Fever Special Pathogens Panel
Controls	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis.	Same as BioFire Global Fever Special Pathogens Panel	Same as BioFire Global Fever Special Pathogens Panel
User Complexity	High	Moderate	Moderate

[1] The BioFire FilmArray 2.0 [K143178] and BioFire FilmArray Torch [K160068] instruments are based on the same technological principles.

VII. Summary of Performance Data

Clinical Performance

Prospective Clinical Study

The prospective clinical study was designed to evaluate the sensitivity/positive percent agreement (PPA) and specificity/negative percent agreement (NPA) for each analyte of the BioFire Global Fever Special Pathogens Panel when testing prospectively collected whole blood specimens in the intended use setting.

Between March 2018 and March 2021, 11 sites contributed 2139 prospectively collected whole blood specimens from individuals who had a recorded or self-reported fever within the past two days. A summary of demographic information for the 2139 specimens included in the prospective study is given in Table 3.

Table 3. Demographic Summary for Prospective BioFire Global Fever Special Pathogens Panel Clinical Evaluation

Demographic		Overall
Sex	Female	1095 (51.2%)
	Male	1044 (48.8%)
Age	<5	178 (8.3%)
	5 to 21	822 (38.4%)
	22 to 50	779 (36.4%)
	>50	360 (16.8%)
Total		**2139**

PPA for each analyte was calculated as 100% × (TP / (TP + FN)). True positive (TP) indicates that both the BioFire Global Fever Special Pathogens Panel and the comparator method had a positive result for this specific analyte, and false negative (FN) indicates that the BioFire Global Fever Special Pathogens Panel result was negative while the comparator result was positive. NPA was calculated as 100% × (TN / (TN + FP)). True negative (TN) indicates that both the BioFire Global Fever Special Pathogens Panel and the comparator method had negative results and a false positive (FP) indicates that the BioFire Global Fever Special Pathogens Panel result was positive, but the comparator result was negative. Results are summarized in Tables 4 (viruses), 5 (bacteria), and 6 (protozoa).

Table 4. BioFire Global Fever Special Pathogens Panel Clinical Performance Summary – Viruses

BioFire Global Fever Special Pathogens Panel Detected Result	Number Tested	PPA			NPA		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Chikungunya virus[a]	1875[c]	25/25	100%	86.7-100%	1848/1850	99.9%	99.6-100%
Crimean-Congo hemorrhagic fever virus	2139	1/1	100%	20.7-100%	2138/2138	100%	99.8-100%
Dengue virus[b]	1875[c]	266/283	94.0%	90.6-96.2%	1592/1592	100%	99.8-100%
Ebola virus	2139	0/0	-	-	2139/2139	100%	99.8-100%
Lassa virus	2139	0/0	-	-	2139/2139	100%	99.8-100%
Marburg virus	2139	0/0	-	-	2139/2139	100%	99.8-100%
West Nile virus	2139	1/1	100%	20.7-100%	2138/2138	100%	99.8-100%
Yellow fever virus	2139	0/0	-	-	2139/2139	100%	99.8-100%

[a] Evidence of Chikungunya virus was found in 2/2 FP specimens by additional PCR.
[b] Evidence of Dengue virus was found in 15/17 FN specimens: five specimens were positive upon BioFire Global Fever Special Pathogens Panel retest and by additional PCR, two were positive only upon BioFire Global Fever Special Pathogens Panel retest, and eight were detected only by additional PCR.
[c] Comparator analysis was not performed for Chikungunya virus or Dengue virus on specimens collected after September 2019.

Table 5. BioFire Global Fever Special Pathogens Panel Clinical Performance Summary – Bacteria

BioFire Global Fever Special Pathogens Panel Detected Result	Number Tested	PPA			NPA		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Bacillus anthracis	2139	0/0	-	-	2139/2139	100%	99.8-100%
Francisella tularensis	2139	0/0	-	-	2139/2139	100%	99.8-100%
Leptospira spp.[a]	1875[b]	15/16	93.8%	71.7-98.9%	1855/1859	99.8%	99.4-99.9%
Yersinia pestis	2139	0/0	-	-	2139/2139	100%	99.8-100%

[a] Evidence of *Leptospira* spp. was found in 1/1 FN specimens by BioFire Global Fever Special Pathogens Panel retest and by additional PCR, and in 3/4 FP specimens by additional PCR.
[b] Comparator analysis was not performed for *Leptospira* on specimens collected after September 2019.

Table 6. BioFire Global Fever Special Pathogens Panel Clinical Performance Summary – Protozoa

BioFire Global Fever Special Pathogens Panel Detected Result	Number Tested	PPA			NPA		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Leishmania spp.	2139	10/10	100%	72.2-100%	2129/2129	100%	99.8-100%
Plasmodium spp.[a,b]	1875[e]	338/343	98.5%	96.6-99.4%	1519/1532	99.2%	98.6-99.5%
Plasmodium falciparum[c]	1875[e]	230/248	92.7%	88.8-95.4%	1624/1627	99.8%	99.5-99.9%
Plasmodium vivax/ovale[d]	1875[e]	115/124	92.7%	86.8-96.1%	1751/1751	100%	99.8-100%

[a] Four (4/5) *Plasmodium* FN specimens were also *P. falciparum* FN and one (1/5) was *P. vivax/ovale* FN. Three (3/13) *Plasmodium* FP specimens were also *P. falciparum* FP.

[b] Evidence of *Plasmodium* spp. was found in 2/5 FN specimens: one specimen was positive upon BioFire Global Fever Special Pathogens Panel retest and by additional PCR, and one was positive only upon BioFire Global Fever Special Pathogens Panel retest. Evidence of *Plasmodium* spp. was found in 11/13 FP specimens by additional PCR (10/13) or by species-level comparator assay (1/13).

[c] Evidence of *P. falciparum* was found in 13/18 FN specimens: three specimens were positive upon BioFire Global Fever Special Pathogens Panel retest and by additional PCR, one was positive only upon BioFire Global Fever Special Pathogens Panel retest, and nine were positive only by additional PCR. Evidence of *P. falciparum* was found in 2/3 FP specimens by additional PCR.

[d] Evidence of *P. vivax/ovale* was found in 7/9 FN specimens: two specimens were positive upon BioFire Global Fever Special Pathogens Panel retest and by additional PCR, two were positive only upon BioFire Global Fever Special Pathogens Panel retest, and three were positive only by additional PCR.

[e] Comparator analysis was not performed for *Plasmodium, P. falciparum,* or *P. vivax/ovale* on specimens collected after September 2019.

Archived Specimen Study

Many of the analytes detectable by the BioFire Global Fever Special Pathogens Panel were not observed or were not encountered in large enough numbers in the prospective clinical evaluation to adequately demonstrate system performance. In this study, archived specimens with known analyte content and/or archived specimens with a high likelihood of containing a given analyte were tested with the BioFire Global Fever Special Pathogens Panel to supplement the prospective clinical evaluation data. Wherever possible, archived whole blood specimens were tested. Where no whole blood specimens could be obtained, blood plasma and blood serum were tested instead. Although plasma and serum are not the intended specimen type for the BioFire Global Fever Special Pathogens Panel, these blood components provide similar results to whole blood specimens in a matrix equivalency study. Archived specimens were collected from a range of ages and sexes (Table 7).

Table 7. Overall and Per Site Archived Demographic Analysis

		Overall	Site 01 [a]	Site 02	Site 03 [a]
Sex	Female	160 (38.5%)	79 (39.7%)	49 (59.8%)	32 (23.7%)
	Male	148 (35.6%)	97 (48.7%)	33 (40.2%)	18 (13.3%)
	Unknown	108 (26.0%)	23 (11.6%)	0 (0%)	85 (63%)
Age	<5	14 (3.4%)	14 (7.0%)	0 (0%)	0 (0%)
	5 to 21	37 (8.9%)	19 (9.5%)	14 (17.1%)	4 (3%)
	22 to 50	200 (48.1%)	121 (60.8%)	56 (68.3%)	23 (17%)
	>50	57 (13.7%)	22 (11.1%)	12 (14.6%)	23 (17%)
	Unknown	108 (26.0%)	23 (11.6%)	0 (0%)	85 (63%)
Total		416	199	82	135

[a] Demographic data was not available for 23 specimens from Site 01 and 85 specimens from Site 03.

Specimens were coded and either tested at the clinical site or shipped to BioFire Defense for testing on the BioFire Global Fever Special Pathogens Panel. Nucleic acid was extracted from each specimen and tested using plate-based PCR comparator methods. Samples for which false positive and/or false negative results (i.e., discrepant results) were obtained when comparing the BioFire Global Fever Special Pathogens Panel results to the comparator method results were further investigated. Results are summarized in Table 8.

Table 8. BioFire Global Fever Special Pathogens Panel Archived Performance Summary

Pathogen[a]	PPA			NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
Crimean-Congo hemorrhagic fever virus	0/0	-	-	281/281	100%	98.7-100%
Ebolavirus spp.[b]	0/0	-	-	279/279	100%	98.6-100%
Lassa virus[b,c]	10/12	83.3%	55.2-95.3	265/267	99.2%	97.3-99.8%
Marburgvirus sp.[b]	0/0	-	-	279/279	100%	98.6-100%
West Nile virus[b,d,e,f]	59/65	90.8%	81.3-95.7%	345/347	99.4%	97.9-99.8%
Yellow fever virus[b]	0/0	-	-	279/279	100%	98.6-100%
Bacteria						
Bacillus anthracis	0/0	-	-	281/281	100%	98.7-100%
Francisella tularensis	0/0	-	-	281/281	100%	98.7-100%
Yersinia pestis	0/0	-	-	281/281	100%	98.7-100%
Protozoa						
Leishmania spp.[g]	0/0	-	-	283/283	283/283	98.7-100%

[a] Results are not shown for assays previously granted in DEN200043 (i.e., chikungunya virus, dengue virus, *Leptospira* spp., and *Plasmodium* spp.).

[b] Due to low specimen volume, comparator results for most pathogens were not obtained for two specimens. These specimens were only tested for a subset of pathogens including Crimean-Congo hemorrhagic fever virus, *Bacillus anthracis*, *Francisella tularensis, Yersinia pestis,* and *Leishmania* spp.

[c] Evidence of Lassa virus was found in ½ FN specimens and ½ FP specimens by additional PCR.

[d] A set of 133 specimens tested at Site 03 had been previously characterized and were expected to be negative for all panel targets or positive for West Nile virus. These specimens were only evaluated by comparator methods for West Nile virus.

[e] Archived specimens included blood serum and blood plasma.

[f] Evidence of West Nile virus was found in 6/6 FN specimens: four specimens were positive upon Global Fever Special Pathogens Panel retesting and three of these were also positive by additional PCR. The other two FN specimens were positive only by additional PCR testing. Evidence of West Nile virus was detected in 1/2 FP specimens by additional PCR testing.

[g] Two specimens tested at Site 03 had been previously characterized and were only tested on comparator assays for *Leishmania* spp.

Contrived Specimen Study

For analytes for which no archived specimens were available, or for which there were an insufficient number of archived specimens, testing was performed using contrived specimens. This study evaluated BioFire Global Fever Special Pathogens Panel sensitivity and specificity when testing whole-blood specimens contrived with *Bacillus anthracis*, Crimean-Congo hemorrhagic fever virus, *Ebolavirus* spp., *Francisella tularensis*, Lassa fever virus, *Leishmania* spp., *Marburgvirus* sp., West Nile virus, Yellow fever virus, and *Yersinia pestis*.

Contrived specimens were prepared using residual human whole blood specimens from patients with signs/symptoms of acute febrile illness. For each analyte, fifty (50) replicates were contrived using quantified isolates at a range of concentrations relative to the limit of detection (LoD). If known, clinically relevant concentrations were used to adjust testing levels. The contrived specimens also served as a negative replicate for all other analytes evaluated. Specimens were prepared and randomized such that the analyte status of each contrived specimen was blinded to the users performing testing. The positive percent agreement (PPA) and negative percent agreement (NPA) were defined as agreement between the BioFire Global Fever Special Pathogens Panel result and the known composition of the contrived specimen. A summary of the results is shown in Table 9.

Table 9. Summary of BioFire Global Fever Special Pathogens Panel Contrived Specimen Performance Data

Analyte	PPA			NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Bacillus anthracis	50/50	100	92.9-100	332/332	100	98.9-100
CCHF virus[a]	98/100[b]	98	93.0-99.5	282/282	100	98.7-100
Ebolavirus spp.	50/50	100	92.9-100	332/332	100	98.9-100
Francisella tularensis	50/50	100	92.9-100	332/332	100	98.9-100
Lassa virus	50/50	100	92.9-100	332/332	100	98.9-100
Leishmania spp.	50/50	100	92.9-100	332/332	100	98.9-100
Marburgvirus sp.[a]	99/100[c]	99	94.6-99.8	282/282	100	98.7-100
West Nile virus	50/50	100	92.9-100	331/332[d]	99.7	98.3-100
Yellow fever virus	49/50[e]	98	89.5-99.7	332/332	100	98.9-100
Yersinia pestis	50/50	100	92.9-100	332/332	100	98.9-100

[a] Tested at additional concentrations to better represent clinically relevant range.
[b] Two false negative CCHF virus at 2× LoD.
[c] Single false negative *Marburgvirus* sp. at 10× LoD.
[d] Discrepancy testing showed near LoD levels of WNV in a single whole blood sample.
[e] Single false negative yellow fever virus at 5× LoD.

Select Analytical Studies

Limit of Detection

The Limit of Detection (LoD) for each analyte on the BioFire Global Fever Special Pathogens Panel was determined using quantified stocks within the BioFire Defense BioSafety Level 2 (BSL2) laboratory. For analytes that required BSL3/4 containment, inactivated strains were used. Contrived samples were prepared at known concentrations in a whole blood background. An estimated LoD concentration for each analyte was determined based on results of serial dilutions spanning at least four concentrations bracketing the anticipated LoD. Four replicates were tested at each dilution, with additional dilutions if needed, to reach a concentration at which loss of detection could be observed. The LoD concentration was confirmed by testing 20 replicates at the estimated LoD concentration and another 20 replicates at a ten-fold lower concentration. The required criteria for confirmation of LoD was a detection rate of at least 95% at LoD ((≥19/20) and a detection rate of less than 95% below LoD (<19/20).

The confirmed LoD concentrations for BioFire Global Fever Special Pathogens Panel analytes along with the detection rate at 1× LoD are shown in Table 10. The LoD concentration for each analyte is reported as target copies/mL determined using commercially available quantitative real-time PCR assay kits.

Table 10. Limit of Detection for BioFire Global Fever Special Pathogens Panel Test Results

Global Fever Special Pathogens Panel Analyte	Isolate Tested	Live/Inactivated	LoD Concentration	
			Copies/mL[1]	Units/mL
BACTERIA				
Bacillus anthracis	Ames35	Live	4.2E+01	N/A
Francisella tularensis	SCHU S4	Inactivated	1.2E+03	N/A
Leptospira spp.	*interrogans*: serovar icterohaemorrhagiae, Serotype: Budapest	Live	3.4E+02	N/A
Yersinia pestis	A1122	Live	1.3E+02	N/A
VIRUSES				
Chikungunya virus	R80422	Inactivated	5.5E+02	3.6E-01 units/mL
Crimean-Congo hemorrhagic fever virus	Strain IbAr10200	Inactivated	6.4E+00	N/A
Dengue virus	DENV-1: Hawaii	Live	2.2E+02	N/A
	DENV-2-1: New Guinea C	Live	3.4E+02	N/A
	DENV-2-2: Dak AR A1247	Live	2.7E+03	1.5E+02 $TCID_{50}$/mL
	DENV-3: H87	Live	1.3E+02	3.7E+00 units/mL
	DENV-4: H241	Live	6.4E+01	1.8E+02 units/mL
Ebolavirus spp.	Bundibugyo: 200706291 Uganda	Inactivated	7.0E+04	N/A
	Taï Forest: Cote d'Ivoire 11/27/94	Inactivated	8.3E+03	N/A
	Reston: 119810 RIID (MKY 53) (prototype 1989)	Inactivated	2.7E+04	N/A
	Sudan: Boniface	Inactivated	1.1E+04	N/A
	Zaire: Guéckédou/Guinea C07	Inactivated	1.0E+02	1.5E+02 PFU/mL
Lassa virus	Josiah	Inactivated	5.6E+04	N/A
Marburgvirus	Marburg virus: Musoke	Inactivated	5.0E+02	N/A
	Ravn virus: Kenya Ravn	Inactivated	2.6E+02	N/A
West Nile virus	NY 2001-6263 (Lineage 1)	Inactivated	1.1E+03	2.7E+01 units/mL
	B-956 Uganda (Lineage 2)	Inactivated	2.3E+04	6.2E+00 units/mL
Yellow fever virus	Strain 17D	Live attenuated	1.2E+02	1.2E+01 $TCID_{50}$/mL
PROTOZOA				
Leishmania spp.	*donovani*: 9515 (MHOM/IN/95/9515)	Live	1.0E+01	2.2E+01 cells/mL
Plasmodium spp.	*falciparum*, IPC 4884 Pursat Cambodia 2011	Live	1.8E+02	N/A
	knowlesi, H strain	gDNA	2.4E+01	20 pg/mL
	malariae (Clinical Specimen)	Live	1.9E+02	2.3E-01 cells/mL
	ovale, *wallikeri* (Clinical Specimen)	Live	2.4E+02	N/A
	vivax, Strain Chesson	Live	1.5E+02	N/A
Plasmodium falciparum	IPC 4884 Pursat Cambodia 2011	Live	1.8E+02	N/A
Plasmodium vivax/ovale	*ovale*, *wallikeri* (Clinical Specimen)	Live	2.4E+02	N/A
	vivax, Strain Chesson	Live	1.5E+02	N/A

[1] Stock concentrations determined using commercially available quantitative real-time PCR assay kits.

Since decreased sensitivity may be observed due to nucleic acid damage when evaluating inactivated BSL3/4 pathogens, additional testing was performed using live strains within a subcontracted BSL3/4 laboratory.

The concentrations tested were based on the confirmed LoD for inactivated/attenuated material. In general, four test concentrations were tested for each analyte: 10×, 1×, 0.1×, and 0.01× the LoD for inactivated/attenuated stock. Some analytes required additional concentrations. For each concentration, four unique blood draws were individually spiked and tested on the BioFire Global Fever Special Pathogens Panel. The estimated LoD was identified as the lowest concentration at which 4/4 replicates were positive and fewer than four replicates were positive at the next lower concentration. The estimated LoD values for infectious material are provided in Table 11.

Table 11. BioFire Global Fever Special Pathogens Panel Estimated LoD in Whole Blood for Live BSL3/4 Agents

BioFire Global Fever Special Pathogens Panel Analyte	Species/Strain	Estimated LoD Concentration	
		Copies/mL	Units/mL
Bacteria			
Bacillus anthracis	Ames	6.4E+01	3.5E+00 cfu/mL
Francisella tularensis	subsp. *tularensis* Schu	1.2E+01	2.1E+02 cfu/mL
Yersinia pestis	CO92	1.5E+02	3.0E+01 cfu/mL
Viruses			
CCHF Virus	IbAr10200	6.4E+02	2.9E+03 pfu/mL
Chikungunya virus	B8635	5.5E+02	4.4E+01 pfu/mL
	Indo23574	5.5E+02	9.0E+01 pfu/mL
Ebolavirus spp.	Bundibugyo virus / Uganda (811250)	7.0E+02	5.6E+00 pfu/mL
	Tai Forest virus / Taï Forest (Ivory Coast)	1.8E+02	N/A
	Reston virus / H-28	2.7E+03	4.7E+01 pfu/mL
	Sudan virus / Boniface	1.1E+02	5.3E+00 pfu/mL
	Zaire ebolavirus / Makona	1.1E+03	1.6E+01 pfu/mL
Lassa Virus	Josiah	5.6E+03	2.6E+01 pfu/mL
Marburg marburgvirus	Ci67	5.0E+02	1.5E+03 pfu/mL
	Ravn	2.6E+02	4.7E+01 pfu/mL
West Nile virus	Bz NY99 (lin. 1)	1.6E+02	9.5E+00 pfu/mL
Yellow fever virus	Asibi	1.2E+01	9.2E+00 pfu/mL

Inclusivity (Reactivity)

The analytical reactivity (inclusivity) of the BioFire Global Fever Special Pathogens Panel was evaluated by testing a diverse collection of strains/species/serotypes representing temporal, geographic, and genetic diversity. Available isolates were prepared as contrived whole blood samples with the isolate at a concentration near the LoD of the analyte. If the isolate was detected within 3× the LoD the BioFire Global Fever Special Pathogens Panel was considered inclusive for that isolate and those with similar sequences. Analytes that were detected at ≥10× the established LoD were considered to have reduced sensitivity.

Table 12 shows a summary of the BioFire Global Fever Special Pathogens Panel reactivity based on empirical data. Isolates used to determine LoD are bolded.

Table 12. BioFire Global Fever Special Pathogens Panel Analytical Reactivity (Inclusivity)

Analyte	# Isolates Detected / Tested	Isolates Tested		Source / ID	Concentration Detected Tested up to 100× LoD[1]	Limitations
BACTERIA						
Bacillus anthracis	4/4	**Ames35**		**BEI / NR-10355**	**4.2E+01 copies/mL**	None
		Sterne 34Fs		BEI / NR-1400	1.3E+02 copies/mL	
		UM23		BEI / NR-10351		
		Weybridge		BEI / NR-10350		
Francisella tularensis	5/5	**Subspecies**	**Strain**			None
		tularensis	**SCHU S4**	**BEI / NR-15753**	**1.2E+03 copies/mL**	
		holarctica	LVSR	BEI / NR-597	3.6E+03 copies/mL	
			Type B LVS (CDC)	BEI / NR-646		
		novicida	CG62	BEI / NR-580		
			KM14S	BEI / NR-573		
Leptospira spp.	19/19	**Species**	**Strain**			None
		interrogans	**Serovar (Budapest)**	**ATCC / 23581**	**3.4E+02 copies/mL**	
			HAI0156 (Copenhageni)	BEI / NR-19891	1.2E+03 copies/mL	
			L495 (Manilae)	BEI / NR-19816		
		alexanderi	L60 (Manhao 3)	ATCC / 700520	1.0E+03 copies/mL	
		alstonii	Sichuan 79601	ATCC / BAA-2439	1.2E+03 copies/mL	
		borgpetersenii	Castellon 3 (Castellonis)	ATCC / 23580		
			Veldrat Bataviae 46 (Javanica)	ATCC / 43292	1.0E+03 copies/mL	
		kirschneri	200701401 (Bogvere)	BEI /NR-19942	1.2E+03 copies/mL	
			3522 C (Cynopteri)	ATCC / 49945	5.3E+02 copies/mL	
		kmetyi	Bejo-Iso9T (Malaysia)	BEI / NR-22254	1.2E+03 copies/mL	
		mayottensis	200901116 (undesignated)	KIT / 0254		
		noguchii	CZ 214T (Panama)	BEI / NR-22283		
		santarosai	LT 821 (Shermani)	ATCC / 43286	8.7E+02 copies/mL	

Analyte	# Isolates Detected / Tested	Isolates Tested		Source / ID	Concentration Detected Tested up to 100× LoD[1]	Limitations
		weilii	6712	KIT / 0220	1.2E+03 copies/mL	
			94-79970/3 (Topaz)	KIT / 0237		
			A 102 (Mengrun)	KIT / 0023		
			Celledoni 20160426	ATCC / 43285		
			H 27 (Hekou)	KIT / 0074		
			LT 89-68 (Vughia)	KIT / 0127		
Yersinia pestis	3/3	**Biovar**	**Strain**			None
		Orientalis	**A1122**	**BEI / NR-636**	**1.3E+02 copies/mL**	
		Orientalis	PY-013	BEI / NR-51666	3.9E+02 copies/mL	
		Antiqua	PH 80/63	BEI / NR-51667		
VIRUSES						
Chikungunya virus	3/3	**R80422**		**ZeptoMetrix / 0810105CFHI**	**5.5E+02 copies/mL**	None
		DHS4263		BEI / NR-50884 (formerly NR-50055)	1.7E+03 copies/mL	
		St. Martin 2013		BEI / NR-50883 (formerly NR-49901)	1.4E+03 copies/mL	
Crimean–Congo hemorrhagic fever virus	1/1	**Nigeria / IbAr10200**		**BEI / NR-37383**	**6.4E+00 copies/mL**	None
Dengue virus	27/28	**Serotype**	**Strain**	**ZeptoMetrix / 0810088CF**	**2.2E+02 copies/mL**	None
		Serotype 1	**Hawaii**			
			Strain 12150	BEI / NR-3785	6.6E+02 copies/mL	
			228690	BEI / NR-3786		
			276RK1	BEI / NR-3782		
			BC89/94	BEI / NR-3787		
			SL-6-6-04	BEI / NR-49744		
			UIS 1162	BEI / NR-49707		
			VN/BID-V1792/2007	BEI / NR-44083		
		Serotype 2	**New Guinea C (DENV 2_1)**	**ZeptoMetrix / 0810089CF**	**3.4E+02 copies/mL**	Not inclusive of strain DKA 811
			DakArA1247 (DENV 2_2)	**BEI / NR-12221**	**2.7E+03 copies/mL**	
			1349	BEI / NR-12219	1.1E+03 copies/mL	
			429557	BEI / NR-12216		
			ArA6894	BEI / NR-12220		
			BC102/94	BEI / NR-3789	9.4E+02 copies/mL	
			DKA 811	BEI / NR-49747	Not Detected	
			VN/BID-V1002/2006	BEI / NR-44085	1.1E+03 copies/mL	
		Serotype 3	**H87**	**ZeptoMetrix / 0810090CF**	**1.3E+02 copies/mL**	Reduced sensitivity for
			271242	BEI / NR-3802	3.9E+02 copies/mL	

Analyte	# Isolates Detected / Tested	Isolates Tested		Source / ID	Concentration Detected Tested up to 100× LoD[1]	Limitations
			BC188/97	BEI / NR-3801	1.6E+04 copies/mL	strain BC188/97
			C0360/94	BEI / NR-48800	3.9E+02 copies/mL	
			VN/BID-V1329/2006	BEI / NR-44087		
		Serotype 4	**H241**	**ZeptoMetrix / 0810091CF**	**6.4E+01 copies/mL**	Reduced sensitivity for strain D85-019
			703	BEI / NR-48801	1.9E+02 copies/mL	
			BC13/97	BEI / NR-3805		
			BC287/97	BEI / NR-3806		
			BC258/97	BEI / NR-3807		
			D85-019	BEI / NR-3804	7.6E+03 copies/mL	
			PR 06-65-740	BEI / NR-49757	2.2E+02 copies/mL	
Ebolavirus	6/6	**Bundibugyo Uganda**		**BEI / NR-31813**	**7.0E+04 copies/mL**	Reduced sensitivity for inactivated *Zaire ebolavirus* Mayinga
		Reston MKY 53		**BEI / NR-44238**	**2.7E+04 copies/mL**	
		Sudan		**BEI / NR-31810**	**1.1E+04 copies/mL**	
		Taï Forest		**BEI / NR-44241**	**8.3E+03 copies/mL**	
		Zaire	**Guéckédou**	**BEI / NR-49462**	**1.0E+02 copies/mL**	
			Mayinga	BEI / NR-31807	1.1E+04 copies/mL	
Lassa Virus	1/1	**Josiah**		**BEI / NR-31822**	**5.6E+04 copies/mL**	None
Marburgvirus	3/3	**Marburg virus Musoke**		**BEI / NR-48951**	**5.0E+02 copies/mL**	None
		Marburg virus Voege		BEI / NR-31816	1.5E+03 copies/mL	
		Ravn virus Kenya		**BEI / NR-31819**	**2.6E+02 copies/mL**	
West Nile virus	5/5	**B-956 Uganda (lin. 2)**		**Zeptometrix/ 0810081cfhi**	**2.3E+04 copies/mL**	None
		B-956 (lin. 2)		BEI / NR-50885	6.9E+04 copies/mL	
		NY 2001-6263 (lin. 1)		**Zeptometrix/ 0810033cfhi**	**1.1E+03 copies/mL**	
		1986 (lin. 1)		Zeptometrix/ 0810082cfhi	2.2E+03 copies/mL	
		Bird 114 (lin. 1)		BEI / NR-50886	3.3E+03 copies/mL	
Yellow fever virus	1/1	**17D**		**BEI / NR-116**	**1.2E+02 copies/mL**	None
PROTOZOA						
Leishmania spp.	12/12	***donovani* 9515**		**BEI / NR-48822**	**1.0E+01 copies/mL**	Reduced sensitivity for *braziliensis* and *infantum* strains
		amazonensis		BEI / NR-49247	3.0E+01 copies/mL	
		braziliensis Vianna		ATCC / 30879	1.0E+02 copies/mL	
		donovani, 1S		BEI / NR-48821	3.4E+01 copies/mL	
		donovani chagasi		ATCC / 50133	3.0E+01 copies/mL	
		infantum		ATCC / 50134	1.0E+02 copies/mL	
		major IR173		BEI / NR-48816	3.0E+01 copies/mL	
		mexicana MHOM/BZ/82/BEL21		ATCC / 50157	2.9E+01 copies/mL	
		panamensis PSC-1		BEI / NR-50162	3.0E+01 copies/mL	
		tropica (MHOM/AF/87/RUP)		BEI / NR-48820		
		tropica (MHOM/SU/58/strain-OD)		ATCC / 50130		
		venezuelensis MHOM-VE/80/H-16		BEI / NR-29184	3.4E+01 copies/mL	
Plasmodium spp.	10/10	***falciparum***	**IPC 4884**	**BEI / MRA-1238**	**1.8E+02 copies/mL**	None
			SenTh021.09	BEI / MRA-1182	3.0E+02 copies/mL	

Analyte	# Isolates Detected / Tested	Isolates Tested		Source / ID	Concentration Detected Tested up to 100× LoD[1]	Limitations
(Plasmodium spp. assay)			St. Lucia	BEI / MRA-331		
			Tanzania, 02000708	BEI / MRA-1169		
		vivax	**Chesson**	**BEI / MRA-383**	**1.5E+02 copies/mL**	
			Panama	ATCC / 30138	3.1E+02 copies/mL	
		ovale	**Wallikeri**	**CDC / N8K9QKI9**	**2.4E+02 copies/mL**	
			Curtisi	CDC / N8K9QL0S	7.2E+02 copies/mL	
		knowlesi	**Strain H**	**BEI / MRA-456G**	**2.4E+01 copies/mL**	
		malariae	**Clinical Sample**	**DLS / DLS17-026015**	**1.9E+02 copies/mL**	
Plasmodium falciparum (Plasmodium falciparum assay)	4/4	***falciparum***	**IPC 4884**	**BEI / MRA-1238**	**1.8E+02 copies/mL**	Reduced sensitivity for SenTh021.09
			SenTh021.09	BEI / MRA-1182	1.8E+03 copies/mL	
			St. Lucia	BEI / MRA-331	3.0E+02 copies/mL	
			Tanzania, 02000708	BEI / MRA-1169		
Plasmodium vivax/ovale (Plasmodium vivax/ovale assay)	4/4	***vivax***	**Chesson**	**BEI / MRA-383**	**1.5E+02 copies/mL**	None
			Panama	ATCC / 30138	3.1E+02 copies/mL	
		ovale	**Wallikeri**	**CDC / N8K9QKI9**	**2.4E+02 copies/mL**	
			Curtisi	CDC / N8K9QL0S	7.2E+02 copies/mL	

[1] Test concentrations were based on the LoD for the analyte in copies/mL with stock concentrations determined using a commercial qPCR assay.

Additionally, detection of BSL 3/4 infectious strains/isolates/serovars/species of *Bacillus anthracis*, *Francisella tularensis*, *Yersinia pestis*, West Nile virus, *Zaire ebolavirus*, Lassa virus, *Marburgvirus*, Yellow fever virus were evaluated by spiking whole blood at near infectious estimated LoD levels. Analytes detected only at ≥10-fold the estimated LoD were considered to have reduced sensitivity. Results are summarized in Table 13. Isolates used to evaluate the infectious estimated LoD are bolded.

Table 13. Summary of BioFire Global Fever Special Pathogens Panel Reactivity (Inclusivity) for Infectious BSL3/4 Analytes

Analyte	# Isolates Detected/Tested	Isolates Tested		UCC [1] ID	Concentration Detected	Limitations
Bacillus anthracis	10/12	**Ames**		**BACI008**	**6.4E+01 copies/mL**	Reduced sensitivity for strains SK-102 and Vollum 1B
		108		BACI226	1.9E+02 copies/mL	
		2002013094		BACI293	1.9E+02 copies/mL	
		Canadian Bison		BACI153	1.9E+02 copies/mL	
		K3		BACI261	1.9E+02 copies/mL	
		Ohio ACB		BACI259	1.9E+02 copies/mL	
		PAK-1		BACI309	1.9E+02 copies/mL	
		RA3		BACI225	1.9E+02 copies/mL	
		SK-102 (Pakistan)		BACI126	6.4E+02 copies/mL	
		South Africa (BA 1035)		BACI207	1.9E+02 copies/mL	
		Sterne		BACI012	1.9E+02 copies/mL	
		Turkey #32		BACI260	1.9E+02 copies/mL	
		Vollum 1B		BACI124	6.4E+02 copies/mL	
Francisella tularensis	9/9	Subspecies	Strain			None
		tularensis	**Schu4**	**FRAN016**	**1.2E+01 copies/mL**	
			Scherm	FRAN031	3.6E+01 copies/mL	
			WY96	FRAN072	3.6E+01 copies/mL	
		holarctica	Holarctica, LVS	FRAN004	3.6E+01 copies/mL	
			Holarctica	FRAN012	3.6E+01 copies/mL	
			Holarctica, 425	FRAN029	3.6E+01 copies/mL	
			HD, DB082106G	FRAN035	2.3E+01 copies/mL	
			VT68	FRAN025	3.6E+01 copies/mL	
		novicida	F6168	FRAN134	3.6E+01 copies/mL	
			U112, GA993550	FRAN003	3.6E+01 copies/mL	
Yersinia Pestis	12/12	Biovar	Strain			None
		Orientalis	**CO92**	**YERS023**	**1.5E+02 copies/mL**	
			A1122	YERS078	4.5E+02 copies/mL	
			Dodson [2]	YERS073	3.6E+02 CFU/mL	
			Java 9	YERS022	4.5E+02 copies/mL	
			PBM19	YERS018	4.5E+02 copies/mL	
			Shasta	YERS074	4.5E+02 copies/mL	
		Antiqua	Angola	YERS080	4.5E+02 copies/mL	
			Antigua	YERS016	4.5E+02 copies/mL	
			Nairobi	YERS017	4.5E+02 copies/mL	
			Pestoides F [2]	YERS020	3.6E+02 CFU/mL	
		Medievalis	Harbin 35	YERS021	5.3E+02 copies/mL [3]	
			KIM5	YERS082	6.4E+02 copies/mL [3]	
			Nicholisk 41	YERS083	4.5E+02 copies/mL	
Zaire ebolavirus	4/4	Strain/Isolate				None
		Makona – SL3864.1		**Ebola027**	**1.1E+03 copies/mL**	
		Mayinga (Zaire 76)		Ebola001	2.1E+03 copies/mL	
		Gabon		Ebola034	3.2E+03 copies/mL	
		Kikwit '95		Ebola007	3.1E+03 copies/mL	
		Luebo		Ebola035	3.1E+03 copies/mL	
Lassa virus	2/2	**Josiah**		Arena002	2.4E+01 PFU/mL	None
		Macenta [4]		Arena009	7.2E+01 PFU/mL	
		Pinneo [4]		Arena003	7.2E+01 PFU/mL	

Analyte	# Isolates Detected/Tested	Isolates Tested		UCC[1] ID	Concentration Detected	Limitations
Marburg Marburgvirus	2/2	Lineage	Strain			None
		Ravn virus	**RAVN**	**Marb002**	**2.6E+02 copies/mL**	
		Marburg virus	**Ci67**	**Marb003**	**5.0E+02 copies/mL**	
			Angola	Marb005	1.5E+03 copies/mL	
			Musoke	Marb001	7.8E+02 copies/mL	
West Nile virus (Lineage 1)	1/1	**Bz NY99**		**Flavi022**	**1.6E+02 copies/mL**	None for West Nile Virus Lineage 1
		Eg101		Flavi016	4.8E+02 copies/mL	
Yellow fever virus	4/4	**Asibi**		**FLAVI005**	**1.2E+01 copies/mL**	None
		SVM 3-18-09		BEI NR-49799	1.2E+04 copies/mL	Reduced sensitivity[5]
		CAREC M2-09		BEI NR-50062	1.2E+03 copies/mL	
		INHRR 7a-05		BEI NR-50071	1.2E+03 copies/mL	
		INHRR 10a-10		BEI NR-50063	1.2E+03 copies/mL	

[1] U.S. Department of Defense Unified Culture Collection

[2] The *Y. pestis* bv. Orientalis Dodson and *Y. pestis* bv. Antiqua Pestoides F strains lack the pPCP plasmid targeted by the qPCR assay, and as a result the nucleic acid concentration could not be quantified. Inclusivity for these two strains was therefore based on concentrations obtained for these two strains, and the CO92 reference strain, by enumeration in Colony Forming Units (CFU)/mL.

[3] After testing was completed, it was determined that *Yersinia pestis* bv. Medievalis strains Harbin35 and KIM5 were evaluated at higher than 3× the estimated LoD, at 3.5× and 4.2×, respectively. Based on amplification data it is expected that these two *Y. pestis* strains will be detected at 3×LoD.

[4] The Lassa virus Macenta and Pinneo strains were not detected by the qPCR assay, only the Josiah strain evaluated in the Estimated LoD study was. Therefore, inclusivity testing for Lassa virus was performed based on concentrations obtained by enumeration in Plaque Forming Units (PFU)/mL.

[5] YFV strains SVM 3-18-09, CAREC M2-09, NHRR 7a-05, and INHRR 10a-10 were isolated from neighboring regions (Trinidad and Venezuela). In silico analyses indicate these strains are closely related and do not represent a broad diversity of sequences.

Exclusivity (Specificity)

Analytical specificity (exclusivity) of the BioFire Global Fever Special Pathogens Panel assays was evaluated by challenging the system with a large collection of organisms and viruses prepared at high concentrations. On-panel organisms and viruses were tested to assess the potential for intra-panel cross-reactivity. Off-panel organisms and viruses (those not intended to be detected by the panel) were tested to assess the potential for non-specific amplification of other pathogens or potential contaminants. Organisms and viruses for off-panel testing were selected based on a combination of several factors including: relatedness to specific species detected by the BioFire Global Fever Special Pathogens Panel (near-neighbors); clinical relevance (cause illness or symptoms similar to the panel pathogens); likelihood of being present in blood as a co-infection based on a geographical region or specific population to which a panel pathogen is endemic; and genetic similarity to BioFire Global Fever Special Pathogens Panel assay primers, as determined by in silico analyses.

On-panel and off-panel testing included 217 isolates of bacteria, viruses, fungi, and protozoa tested at high concentration (typically $>10^6$ genomic copies/mL). Table 14 shows a complete list of the on- and off-panel bacteria, viruses, protozoa, and fungi that were tested and received the expected BioFire Global Fever Panel Special Pathogens test result (negative for all assays; no cross-reactivity) or for which in silico analysis does not predict cross-reactivity.

Table 14. On and Off Panel Organisms and Viruses with No Cross-Reactivity with BioFire Global Fever Special Pathogens Panel Assays

ON-PANEL		
Bacteria		
Bacillus anthracis	*Leptospira interrogans*	
Francisella tularensis subsp. *tularensis*	*Yersinia pestis* (2 strains: A1122 and CO92)	
Viruses		
Crimean-Congo hemorrhagic fever virus	*Zaire ebolavirus*	*Marburg Marburgvirus* variant Musoke
Chikungunya virus	*Sudan ebolavirus*	*Marburg marburgvirus* (RAVN)
Dengue virus Serotype 1	*Bundibugyo ebolavirus*	West Nile virus Lineage 1
Dengue virus Serotype 2	*Taï Forest ebolavirus*	West Nile virus Lineage 2
Dengue virus Serotype 3	*Reston ebolavirus*	Yellow fever virus
Dengue virus Serotype 4	Lassa virus	
Protozoa		
Leishmania donovani	*Plasmodium vivax*	*Plasmodium ovale*
Plasmodium falciparum		
OFF-PANEL		
Bacteria		
Tested		
Acinetobacter baumannii	*Enterococcus faecium*	*Salmonella enterica* subs. *enterica* serovar Muenchen
Bacillus brevis	*Francisella persica* (formerly *Wolbachia persica*)	*Salmonella enterica* subs. *enterica* serovar Newport
Bacillus cereus	*Francisella philomiragia* (formerly *Yersinia*)	*Salmonella enterica* subs. *enterica* serovar Rubislaw
Bacillus circulans	*Klebsiella oxytoca*	*Salmonella enterica* susb. *enterica* serovar Saintpaul
Bacillus coagulans	*Legionella pneumophila*	*Salmonella enterica* subs. *enterica* serovar Tennessee
Bacillus halodurans	*Leptospira biflexa*	*Salmonella enterica* subs. *enterica* serovar Thompson
Bacillus licheniformis	*Leptospira meyeri*	*Salmonella enterica* subs. *enterica* serovar Typhimurium
Bacillus megaterium	*Leptospira terpstrae* genomospecies 4	*Salmonella enterica* subs. *houtenae*
Bacillus mycoides	*Leptospira vanthielii* genomospecies 3	*Salmonella enterica* subs. *indica*
Bacillus pumilus	*Leptospira wolbachii*	*Salmonella enterica* subs. *salamae*
Bacillus subtilis	*Leptospira yanagawae* genomospecies 5	*Salmonella enterica* subsp. *enterica* Paratyphi A
Bacillus thuringiensis	*Listeria monocytogenes*	*Salmonella enterica* subsp. *enterica* Typhi
Bacteroides fragilis	*Mycobacterium tuberculosis*	*Serratia marcescens*
Bordetella bronchiseptica	*Mycoplasma pneumoniae*	*Staphylococcus aureus*
Borrelia burgdorferi	*Neisseria meningitidis*	*Streptococcus agalactiae*
Brucella melitensis	*Orientia chuto (tsutsugamushi)*	*Streptococcus pneumoniae*
Burkholderia cepacia	*Proteus mirabilis*	*Streptococcus pyogenes*

Burkholderia mallei	*Pseudomonas aeruginosa*	*Treponema pallidum pallidum*
Burkholderia pseudomallei	*Rickettsia prowazekii*	*Vibrio cholerae*
Chlamydophila pneumoniae	*Rickettsia ricketsii*	*Yersinia aldovae*
Chlamydophila psittaci	*Rickettsia typhi*	*Yersinia bercovieri*
Citrobacter koseri	*Salmonella enterica* subs.*bongori*	*Yersinia entericolitica*
Clostridium bifermentans	*Salmonella enterica* subs. *arizonae*	*Yersinia fredericksenii*
Clostridium botulinum/sporogenes	*Salmonella enterica* subs. *diarizoniae*	*Yersinia intermedia*
Clostridium perfringens	*Salmonella enterica* subs. *enterica* serovar Enteritidis	*Yersinia kristensenii*
Clostridium sordellii	*Salmonella enterica* subs. *enterica* serovar Heidelberg	*Yersinia mollaretii*
Coxiella burnetii	*Salmonella enterica* subs. *enterica* serovar Javiana	*Yersinia pseudotuberculosis*
Enterobacter aerogenes	*Salmonella enterica* subs. *enterica* serovar Montevideo	*Yersinia rohdei*
Enterococcus faecalis	*Yersinia similis*	*Yersinia ruckeri*
***In silico* Analysis Only**		
Bacillus luciferensis		
Viruses		
Tested		
Adenovirus 1	HPIV-1	Omsk hemorrhagic fever
Adenovirus 3	HPIV-3	Parvovirus
Adenovirus 5	Hughes virus	Powassan virus
Aura virus	Human herpesvirus 6B	Rabies virus
Barmah Forest virus	Human immunodeficiency virus, type 1	Rift Valley fever virus
Bunyamwera virus	Human immunodeficiency virus, type 2	Zika virus
Coronavirus NL63	Human T-lymphotropic virus, type 1	Ross River virus
Cytomegalovirus	Human T-lymphotropic virus, type 2	Human respiratory syncytial virus
Dugbe virus	Influenza A H1N1-2009	Rubella virus
Eastern equine encephalitis virus	Influenza A H3N2	SARS-CoV-2
Enterovirus, HEV-71	Influenza B virus	Saint Louis encephalitis virus
Epstein Barr virus	Japanese encephalitis virus	Semliki Forest virus
Flexal virus	Junin virus (2 strains: XJ and Candid)	Sindbis virus
Guanarito virus	Machupo virus	Spondweni virus
Hantaan virus	Mayaro virus	Tickborne encephalitis virus
Hazara virus	Measles virus	Tonate virus
Hendra virus	Metapneumovirus	Una virus
Hepatitis A virus	Middelburg virus	Usutu virus
Hepatitis B virus	Mopeia virus	Vaccinia virus
Hepatitis C virus	Mumps virus	Varicella zoster virus
Herpes simplex virus type 2	Murray Valley encephalitis virus	Venezuelan equine encephalomyelitis virus

***In silico* Analysis Only**		
Avalon virus	Lymphocytic choriomeningitis virus	Sabia virus (Brazilian hemorrhagic fever)
Bas-Congo virus	Pirital virus	Variola major
Protozoa		
Tested		
Babesia microti	*Toxoplasma gondii*	*Trypanosoma cruzi*
Cyclospora cayetanensis	*Trypanosoma brucei*	*Trypanosoma cruzi*
Fungus		
Tested		
Aspergillus fumigatus	*Cryptococcus neoformans* var. *grubii*	
Helminths		
Tested		
Schistosoma mansoni		

Table 15 lists the BioFire Global Fever Special Pathogens Panel assays and corresponding organisms and viruses for which some cross-reactivity was identified (either empirically observed in testing or predicted by in silico analyses).

Table 15. Observed or Predicted Cross-Reactivity of the BioFire Global Fever Special Pathogens Panel

CROSS-REACTIVE ORGANISM	BIOFIRE GLOBAL FEVER SPECIAL PATHOGENS PANEL TEST RESULT
On-Panel	
Plasmodium knowlesi [1]	*Plasmodium vivax/ovale* Detected
Plasmodium malariae [2]	
Off-Panel	
Francisella hispaniensis [3]	*Francisella tularensis* Detected
Francisella tularensis subsp. *mediasiatica* [4]	
O'nyong-nyong virus	Chikungunya virus Detected
Crithidia fasciculata [5]	*Leishmania* spp. Detected
Leptomonas seymouri [6]	
Plasmodium berghei [7]	*Plasmodium spp.* and *Plasmodium vivax/ovale* Detected
Plasmodium brasilianum [7]	
Plasmodium cynomolgi [7]	
Plasmodium fieldi [7]	
Plasmodium fragile [7]	
Plasmodium inui [7]	
Plasmodium simiovale [7]	

[1] Cross-reactive with the Plasmodium vivax/ovale assay at concentrations ≥2.2E+04 copies/mL (~100×LoD).
[2] In silico analysis predicts potential cross-reactivity with the *Plasmodium vivax* assay; cross-reactivity was not observed at the concentration evaluated, 1.9E+05 copies/mL.
[3] Cross-reactivity was predicted by in silico analysis and observed during wet testing; *Francisella hispaniensis* is a pathogenic *Francisella* species.
[4] In silico analysis predicts cross-reactivity; the potential for *Francisella tularensis* subsp. *mediasiatica* to cause disease in humans is unknown.
[5] *Crithidia fasciculata* is a non-human infective trypanosomatid, however there have been very rare cases where a closely related *Crithidia* strain was found to be infective and act much like *Leishmania* spp.
[6] *Leptomonas seymouri* is an opportunistic parasite in immunocompromised individuals, particularly those infected with visceral leishmaniasis.
[7] *Plasmodium* spp. that typically infect non-human primates and rodents but are rarely found in humans.

Interference

Potentially interfering substances were selected for evaluation based upon whether the substance may normally be found in blood or may be introduced into blood specimens during collection, handling, or testing. These substances included endogenous substances (e.g., albumin, immunoglobulin, etc.), exogenous substances (e.g., antibiotic and antiviral drugs), microorganisms such as viral and bacterial species that may be present as co-infections, and technique-specific substances that may be introduced into a sample during routine laboratory handling including collection tubes.

The concentrations of endogenous and exogenous substances tested on the BioFire Global Fever Special Pathogens Panel were selected to represent a worst-case scenario based on a reference concentration of normal to high levels expected to be present in clinical specimens. Potentially competing microorganisms were tested at the highest levels possible based on stock concentrations. Technique-specific substances were tested at levels exceeding what would be expected to be found due to incidental contact, accidental addition, or during their use as solvents. Results are shown in Table 16.

Potential interference was only observed for heparin and TRIzol when testing analytes at near-LoD concentrations.

Table 16. Results for Potentially Interfering Substances Tested on the BioFire Global Fever Special Pathogens Panel

Potentially Interfering Substance	Concentration Tested	Results
Endogenous Substances		
Albumin	60.0 mg/mL	No interference
Bilirubin (Conjugated)	0.41 mg/mL	No interference
Bilirubin (Unconjugated)	0.41 mg/mL	No interference
Cholesterol (total)	4.2 mg/mL	No interference
Glucose	10.1 mg/mL	No interference
Hemoglobin	137.0 mg/mL	No interference
Immunoglobulins	60.0 mg/mL	No interference
Triglycerides	15.1 mg/mL	No interference
White Blood Cells	6.1E+06 cells/mL	No interference
Exogenous Substances		
Artemether-Lumefantrine	0.0004 mg/mL	No interference
Atovaquone	0.005 mg/mL	No interference
Proguanil	0.001 mg/mL	No interference
Mefloquine	0.0017 mg/mL	No interference
Amphotericin B	0.002 mg/mL	No interference
Pentamidine	0.0015 mg/mL	No interference
Fluconazole	0.026 mg/mL	No interference
Amoxicillin	0.062 mg/mL	No interference
Azithromycin	0.011 mg/mL	No interference
Ceftriaxone	1.0 mg/mL	No interference
Ciprofloxacin	0.012 mg/mL	No interference
Clindamycin	0.055 mg/mL	No interference
Doxycycline	0.02 mg/mL	No interference

Potentially Interfering Substance	Concentration Tested	Results
Gentamicin	0.036 mg/mL	No interference
Meropenem	0.39 mg/mL	No interference
Sulfamethoxazole	0.38 mg/mL	No interference
Vancomycin	0.12 mg/mL	No interference
Cycloserine	75.0 mg/mL	No interference
Isoniazid	0.06 mg/mL	No interference
Oseltamivir	0.0005 mg/mL	No interference
Ribavirin	0.011 mg/mL	No interference
Tenofovir	0.001 mg/mL	No interference
Acetaminophen	0.16 mg/mL	No interference
Aspirin (Acetylsalicylic Acid)	0.03 mg/mL	No interference
Ibuprofen	0.22 mg/mL	No interference
Prednisone	0.0001 mg/mL	No interference
Prednisolone	1.2 mg/mL	No interference
Cortisone	0.001 mg/mL	No interference
Artesunate	0.1 mg/mL	No interference
Competitive Microorganisms		
Corynebacterium diphtheriae	1:10 of Stock	No interference
Staphylococcus epidermidis	3.8E+06 CFU/mL	No interference
Escherichia coli	1:10 of Stock	No interference
Klebsiella pneumoniae	5.5E+04 CFU/mL	No interference
Haemophilus influenzae	1.0E+08 CFU/mL	No interference
Herpes Simplex virus	1.2E+05 PFU/mL	No interference
Epstein-Barr virus	3.3E+07 copies/mL	No interference
Cytomegalovirus (CMV) AD-169	1:10 of Stock	No interference
Human Immunodeficiency virus (HIV-1 and HIV-2)	1:10 of Stocks	No interference
Plasmodium vivax	1.5E+06 copies/mL	No interference
Technique Specific Substances		
Bleach	1% v/v	No interference
Povidone-iodine	1% v/v	No interference
Ethanol	2% v/v	No interference
TRIzol	2-3% v/v	Potentially Interfering
DMSO	2% v/v	No interference
Methanol	2% v/v	No interference
Saline	2% v/v	No interference
Chloroform	2% v/v	No interference
Acetone	2% v/v	No interference
Hydrochloric Acid (HCl)	0.0005N	No interference
Blood Collection Tubes		
Citrate (sodium)	~0.32%	No interference
EDTA in excess (5x)	~9.0 mg/mL	No interference
Heparin	~19.0 USP/mL	Potentially Interfering
Acid-citrate-dextrose (ACD)	2.2mg/mL (trisodium citrate) 0.8 mg/mL (citric acid) 2.5 mg/mL (dextrose)	No interference
Sodium polyanethenole sulfonate (SPS)	0.72 mg/mL	No interference
Serum Separation Tubes	N/A	No interference

Reproducibility

Assay reproducibility was evaluated using three contrived whole blood samples with a mixture of four representative panel analytes: one bacterium, one virus, and two protozoa. For each analyte, one sample was spiked at a moderate positive (3×LoD) level, another sample at a low positive (1×LoD) level, and the third sample was not spiked (negative). Six replicates of each sample were tested at three locations on five different days, providing a total of 90 replicate test results per sample. On each test day at each site, two different operators used three BioFire FilmArray 2.0 instruments; the BioFire Global Fever Special Pathogens Panel pouch lot was rotated daily. In total, 270 valid test results were obtained for the reproducibility evaluation of the BioFire Global Fever Special Pathogens Panel.

The primary assessment of reproducibility is based on a comparison of the observed test results (Detected/Not Detected) to the expected test results (Detected for spiked samples, Not Detected for un-spiked samples). The detection rate and percent agreement between observed and expected test results are shown in Table 17. The expected percent agreement was ≥95%. Based on the percent agreement between observed and expected test results, the reproducibility evaluation demonstrates that the BioFire Global Fever Special Pathogens Panel can provide accurate and highly reproducible test results in the context of multiple variables that may be expected in a clinical testing environment, including analyte concentration, location, test day, operator, instruments, and reagent lot.

Table 17. Reproducibility of the BioFire Global Fever Special Pathogens Panel

Analyte (Source / ID)		Concentration Tested (copies/mL)	Expected Result	Detection Rate (n/N) % Agreement with Expected Result [95% Confidence Interval]			
				Site 1	Site 2	Site 3	All Sites
Leptospira interrogans* serovar *icterohaemorrhagiae (ATTC / 23581)		Moderate Positive 3× LoD (1.0E+03)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
		Low Positive 1× LoD (3.4E+02)	Detected	27/30 90.0%	28/30 93.3%	26/30 86.7%	**81/90 90.0%** [82.1-94.6%]
		Negative (No Analyte)	Not Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
Dengue virus DENV-2 New Guinea C (Zeptometrix / 0810089CF)		Moderate Positive 3× LoD (1.0E+03)	Detected	29/30 96.7%	30/30 100%	30/30 100%	**89/90 98.9%** [94.0-99.8%]
		Low Positive 1× LoD (3.4E+02)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
		Negative (No Analyte)	Not Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
Leishmania donovani 1S (MHOM/SD/62/1S) (BEI / NR-48821)		Moderate Positive 3.4× LoD [1] (3.4E+01)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
		Low Positive 1.1× LoD [1] (1.1E+01)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
		Negative (No Analyte)	Not Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
Plasmodium falciparum	***Plasmodium* spp.**	Moderate Positive 1.5× LoD [2] (2.7E+02)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]

Analyte (Source / ID)		Concentration Tested (copies/mL)	Expected Result	Detection Rate (n/N) % Agreement with Expected Result [95% Confidence Interval]			
				Site 1	Site 2	Site 3	All Sites
IPC 4884 (BEI / MRA-1238)	**Detection Results**	Low Positive 0.5× LoD [2] (9.0E+01)	Detected	28/30 93.3%	30/30 100%	29/30 96.7%	**87/90 96.7%** [90.7-98.9%]
		Negative (No Analyte)	Not Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
	***Plasmodium falciparum* Detection Results**	Moderate Positive 1.5× LoD [2] (2.7E+02)	Detected	29/30 96.7%	30/30 100%	28/30 93.3%	**87/90 96.7%** [90.7-98.9%]
		Low Positive 0.5× LoD [2] (9.0E+01)	Detected	18/30 60.0%	24/30 80.0%	21/30 70.0%	**63/90 70.0%** [59.9-78.5%]
		Negative (No Analyte)	Not Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
Overall Agreement with Expected Result		**All Concentrations**	**All Results**	**1307/1350 96.8%** [95.7-97.2%]			

[1] Due to a correction in the stock concentration, *L. donovani* was evaluated at 3.4×LoD and 1.1×LoD.
[2] Due to a correction in the stock concentration, *P. falciparum* was evaluated at 1.5×LoD and 0.5×LoD.

Reproducibility Comparison Between BioFire FilmArray 2.0 and BioFire FilmArray Torch

Performance of the BioFire Global Fever Special Pathogens Panel was compared between the BioFire FilmArray 2.0 and BioFire FilmArray Torch systems by examining analyte detection of contrived whole blood samples. The testing incorporated a range of potential variation introduced by operator, instrument system, analyte concentration, and reagent lot for a total of 90 replicates for each analyte concentration distributed equally over three BioFire FilmArray 2.0 systems and three BioFire FilmArray Torch systems.

A summary of results (percent (%) agreement with the expected Detected or Not Detected result) for the analytes by BioFire FilmArray system is provided in Table 18**.** Overall agreement between observed and expected results was 99.5% for the BioFire FilmArray 2.0 system and 99.1% for the BioFire FilmArray Torch system demonstrating similar performance between both instrument systems.

Table 18. Reproducibility of the BioFire Global Fever Special Pathogens Panel Test Results on BioFire FilmArray 2.0 and BioFire FilmArray Torch

Analyte (Source / ID)		Concentration Tested (copies/mL)	Expected Result	Detection Rate (n/N) % Agreement with Expected Result							
				BioFire FilmArray 2.0 Platform				BioFire FilmArray Torch Platform			
				System 1	System 2	System 3	All FA 2.0 Systems [95% CI]	System 1	System 2	System 3	All FA Torch Systems [95% CI]
Leptospira interrogans* serovar *icterohaemorrhagiae (ATTC / 23581)		Moderate Positive 3×LoD (1.0E+03)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100]
		Low Positive 1×LoD (3.4E+02)	Detected	29/30 96.7%	29/30 96.7%	28/30 93.3%	**86/90 95.6%** [89.1-98.3%]	29/30 96.7%	28/30 93.3%	28/30 93.3%	**85/90 94.4%** [87.6-97.6I]
		Negative (No Analyte)	Not Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100]
Dengue virus DENV-2 New Guinea C (Zeptometrix / 0810089CF)		Moderate Positive 3×LoD (1.0E+03)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
		Low Positive 1×LoD (3.4E+02)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	29/30 96.7%	30/30 100%	**89/90 98.9%** [94.0-99.8%]
		Negative (No Analyte)	Not Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	29/30 96.7%	**89/90 98.9%** [94.0-99.8%]
Leishmania donovani 1S (MHOM/SD/62/1S) (BEI / NR-48821)		Moderate Positive 3×LoD (3.0E+01)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
		Low Positive 1×LoD (1.0E+01)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
		Negative (No Analyte)	Not Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
Plasmodium falciparum IPC 4884 (BEI / MRA-1238)	***Plasmodium* spp. Detection Results**	Moderate Positive 3×LoD (5.4E+02)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
		Low Positive 1×LoD (1.8E+02)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
		Negative (No Analyte)	Not Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
	***Plasmodium falciparum* Detection Results**	Moderate Positive 3×LoD (5.4E+02)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	29/30 96.7%	30/30 100%	**89/90 98.9%** [94.0-99.8%]
		Low Positive 1×LoD (1.8E+02)	Detected	30/30 100%	28/30 93.3%	29/30 96.7%	**87/90 96.7%** [90.7-98.9%]	30/30 100%	28/30 93.3%	28/30 93.3%	**86/90 95.6%** [89.1-98.3%]
		Negative (No Analyte)	Not Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]

Analyte (Source / ID)	Concentration Tested (copies/mL)	Expected Result	Detection Rate (n/N) % Agreement with Expected Result							
			BioFire FilmArray 2.0 Platform				BioFire FilmArray Torch Platform			
			System 1	System 2	System 3	All FA 2.0 Systems [95% CI]	System 1	System 2	System 3	All FA Torch Systems [95% CI]
Overall Agreement with Expected Result	**All Concentrations**	**All Results**	**1343/1350 99.5%** [98.9-99.8%]				**1338/1350 99.1%** [98.4-99.5%]			

Abbreviations: FA – FilmArray; 95% CI – 95% Confidence Interval

Specimen Storage

Stability of whole blood specimens was evaluated to support labeling recommendations for storage of samples at room temperature for up to 24 hours or at 2−8°C for up to seven days. These conditions were selected to be consistent with, or exceed, standard storage and transport conditions for most laboratory testing of clinical human whole blood specimens. Evaluation of frozen samples at ultra-low temperatures prior to testing was performed to support other analytical and clinical testing.

Testing was conducted using contrived samples composed of human whole blood and four representative BioFire Global Fever Special Pathogens Panel analytes in two mixes at a concentration of 3× their respective LoD or less. Organisms were selected to be representative of the analytes detected by the BioFire Global Fever Special Pathogens Panel, including bacteria, viruses, and protozoa. A time point zero was tested immediately after sample preparation as a no-storage control. Ten replicates were tested at each of the storage conditions. Two different room temperature conditions (18-21°C and 30°C) were also tested. The results are summarized in Table 19.

Table 19. Summary of Analyte Detections Over Total Number Tested by Storage Condition

Analyte	No Storage (Control)	Room Temperature		Refrigerated Storage 2-8°C			Ultra-low Freezing ≤ -70°C
		Ambient 18-21°C for 24 Hours	Upper Limit 30°C for 24 Hours[1]	Day 1	Day 3	Day 7	
Leptospira interrogans	10/10	10/10		10/10	10/10	10/10	10/10
	10/10		10/10				
Dengue virus	10/10	10/10		10/10	10/10	10/10	10/10
	10/10		10/10				
Leishmania donovani	10/10	10/10		10/10	10/10	10/10	10/10
	10/10		10/10				
Plasmodium falciparum	10/10	10/10		10/10	10/10	10/10	10/10
	10/10		10/10				

[1] Testing of the upper limit of room temperature storage, 30°C, was performed separately and required a separate no storage control.

VIII. Assayed External Controls

Name of Device: BIOFIRE® SHIELD™ Control Kit for the BioFire Global Fever Special Pathogens Panel
Common or Usual Name: Same
Product Code: PMN
Regulation: 21 CFR 866.3920
Classification Name: Assayed quality control material for clinical microbiology assays
Regulatory Class: Class II (Special Controls)
Panel: Microbiology – 83
Predicate Device: BIOFIRE® SHIELD™ Control Kit for the Global Fever Panel (BioFire Defense, LLC) [K202382]
This predicate has not been subject to a design-related recall.

Device Description

The BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens (GF SP) Panel is a surrogate control to monitor performance of the BioFire GF SP Panel assays. The BIOFIRE SHIELD Control Kit for the BioFire GF SP Panel is designed to mitigate the risk of control contamination or misuse when evaluating clinical specimens on BioFire FilmArray Systems. Good laboratory practice recommends running positive and negative external controls regularly. Evaluation of external controls is recommended prior to using a new shipment or new lot of BioFire GF SP Panel kits, when there is a new operator, and following replacement or repair of a BioFire FilmArray System. It is the responsibility of each laboratory to determine the frequency of external control testing with the BioFire GF SP Panel as part of the laboratory's Quality Control program. Quality control materials should be used in accordance with local, state, federal regulations and accreditation requirements.

Materials provided in each BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel:

- Six individually packaged Positive External Control Injection Vials
- Six individually packaged Negative External Control Injection Vials
- Instructions available online at *www.biofiredefense.com*:
    - *BIOFIRE® SHIELD™ Control Kit for the BioFire Global Fever Special Pathogens Panel – Instructions for Use*
    - *BIOFIRE® SHIELD™ Control Kit for the BioFire Global Fever Special Pathogens Panel – Quick Guide*

Materials required but not provided:

- BioFire® FilmArray® System including:
    - BioFire® FilmArray® 2.0 or BioFire® FilmArray® Torch Instrument System including accompanying platform-specific core software
    - BioFire® FilmArray® Pouch Loading Station

- BioFire® Global Fever Special Pathogens Panel (Part No. DFA2-ASY-0018) and accompanying pouch module software
- 10% bleach solution or a similar disinfectant

Intended Use

The BIOFIRE® SHIELD™ Control Kit for the BioFire® Global Fever Special Pathogens Panel contains Positive and Negative External Controls intended for use as assayed quality controls to monitor the performance of *in vitro* diagnostic laboratory nucleic acid testing procedures for the qualitative detection of *Bacillus anthracis*, chikungunya virus, Crimean-Congo hemorrhagic fever virus, dengue virus (serotypes 1, 2, 3, and 4), *Ebolavirus* spp., *Francisella tularensis*, Lassa virus, *Leishmania* spp., Leptospira spp., *Marburgvirus, Plasmodium* spp. (including species differentiation of *Plasmodium falciparum* and *Plasmodium vivax/ovale*), West Nile virus, yellow fever virus, and *Yersinia pestis* when using the BioFire® Global Fever Special Pathogens Panel on BioFire® FilmArray® 2.0 and BioFire® FilmArray® Torch Systems. The BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel is designed for and intended to be used solely with the BioFire Global Fever Special Pathogens Panel. This product does not replace manufacturer internal controls provided as part of the BioFire Global Fever Special Pathogens Panel.

Both the Positive and Negative External Controls are provided in a FilmArray Control Injection Vial format. The Positive Control Injection Vial contains dried synthetic DNA segments in buffer and stabilizer to assess the presence of each individual assay on the BioFire Global Fever Special Pathogens Panel. The Negative Control Injection Vial contains no DNA and is non-reactive with the BioFire Global Fever Special Pathogens Panel assays.

For In Vitro Diagnostic Use.

Substantial Equivalence

Element	Subject Device: BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel	Predicate: BIOFIRE SHIELD Control Kit for the BioFire Global Fever Panel [K202382]
Device Description	Positive and negative external assayed quality controls to monitor assay performance in the BioFire Global Fever Special Pathogens Panel	Positive and negative external assayed quality controls to monitor assay performance in the BioFire Global Fever Panel
Physical Format	External control material dried on Control Injection Vial filter	Same as subject device
Composition	Tm-shifted synthetic DNA (positive control only)	Same as subject device
Targets Monitored	*Bacillus anthracis*, chikungunya virus, Crimean-Congo hemorrhagic fever virus, dengue virus (serotypes 1, 2, 3, and 4), *Ebolavirus* spp., *Francisella tularensis*, Lassa virus, *Leishmania* spp., *Leptospira* spp., *Marburgvirus*, *Plasmodium* spp. (including species differentiation of *P. falciparum* and *P. vivax/ovale*), West Nile virus, yellow fever virus, and *Yersinia pestis*	Chikungunya virus, dengue virus (serotypes 1, 2, 3, and 4), *Leptospira* spp., *Plasmodium* spp. (including species differentiation of *P. falciparum* and *P. vivax/ovale*)
Instrumentation	BioFire Global Fever Special Pathogens Panel run on BioFire FilmArray 2.0 or BioFire FilmArray Torch systems	BioFire Global Fever Panel run on BioFire FilmArray 2.0
Test Interpretation	Automated test interpretation and report generation; user cannot access raw data	Same as subject device
Reagent Storage	Room temperature	Same as subject device

Summary of Performance Data

Reproducibility

Reproducibility for the BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel was evaluated both for the BioFire FilmArray 2.0 System and the BioFire FilmArray Torch System. Reproducibility on the BioFire FilmArray 2.0 System was evaluated using SHIELD lots from three different manufacturing events at three test sites by two operators and three instruments per test site using three different BioFire GF SP Panel reagent lots. Testing took place over 5 days at each site. Reproducibility on the BioFire FilmArray Torch was performed in a similar manner with the exception that sites were simulated using three different BioFire FilmArray Torch Systems. Reproducibility was evaluated by calculating the percent agreement between observed test results (Passed or Failed) and expected test results (Passed) for both negative and positive SHIELD controls. Table 20 and Table 21 show summarized results for BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems, respectively.

Table 20. Multi-Site Reproducibility Test Results of the BIOFIRE SHIELD Control Kit for the BioFire GF SP Panel on BioFire FilmArray 2.0 Systems

SHIELD Control Type	Expected Result	Observed/Expected (Percent Agreement) [95% Confidence Interval]			
		Site 1	Site 2	Site 3	All Sites
Positive	Passed [1]	42/45 (93.3%)	45/45 (100%)	45/45 (100%)	**132/135 (97.8%)** [93.7-99.2%]
Negative	Passed [2]	45/45 (100%)	44/45 [3] (97.8%)	44/45 [4] (97.8%)	**133/135 (98.5%)** [94.8-99.6%]
Overall Agreement with Expected Result		**265/270 (98.1%)** [95.7-99.2%]			

[1] All BioFire GF SP Panel assays have a positive amplicon melt in the SHIELD control melt range.
[2] All BioFire GF SP Panel assays have no melt in both the SHIELD control melt range and in the pathogen melt range.
[3] Unexpected detection of pathogen amplicon for *Leishmania* spp.
[4] Unexpected detection of pathogen amplicon for dengue virus.

Table 21. Reproducibility Test Results of the BIOFIRE SHIELD Control Kit for the BioFire GF SP Panel on BioFire FilmArray Torch Systems

SHIELD Control Type	Expected Result	Observed/Expected (Percent Agreement)			
		FA Torch System 1	FA Torch System 2	FA Torch System 3	Overall [95% Confidence Interval]
Positive	Passed [1]	43/45 (95.6%)	43/45 (95.6%)	44/45 (97.8%)	**130/135 (96.3%)** [91.6-98.4%]
Negative	Passed [2]	43/45 (95.6%)	45/45 (100%)	45/45 (100%)	**133/135 (98.5%)** [94.8-99.6%]
Overall Agreement with Expected Result		**263/270 (97.4%)** [94.7-98.7%]			

[1] All BioFire GF SP Panel assays have a positive amplicon melt in the positive SHIELD control melt range.
[2] All BioFire GF SP Panel assays have no melt in either the positive SHIELD control melt range or the pathogen melt range.

Repeatability

Repeatability was performed by a single operator testing 45 Positive and 45 Negative External Controls from a single BIOFIRE SHIELD Control Kit, using a single BioFire GF SP Panel reagent lot on one BioFire FilmArray 2.0 instrument over a period of 14 days. The primary assessment is based on a comparison of the observed External Control test results (Passed/Failed) to the expected test results (Passed). Table 22 shows the results for the Repeatability evaluation.

Table 22. Summary of BIOFIRE SHIELD Control Kit for the BioFire GF SP Panel Repeatability Test Results

SHIELD Control Type	Expected Result	Observed/Expected (Percent Agreement)
Positive	Passed [1]	45/45 (100%)
Negative	Passed [2]	45/45 (100%)

[1] All BioFire GF SP Panel assays have a positive amplicon melt in the positive SHIELD control melt range.
[2] All BioFire GF SP Panel assays have no melt in either the positive SHIELD control melt range or the pathogen melt range.

Clinical Evaluation

Six clinical sites evaluated the BIOFIRE SHIELD Control Kit by testing a Positive or Negative External Control each day prior to testing clinical specimens. Results for the BioFire GF SP Panel on BioFire FilmArray 2.0 Systems are shown in Table 23.

Table 23. Summary of BIOFIRE SHIELD Control Kit for the BioFire GF SP Panel Clinical Evaluation Results

SHIELD Control Type	Completed with Passed Result	Total Completed	Percent Passed (%)
Positive	158 [a]	160	98.8%
Negative	157 [a]	159	98.7%
Overall	**315**	**319**	**98.7%**

[a] Site tested a Positive and a Negative External Control on the same day. Controls were most likely swapped as the Negative External Control failed because all External Control targets were Detected, and the Positive External Control failed because all External Control targets were Not Detected.