REF DFA2-ASY-0018

BioFire® Global Fever Special Pathogens Panel Instructions for Use

IVD

℞ Only

The Symbols Glossary is provided on Page 61 of this booklet.

For In Vitro Diagnostic Use

Manufactured by

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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.

SUMMARY AND EXPLANATION OF THE TEST

The BioFire Global Fever Special Pathogens (GF SP) Panel conducts sixteen tests for the simultaneous identification of bacterial, viral, and protozoan pathogens directly from whole blood specimens (**Table 1**Table 1). Results are available in about one 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.

Summary of Detected Organisms

Bacteria

Bacillus anthracis is a gram-positive spore-forming bacterium and is the etiological agent of anthrax. Anthrax is endemic to the United States but is uncommon[1]. Outside the U.S. the disease occurs most commonly as a cutaneous infection among persons working closely with animals or animal products through the introduction of spores subcutaneously. Anthrax may also occur through inhalation or ingestion of spores.[2,3] *B. anthracis* has been a focus of biological warfare and is classified as a select agent per the Federal Select Agent Program.[1]

Francisella tularensis is an aerobic, gram-negative coccobacillus and is the causative agent of tularemia. Tularemia occurs throughout North America in addition to Europe, Asia, and the Middle East although it is relatively uncommon. The disease affects both animals and humans and is transmitted primarily through contact with infected animals or bites from ticks and flies.[4] Transmission may also occur through the inhalation of dust or aerosols containing the bacterium.[5] Use of *F. tularensis* as a bioweapon has historically been investigated and *F. tularensis* is designated as a select agent per the Federal Select Agent Program due to the potential threat to national security.[1]

***Leptospira* spp.** (Family *Leptospiraceae*) are spirochete bacteria and the causative agents of leptospirosis. Leptospirosis is a zoonotic disease with worldwide distribution. *Leptospira* bacteria are transmitted through direct contact with urine or tissues from infected animals, or indirectly through contaminated soil or water. The exposure may occur through abrasions and cuts in the skin, or mucous membranes.[6–8] The genus is currently divided into three subgroups, however only one subgroup of the species is known to be pathogenic. The pathogenicity of the Group I members ranges from subclinical infections to severe disease and death. The Group I species most responsible for severe leptospirosis include *L. interrogans*, *L. kirschneri*, and *L. noguchii*.[8]

Yersinia pestis is an aerobic gram-negative bacterium which causes plague. The disease is spread to humans from rodents and other wild animals by flea bites, although contact with contaminated fluids or tissue are also modes of transmission.[9] Within the United States *Y. pestis* is endemic to rural areas in all Western states and the range is expanding.[9] However, human disease within the U.S. is uncommon. The disease manifests as three major clinical syndromes: bubonic plague, septicemic plague, and pneumonic plague. Of the three, bubonic plague is the most common and is characterized by swelling in lymph nodes (bubo).[10] Historically there have been three major pandemics of plague, and small outbreaks of plague continue to occur.[11] *Y. pestis* has been a focus of biological warfare efforts and has been designated as a select agent per the Federal Select Agent Program.[1]

Viruses

Chikungunya virus is a positive-sense single-stranded RNA virus (genus *Alphavirus*). The chikungunya virus is transmitted to humans by infected mosquitoes of the species *Aedes aegypti* and *Aedes albopictus*. The infection causes severe joint pain; chikungunya virus is closely related to several other alphaviruses that are known to cause arthritis including o'nyong-nyong virus and Mayaro virus.[12,13] Chikungunya infections are rarely lethal, but symptoms may be severe and disabling. Persons at risk for more severe disease include infants, and older adults, as well as persons with underlying medical conditions such as high blood pressure, diabetes, or heart disease.[14] Differential diagnosis of chikungunya disease can be difficult due to overlapping symptoms, transmission, and geographic distribution of other viruses such as dengue virus and Zika virus.[14,15]

Crimean-Congo hemorrhagic fever (CCHF) virus is a negative-stranded, enveloped RNA virus and is a member of the *Nairovirus* genus. CCHF is a zoonotic disease transmitted primarily by bites from ticks of the genus *Hyalomma.* Contact with blood and bodily fluids from infected animals or persons is also a mode of transmission.[16] Wild animals and domestic livestock are the primary reservoirs of the virus which is endemic to the Middle East, southeastern Europe, and parts of Africa, and Asia.[17,18] Infections are uncommon within the United States. CCHF is classified as a select agent per the Federal Select Agent Program. [1]

Dengue virus is a positive-sense single-stranded RNA virus (genus *Flavivirus*). Four serotypes of dengue virus have been identified. The serotypes are both phylogenetically and antigenically distinct and acquired long-term immunity from one serotype does not extend to the other three. The viruses are transmitted through the mosquito species *Aedes aegypti* and *Aedes albopictus.* [19,20] While the majority of dengue infections are asymptomatic, the most severe cases may result in life-threatening dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS).[21] The DHF and DSS forms of the disease are most often associated with secondary infections of a different serotype, which is especially of concern in regions where all four serotypes are co-circulating. [19,22] Other endemic viruses in these regions such as chikungunya virus and Zika virus have similar symptoms and transmission which complicate differential diagnosis. [15,21]

***Ebolavirus* spp.** are linear negative-sense, single-stranded RNA viruses of the family *Filoviridae* and are the etiological agent of Ebola virus disease. The *Ebolavirus* genus contains six species, and these species are named for the regions in which they were recognized: Bombali, Bundibugyo, Reston, Sudan, Taï Forest, and Zaire.[23,24] Of these species only Bundibugyo, Sudan, Taï Forest, and Zaire are known to cause disease in humans. The Zaire species has been responsible for the majority of Ebola virus disease outbreaks.[23] The Reston species causes disease in non-human primates and pigs.[25] Ebola virus disease is uncommon in the United States. The viruses spread through direct contact with blood, body fluids, or tissues from infected persons or animals. Transmission through sexual contact with an infected individual is also possible.[25] Due to the risk to national security ebolaviruses are designated as a select agent per the Federal Select Agent Program.[1]

Lassa virus causes Lassa fever, a zoonotic viral illness endemic to regions of West Africa and uncommon within the United States.[26] The virus is a bi-segmented single-stranded RNA virus of the *Arenaviridae* family. Transmission occurs primarily through direct or indirect contact with urine and feces from the rodent *Mastomys natalensis*, which is the primary reservoir of the virus. Transmission from an infected individual is also possible through direct contact with bodily fluids.[27] Lassa fever manifests as a broad range of symptoms, and approximately 80% of infected individuals have only mild symptoms.[28] Lassa virus is categorized as a select agent in the Federal Select Agent Program due to the potential threat to national security.[1]

Marburgviruses are linear, non-segmented, negative-sense, single-stranded RNA viruses that belong to the same family as Ebola virus (*Filoviridae*). Two lineages have been characterized to date: Marburg virus (MARV) and Ravn virus (RAVV). Viruses from both lineages cause Marburg virus disease and infections are rare.[29] The primary reservoir of *Marburgvirus* is believed to be the African fruit bat (*Rousettus aegyptiacus*), and the viruses are suspected to be endemic throughout most of sub-Saharan Africa.[30] The mechanism of transmission to humans is not currently known. However, person-to-person transmission occurs through direct contact with blood and body fluids. Sexual transmission is also possible.[31] *Marburgvirus* has been designated as a select agent in the Federal Select Agent Program due to the potential threat to national security.[1]

West Nile virus is a positive-sense, single-stranded RNA virus of the *Flavivirus* genus (*Flaviviridae*). The virus is widely distributed throughout the world.[32] The virus causes West Nile fever and is primarily transmitted to humans through mosquito bites. Up to 80% of West Nile virus infections are asymptomatic in humans.[33,34] Mild cases of West Nile fever are clinically indistinguishable from disease caused by other *Flaviviruses* such as dengue fever. In rare cases a more severe illness affecting the central nervous system may develop.[34,35] West Nile virus has been identified in multiple mosquito species. However, mosquitos of the *Culex* genus are believed to be responsible for most transmission. *Culex* species primarily feed on birds which are a natural reservoir of the virus.[36]

Yellow fever virus is a positive-sense, single-stranded RNA virus. The virus belongs to the *Flavivirus* genus (*Flaviviridae*) and is primarily transmitted through the mosquito of the *Aedes* or *Haemagogus* genuses.[37] The mosquito vectors facilitate both sylvatic transmission as well as urban human-to-human transmission.[38] Yellow fever disease is endemic to tropical regions of South America and sub-Saharan Africa and cases within the United States are typically travelers to these regions.[39] Most patients have no symptoms or only mild febrile symptoms. In a small number of patients more severe disease may develop including renal dysfunction and hemorrhage which is often fatal.[40,41]

Protozoa

***Leishmania* spp.,** a group of protozoan parasites belonging to the genus *Leishmania*, cause leishmaniasis. The parasites are transmitted through the bites of phlebotomine sand flies and are widely distributed throughout the world, although uncommon within the United States.[42] Leishmaniasis may manifest as either cutaneous leishmaniasis, which causes skin lesions, or visceral leishmaniasis, which affects the internal organs.[42] Visceral infections are often asymptomatic but can also cause severe disease which may be fatal.[43,44] Visceral leishmaniasis is primarily caused by infections of the species *L. donovani* and *L. infantum.*[45] However, *Leishmania* spp. associated with cutaneous leishmaniasis may also develop as visceral leishmaniasis in rare cases.[46,47] The BioFire Global Fever Special Pathogens Panel detects all species within the *Leishmania* genus, including visceral and cutaneous species; however, reduced sensitivity was observed for *L. infantum*. Note that only visceral leishmaniasis is expected to be detected in whole blood specimens tested with the BioFire Global Fever Special Pathogens Panel.

Plasmodium – Malaria in humans is primarily caused by five species of the *Plasmodium* genus (Family *Plasmodiiae*); *P. falciparum, P. knowlesi, P. malariae, P. ovale, P. vivax.* Transmission of these parasitic protozoans occurs through bites from female mosquitoes of the *Anopheles* genus. The five species are found in various geographical locations, and treatments vary depending on the species and whether there is known drug resistance in the area. Early identification of the *Plasmodium* species is important for selecting the appropriate treatment.[48] The most common malaria infections are caused by *P. falciparum* and *P. vivax,* whereas infections of *P. ovale* and *P. malariae* are less common.[48,49] *P. knowlesi* is emerging as a significant cause of zoonotic malaria in Southeast Asia. [48,50,51] Co-infection with multiple *Plasmodium* species is possible and should always be considered.[48,50] The GF SP Panel detects all *Plasmodium* species known to infect humans and provides species identification for *P. falciparum* and *P. vivax/ovale*.

Plasmodium falciparum is found in tropical and subtropical areas worldwide but predominates in Africa. The species is capable of rapid multiplication in the blood which can result in rapidly progressive severe illness.[49,50] In the most severe cases the infection can affect the brain and cause cerebral malaria which may be fatal.[48,50] In some areas *P. falciparum* has developed resistance to the malaria treatment drugs chloroquine and mefloquine.[50,52]

Plasmodium vivax is primarily found in Asia and Latin America, as well as some parts of Africa. Severe malaria is less common in *P. vivax* infections, but additional treatment for dormant hypnozoites in the liver is required to prevent relapse of disease.[48–50,52] *P. vivax* is generally considered sensitive to chloroquine, however in Papua New Guinea and Indonesia there is a high prevalence of chloroquine-resistance.[50]

Plasmodium ovale is predominantly found in West Africa and infection is less likely to result in severe cases of malaria as compared to *P. falciparum*.[48–50,52] Similar to *P. vivax*, relapse of disease may occur unless infections are treated for dormant hypnozoites in the liver.[48,50,52] No wide-spread resistance to chloroquine has been reported for *P. ovale*.[50]

Plasmodium malariae is distributed worldwide in malaria regions, however its prevalence is lower than that of other species. Because of its slower life cycle and low level of infection, malaria symptoms may be less pronounced, and symptomatic patients should be evaluated for co-infection with other *Plasmodium* species. Untreated, *P. malariae* may result in chronic infections that last many years.[48–50] No widespread resistance to chloroquine has been observed in *P. malariae.*[50,52]

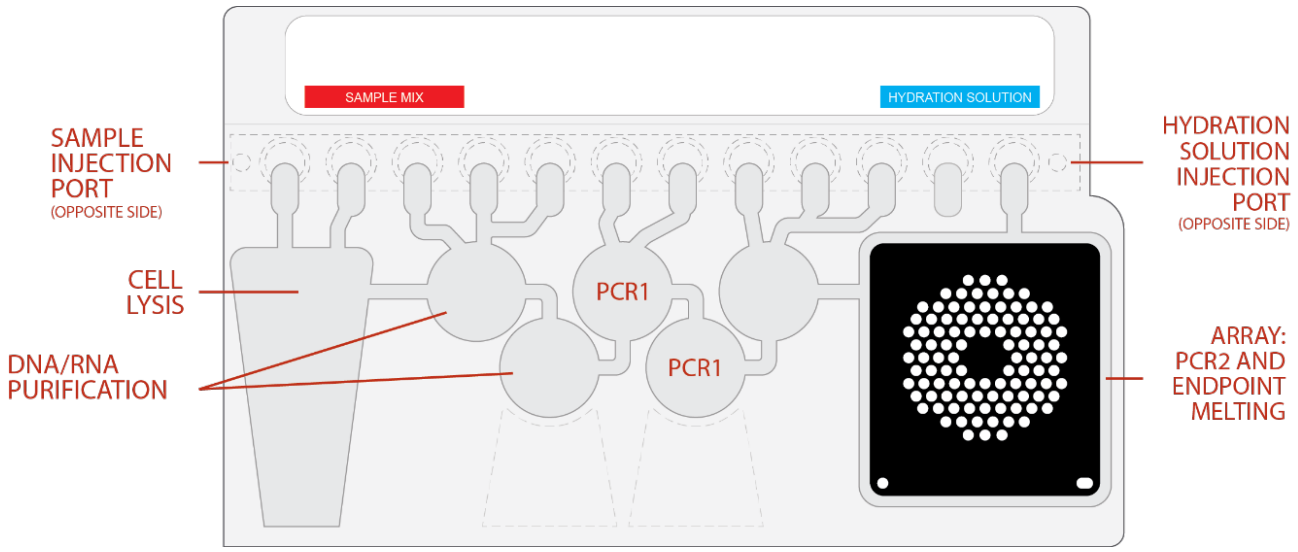
Plasmodium knowlesi is a zoonotic malaria parasite found throughout Southeast Asia, particularly in the states of Sarawak and Sabah in Malaysia. The primary host of *P. knowlesi* is macaques, and transmission of the parasite to humans is limited to persons exposed to mosquitoes that feed on both humans and macaques.[48,51] As with *P. falciparum*, *P. knowlesi* reproduces rapidly in the blood and may result in rapidly progressive severe illness and/or death. No resistance to chloroquine has been documented in *P. knowlesi.*[50,52]

PRINCIPLE OF THE PROCEDURE

The BioFire GF SP Panel pouch is a closed-system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple pathogens within a single whole blood specimen. After sample collection, the user injects BIOFIRE® FILMARRAY® Hydration Solution and sample combined with BIOFIRE® FILMARRAY® Sample Buffer into the pouch, places the pouch into a BIOFIRE® FILMARRAY® System instrument module and starts a run. The run process takes about an hour. Additional details can be found in the appropriate BIOFIRE® FILMARRAY® operator's manual.

During a Run, the BIOFIRE FILMARRAY System:

- Lyses the sample by agitation (bead beating) in addition to chemical lysis mediated by the Sample Buffer.
- Extracts and purifies all nucleic acids from the sample using magnetic bead technology.
- Performs nested multiplex PCR by:
  - First performing reverse transcription and a single, large volume, highly-multiplexed reaction (PCR1).
  - Then performing multiple singleplex second-stage PCR reactions (PCR2) to amplify sequences within the PCR1 products.
- Uses endpoint melting curve data to detect and generate a result for each target on the BioFire GF SP Panel.



MATERIALS PROVIDED

Each BioFire GF SP Panel kit contains sufficient reagents to test 6 samples (Part No. DFA2-ASY-0018). Materials include:

- Individually packaged BioFire GF SP Panel pouches
- Single-use (1.0 mL) BIOFIRE FILMARRAY Sample Buffer tubes
- Single-use pre-filled (1.5 mL) BIOFIRE FILMARRAY Hydration Injection Vials (blue)
- Individually packaged BIOFIRE FILMARRAY Sample Injection Vials (red)
- Individually packaged Transfer Pipettes
- Instructions available online at: *www.biofiredefense.com/gfspecialpathogens/*
  - *BioFire Global Fever Special Pathogens Panel – Instructions for Use*
  - *BioFire Global Fever Special Pathogens Panel – Quick Guide*

NOTE: *Additional documentation is available online at www.biofiredefense.com*

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 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

ADDITIONAL AVAILABLE MATERIALS

- BIOFIRE® SHIELD™ Control Kit for the BioFire Global Fever Special Pathogens Panel (Part No. DFA2-ASY-0019)
  - The BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel provides assayed positive and negative external controls for use with BioFire Global Fever Special Pathogens Panel.
  - See the *BIOFIRE® SHIELD™ Control Kit for the BioFire Global Fever Special Pathogens Panel Instructions for Use* for further information.

WARNINGS AND PRECAUTIONS

Precautions Related to Public Health

A health care professional, with training in principles and use of infectious disease diagnostics and reporting of results, should carefully interpret the results from the BioFire GF SP Panel in conjunction with a patient's signs and symptoms, and results from other diagnostic tests.

All pathogens detected on this panel except for *Leishmania* spp. must be reported to the Centers for Disease Control and Prevention (CDC). Local, state, and federal regulations for notification of reportable disease are continually updated and include a number of pathogens for surveillance and outbreak investigations.[23,24] Laboratories are responsible for following their state and/or local regulations and should consult their local and/or state public health laboratories for isolate and/or clinical sample submission guidelines.

Diagnostic testing for viral hemorrhagic fevers should be performed only after consultation with public health officials including state or local health departments and the Centers for Disease Control (CDC).

The following pathogens detected by the BioFire GF SP Panel are select agents: *Bacillus anthracis*, Crimean-Congo hemorrhagic fever virus, *Ebolavirus* spp., *Francisella tularensis*, Lassa virus, *Marburgvirus*, and *Yersinia pestis*. Positive results for select agent pathogens are presumptive. If a Detected result is obtained for one or more of these pathogens, follow national public health requirements for reporting and follow-up testing. In the United States, the Centers for Disease Control and Prevention (CDC) should be notified immediately for further characterization and confirmation.

General Precautions

1. Always check the expiration date on the pouch. Do not use a pouch after its expiration date.
2. BIOFIRE FILMARRAY pouches are stored under vacuum in individually wrapped canisters. To preserve the integrity of the pouch vacuum for proper operation, be sure that a BIOFIRE FILMARRAY instrument/module will be available and operational before unwrapping any pouches for loading.
3. Bleach introduced in a sample may damage nucleic acids in the sample, which may lead to a false negative result.

Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable clean powder-free gloves and lab coats. Protect skin, eyes, and mucus membranes. Change gloves often when handling reagents or samples.
2. Handle all samples and waste materials as if they are capable of transmitting infectious agents. Observe safety guidelines such as those outlined in:
   - CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*[53]
   - CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections* [54]
3. Follow your institution's safety procedures for handling biological samples.
4. Dispose of materials used in this test (including reagents, samples, and used buffer tubes) according to federal, state, and local regulations.
5. Sample Buffer is assigned the following classifications:
   - Acute toxicity (Category 4),
   - Serious eye damage (Category 1), and
   - Skin irritation (Category 2).

   Please refer to the *BioFire Global Fever Panel Safety Data Sheet* (SDS) for more information.
6. Sample Buffer will form hazardous compounds and fumes when mixed with bleach or other disinfectants.

   WARNING: To avoid generating chlorine gas, never add bleach to Sample Buffer or sample waste.
7. Bleach, a recommended disinfectant, is corrosive and may cause severe irritation or damage to eyes and skin. Vapor or mist may irritate the respiratory tract. Bleach is harmful if swallowed or inhaled.
   - Eye contact: Hold eye open and rinse with water for 15-20 minutes. Remove contact lenses after the first 5 minutes and continue rinsing eye. Seek medical attention.
   - Skin contact: Immediately flush skin with plenty of water for at least 15 minutes. If irritation develops, seek medical attention.
   - Ingestion: Do not induce vomiting. Drink a glassful of water. If irritation develops, seek medical attention.
   - Please refer to the appropriate Safety Data Sheet (SDS) for more information.

Laboratory Precautions

1. Preventing Sample Contamination

Due to the sensitive nature of the BioFire GF SP Panel, it is important to guard against contamination of the sample and work area by carefully following the testing process outlined in this instruction document, including these guidelines:

- To avoid potential contamination, handle specimens in a biosafety cabinet. If a biosafety cabinet is not available, a dead air box, a splash shield, or a face shield should be used when preparing specimens for testing.
- Do not handle specimens or pouches in a biosafety cabinet which is used for manipulating pathogen cultures.
- Clinical specimens should not be centrifuged before testing.
- Prior to processing samples, thoroughly clean both the work area and the BIOFIRE FILMARRAY Pouch Loading Station using a suitable cleaner such as freshly prepared 10% bleach or a similar disinfectant. To avoid residue buildup and potential damage to the sample or interference from disinfectants, wipe disinfected surfaces with water.
- Samples and pouches should be handled and/or tested one-at-a-time. Always change gloves and clean the work area between each pouch and sample.
- Use clean gloves to remove materials from bulk packaging bags and reseal bulk-packaging bags when not in use.
- In the case of a select agent Detected result or a pouch leak, refer to Decontamination Procedures below.

2. Preventing Amplicon Contamination

A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicon. Because the BioFire GF SP Panel pouch is a closed system, the risk of amplicon contamination is low, provided that the recommended procedures are followed, and pouches remain intact after the test is completed. Adhere to the following guidelines, in addition to those above, to prevent amplicon contamination:

- Discard used pouches in a biohazard container immediately after the run has completed.
- Avoid excessive handling of pouches after test runs.
- Change gloves after handling a used pouch.
- Avoid exposing pouches or Sample Injection Vials to sharp edges or anything that might cause a puncture.

WARNING: If liquid is observed on the exterior of a pouch, the liquid and pouch should be immediately contained and discarded in a biohazard container. The instrument and workspace must be decontaminated. Refer to the Decontamination Procedures section in this document and the appropriate BIOFIRE FILMARRAY operator's manual.
DO NOT PERFORM ADDITIONAL TESTING UNIL THE AREA HAS BEEN DECONTAMINATED

REAGENT STORAGE, HANDLING, AND STABILITY

1. Store the test kit, including reagent pouches and buffers, at room temperature (18-30°C). **DO NOT REFRIGERATE**.
2. Avoid storage of any materials near heating or cooling vents, or in direct sunlight.
3. All kit components should be stored and used together. Do not use components from one kit with those of another kit. Discard any extra components from the kit after all pouches have been consumed.
4. Do not remove pouches from their packaging until a sample is ready to be tested. Once the pouch packaging has been opened, the pouch should be loaded as soon as possible (within approximately 30 minutes).
5. Once a pouch has been loaded, the test run should be started as soon as possible (within approximately 60 minutes). Do not expose a loaded pouch to temperatures above 40°C (104°F) prior to testing.
6. Always check the kit expiration date and do not use reagents beyond the expiration date printed on the pouch or kit.

SAMPLE REQUIREMENTS

The following table describes the recommended requirements for sample collection, preparation, and handling that will help ensure accurate test results.

Recommended Specimen Type	Human Whole Blood collected in EDTA tubes
Minimum Sample Volume	~0.2 mL (200 µL) of whole blood
Specimen Transport and Storage	Specimens should be tested with the BioFire GF SP Panel as soon as possible. If storage is required, samples can be held: • At room temperature for up to 1 day (15-30ºC) • Refrigerated for up to 7 days (2-8ºC)

NOTE: *Bleach can damage organisms/nucleic acids within the specimen, potentially causing false negative results. Contact between bleach and specimens during collection, disinfection, and testing procedures should be avoided.*

PROCEDURE

Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one BioFire GF SP Panel pouch at a time and change gloves between samples and pouches. Once sample is added to the pouch, promptly transfer to the instrument to start the run. After the run is complete, discard the pouch in a biohazard container.

There is a risk of false positive results due to contamination of the specimen or testing area with organisms, their nucleic acid, or amplified product. Particular attention should be given to the laboratory precautions noted under the *Warnings and Precautions* section.

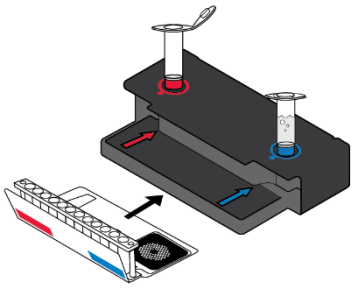
Refer to the *BioFire Global Fever Special Pathogens Panel Quick Guide* or the appropriate BIOFIRE FILMARRAY operator's manual for more details.

Step 1: Prepare Pouch

1. Thoroughly clean the work area and the BIOFIRE FILMARRAY Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.
2. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.

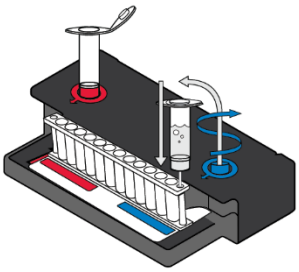
   NOTE: *The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.*

3. Check the expiration date on the pouch. Do not use expired products.
4. Insert the pouch into the Pouch Loading Station, aligning the red and blue labels on the pouch with the red and blue arrows on the Pouch Loading Station.
5. Remove the Sample Injection Vial from its package by tearing or cutting the notched outer packaging. Place the Sample Injection Vial (with red cover) into the red well of the Pouch Loading Station.



6. Place a Hydration Injection Vial (with blue cover) into the blue well of the Pouch Loading Station.

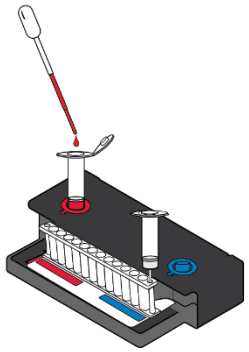
Step 2: Hydrate Pouch



1. Unscrew the Hydration Injection Vial from the blue cover.
2. Remove the Hydration Injection Vial, leaving the blue cover in the Pouch Loading Station.
3. Insert the Hydration Injection Vial cannula tip into the pouch hydration port located directly below the blue arrow of the Pouch Loading Station.
4. Forcefully push down in a firm and quick motion to puncture seal until a faint "pop" is heard and there is an ease in resistance. Wait as the correct volume of Hydration Solution is pulled into the pouch by vacuum.
    - If the Hydration Solution is not automatically drawn into the pouch, re-insert Hydration Injection Vial to ensure that the seal of the pouch hydration port was broken. If Hydration Solution is again not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.
5. Verify that the pouch has been hydrated.
    - Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen.
    - If the pouch fails to hydrate (dry reagents appear as white pellets), re-insert Hydration Injection Vial to ensure that the seal of the pouch hydration port was broken. If Hydration Solution is still not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.

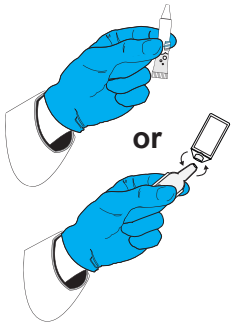
Step 3: Prepare Sample Mix

NOTE: *Gently invert whole blood container until thoroughly mixed. Do not centrifuge specimens as this may affect sensitivity of the test. Do not use a specimen if it has clotted.*



1. Use the Transfer Pipette provided in the test kit to draw the specimen to the second line (approximately 0.2 mL) of the Transfer Pipette.
2. Add the sample to the Sample Injection Vial.
3. Discard the Transfer Pipette in a biohazard waste container.

   NOTE: *DO NOT use the Transfer Pipette to mix the sample once it is loaded into the Sample Injection Vial.*



4. Add Sample Buffer to the Sample Injection Vial.

   NOTE: *There are 2 possible designs of the Sample Buffer Ampoule.*

   - Hold the Sample Buffer Ampoule with the tip facing up.

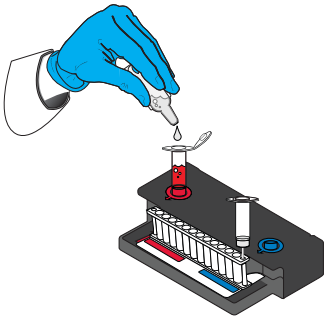
   NOTE: *Avoid touching the ampoule tip during handling, as this may introduce contamination.*

   - If the ampoule has a textured tab on the side of it: firmly pinch the tab on the ampoule until the seal snaps.

   or

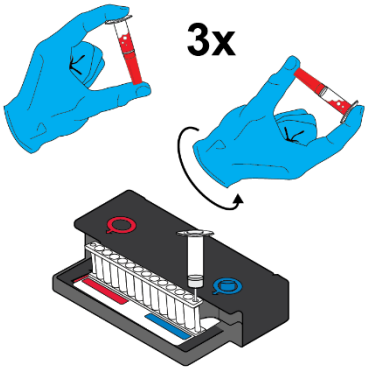
   - If the ampoule has a plastic tab on the tip: gently twist and remove the tab at the tip of the ampoule.
   - Invert the ampoule over the Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

   NOTE: *Avoid squeezing the ampoule additional times. This will generate foam, which should be avoided.*

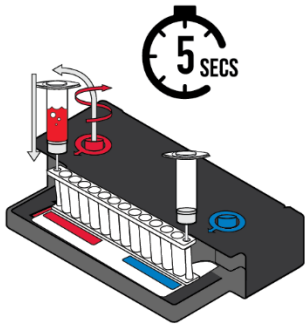


WARNING: Contact with sample buffer can cause serious eye damage and skin irritation and is harmful if swallowed.

5. Tightly close the lid of the Sample Injection Vial.
6. Remove the Sample Injection Vial from the Pouch Loading Station and invert the vial at least **3 times** to mix.
7. Return the Sample Injection Vial to the red well of the Pouch Loading Station.



Step 4: Load Sample Mix



1. Slowly twist to unscrew the Sample Injection Vial from the red cover and wait for **5 seconds** with the vial resting in the cover.

   NOTE: *Waiting **5 seconds** decreases the risk of dripping and contamination from the sample.*

2. Lift the Sample Injection Vial, leaving the red cover in the well of the Pouch Loading Station, and insert the Sample Injection Vial cannula tip into the pouch sample port located directly below the red arrow of the Pouch Loading Station.
3. Forcefully push down in a firm and quick motion to puncture seal (a faint "pop" is heard) and sample is pulled into the pouch by vacuum.
4. Verify that the sample has been loaded.
   - Flip the barcode label down and check to see that fluid has entered the reagent well next to the pouch sample port.
   - If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded. Discard the Sample Injection Vial and the Hydration Injection Vial in a biohazard container. Retrieve a new pouch and repeat from *Step 1: Prepare Pouch*.
5. Screw the injection vials back into their plastic covers in the Pouch Loading Station before disposing of them in a biohazard container.
6. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the Pouch Loading Station.

   NOTE: *Optional added operator protection: Before removal from biosafety cabinet, run a bleach wipe, a paper towel with 10% bleach (one part bleach to nine parts water), across the top of the pouch from the pouch hydration port to the pouch sample port, and follow with a water wipe. This reduces the potential for contact with small amounts of sample mixed with Sample Buffer that may be retained at the pouch sample port.*

Step 5: Run Pouch

The BIOFIRE FILMARRAY Software includes step-by-step on-screen instructions that guide the operator through performing a run. Brief instructions for BIOFIRE FILMARRAY 2.0 and BioFire BIOFIRE FILMARRAY Systems are given below. Refer to the appropriate BIOFIRE FILMARRAY operator's manual for more detailed instructions.

BIOFIRE FILMARRAY 2.0

1. Ensure that the BIOFIRE FILMARRAY 2.0 System (instrument and computer) is powered on and the software is launched.
2. Follow on-screen instructions and procedures described in the appropriate BIOFIRE FILMARRAY 2.0 operator's manual to place the pouch in an instrument and enter pouch, sample, and operator information.
3. Pouch identification (Lot Number and Serial Number) and Pouch Type information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number and Pouch Type can be manually entered from the information provided on the pouch label into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

   NOTE: *When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the BioFire Global Fever Special Pathogens Panel pouch.*
4. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.
5. Select and confirm the appropriate Protocol from the Select Protocol dialogue box. The BioFire GF SP Panel will display three Protocols in the dialogue box: GF Blood, Positive External Control, and Negative External Control. The GF Blood protocol should be used for clinical specimen testing.

   NOTE: *Two additional protocols are provided for use with the BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel. It is necessary to select the appropriate protocol prior to running the test. The Positive External Control and the Negative External Control protocols are only for use with the BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel and should not be used to test clinical specimens or other types of controls. Refer to the BIOFIRE SHIELD Control Kit for the BioFire GF SP Panel Instructions for Use for procedures to prepare and run BIOFIRE SHIELD Controls.*
6. Enter a username and password in the Name and Password fields.

   NOTE: *The font color of the username is red until the username is recognized by the software.*
7. Review the entered run information on the screen. If correct, select Start Run. Once the run has started, the screen displays a list of the steps being performed by the instrument and the number of minutes remaining in the run.

   NOTE: *The bead-beater apparatus makes an audible, high-pitched noise during the first minute of operation.*
8. When the run is finished, follow the on-screen instructions to remove the pouch, then immediately discard it in a biohazard waste container.
9. The run file is automatically saved in the BIOFIRE FILMARRAY Software database, and the test report can be viewed, printed, and/or saved as a PDF file.
10. To view run data, double click on a run file, select the interpretation tab and click on an analyte for a specific assay.

BIOFIRE FILMARRAY Torch

1. Ensure that the BIOFIRE FILMARRAY Torch system is powered on.
2. Select an available module on the touch screen or scan the barcode on the pouch using the barcode scanner.
3. Pouch identification (Lot Number and Serial Number) and Pouch Type information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, and Pouch Type can be manually entered from the information provided on the pouch label into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

   NOTE: *When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the BioFire GF SP Panel pouch.*
4. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.
5. Insert the pouch into the available Module (instrument).
   - Ensure that the pouch fitment label is lying flat on top of pouch and not folded over. As the pouch is inserted, the module will grab onto the pouch and pull it into the chamber.
6. Select and confirm the appropriate protocol. The BioFire GF SP Panel has three Protocols available: GF Blood, Positive External Control, and Negative External Control. The GF Blood protocol should be used for clinical specimen testing.

   NOTE: *Two additional protocols are provided for use with the BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel. It is necessary to select the appropriate protocol prior to running the test. The Positive External Control and the Negative External Control protocols are only for use with the BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel and should not be used to test clinical specimens or other types of controls. Refer to the BIOFIRE SHIELD Control Kit for the BioFire GF SP Panel Instructions for Use for procedures to prepare and run BIOFIRE SHIELD Controls.*
7. Enter operator username and password, then select Next.

   NOTE: *The font color of the username is red until the username is recognized by the software.*
8. Review the entered run information on the screen. If correct, select Start Run. Once the run has started, the screen displays a list of the steps being performed by the module and the number of minutes remaining in the run.

   NOTE: *The bead-beater apparatus makes an audible, high-pitched noise during the first minute of operation.*
9. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.
10. The run file is automatically saved in the FilmArray Software database, and the test report can be viewed, printed, and/or saved as a PDF file.

QUALITY CONTROL

Process Controls

Two process controls are included in each pouch:

1. **RNA Process Control**
   The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, PCR1, dilution, PCR2, and DNA melting. A positive control result indicates that all steps carried out in the BioFire GF SP Panel pouch were successful.

2. **PCR2 Control**
   The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive control result indicates that PCR2 was successful.

Both control assays must be positive for the test run to pass. If controls fail the results are invalid and the sample should be retested using a new pouch.

Monitoring Test System Performance

The BIOFIRE FILMARRAY Software will automatically fail the run if the melting temperature (Tm) for either the RNA Process Control or the PCR2 Control is outside of an acceptable range (80.0-84.0°C for the RNA Process Control and 74.0-78.0°C for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices.[55,56] Refer to the appropriate BIOFIRE FILMARRAY operator's manual for instructions on obtaining control assay Tm values.

External Controls

Good laboratory practice recommends running positive and negative external controls regularly in accordance with laboratory protocols and the appropriate accrediting organization requirements, as applicable.

Evaluation of external controls is recommended prior to using a new shipment or new lot of BioFire GF SP Panel Kits. Evaluation of external controls is also recommended when there is a new operator and following replacement/repair of a BIOFIRE FILMARRAY System. The BioFire GF SP Panel should not be used in patient testing if the external controls do not produce the expected results. 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.

Molecular grade water or saline can be used as a negative external control. Previously characterized positive samples or negative samples spiked with well-characterized organisms may also be used as positive external controls. The GF Blood protocol must be used when running external controls of these types.

An assayed external quality control kit is also available from BioFire Defense to monitor the performance of in vitro laboratory nucleic acid testing procedures for the qualitative detection of the BioFire GF SP Panel

performed on BIOFIRE FILMARRAY Systems. The BIOFIRE® SHIELD™ Control Kit for the BioFire GF SP Panel is composed of two controls, Positive External Control, and Negative External Control. The Positive External Control is a surrogate assayed quality control material comprised of dried synthetic DNA in buffer and stabilizer. Both the Positive and Negative External Controls are supplied as a FilmArray Control Injection Vial that is used directly with the BioFire GF SP Panel.

The BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel is available for purchase directly from BioFire Defense. Contact BioFire Defense Customer Support for more information.

BIOFIRE® SHIELD™ Control Kit for the BioFire® Global Fever Special Pathogens Panel
Part Number: DFA2-ASY-0019

INTERPRETATION OF RESULTS

Assay Interpretation

When PCR2 is complete, the BIOFIRE FILMARRAY instrument performs a DNA melting analysis on the PCR products and measures the fluorescence signal generated in each PCR2 array well (for more information see the appropriate BIOFIRE FILMARRAY operator's manual). The BioFire FilmArray Software then performs several analyses and assigns a final assay result for every well. The steps in the analyses are described below.

Analysis of Melt Curves. The BIOFIRE FILMARRAY Software evaluates the DNA melt curve for each well of the PCR2 array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve and compares it against the expected Tm range for the assay in that well. If the software determines that the Tm of the curve is within the assay Tm range, the melt curve is called positive. If the software determines that the Tm of the curve is not in the appropriate Tm range, the melt curve is called negative.

Analysis of Replicates. Once positive melt curves have been identified, the software evaluates the replicates for each assay to determine the assay result. For an assay to be called positive, at least two associated melt curves must be called positive, and both Tm values must be similar. Assays that do not meet these criteria are called negative.

Organism Interpretation

The BioFire GF SP Panel automatically interprets and returns results (Detected or Not Detected) for each pathogen. The interpretation is based on the results of one or more assays for each pathogen as shown in **Table 2**. In cases where either or both control assays have failed all analyte results are reported as Invalid (**Figure 1**).

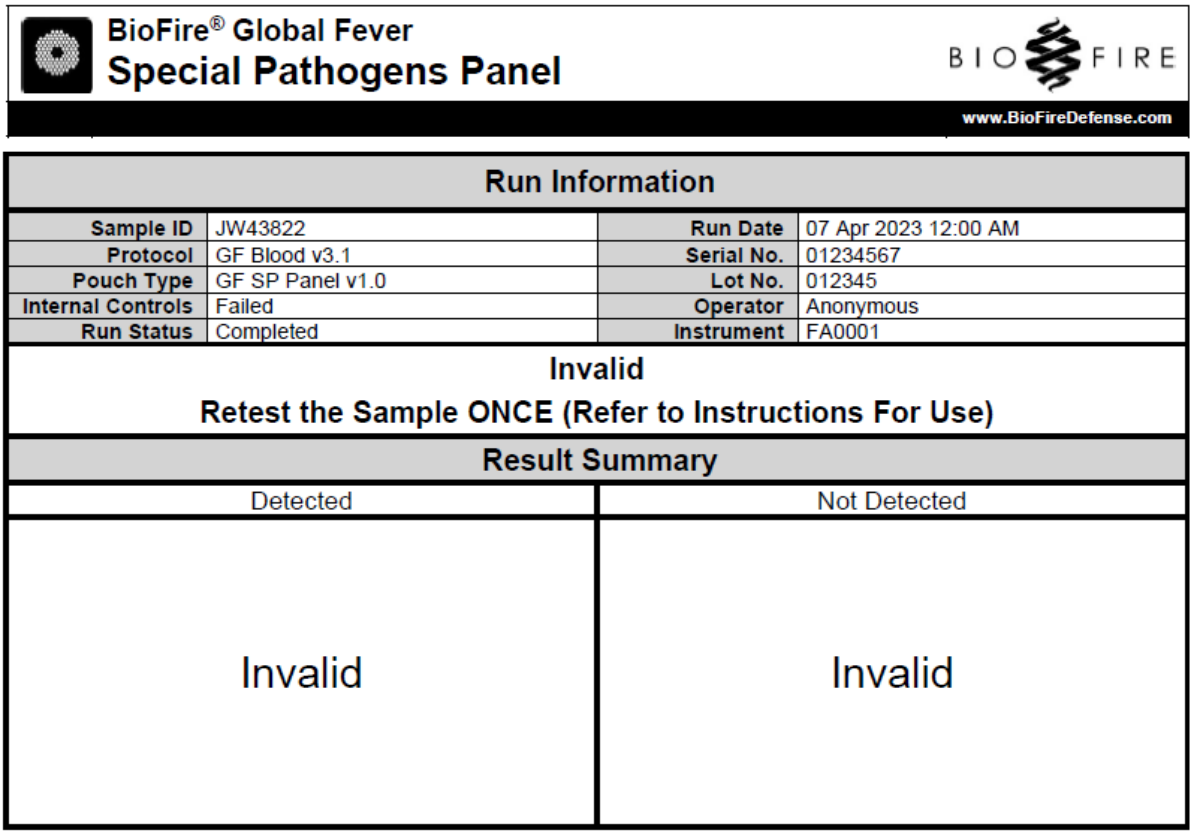
Table 2. Assay Number and Interpretation Rules for the BioFire GF SP Panel

Pathogen	No. of Assays	Assay Interpretation Rules
BACTERIA		
Bacillus anthracis	1	Positive = Detected
Francisella tularensis	2	Any Positive = Detected
Leptospira spp.	1	Positive = Detected
Yersinia pestis	2	Any Positive = Detected
VIRUSES		
Chikungunya virus	2	Any Positive = Detected
Crimean-Congo hemorrhagic fever virus	2	Any Positive = Detected
Dengue virus (serotypes 1, 2, 3, and 4)	5 [a]	Any Positive = Detected
Ebolavirus spp. (Bundibugyo, Reston, Sudan, Taï Forest, and Zaire)	3 [b]	Any Positive = Detected
Lassa virus	2	Any Positive = Detected
Marburgvirus	1	Positive = Detected
West Nile virus	2	Any Positive = Detected
Yellow fever virus	1	Positive = Detected
PROTOZOAN		
Leishmania spp.	1	Positive = Detected
Plasmodium spp.	1	Positive = Detected
Plasmodium falciparum	1	Positive = Detected
Plasmodium ovale/ Plasmodium vivax	1 [c]	Positive = Detected

[a] The BioFire GF SP Panel contains multiple assays for the detection of the four dengue virus serotypes. See dengue virus reporting section below for more information.

[b] The BioFire GF SP Panel contains three multiplexed assays for the detection of all five known *Ebolavirus* spp. which is reported with a single interpretation call. See *Ebolavirus* spp. reporting section below for more information.

[c] The BioFire GF SP Panel contains one multiplexed assay for the detection of both *Plasmodium vivax* and *Plasmodium ovale*, which is reported with a single interpretation call. See *Plasmodium* spp. reporting section below for more information.



BioFire® Global Fever
Special Pathogens Panel

BIOFIRE

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Run Information

Sample ID	JW43822	**Run Date**	07 Apr 2023 12:00 AM
Protocol	GF Blood v3.1	**Serial No.**	01234567
Pouch Type	GF SP Panel v1.0	**Lot No.**	012345
Internal Controls	Failed	**Operator**	Anonymous
Run Status	Completed	**Instrument**	FA0001

Invalid

Retest the Sample ONCE (Refer to Instructions For Use)

Result Summary

Detected	Not Detected
Invalid	Invalid

Figure 1. BioFire GF SP Panel Report with failed Internal Controls and Invalid results

Bacteria Reporting

Bacillus anthracis

The BioFire GF SP Panel contains one assay for the detection of *Bacillus anthracis*. A positive *Bacillus anthracis* assay will result in a *Bacillus anthracis* Detected test result.

Francisella tularensis

The BioFire GF SP Panel contains two assays for the detection of *Francisella tularensis*. One or more positive *Francisella tularensis* assays will result in a Detected call for *Francisella tularensis*.

Leptospira spp.

The BioFire GF SP Panel contains a single pan assay for genus-level detection of all *Leptospira* Group 1 species. A positive Leptospira pan assay will result in a Detected call for *Leptospira* spp.

Yersinia pestis

The BioFire GF SP Panel contains two assays for the detection of *Yersinia pestis*. A positive result for either assay will result in a Detected test result for *Yersinia pestis*.

Virus Reporting

Chikungunya virus
The BioFire GF SP Panel contains two assays for species-level detection of all chikungunya virus strains, and one or more positive chikungunya virus assay(s) will result in a Detected call for chikungunya virus.

Crimean-Congo hemorrhagic fever virus
The BioFire GF SP Panel contain two assays for the detection of the Crimean-Congo hemorrhagic fever virus. A positive call for either or both assays will result in a Crimean-Congo hemorrhagic fever virus Detected test result.

Dengue virus
The BioFire GF SP Panel contains five assays for the detection of the four dengue virus serotypes. Any positive assay call will result in a Detected call for dengue virus.

Ebolavirus spp.
The BioFire GF SP Panel contains three *Ebolavirus* spp. assays designed to detect five *Ebolavirus* spp. (*Bundibugyo ebolavirus, Reston ebolavirus, Sudan ebolavirus*, *Taï Forest ebolavirus*, and *Sudan ebolavirus*). Any positive Ebolavirus assay will result in an *Ebolavirus* Detected test result. The BioFire GF SP Panel does **not** detect *Bombali ebolavirus*; this species is not known to infect humans.

Lassa virus
The BioFire GF SP Panel contains two assays for the detection of Lassa virus. A positive call for either or both assays will result in a Lassa virus Detected result.

Marburgvirus
The BioFire GF SP Panel contains one assay for the detection of Marburgviruses. A positive Marburgvirus assay will result in a *Marburgvirus* Detected test result.

Yellow fever virus
The BioFire GF SP Panel contains a single assay for detection of the yellow fever virus. A positive result for the assay will result in a Detected call for yellow fever virus.

West Nile virus
The BioFire GF SP Panel contains two assays for the detection of West Nile virus. A positive call for either or both assays will result in a West Nile virus Detected result.

Protozoa Reporting

Leishmania spp.

The BioFire GF SP Panel contains a single assay for the genus-level detection of *Leishmania spp.* that cause visceral leishmaniasis (e.g., *L. donovani* and *L. infantum).* A positive Leishmania assay will result in a Detected *Leishmania* spp. test result.

Plasmodium spp.

The BioFire GF SP Panel contains three *Plasmodium* assays, one genus-level assay and two species-level assays. The genus-level assay (Plasmodium spp. assay) detects *Plasmodium* species including the five *Plasmodium* species known to infect humans (*P. falciparum, P. vivax, P. ovale, P. malariae*, and *P. knowlesi*). In addition, one species-level assay detects *Plasmodium falciparum*, and a combined species-level assay detects *Plasmodium vivax* and *Plasmodium ovale*. **Table 3** shows the reporting scheme for the three *Plasmodium* assays.

This test cannot differentiate co-infections other than *Plasmodium falciparum* and *Plasmodium vivax*/*ovale*. Infection with additional *Plasmodium* species is always possible and should be considered.

WARNING: Before consideration of treatment for the hypnozoite liver form, confirm the presence of a *P. vivax* or *P. ovale* infection.

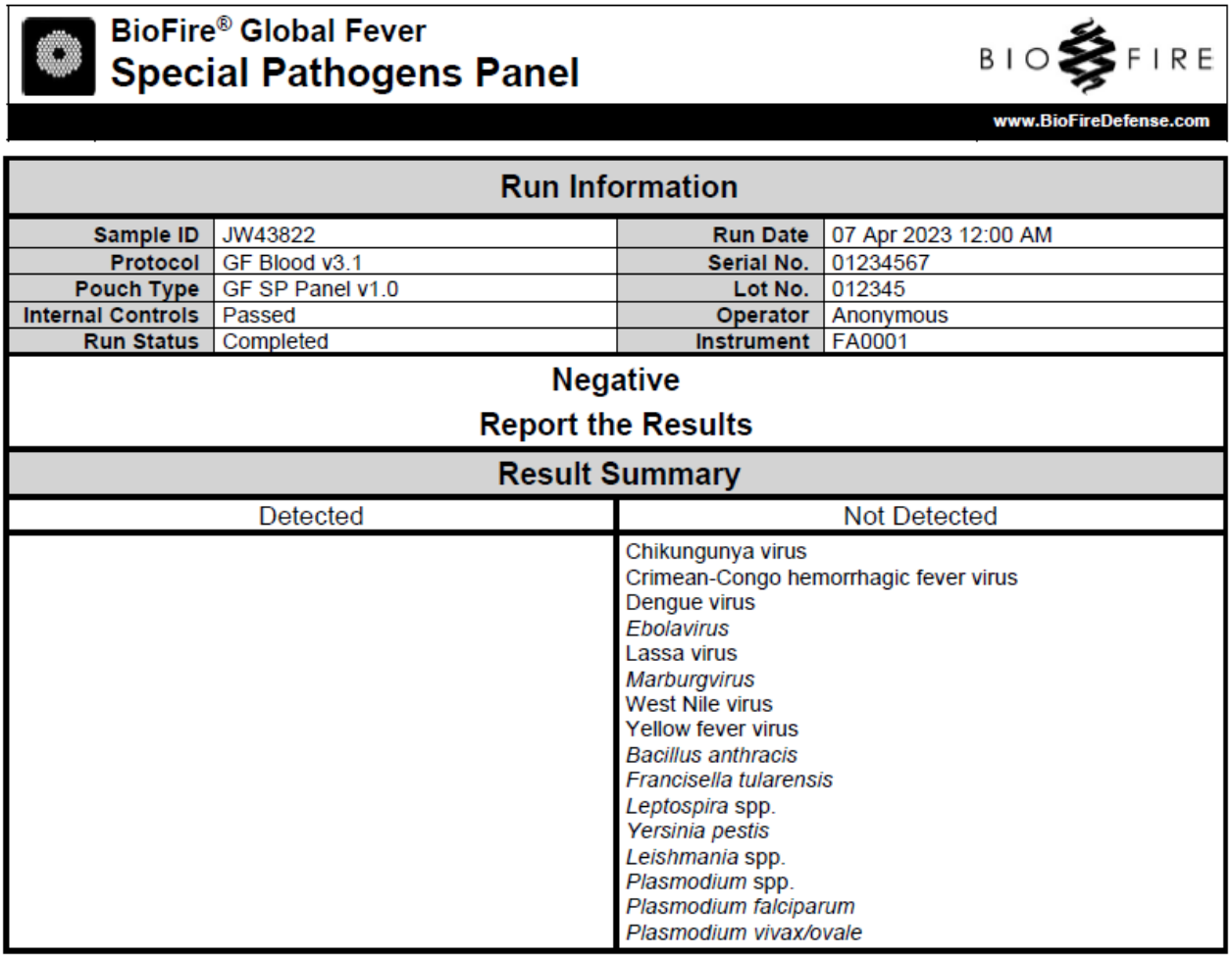
Table 3. *Plasmodium* Report Scheme

Assay Detection Outcomes			Result Banner	Result Summary [1]
***Plasmodium* spp.**	***Plasmodium falciparum***	***Plasmodium vivax/ovale***		
Negative	Negative	Negative	N/A - No *Plasmodium* species detected in the sample (no results shown in Report).	*Plasmodium* spp. Not Detected *Plasmodium falciparum* Not Detected *Plasmodium vivax/ovale* Not Detected
Positive	Negative	Negative	***Plasmodium* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Report the Results.	***Plasmodium* spp. Detected** *Plasmodium falciparum* Not Detected *Plasmodium vivax/ovale* Not Detected
Positive	**Positive**	Negative	***Plasmodium falciparum* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Report the Results.	***Plasmodium* spp. Detected** ***Plasmodium falciparum* Detected** *Plasmodium vivax/ovale* Not Detected
Positive	Negative	**Positive**	***Plasmodium vivax/ovale* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Report the Results.	***Plasmodium* spp. Detected** *Plasmodium falciparum* Not Detected ***Plasmodium vivax/ovale* Detected**
Positive	**Positive**	**Positive**	***Plasmodium falciparum* and *Plasmodium vivax/ovale* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Note: The detection of 2 or more organisms is uncommon. Retest the Sample ONCE then Report the Results.	***Plasmodium* spp. Detected** ***Plasmodium falciparum* Detected** ***Plasmodium vivax/ovale* Detected**
Negative	**Positive**	**Positive**	***Plasmodium falciparum* and *Plasmodium vivax/ovale* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Note: The detection of 2 or more organisms is uncommon. Retest the Sample ONCE then Report the Results.	*Plasmodium* spp. Not Detected ***Plasmodium falciparum* Detected** ***Plasmodium vivax/ovale* Detected**
Negative	**Positive**	Negative	***Plasmodium falciparum* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Report the Results.	*Plasmodium* spp. Not Detected ***Plasmodium falciparum* Detected** *Plasmodium vivax/ovale* Not Detected
Negative	Negative	**Positive**	***Plasmodium vivax/ovale* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Report the Results.	*Plasmodium* spp. Not Detected *Plasmodium falciparum* Not Detected ***Plasmodium vivax/ovale* Detected**

[1] Bolded results are displayed in the Detected column of the Result Summary. Non-bolded results are shown in the Not Detected column of the Result Summary.

BioFire Global Fever Special Pathogens Panel Test Report

The BioFire GF SP Panel test report is automatically displayed upon completion of a run and contains two sections: Run Information and Result Summary (**Figure 2**). The test report can be saved as a PDF file or printed.



BioFire® Global Fever
Special Pathogens Panel

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Run Information

Sample ID	JW43822	**Run Date**	07 Apr 2023 12:00 AM
Protocol	GF Blood v3.1	**Serial No.**	01234567
Pouch Type	GF SP Panel v1.0	**Lot No.**	012345
Internal Controls	Passed	**Operator**	Anonymous
Run Status	Completed	**Instrument**	FA0001

Negative

Report the Results

Result Summary

Detected	Not Detected
	Chikungunya virus Crimean-Congo hemorrhagic fever virus Dengue virus *Ebolavirus* Lassa virus *Marburgvirus* West Nile virus Yellow fever virus *Bacillus anthracis* *Francisella tularensis* *Leptospira* spp. *Yersinia pestis* *Leishmania* spp. *Plasmodium* spp. *Plasmodium falciparum* *Plasmodium vivax/ovale*

Figure 2. Example BioFire GF SP Panel Run Report

The **Run Information** section of the test report is displayed at the top of the page. It provides information about the run including: Sample ID, Protocol, pouch information (including Pouch Type, Serial Number, and Lot Number), Run Date, Run Status (Completed, Aborted, Instrument Error, or Software Error), the identity of the operator who performed the test (Operator), and the instrument used to perform the test. Internal Control results are reported as Passed, Failed, or Invalid. The section also contains a Result Banner that lists the Detected results and required actions. If there are no Detected results, the Result Banner shown is Negative.

Table 4 provides additional information for each of the possible Internal Controls field results. See **Table 5** and **Table 6** for complete results interpretation and required actions.

Table 4. Interpretation of Internal Controls Field on the BioFire GF SP Panel Test Report

Internal Controls Result	Explanation	Action Required
Passed	The run was successfully completed AND Both pouch controls (RNA Process Control and PCR2 Control) were successful.	Follow any instructions provided in the Result Banner.
Failed	The run was successfully completed BUT At least one of the pouch controls (RNA Process Control and/or PCR2 Control) failed.	Repeat the test using a new pouch. If the error persists, call BioFire Defense Technical Support for further instructions.
Invalid	The controls are invalid because the run did not complete. (This typically indicates a software or hardware error.)	Note any error codes displayed by the software during the run. Refer to the appropriate BIOFIRE FILMARRAY operator's manual or call BioFire Defense Technical Support for further instruction. If the error can be resolved, repeat the test once using a new pouch.

The **Result Summary** section of the test report lists each target tested as either Detected or Not Detected. Within the Results Summary section, targets that are detected are listed in the left-hand column, and targets that are not detected are listed in the right-hand column. For runs with failed internal controls or other run errors, the Result Summary columns display Invalid. See the Results Explanation section below for detailed information about interpretation of test results and appropriate follow-up for Invalid results.

Once a run has completed, it is possible to edit the Sample ID. If this information has been changed, an additional section called **Change Summary** will be added to the test report. This Change Summary section lists the field that was changed, the original entry, the revised entry, the operator that made the change and the date that the change was made (**Figure 3**). Sample ID is the only field of the report that can be changed.

Change Summary

Field	Changed To	Changed From	Operator	Date
[1]Sample ID	New Example Id	Old Example Id	Anonymous	24 Mar 2021

Figure 3. Change Summary Field

Results Explanation and Required Actions

The **Result Summary** section provides a complete list of all test results. Possible results for each pathogen include Detected, Not Detected, and Invalid. **Table 5**Table 5 provides explanations for each interpretation and any follow-up necessary to obtain a final result. Table 6 **Table 6** shows test Result Banner text in cases where more than one pathogen is detected and/or in the case of the detection of a select agent.

Table 5. Interpretation of Results on the BioFire GF SP Panel Test Report

Results	Explanation	Action Required
Not Detected	The run was successfully completed AND The pouch controls were successful (Pass) AND The assay(s) for the pathogen were NEGATIVE	Results are valid. Follow any instructions provided in the Result Banner.
Detected	The run was successfully completed AND The pouch internal controls were successful (Pass) AND The assay(s) for the pathogen were POSITIVE	Results are valid. Follow any instructions provided in the Result Banner. **For select agents, detection is presumptive. Retest once and report following national public health requirements for reporting and follow-up testing requirements; in the US, the CDC should be notified for further characterization and confirmation.**
Invalid	Run completed and pouch internal controls failed OR Run did not complete	All results are invalid because the run failed. Note any error codes displayed and refer to the appropriate BIOFIRE FILMARRAY operator's manual for more information. If the error persists, contact BioFire Defense Technical Support for further instruction. Retest the sample.

Table 6. Result Banners for Multiple Analyte and/or Select Agent Detections

Detection Results	Result Banner Text
Two pathogens Detected	<Pathogen 1> and <Pathogen 2> Detected Note: The detection of 2 or more organisms is uncommon Retest the Sample ONCE then Report the Results
Three or more pathogens Detected	Multiple Organisms Detected Note: The detection of 2 or more organisms is uncommon Retest the Sample ONCE then Report the Results
One or more of the following pathogens are detected: • *Bacillus anthracis* • Crimean-Congo hemorrhagic fever virus • *Ebolavirus* spp. • *Francisella tularensis* • Lassa virus • *Marburgvirus* • *Yersinia pestis*	**Note:** All positive select agent results are presumptive. Presumptive results must not be reported to the ordering physician, but require additional testing and confirmation in consultation with appropriate public health authorities; In the United States, the Centers for Disease Control and Prevention (CDC) should be notified for further characterization and confirmation.

DECONTAMINATION PROCEDURES

The decontamination and cleaning procedures listed are intended to limit spread of contaminants and mitigate the risk of false positive results in subsequent runs. Decontamination is necessary if a select agent pathogen is Detected, the pouch is loaded with a suspected positive select agent specimen, or if the pouch leaks or is punctured.

If a pouch leak or breakage occurs, change gloves and other potentially contaminated personal protective equipment (PPE). Change gloves often during the decontamination process, especially during the first steps of decontamination and before touching any clean surface. All PPE should be disposed of after decontamination.

CAUTION: It is important that contamination from leaking and/or punctured pouches be contained and cleaned immediately. Pouches that break after PCR contain amplified nucleic acid material that can contaminate future pouch runs. This material, although noninfectious, is easily spread if precautions are not taken. Very small (molecular) quantities can be amplified by PCR in future runs, which can result in false positives. Treat all broken pouches as capable of contaminating the work area.

BIOLOGICAL RISKS: If the pouch contains potentially infectious material, the risk of biohazard contamination exists in addition to sample contamination.

Cleaning Materials

This list provides items that are necessary in a laboratory to keep contamination to a minimum.

- 10% bleach solution in a squeeze or spray bottle (1 part bleach to 9 parts water)
- Distilled, de-ionized, sterile, or molecular grade water in a squeeze or spray bottle
- DNAZap™ or equivalent DNA degrading system
- Paper towels
- Bleach wipes

Pouch Loading Station Decontamination

Routine cleaning of the Pouch Loading Station includes a 10% bleach wipe followed by two water wipes before each new pouch is loaded. In the event of a sample spill, or a pouch leak, perform the following decontamination procedures:

1. Put on clean PPE, such as lab coat, gloves, and eye protection.
2. Fill a sink or bin with water and add bleach to create a 10% bleach solution.
3. Submerge the Pouch Loading Station until completely covered with bleach solution. Soak for 15 minutes.
4. Remove the Pouch Loading Station from sink or bin. Replace bleach solution with distilled water.
5. Rinse the Pouch Loading Station by completely submerging in water two additional times.

Contact *BioFire Defense Technical Support* to obtain a replacement Pouch Loading Station, if necessary.

Decontamination Related to Pouch Leakage or Detected/Suspected Select Agent Specimen

Change gloves and other potentially contaminated personal protective equipment (PPE) prior to initiating decontamination. Change gloves often during the decontamination process, especially during the first steps of decontamination and before touching any clean surface. All PPE should be disposed of after decontamination.

If a pouch was loaded with a Detected select agent pathogen, a suspected positive select agent specimen, or if the pouch leaks, take the following precautions to avoid contamination:

1. Put on clean PPE as appropriate.
2. Ensure no one uses the instrument or potentially contaminated areas until the decontamination is complete.
3. Decontaminate the instrument and work area and dispose of the pouch using the following steps:
   i. Dispose of potentially contaminated gloves and put on clean gloves.
   ii. Dispose of the potentially contaminated lab coat and put on a clean lab coat.
   iii. Discard the pouch in a biohazard container.
   iv. Change gloves.
   v. Clean the instrument and affected work areas per the guidelines below.

CAUTION: Use only 10% bleach solution, distilled water, and/or DNAZap™ to decontaminate the instrument and Pouch Loading Station.

BIOFIRE FILMARRAY 2.0 Instrument Decontamination

Pouch Loading Chamber Decontamination

1. Put on clean PPE as appropriate.
2. Remove pouch from instrument and discard in biohazard waste container. Change gloves and any contaminated PPE.
3. Wet a paper towel with 10% bleach (one part bleach to nine parts water) and wipe the inner chamber and under the lid. Let it stand for at least 3 minutes to allow the bleach solution to react with any contaminants. Discard paper towel in biohazard waste. Change gloves.
4. Repeat Step 3 twice with fresh paper towels for a total of three bleach wipes.
5. Wet a paper towel with water and wipe the inner chamber.
6. Repeat Step 5 with fresh gloves and paper towel.

Instrument Exterior Decontamination

1. Put on clean PPE as appropriate.
2. Wet a paper towel with the 10% bleach solution and wipe all exterior surfaces of the instrument, including the bottom and the bench top where the instrument had contact. Let it stand for at least 3 minutes to allow the bleach solution to react with any contaminants. Discard paper towel in biohazard waste. Change gloves.
3. Repeat Step 2 twice with fresh paper towels and clean gloves, for a total of three bleach wipes.
4. Change gloves, then wet a new paper towel with distilled water and wipe the surfaces of the inner chamber, including under the lid, and the entire exterior of the instrument, including the bottom and the bench top where the instrument had contact.
5. Repeat Step 4 with fresh gloves and paper towel.

BIOFIRE FILMARRAY Torch Module Decontamination

1. Put on clean PPE, such as a lab coat and gloves.
2. Remove pouch from instrument and discard in biohazard waste container.
3. Dispose of potentially contaminated gloves and lab coat and put on clean gloves and lab coat.
4. Wet a paper towel with 10% bleach and wipe all exterior surfaces of the BioFire Torch, including the bottom and the bench top where the BioFire Torch Module had contact. Let it stand for at least 3 minutes to allow the bleach solution to react with any contaminants. Discard paper towel in biohazard waste. Change gloves.

   NOTE: *When cleaning the touch screen, put the BIOFIRE FILMARRAY Torch into Cleaning Mode. The Cleaning Mode allows 30 seconds for the touch screen to be cleaned. Access this feature from the Settings toolbar (See the BIOFIRE FILMARRAY Torch Operator's Manual for more information).*

CAUTION: The interior of the pouch slot and Module(s) should not be cleaned. Do not spray or insert any cleaning materials into the Module.

5. Repeat Step 4 twice with fresh paper towels for a total of three bleach wipes.
6. Change gloves, then wet a new paper towel with distilled water and wipe all exterior surfaces of the BioFire Torch. Dispose of the pare towel in biohazard waste. Change gloves.
7. Repeat Step 6 with a new paper towel.

8. Remove Module front cover. Repeat Steps 3 through 7 for inner front cover and pouch slot surfaces.
9. Wet a paper towel with water and wipe the inner chamber.

Decontamination of Bench Tops and Other Areas

1. Put on clean PPE, such as a lab coat and gloves.
2. Spray the 10% bleach solution on the area that may have been contaminated. Let it stand for at least three minutes to allow the bleach solution to react with any contaminants on the surface.
3. Wipe the area with a clean paper towel. Change gloves.
4. Repeat Steps 2 and 3 twice, for a total of three wipes.
5. Change gloves. Spray the area with distilled water.
6. Wipe the area dry with a new paper towel. Change gloves.
7. Spray the area with DNAZap™ or an equivalent product. Follow the product's instructions for correct use. Change gloves.
8. Rinse the area by spraying it with distilled water and wiping it dry.

Check Function of Decontaminated Instrument

1. Test a negative sample by preparing a pouch, using water as the sample. Use distilled, sterile, or molecular grade water for the test.
2. If run is successful and all results are negative, continue using the instrument as normal.
3. If unexpected positive results are obtained or the run fails, please contact BioFire Defense Technical Support for further instructions.

Check for Environmental Contamination

After decontaminating the work area and instrument as described above, use environmental swabs to check for contamination by following the protocol below:

1. Prepare four aliquots of 0.2 mL of molecular grade water.
2. Place one environmental swab in each aliquot and let soak for five minutes.
3. Thoroughly swab exterior of instrument and accessories, including laptop, especially areas of operator contact.
4. Return each swab to its original aliquot and mix the sample well.
5. Dispose of swabs and combine the four aliquots into one.
6. Load pouch as described in Procedure section of this document.
    i. Load 0.2 mL of combined swabbing aliquot as the sample using Transfer Pipette, by drawing liquid up to the 2nd line.
    ii. Add sample to Sample Injection Vial.
    iii. Proceed with normal pouch loading procedure.
7. Run pouch using the Negative External Control Protocol.

    NOTE: *The Negative External Control Protocol detects analyte and Positive External Control contamination.*

8. If the Negative External Control run fails, repeat decontamination step and contamination testing until no contamination is detected. To determine type of contamination on the BIOFIRE FILMARRAY 2.0, double click on the run file, select the interpretation tab and view specific assay results. If using BIOFIRE

FILMARRAY Torch, select a run and click the melt curve icon to open the Melt Curve Viewer. If the Melt Curve Viewer is not available, contact BioFire Defense Technical Support.

9. If problems persist, contact BioFire Defense Technical Support for further instructions.

LIMITATIONS

1. For prescription use only.
2. BioFire Global Fever Special Pathogens Panel test performance has only been established on BIOFIRE FILMARRAY 2.0 and BIOFIRE FILMARRAY Torch Systems.
3. Use of the BioFire Global Fever Special Pathogens Panel is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet the requirements to perform high complexity tests, and similarly qualified U.S. Department of Defense (DoD) and non-U.S. laboratories.
4. This test is a qualitative test and does not provide a quantitative value for the analyte(s) in the sample.
5. Performance of this test has not been established for monitoring treatment of infection with any of the panel analytes.
6. This test is not intended for screening asymptomatic individuals.
7. A false negative BioFire Global Fever Special Pathogens Panel result may occur when the concentration of analyte(s) in the sample is near or below the device limit of detection.
8. The detection of pathogen nucleic acid is dependent upon proper sample collection, handling, transportation, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive and false negative results caused by improperly collected, transported, or handled samples. The RNA process control and the PCR2 control will not indicate nucleic acid loss due to inadequate collection, transport, or storage of samples.
9. Recent administration of a vaccine for a BioFire Global Fever Special Pathogens Panel pathogen prior to whole blood specimen collection may lead to a false positive result.
10. The performance of the BioFire Global Fever Special Pathogens Panel was evaluated only with human whole blood collected in EDTA tubes.
11. A false negative BioFire Global Fever Special Pathogens Panel result may occur in the presence of heparin or TRIzol.
12. A dengue virus Detected result does not provide information on the severity of the disease. The potential for hemorrhagic dengue fever should be considered.
13. A *Plasmodium* spp. Detected result does not provide parasitemia level information. The potential for severe malaria should be considered.
14. All *Plasmodium* spp. Detected results from patients potentially exposed in Southeast Asia should be further investigated for possible *P. knowlesi* infection, which may require intensive immediate monitoring and treatment.
15. *Plasmodium malariae* and *Plasmodium knowlesi* may cross-react with the *Plasmodium vivax/ovale* assay. A *Plasmodium vivax/ovale* Detected result should be confirmed as infection due to *P. vivax* or *P. ovale.* Neither *P. malariae* nor *P. knowlesi* have a hypnozoite liver form requiring additional monitoring and treatment.
16. BioFire Global Fever Special Pathogens Panel results for *Plasmodium* may not directly correlate with microscopy performed on the same specimen.
17. A chikungunya virus Detected result may be due to o'nyong-nyong virus cross-reactivity. O'nyong-nyong and chikungunya virus infections can occur in the same geographic locations, and present with similar symptoms.

18. In the rare instance of a *Francisella hispaniensis or Francisella novicida* infection, the BioFire Global Fever Special Pathogens Panel may report a *Francisella tularensis* Detected result due to cross-reactivity with closely related *Francisella* species .
19. In silico analysis suggests the rare Central Asian *F. tularensis* subsp. *mediasiatica* is a cross-reactive species and may return a *Francisella tularensis* Detected result on the BioFire Global Fever Special Pathogens Panel.
20. Infection with *Leptomonas seymouri* may return a *Leishmania* spp. Detected result on the Global Fever Special Pathogens Panel due to cross-reactivity. Immunocompromised individuals with visceral leishmaniasis are commonly coinfected with *L. seymouri*.
21. The BioFire Global Fever Special Pathogens Panel may have reduced sensitivity for the detection of *Bacillus anthracis* strains SK-102 (Pakistan) and Vollum 1B.
22. The BioFire Global Fever Special Pathogens Panel may have reduced sensitivity for *Leishmania infantum.*
23. The BioFire Global Fever Special Pathogens Panel may have reduced sensitivity for the following isolates of yellow fever virus: SVM 3-18-09, CAREC M2-09, INHRR 7a-05, and INHRR 10a-10.
24. Dengue virus inclusivity testing and in silico analyses demonstrated that the BioFire Global Fever Special Pathogens Panel may have variable detection or reduced sensitivity for some strains detected by the dengue virus assays.
25. Due to the genetic diversity of Lassa virus not all sequences may be detected by the BioFire Global Fever Special Pathogens Panel. In silico analysis indicated potentially reduced sensitivity for Lassa virus Lineage VI. Additional testing of negative results may be warranted if Lassa virus infection is suspected.
26. Negative results do not rule out infection with *Leishmania* spp. Diagnosis of Leishmania infection should consider other clinical and diagnostic findings as well as patient history and epidemiological information.
27. Due to the rarity of *Bacillus anthracis*, Crimean-Congo Hemorrhagic Fever Virus, *Ebolavirus*, *Francisella tularensis*, *Marburgvirus*, Yellow fever virus, and *Yersinia pestis* clinical specimens, performance characteristics were established primarily using contrived clinical specimens.
28. Detection of two or more pathogens on the Global Fever Special Pathogens Panel is uncommon and results should be verified by a single retest.
29. Detection of any select agent (*Bacillus anthracis*, *Francisella tularensis*, Lassa virus, *Yersinia pestis*, Crimean-Congo Hemorrhagic Fever virus, *Ebolavirus* spp., and *Marburgvirus*) on the BioFire Global Fever Special Pathogens Panel is rare and presumptive and must be reported to public health authorities. Retesting is recommended.

EXPECTED VALUES

In the prospective clinical evaluation of the BioFire GF SP Panel, 2139 whole blood specimens were collected and tested at eleven study sites across the world over approximately three years (March 2018 – March 2021). Expected value summaries (as determined by the BioFire GF SP Panel), stratified by region and site, are presented in **Table 7**Table 7. Also provided is the prevalence of the BioFire GF SP Panel pathogens in the United States and Territories since the beginning of 2010 (as reported by the Centers for Disease Control and Prevention). Total number of cases in the United States are provided in **Table 8**Table 8 and in the United States Territories in **Table 9**Table 9.

Table 7. Expected Value (As Determined by the BioFire GF SP Panel) Summary by Region and Study Site (Specimens Acquired March 2018 – March 2021); # = Number; EV= Expected Value

Analyte	Overall (n=2139)		USA				Africa								Southeast Asia				Europe		Central & South America			
			Site 07 (n=179)		Site 14 (n=79)		Site 01 (n=134)		Site 02 (n=189)		Site 05 (n=199)		Site 11 (n=158)		Site 08 (n=249)		Site 09 (n=406)		Site 04 (n=113)		Site 12 (n=297)		Site 13 (n=136)	
	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV
Chikungunya virus[a]	**27**	**1.3%**	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	27	6.7%	0	0.0%	0	0.0%	0	0.0%
Crimean-Congo hemorrhagic fever virus	**1**	**0.05%**	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	0.5%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Dengue virus[b] (serotypes 1, 2, 3, 4)	**266**	**12.4%**	0	0.0%	0	0.0%	1	0.7%	0	0.0%	0	0.0%	0	0.0%	90	36.1%	54	13.3%	0	0.0%	20	6.7%	101	74.3%
West Nile virus	**1**	**0.05%**	1	0.6%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Leptospira spp.	**19**	**0.9%**	1	0.6%	0	0.0%	0	0.0%	1	0.5%	0	0.0%	0	0.0%	4	1.6%	4	1.0%	0	0.0%	9	3.0%	0	0.0%
Leishmania spp.	**10**	**0.5%**	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	10	8.8%	0	0.0%	0	0.0%
Plasmodium spp.	**407**	**19.0%**	0	0.0%	0	0.0%	16	11.9%	105	55.6%	141	70.9%	49	31.0%	7	2.8%	4	1.0%	1	0.9%	84	28.3%	0	0.0%
P. falciparum	**283**	**13.2%**	0	0.0%	0	0.0%	14	10.4%	94	49.7%	125	62.8%	42	26.6%	3	1.2%	0	0.0%	0	0.0%	5	1.7%	0	0.0%
P. vivax/ovale	**118**	**5.5%**	0	0.0%	0	0.0%	3	2.2%	3	1.6%	12	6.0%	12	7.6%	4	1.6%	4	1.0%	0	0.0%	80	26.9%	0	0.0%

[a] Chikungunya virus was detected in specimens collected in Southern Thailand, between February and September 2019, during a local chikungunya virus outbreak.
[b] Dengue virus was detected at five study sites between November 2018 and October 2019 during a multi-country dengue virus outbreak.

Table 8. Number of Cases of BioFire GF SP Panel Diseases Reported in the United States since 2010 as Reported by the CDC (MMWR) (Leishmaniasis is not a CDC reportable disease and is not included in this table)

Disease	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019 [a]	2020 [b]	2021 [b,c]
Anthrax	0	1	0	0	0	0	0	0	1	1	0	1
Chikungunya virus disease [d]	N/A	N/A	N/A	N/A	N/A	896	247	156	117	192	22	6
Crimean-Congo hemorrhagic fever virus	0	0	0	0	0	0	0	0	0	0	0	0
Dengue virus infections [e]	700	254	547	843	680	951	953	454	474	1,414	332	32
Ebola virus	0	0	0	0	4 [f]	0	0	0	0	0	0	0
Lassa virus	1	0	0	0	1 [f]	1	0	0	0	0	0	0
Leptospirosis [g]	N/A	N/A	N/A	N/A	38	40	78	72	91	94	31	23
Malaria	1,773	1,724	1,503	1,594	1,653	1,390	1,955	2,056	1,748	1,936	360	569
Marburg virus	0	0	0	0	0	0	0	0	0	0	0	0
Plague	2	3	4	4	10	16	4	5	1	1	7	0
Tularemia	124	166	149	203	180	314	230	239	229	274	78	52
West Nile virus disease [h]	1,021	712	5,673	2,469	2,205	2,175	2,149	2,097	2,646	974	664	210
Yellow fever	0	0	0	0	0	0	1	0	0	0	0	0

[a] The following 24 jurisdictions may have incomplete data, due to the coronavirus disease 2019 (COVID-19) pandemic: Alaska, California, Connecticut, Delaware, District of Columbia, Florida, Idaho, Indiana, Kansas, Massachusetts, Minnesota, Missouri, Montana, Nebraska, New Hampshire, New York (excluding New York City), New York City, North Dakota, Ohio, Oklahoma, South Carolina, Tennessee, Texas, and West Virginia.
[b] Case counts for reporting years 2020 and 2021 are provisional and subject to change.
[c] To week of August 28, 2021.
[d] Chikungunya virus disease added to the list of notifiable diseases in 2015.
[e] Note that the case definitions of dengue fever were updated in 2015.
[f] In addition to the four cases of Ebola Virus Disease diagnosed in the United States in 2014, six patients were medically evacuated to the United States for care after being diagnosed with Ebola Virus Disease in West Africa. In total, 11 VHF cases were reported for 2014, 10 confirmed infections with Ebola virus and one confirmed infection with Lassa virus.
[g] Leptospirosis data became available in 2014.
[h] West Nile virus includes neuroinvasive and non-neuroinvasive.

Table 9. Number of Cases of BioFire GF SP Panel Diseases Reported in the United States Territories since 2010 as Reported by the CDC (MMWR) (Leishmaniasis is not a CDC reportable disease and is not included in this table).

Disease	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020 [a]	2021 [a,b]
Anthrax	0	0	0	0	0	0	0	0	0	0	0	0
Chikungunya virus disease [c]	N/A	N/A	N/A	N/A	N/A	237	180	39	8	2	0	0
Crimean-Congo hemorrhagic fever virus	0	0	0	0	0	0	0	0	0	0	0	0
Dengue virus infections [d]	10,911 [e]	1,541	6,167 [e]	9,884 [f]	544	61	218	512	157	118	295	287
Ebola virus	0	0	0	0	0	0	0	0	0	0	0	0
Lassa virus	0	0	0	0	0	0	0	0	0	0	0	0
Leptospirosis [g]	N/A	N/A	N/A	N/A	69	56	77	123	92	95	23	3
Malaria	5	2	1	0	1	7	3	0	0	2	1	0
Marburg virus	0	0	0	0	0	0	0	0	0	0	0	0
Plague	0	0	0	0	0	0	0	0	0	0	0	0
Tularemia	0	0	0	0	0	0	0	0	0	0	0	0
West Nile virus disease [h]	0	0	1	0	0	0	0	0	0	0	0	0
Yellow fever	0	0	0	0	0	0	0	0	0	0	0	0

[a] Case counts for reporting years 2020 and 2021 are provisional and subject to change.
[b] To week of August 28, 2021.
[c] Chikungunya virus disease added to the list of notifiable diseases in 2015.
[d] Note that the case definitions of dengue fever were updated in 2015.
[e] Rise in cases marked by Dengue virus outbreak in Puerto Rico.
[f] Rise in cases marked by a Dengue virus outbreak in Puerto Rico and the U.S. Virgin Islands
[g] Leptospirosis data became available in 2014.
[h] West Nile virus includes neuroinvasive and non-neuroinvasive.

Testing of Prospective Clinical Specimens

The clinical performance of the BioFire GF SP Panel was established during a multi-center study conducted at eleven geographically distinct study sites, including two in the United States, from March 2018 to March 2021. A total of 2249 subjects were enrolled in the prospective clinical study; 110 subjects or their specimens were excluded from the final data analysis.

The most common reasons for specimen exclusion were difficulty drawing blood, procedural errors by laboratory personnel, or inability to obtain a BioFire GF SP Panel or comparator result. The final data set consisted of 2139 whole blood specimens. Table 10**Table 10** provides a summary of demographic information for the specimens included in the prospective study (note: skipped site numbers are due to some potential sites ultimately not participating in the BioFire GF SP Panel Study).

Table 10. Demographics: Overall and Per Site Enrollment

		Overall	Site 01	Site 02	Site 04	Site 05	Site 07	Site 08	Site 09	Site 11	Site 12	Site 13	Site 14
Sex	Female	**1095 (51.2%)**	58 (43.3%)	115 (60.8%)	31 (27.4%)	125 (62.8%)	113 (63.1%)	107 (43.0%)	206 (50.7%)	87 (55.1%)	132 (44.4%)	80 (58.8%)	41 (51.9%)
	Male	**1044 (48.8%)**	76 (56.7%)	74 (39.2%)	82 (72.6%)	74 (37.2%)	66 (36.9%)	142 (57.0%)	200 (49.3%)	71 (44.9%)	165 (55.6%)	56 (41.2%)	38 (48.1%)
Age	<5	**178 (8.3%)**	44 (32.8%)	35 (18.5%)	0 (0%)	0 (0%)	0[a] (0%)	25 (10.0%)	0[b] (0%)	66 (41.8%)	3 (1.0%)	5 (3.7%)	0 (0%)
	5 to 21	**822 (38.4%)**	21 (15.7%)	78 (41.3%)	14 (12.4%)	128 (64.3%)	14[a] (7.8%)	127 (51.0%)	204[b] (50.2%)	70 (44.3%)	102 (34.3%)	61 (44.9%)	3 (3.8%)
	22 to 50	**779 (36.4%)**	42 (31.3%)	46 (24.3%)	62 (54.9%)	59 (29.6%)	106 (59.2%)	63 (25.3%)	141 (34.7%)	21 (13.3%)	146 (49.2%)	61 (44.9%)	32 (40.5%)
	>50	**360 (16.8%)**	27 (20.1%)	30 (15.9%)	37 (32.7%)	12 (6.0%)	59 (33.0%)	34 (13.7%)	61 (15.0%)	1 (0.6%)	46 (15.5%)	9 (6.6%)	44 (55.7%)
Total		**2139**	**134**	**189**	**113**	**199**	**179**	**249**	**406**	**158**	**297**	**136**	**79**

[a] Site was not enrolling subjects <18 years old.
[b] Site was not enrolling subjects <7 years old.

Specimens were evaluated with the BioFire GF SP Panel at clinical study sites. Nucleic acids were also extracted at clinical study sites and shipped to BioFire Defense for comparator testing using analytically validated polymerase chain reaction (PCR)/sequencing-based comparator methods. A BioFire GF SP Panel result (Detected or Not Detected) was considered a True Positive (TP) or True Negative (TN) only when it agreed with the comparator result.

Positive Percent Agreement (PPA) for each analyte was calculated as 100% × (TP / (TP + FN)). False Negative (FN) indicates that the BioFire GF SP Panel result was Not Detected, while the comparator result was positive. Negative Percent Agreement (NPA) was calculated as 100% × (TN / (TN + FP)). False Positive (FP) indicates that the BioFire GF SP Panel result was Detected, but the comparator result was negative. The Wilson Score two-sided 95% confidence interval was calculated.

Specimens for which false positive and/or false negative results (i.e., discrepant results) were obtained when comparing the BioFire GF SP Panel results to the comparator method results were further investigated. The discrepancy investigations were typically performed as follows: 1) Discrepancies between the BioFire GF SP Panel and comparator assays were examined and additional testing performed to determine whether the analyte was initially reported as Negative or Not Detected because it was near or below the detection threshold; 2) FP and FN results were evaluated by at least one additional PCR test that used different primers than the BioFire GF SP Panel assays or the comparator assays; 3) When possible, unresolved discrepancies were evaluated with additional PCR testing that could be verified by sequence analysis. The prospective clinical study results are summarized in **Table 11**Table 11 through **Table 14**Table 13.

The majority of specimens (1720/2139; 80.4%) were tested fresh, while 419 (19.6%) specimens were frozen before later being thawed and tested. The validity of testing frozen specimens was evaluated in a separate study in which contrived and clinical specimens were tested fresh and then retested after freezing. Equivalence was observed for BioFire GF SP Panel performance with frozen compared to fresh specimens. Similarly, a comparison of BioFire GF SP Panel performance on fresh and frozen specimens tested in this study also demonstrated equivalence (Table 11**Table 11**Table 11 through **Table 13**Table 13). Thus, all data for fresh and frozen specimens are combined for all analyses.

Table 11. BioFire GF SP Panel Clinical Performance Summary – Viruses

BioFire GF SP 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%
Ebolavirus	2139	0/0	-	-	2139/2139	100%	99.8-100%
Lassa virus	2139	0/0	-	-	2139/2139	100%	99.8-100%
Marburgvirus	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 12. BioFire GF SP Panel Clinical Performance Summary – Bacteria

BioFire GF SP 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 13. BioFire GF SP Panel Clinical Performance Summary – Protozoa

BioFire GF SP 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.

The prevalence of unique analytes in co-detections is presented in **Table 14**Table 14**.** The BioFire GF SP Panel reported a total of 31 specimens with discernible multiple analyte detections (1.4% of all specimens, 31/2139; 4.3% of positive specimens, 31/725). The majority of co-detections (29/31; 93.5%) contained *Plasmodium* spp. No co-detections contained more than two unique analytes. The most frequently occurring combination of analytes was *P. falciparum* with *P. vivax/ovale* (3.4% of all positive specimens; 25/725).

Table 14. Prevalence of Analytes in Co-Detections as Determined by the BioFire GF SP Panel

FilmArray Detected Result [a]	Prevalence in Overall Detections for the Analyte		Prevalence in All Co-Detections	
Chikungunya virus	2/27	7.4%	2/31	6.4%
Dengue virus (serotypes 1, 2, 3, and 4)	4/266	1.5%	4/31	12.9%
Leptospira	2/19	10.5%	2/31	6.4%
Plasmodium [b]	29/407	7.1%	29/31	93.5%
P. falciparum [c]	26/283	9.2%	26/31	83.9%
P. vivax/ovale [c]	27/118	22.9%	27/31	87.1%

[a] Multiple species/strains within a genus are not always discernible as individual detections.
[b] One out of 29 (1/29) *Plasmodium* spp. co-detections did not have a species-level *Plasmodium* assay detection.
[c] Twenty-five (25) co-detections were positive for *P. falciparum* and *P. vivax/ovale*.

The overall success rate for initial specimen tests on the BioFire GF SP Panel was 98.5% (2136/2168); seven tests did not complete (four due to loss of power, two instrument errors, and one software error), and 25 tests had pouch internal control failures. Of the 32 unsuccessful initial tests, all were retested once, and valid results were produced for 27/32 retested specimens. Only 0.2% (5/2168) of eligible specimens were ultimately excluded due to the inability to obtain a BioFire GF SP Panel test result.

Testing of Archived Clinical Specimens

Many of the analytes detectable by the BioFire GF SP Panel were not encountered in large enough numbers 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 GF SP 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 GF SP Panel, these blood components were expected to provide similar results to whole blood specimens. Specimens came from a range of ages and sexes (**Table 15**Table 15).

Table 15. Overall and Per Site 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 also tested by the same PCR comparator methods used in the clinical performance evaluation. Not all comparator assays were used for every specimen in this study. The selection of comparator assays was based on the potential for observing the given analytes in each specimen. Additionally, comparator testing for pathogen targets previously reviewed by the U.S. Food and Drug Administration as part of the BioFire Global Fever Panel were not performed for this study (i.e., chikungunya virus, dengue virus, *Leptospira* spp., and *Plasmodium* spp.). A panel result (Detected or Not Detected) was considered a true positive (TP) or true negative (TN) only when it agreed with the comparator result (**Table 16**Table 16).

Table 16. BioFire GF SP Panel Archived Specimen 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 [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	100%	98.7-100%

[a] Results for chikungunya virus, dengue virus, *Leptospira* spp., and *Plasmodium* spp. were not evaluated with comparator assays and are omitted from this table.

[b] Due to low specimen volume comparator results for most pathogens were not obtained for two specimens. These specimens were only tested on 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 1 out of 2 FN specimens and 1 out of 2 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 out of 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..

Testing of Contrived Clinical Specimens

Testing was performed using contrived clinical specimens for rare 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 GF SP Panel sensitivity and specificity when testing whole blood specimens contrived with *Bacillus anthracis*, Crimean-Congo hemorrhagic fever virus, *Ebolavirus*, *Francisella tularensis*, Lassa fever virus, *Leishmania* spp., *Marburgvirus*, West Nile virus, yellow fever virus, and *Yersinia pestis*.

Contrived specimens were prepared using residual human whole blood specimens from patients with signs and/or 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. If known, clinically relevant concentrations were used to adjust testing levels. The contrived specimens also served as a negative replicate for all other analytes. 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 GF SP Panel result and the known composition of the contrived specimen. A summary of the results is shown in **Table 17**Table 17.

Table 17. Summary of BioFire GF SP 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[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] CCHF virus and Marburg viruses were tested at additional concentrations to better represent clinically relevant range.
[b] Two false negative CCHF virus results were observed at 2× LoD.
[c] One false negative *Marburgvirus* result was observed at 10× LoD.
[d] Discrepancy testing showed near LoD levels of WNV in a single whole blood sample.
[e] One false negative yellow fever virus result was observed at 5× LoD.

Limit of Detection

The Limit of Detection (LoD) was first estimated by testing dilutions of contrived whole blood samples containing known analyte concentrations. Confirmation of the BioFire GF SP Panel LoD was achieved by testing 20 replicates containing analytes at their estimated LoD concentration. LoD was confirmed when the organism was detected in at least 19 of the 20 replicates tested (19/20 = 95%).

The confirmed LoD values are shown in copies/mL based on quantitative PCR testing using commercially available quantitative real-time PCR assay kits, and units when provided by the source (See **Table 18**

Table 18).

Table 18. Summary of Limit of Detection (LoD) for the BioFire GF SP Panel

Global Fever Special Pathogens Panel Analyte	Isolate Tested	Live/Inactivated	LoD Concentration	
			Copies/mL[1]	Units/mL[2]
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 $TCID_{50}$/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 $TCID_{50}$/mL
	DENV-4: H241	Live	6.4E+01	1.8E+02 $TCID_{50}$/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 $TCID_{50}$/mL
	B-956 Uganda (Lineage 2)	Inactivated	2.3E+04	6.2E+00 $TCID_{50}$/mL
Yellow fever virus	Strain 17D	Live attenuated	1.2E+02	1.2E+01 $TCID_{50}$/mL
PROTOZOA				
Leishmania spp.	*L. donovani*: 9515 (MHOM/IN/95/9515)	Live	1.0E+01	2.2E+01 cells/mL
Plasmodium spp.	*P. falciparum*, IPC 4884 Pursat Cambodia 2011	Live	1.8E+02	N/A
	P. knowlesi, H strain	gDNA	2.4E+01	20 pg/mL
	P. malariae (Clinical Specimen)	Live	1.9E+02	2.3E-01 cells/mL
	P. ovale, *wallikeri* (Clinical Specimen)	Live	2.4E+02	N/A
	P. 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	*P. ovale*, *wallikeri* (Clinical Specimen)	Live	2.4E+02	N/A
	P. vivax, Strain Chesson	Live	1.5E+02	N/A

[1] Based on analyte stock concentrations determined using commercially available quantitative real-time PCR assay kits.
[2] Based on analyte stock concentration/titer values provided by vendor.

The LoD for BioFire GF SP Panel analytes that require BioSafety Level (BSL) 3 or 4 facilities when handling infectious material was estimated by testing dilutions of whole blood samples contrived with infectious stocks. The estimated LoD was defined as the lowest concentration in a serial 10-fold dilution with four out of four (4/4) replicates testing positive (See **Table 19**Table 19).

Table 19. Summary of Estimated LoD for Infectious BSL3/4 BioFire GF SP Panel Analytes

BioFire GF SP Panel Interpretation	Species/Strain	UCC[1] ID	Concentration	
			Copies/mL[2]	Units/mL[3]
BACTERIA				
Bacillus anthracis	Ames	BACI008	6.4E+01	3.5E+00 cfu/mL
Francisella tularensis	subsp. *tularensis* Schu	FRAN016	1.2E+01	2.1E+02 cfu/mL
Yersinia pestis	CO92	YERS023	1.5E+02	3.0E+01 cfu/mL
VIRUSES				
CCHF Virus	IbAr10200	Nairo001	6.4E+02	2.9E+03 pfu/mL
Chikungunya Virus	B8635	Alpha031	5.5E+02	4.4E+01 pfu/mL
	Indo23574	Alpha008	5.5E+02	9.0E+01 pfu/mL
Ebolavirus	Bundibugyo virus / Uganda (811250)	Ebola005	7.0E+02	5.6E+00 pfu/mL
	Taï Forest virus / Taï Forest (Ivory Coast)	Ebola004	1.8E+02	N/A
	Reston virus / H-28	Ebola003	2.7E+03	4.7E+01 pfu/mL
	Sudan virus / Boniface	Ebola002	1.1E+02	5.3E+00 pfu/mL
	Zaire ebolavirus / Makona	Ebola027	1.1E+03	1.6E+01 pfu/mL
Lassa Virus	Josiah	Arena002	5.6E+03	2.6E+01 pfu/mL
Marburg marburgvirus	Ci67	Marbrg003	5.0E+02	1.5E+03 pfu/mL
	Ravn	Marbrg002	2.6E+02	4.7E+01 pfu/mL
West Nile Virus	Bz NY99 (lin. 1)	Flavi022	1.6E+02	9.5E+00 pfu/mL
Yellow Fever Virus	Asibi	Flavi005	1.2E+01	9.2E+00 pfu/mL

[1] U.S. Department of Defense Unified Culture Collection.
[2] Based on analyte stock concentrations determined using commercially available quantitative real-time PCR assay kits.
[3] Based on analyte stock titer values provided by vendor.

Analytical Reactivity (Inclusivity)

Analytical reactivity of the BioFire GF SP Panel assays was evaluated in silico and by empirical testing of isolates representing relevant species, serotypes, and strains where available. Each isolate was tested in triplicate at near LoD levels. If an isolate was detected at ≤3.5×LoD, the isolate was considered inclusive. Isolates detected at higher concentrations are considered to be detected with reduced sensitivity.

Isolates with limitations on assay reactivity (based on wet testing observations) are noted in **Table 20**Error! Reference source not found., with results of the in silico analysis for these isolates shown in the footnotes. Results for analytical reactivity testing are summarized for each analyte in Error! Reference source not found.**Table 21** and **Table 22** Error! Reference source not found.with strains used for LoD testing shown in bold text. No additional isolates of CCHF virus were available for testing.

NOTE: *The BioFire GF SP Panel may react with vaccines that contain specific segments of the pathogen or full genome vaccines containing attenuated/inactivated pathogen, including vaccines for yellow fever. Care should be taken to minimize contamination of samples with vaccines, and clinical history of vaccine administration should be considered in the interpretation of results.*

Table 20. Limitations on Analytical Reactivity of the BioFire GF SP Panel Assays

Observed Result	Detection Level	Analyte	Serotype/Strain/Isolate
Detected (may be underreported)	10×LoD	*Leishmania* spp.	*L. braziliensis* Vianna [1]
			L. infantum [1]
		Plasmodium falciparum	SenTh021.09 [1]
		Bacillus anthracis	SK-102 (Pakistan) [2]
			Vollum 1B [2]
	~100×LoD	Dengue virus	Serotype 3 BC188/97 [1]
			Serotype 4 D85-019 [1]
		Ebolavirus	*Zaire ebolavirus* Mayinga (Inactivated) [3]
		Yellow fever virus	CAREC M2-09 [4]
			INHRR 7a-05 [4]
			INHRR 10a-10 [4]
	1000×LoD	Yellow fever virus	SVM 3-18-09 [4]
Not Detected	N/A	Dengue virus	Serotype 2 DKA 811 [5]

[1] The reason for the observed reduced reactivity could not be identified based on in silico sequence analysis. Sequences for these specific strains were not available in public databases.
[2] In silico analysis did not reveal any sequence misalignments under the primers that would explain the observed reduction in sensitivity.
[3] Results of live testing did not show reduced sensitivity for the detection of *Zaire ebolavirus* Mayinga, and therefore the observed reduced sensitivity for inactivated *Zaire ebolavirus* Mayinga material most likely was the result of damage to nucleic acid due to the inactivation process. Therefore, no limitation for detection of *Zaire ebolavirus* Mayinga is listed.
[4] 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. Available sequences for these strains do not contain the full target region of the assay, therefore the reduced sensitivity could not be fully investigated.
[5] In silico analysis predicted reduced sensitivity or missed detection of this isolate due to sequence variation under the primers. Wet testing of this rare sylvatic strain at 10,000×LoD confirmed that detection was significantly impaired.

Table 21. BioFire GF SP 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	

Analyte	# Isolates Detected / Tested	Isolates Tested		Source / ID	Concentration Detected Tested up to 100× LoD[1]	Limitations
		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	
		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**	

Analyte	# Isolates Detected / Tested	Isolates Tested		Source / ID	Concentration Detected Tested up to 100× LoD[1]	Limitations
			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 strain BC188/97
			271242	BEI / NR-3802	3.9E+02 copies/mL	
			BC188/97	BEI / NR-3801	1.6E+04 copies/mL	
			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	***L. donovani* 9515**		**BEI / NR-48822**	**1.0E+01 copies/mL**	Reduced sensitivity for *braziliensis* and *infantum* strains
		L. amazonensis		BEI / NR-49247	3.0E+01 copies/mL	
		L. braziliensis Vianna		ATCC / 30879	1.0E+02 copies/mL	
		L. donovani, 1S		BEI / NR-48821	3.4E+01 copies/mL	
		L. donovani chagasi		ATCC / 50133	3.0E+01 copies/mL	
		L. infantum		ATCC / 50134	1.0E+02 copies/mL	
		L. major IR173		BEI / NR-48816	3.0E+01 copies/mL	

Analyte	# Isolates Detected / Tested	Isolates Tested		Source / ID	Concentration Detected Tested up to 100× LoD[1]	Limitations
		L. mexicana MHOM/BZ/82/BEL21		ATCC / 50157	2.9E+01 copies/mL	
		L. panamensis PSC-1		BEI / NR-50162	3.0E+01 copies/mL	
		L. tropica (MHOM/AF/87/RUP)		BEI / NR-48820		
		L. tropica (MHOM/SU/58/strain-OD)		ATCC / 50130		
		L. venezuelensis MHOM-VE/80/H-16		BEI / NR-29184	3.4E+01 copies/mL	
Plasmodium spp. (Plasmodium spp. assay)	10/10	***P. falciparum***	**IPC 4884**	**BEI / MRA-1238**	**1.8E+02 copies/mL**	None
			SenTh021.09	BEI / MRA-1182	3.0E+02 copies/mL	
			St. Lucia	BEI / MRA-331		
			Tanzania, 02000708	BEI / MRA-1169		
		P. vivax	**Chesson**	**BEI / MRA-383**	**1.5E+02 copies/mL**	
			Panama	ATCC / 30138	3.1E+02 copies/mL	
		P. ovale	**Wallikeri**	**CDC / N8K9QKI9**	**2.4E+02 copies/mL**	
			Curtisi	CDC / N8K9QL0S	7.2E+02 copies/mL	
		P. knowlesi	**Strain H**	**BEI / MRA-456G**	**2.4E+01 copies/mL**	
		P. malariae	**Clinical Sample**	**DLS / DLS17-026015**	**1.9E+02 copies/mL**	
Plasmodium falciparum (Plasmodium falciparum assay)	4/4	***P. 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	***P. vivax***	**Chesson**	**BEI / MRA-383**	**1.5E+02 copies/mL**	None
			Panama	ATCC / 30138	3.1E+02 copies/mL	
		P. 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.

Table 22. BioFire GF SP Panel Analytical 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	
Marburg Marburgvirus	2/2	**Lineage**	**Strain**			None
		Ravn virus	**RAVN**	**Marb002**	**2.6E+02 copies/mL**	

Analyte	# Isolates Detected/Tested	Isolates Tested		UCC [1] ID	Concentration Detected	Limitations
		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 by enumeration in Colony Forming Units (CFU)/mL.

3 After testing was completed, it was determined that *Y. pestis* bv. Medievalis strains Harbin35 and KIM5 were evaluated at higher than 3× the estimated LoD, at 3.5× and 4.2×, respectively.

4 The Lassa virus Macenta and Pinneo strains were not detected by the qPCR assay. 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. Available sequences for these strains do not contain the full target region of the assay, therefore the reduced sensitivity could not be fully investigated.

Analytical Reactivity (Cross-Reactivity and Exclusivity)

The potential for non-specific amplification and detection by the GF SP Panel assays was evaluated by empirical testing of high concentrations of organisms/viruses. Organisms/viruses not available for empirical testing were evaluated by in silico analysis. On-panel organisms were tested to assess the potential for intra-panel cross-reactivity. Organisms and viruses for off-panel testing were selected based on a combination of several factors including (1) relatedness to specific species detected by the GF SP Panel (near-neighbors), (2) clinical relevance (cause illness or symptoms similar to the panel pathogens) (3) 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 (4) genetic similarity to BioFire GF SP Panel assay primers, as determined by in silico analyses.

On-panel and off-panel testing included more than 217 isolates of bacteria, viruses, fungi, and protozoa evaluated at high concentrations (typically >1E+06 genomic copies/mL). **Table 23**Table lists the organisms and virus that showed cross-reactivity with BioFire GF SP Panel assays with results of the in silico analysis shown in the footnotes. Table**Table 24** lists the on-panel and off-panel organisms and viruses that showed no cross-reactivity, respectively (either observed in testing or, when not available for testing, predicted by in silico analyses).

Table 23. Observed Cross-Reactivity of BioFire GF SP Panel Assays

Cross-Reactive Organism/Virus	BioFire GF SP Panel Test Result
On-Panel	
Plasmodium knowlesi [1] *Plasmodium malariae* [2]	*Plasmodium vivax/ovale*
Off-Panel	
Francisella hispaniensis [3] *Francisella tularensis* subsp. *mediasiatica* [4]	*Francisella tularensis*
O'nyong-nyong virus	Chikungunya virus
Crithidia fasciculata [5] *Leptomonas seymouri* [6]	*Leishmania* spp.
Plasmodium berghei [7] *Plasmodium brasilianum* [7] *Plasmodium cynomolgi* [7] *Plasmodium fieldi* [7] *Plasmodium fragile* [7] *Plasmodium inui* [7] *Plasmodium simiovale* [7]	*Plasmodium spp.* and *Plasmodium vivax/ovale*

[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/ovale* 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.

Table 24. Organisms and Viruses with No Cross-Reactivity with BioFire GF SP Panel Assays (Observed or Predicted by in silico Analysis)

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* (Musoke Strain)
Chikungunya virus	*Sudan ebolavirus*	*Marburg marburgvirus* (Ravn Strain)
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 Newport
Bacillus brevis	*Francisella persica* (formerly *Wolbachia persica*)	*Salmonella enterica* subs. *enterica* serovar Rubislaw
Bacillus cereus	*Francisella philomiragia* (formerly *Yersinia*)	*Salmonella enterica* susb. *enterica* serovar Saintpaul
Bacillus circulans	*Klebsiella oxytoca*	*Salmonella enterica* subs. *enterica* serovar Tennessee
Bacillus coagulans	*Legionella pneumophila*	*Salmonella enterica* subs. *enterica* serovar Thompson
Bacillus halodurans	*Leptospira biflexa*	*Salmonella enterica* subs. *enterica* serovar Typhimurium
Bacillus licheniformis	*Leptospira meyeri*	*Salmonella enterica* subs. *houtenae*
Bacillus megaterium	*Leptospira terpstrae* genomospecies 4	*Salmonella enterica* subs. *indica*
Bacillus mycoides	*Leptospira vanthielii* genomospecies 3	*Salmonella enterica* subs. *salamae*
Bacillus pumilus	*Leptospira wolbachii*	*Salmonella enterica* subsp. *enterica* Paratyphi A
Bacillus subtilis	*Leptospira yanagawae* genomospecies 5	*Salmonella enterica* subsp. *enterica* Typhi
Bacillus thuringiensis	*Listeria monocytogenes*	*Serratia marcescens*
Bacteroides fragilis	*Mycobacterium tuberculosis*	*Staphylococcus aureus*
Bordetella bronchiseptica	*Mycoplasma pneumoniae*	*Streptococcus agalactiae*
Borrelia burgdorferi	*Neisseria meningitidis*	*Streptococcus pneumoniae*
Brucella melitensis	*Orientia chuto (tsutsugamushi)*	*Streptococcus pyogenes*
Burkholderia cepacia	*Proteus mirabilis*	*Treponema pallidum pallidum*
Burkholderia mallei	*Pseudomonas aeruginosa*	*Vibrio cholerae*
Burkholderia pseudomallei	*Rickettsia prowazekii*	*Yersinia aldovae*
Chlamydophila pneumoniae	*Rickettsia ricketsii*	*Yersinia bercovieri*
Chlamydophila psittaci	*Rickettsia typhi*	*Yersinia entericolitica*
Citrobacter koseri	*Salmonella enterica* subs.*bongori*	*Yersinia fredericksenii*
Clostridium bifermentans	*Salmonella enterica* subs. *arizonae*	*Yersinia intermedia*
Clostridium botulinum/sporogenes	*Salmonella enterica* subs. *diarizoniae*	*Yersinia kristensenii*
Clostridium perfringens	*Salmonella enterica* subs. *enterica* serovar Enteritidis	*Yersinia mollaretii*
Clostridium sordellii	*Salmonella enterica* subs. *enterica* serovar Heidelberg	*Yersinia pseudotuberculosis*
Coxiella burnetii	*Salmonella enterica* subs. *enterica* serovar Javiana	*Yersinia rohdei*
Enterobacter aerogenes	*Salmonella enterica* subs. *enterica* serovar Montevideo	*Yersinia ruckeri*
Enterococcus faecalis	*Salmonella enterica* subs. *enterica* serovar Muenchen	*Yersinia similis*
***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	Ross River virus
Coronavirus NL63	Human respiratory syncytial virus	Rubella virus
Cytomegalovirus	Human T-lymphotropic virus, type 1	SARS-CoV-2
Dugbe virus	Human T-lymphotropic virus, type 2	Saint Louis encephalitis virus
Eastern equine encephalitis virus	Influenza A H1N1-2009	Semliki Forest virus
Enterovirus, HEV-71	Influenza A H3N2	Sindbis virus
Epstein Barr virus	Influenza B virus	Spondweni virus
Flexal virus	Japanese encephalitis virus	Tickborne encephalitis virus
Guanarito virus	Junin virus (2 strains: XJ and Candid)	Tonate virus
Hantaan virus	Machupo virus	Una virus
Hazara virus	Mayaro virus	Usutu virus
Hendra virus	Measles virus	Vaccinia virus
Hepatitis A virus	Metapneumovirus	Varicella zoster virus
Hepatitis B virus	Middelburg virus	Venezuelan equine encephalomyelitis virus
Hepatitis C virus	Mopeia virus	Western equine encephalomyelitis
Herpes simplex virus type 2	Mumps virus	Zika virus
Hughes virus	Murray Valley encephalitis 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 rangeli*
Fungus		
Tested		
Aspergillus fumigatus	*Cryptococcus neoformans* var. *grubii*	
Helminths		
Tested		
Schistosoma mansoni		

Reproducibility

Reproducibility testing was performed with contrived whole blood samples over multiple days at three sites using the BIOFIRE FILMARRAY 2.0 system. The testing incorporated a range of potential variation introduced by operator, instrument, analyte concentration, and reagent lot for a total of 90 replicates for each analyte concentration distributed equally over three sites.

Three contrived whole blood samples were prepared with different mixtures of six representative panel analytes, two bacteria, two viruses, 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).

A summary of results (percent (%) agreement with the expected Detected or Not Detected result) for the analytes by site is provided in **Table 25**Table **.**

Table 25. Reproducibility of BioFire GF SP Panel Test on BIOFIRE FILMARRAY 2.0 Systems

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%]

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
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 IPC 4884 (BEI / MRA-1238)	***Plasmodium* spp. Detection Results**	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%]
		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 of BIOFIRE FILMARRAY 2.0 and BIOFIRE FILMARRAY Torch Systems

Performance of the BioFire GF SP Panel was compared between the BIOFIRE FILMARRAY 2.0 and BIOFIRE FILMARRAY Torch systems by comparing analyte detection for the same 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 site is provided in **Table 26**Table .

NOTE: *All other performance evaluations were performed using the BIOFIRE FILMARRAY 2.0 system. Performance of the BioFire GF SP Panel when using the BIOFIRE FILMARRAY Torch system was comparable to the BIOFIRE FILMARRAY 2.0 system.*

Table 26. Reproducibility of BioFire GF SP 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**							
			FilmArray 2.0 Platform				**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%** [87.6-97.6%]
	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%]

Analyte (Source / ID)		Concentration Tested (copies/mL)	Expected Result	Detection Rate (n/N) % Agreement with Expected Result							
				FilmArray 2.0 Platform				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]
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%]
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

Interference

Potentially interfering substances were selected 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; (i) endogenous substances native to blood that may vary in concentration as a result of normal or disease physiology, (ii) exogenous substances that may be found in a blood sample as a consequence of therapeutic intervention or ingestion, (iii) microorganisms that may be present as co-infections or that may be unintentionally introduced into samples, and (iv) technique specific substances that may be introduced into a sample during routine laboratory handling. The technique specific substances evaluated included blood collection tubes containing a variety of anticoagulants.

Potential interference of substances on the control assays and analyte detection test results was evaluated by comparing BioFire GF SP Panel test results from a control blood sample, containing representative panel analytes at concentrations near (approximately 3×) LoD, to results from a sample with the same analyte composition plus a test substance. In addition, a negative sample (no analytes) containing only the test substance was evaluated for the potential to result in false positives.

A summary of substances tested and whether interference was observed is provided in **Table 27**Table 27.. Potential interference with analyte detection was only observed for heparin and TRIzol when testing analytes at near-LoD concentrations. Overall, based on acceptance criteria of this study, no other substance tested showed interference with performance of the BioFire GF SP Panel at the concentration tested.

Table 27. Evaluation of Potentially Interfering Substances on the BioFire GF SP 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
Gentamicin	0.036 mg/mL	No interference
Meropenem	0.39 mg/mL	No interference

Potentially Interfering Substance	Concentration Tested	Results
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
Acetone	2% 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
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

APPENDIX A

Symbols Glossary

The following symbols can be found on labeling for the BioFire FilmArray 2.0, BioFire FilmArray Torch, BioFire GF SP Panel kits, kit components, and throughout accompanying packaging.

ISO 15223-1 Graphical symbols for use on equipment – Registered Symbols					
5.1.1 	Manufacturer	5.1.4 	Use-By date (YYYY-MM-DD)	5.1.5 	Batch Code (Lot Number)
5.1.6 	Catalog Number	5.1.7 	Serial Number	5.2.8 	Do Not Use if Package Is Damaged
5.3.2 	Keep Away from Sunlight	5.3.7 	Temperature Limit	5.4.2 	Do not re-use
5.4.3 	Consult Instructions for Use	5.5.1 	*In vitro* Diagnostic Medical Device	5.5.5 	Contains sufficient for <n> tests
United Nations Globally Harmonized System of Classification and Labeling of chemicals (GHS) (ST/SG/AC.10/30)					
	Serious eye damage, Category 1		Acute aquatic hazard, Category 1 & Long-term aquatic hazard, Category 1		Acute toxicity, oral, Category 4 & Skin corrosion, irritation, Category 2
Use of Symbols in Labeling – 81 FR 38911, Docket No. (FDA-2013-N-0125)					
℞ Only	Prescription Use Only CAUTION: Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner.				
Manufacturer Symbols (BioFire Defense, LLC)					
	BioFire Defense Logo		BioFire Global Fever Special Pathogens Panel		

APPENDIX B

Contact and Legal Information

Customer and Technical Support	
Contact Us on the Web http://www.BioFireDefense.com **Contact Us by Mail** 79 West 4500 South, Suite 14 Salt Lake City, Utah USA 84107	**Contact Us by E-mail** support@BioFireDefense.com **Contact Us by Phone** 1-801-262-3592 – US and Canada 1-801-262-3592 – International **Contact Us by Fax** 1-801-447-6907

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79 West 4500 South, Suite 14
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 DFA2-PRT-0136-03, April 2025

APPENDIX C

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Revision History

Version	Revision Date	Description of Revision(s)
01	July 2023	Initial Release
02	May 2024	Removed Clear Cap from Step 1: Sample Processing Instructions
03	April 2025	Added new Ampoule option, updated BIOFIRE FILMARRAY branding

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