REF DFA2-ASY-0004

BioFire®

Global Fever Panel

Instructions for Use

IVD

℞ Only

The Symbols Glossary is provided on Page 46 of this booklet.

For In Vitro Diagnostic Use

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INTENDED USE

The BioFire® Global Fever Panel is a qualitative, multiplexed, nucleic acid-based *in vitro* diagnostic test intended for use with BioFire® FilmArray® 2.0 and BioFire® FilmArray® Torch Systems. The BioFire Global Fever Panel detects and identifies selected 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 following target pathogens: chikungunya virus, dengue virus (serotypes 1, 2, 3 and 4), *Leptospira* spp., and *Plasmodium* spp. (including species differentiation of *Plasmodium falciparum* and *Plasmodium vivax/ovale*). 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. 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.

Positive results do not rule out co-infections with pathogens not included on the BioFire Global Fever Panel. Not all pathogens that cause acute febrile illness are detected by this test, and negative results do not rule out the presence of other infections. In the United States, patient travel history and consultation of the CDC Yellow Book should be considered prior to use of the BioFire Global Fever Panel as some pathogens are more common in certain geographical locations.

For In Vitro Diagnostic Use.

SUMMARY AND EXPLANATION OF THE TEST

The BioFire Global Fever (GF) Panel pouch conducts six tests for the identification of six bacterial, viral, and protozoan pathogens (**Table 1**). The specimen can be tested using the BioFire Global Fever Panel with results available in about one hour.

Table 1. Pathogens Detected by the BioFire Global Fever (GF) Panel

Type	Organism
Bacterial	*Leptospira* spp.
Viral	Chikungunya virus
	Dengue virus (serotypes 1, 2, 3 and 4)
Protozoan	*Plasmodium* spp.
	Plasmodium falciparum
	Plasmodium vivax/ovale

Summary of Detected Organisms

Bacteria

***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[1–3]. 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*[3].

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[4,5]. 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[6]. 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[6,7].

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* [8,9]. 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)[10]. 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 [8,11]. Other endemic viruses in these regions such as chikungunya virus and Zika virus have similar symptoms and transmission which complicate differential diagnosis [7,10].

Protozoa

Malaria in humans is primarily caused by five species of the *Plasmodium* genus (Family *Plasmodiiae*); *P. falciparum, P. vivax, P. malariae, P. ovale,* and *P. knowlesi*. 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[12]. The most common malaria infections are caused by *P. falciparum* and *P. vivax,* whereas infections of *P. ovale* and *P. malariae* are less common[12,13]. *P. knowlesi* is emerging as a significant cause of zoonotic malaria in Southeast Asia [12,14,15]. Co-infection with multiple *Plasmodium* species is possible and should always be considered[12,14].

> ***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[13,14]. In the most severe cases the infection can affect the brain and cause cerebral malaria which may be fatal[12,14]. In some areas *P. falciparum* has developed resistance to the malaria treatment drugs chloroquine and mefloquine[14,16].

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[12–14,16]. *P. vivax* is generally considered sensitive to chloroquine, however in Papua New Guinea and Indonesia there is a high prevalence of chloroquine-resistance[14].

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*[12–14,16]. Similar to *P. vivax*, relapse of disease may occur unless infections are treated for dormant hypnozoites in the liver[12,14,16]. No wide-spread resistance to chloroquine has been reported for *P. ovale*[14].

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[12–14]. No widespread resistance to chloroquine has been observed in *P. malariae*[14,16].

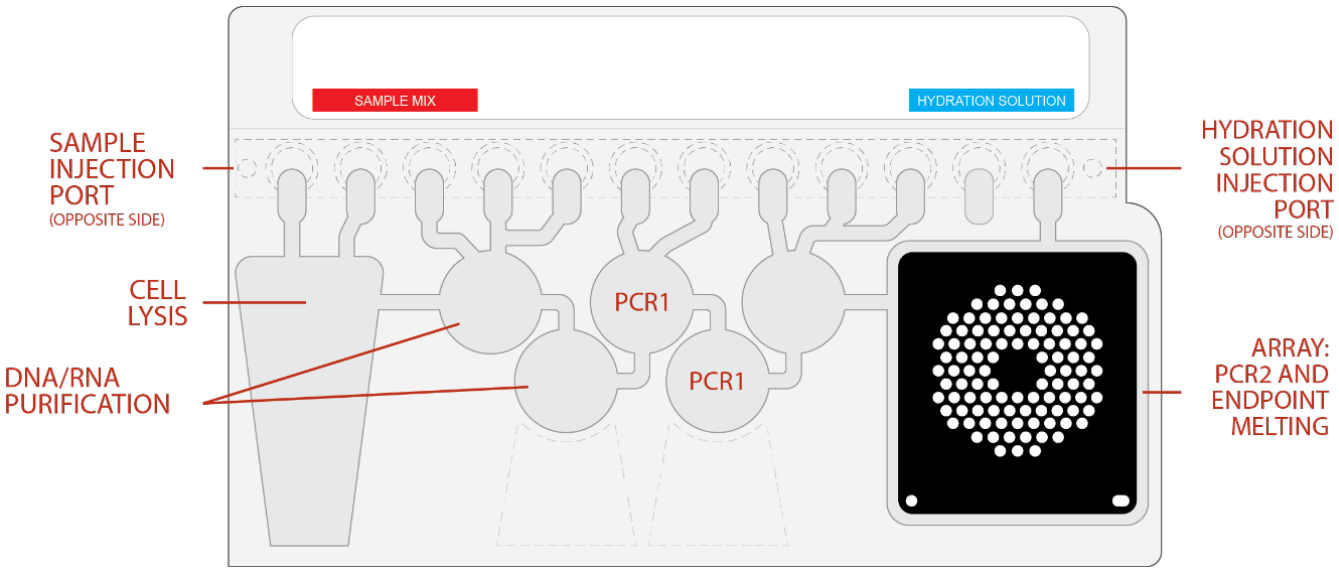
Plasmodium knowlesi is a zoonotic malaria parasite found throughout Southeast Asia, particularly in the states of Sarawak and Sabah in Malaysia. The primary host of *P. knowlesi* is macaques, and transmission of the parasite to humans is limited to persons exposed to mosquitoes that feed on both humans and macaques[12,15]. 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*[14,16].

PRINCIPLE OF THE PROCEDURE

The BioFire Global Fever (GF) 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 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 beading) 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, massively-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 Panel.



MATERIALS PROVIDED

Each BioFire Global Fever Panel kit contains sufficient reagents to test 6 samples (Part No. DFA2-ASY-0004). Materials include:

- Individually packaged BioFire® Global Fever 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/globalfeverpanel
  - *BioFire Global Fever Panel – Instructions for Use*
  - *BioFire Global Fever 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 and accompanying software
  - BioFire® FilmArray® Pouch Loading Station
  - BioFire® Global Fever Panel Pouch Module Software is required to run the BioFire Global Fever 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

OPTIONAL MATERIALS

- BIOFIRE® SHIELD™ Control Kit for the BioFire Global Fever Panel (Part No. DFA2-ASY-0006)
  - The BIOFIRE SHIELD Control kit for the BioFire Global Fever Panel provides assayed positive and negative external controls for use in BioFire Global Fever Panel verifications.
  - See the *BIOFIRE SHIELD Control Kit for the BioFire Global Fever Panel Instructions for Use* for further information.

WARNINGS AND PRECAUTIONS

General Precautions

1. For In Vitro Diagnostic (IVD) Use Only.
2. BioFire Global Fever Panel pouches are only for use with BioFire FilmArray 2.0 and Torch Systems.
3. Always check the expiration date on the pouch. Do not use a pouch after its expiration date.
4. 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.
5. 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 were capable of transmitting infectious agents. Observe safety guidelines such as those outlined in:
   - CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*[17]
   - CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*[18]
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 contains Guanidinium chloride and Triton X100.

   The following statements apply:

   - Health Hazards
     - Acute Toxicity, oral (Category 4)
       - H302 – Harmful if swallowed.
     - Skin corrosion/irritation (Category 2)
       - H315 - Causes skin irritation.
     - Serious eye damage/eye irritation (Category 1)
       - H318 - Causes serious eye damage.

   - Environment Hazards
     - Hazardous to the aquatic environment, acute aquatic hazard (Category 1)
       - H400 - Very toxic to aquatic life.
     - Hazardous to the aquatic environment, long-term aquatic hazard (Category 1)
       - H410 - Very toxic to aquatic life with long lasting effects.

   - Precautionary Statements
     - Prevention
       - P273 – Avoid release to the environment.
       - P280 – Wear protective gloves/protective clothing/eye protections/face protection.

   - Response
       - P332 + P313 - If skin irritation occurs: Get medical advice/attention.

- P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- P301 + P312 - IF SWALLOWED: Call a POISON CENTRE/doctor if you feel unwell.
- P337 + P313 - If eye irritation persists: Get medical advice/attention.

Please refer to the *BioFire Global Fever Panel Safety Data Sheet* (SDS) for more information: https://www.biofiredefense.com/product-support/safety-data-sheets/

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 Global Fever Panel, it is important to guard against contamination of the specimen and work area by carefully following the testing process outlined in this instruction document, including these guidelines:

- Specimens should be processed in a biosafety cabinet. If a biosafety cabinet is not available, a dead air box or protective 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 culture.
- 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.

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 Global Fever 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 as described below and in the appropriate BioFire FilmArray operator's manual. DO NOT PERFORM ADDITIONAL TESTING UNIL THE AREA HAS BEEN DECONTAMINATED

Precaution Related to Public Health

Local and/or national regulations for notification of reportable disease are continually updated and include a number of pathogens for surveillance and outbreak investigations[19]. Laboratories are responsible for following their local and/or national regulations and should consult their local and/or national public health laboratories for isolate and/or sample submission guidelines. In the United States, when Detected, all of the pathogens on this panel must be reported to the Centers for Disease Control and Prevention (CDC).

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 Global Fever 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 Global Fever 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.

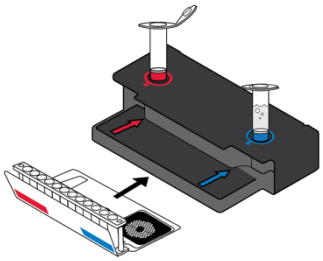
Refer to the *BioFire Global Fever Panel Quick Guide* or the appropriate BioFire FilmArray operator's manual for more details.

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.*

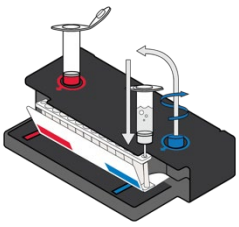
3. Check the expiration date on the pouch. Do not use expired products.
4. Insert the pouch into the Pouch Loading Station, aligning the red and blue labels on the pouch with the red and blue arrows on the Pouch Loading Station.
5. Remove the Sample Injection Vial from its package by tearing or cutting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

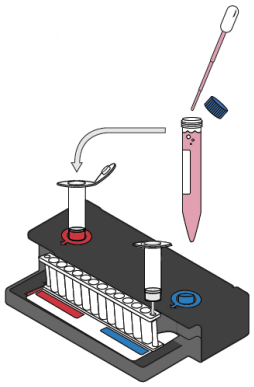
1. Unscrew the Hydration Injection Vial from the blue cover.
2. Remove the Hydration Injection Vial, leaving the blue cover in the Pouch Loading Station.
3. Insert the Hydration Injection Vial cannula tip into the pouch hydration port located directly below the blue arrow of the Pouch Loading Station.



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*.

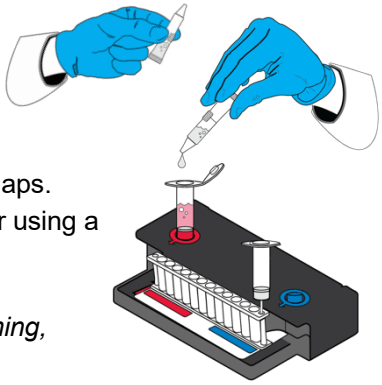
Step 3: Prepare Sample Mix

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



1. Use the Transfer Pipette provided in the test kit to draw the sample 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.*



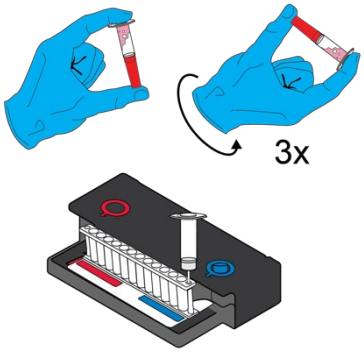
4. Add Sample Buffer to the Sample Injection Vial.
   - Hold the Sample Buffer tube with the tip facing up.

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

   - Firmly pinch at textured plastic tab on the side of the tube until the seal snaps.
   - Invert the tube over the Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

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

WARNING: The Sample Buffer is harmful if swallowed and can cause serious eye damage and skin irritation.

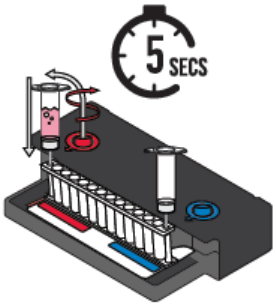


5. Tightly close the lid of the Sample Injection Vial.
6. Remove the Sample Injection Vial from the Pouch Loading Station and invert the vial at least 3 times to mix.
7. Return the Sample Injection Vial to the red well of the Pouch Loading Station.

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 sample loading port.
   - If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded. 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 FilmArray 2.0 and FilmArray Torch 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 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 Global Fever Panel has three Protocols available in the drop-down list: GF Blood, Positive External Control, and Negative External Control.

   NOTE: *Two additional protocols are provided for use with the BIOFIRE SHIELD Control Kit for the BioFire Global Fever Panel. It is necessary to select the appropriate protocol prior to running the test. The Positive External Control and Negative External Control protocols are only for use with the BIOFIRE SHIELD Control Kit and should not be used to test clinical samples or other types of controls. Refer to the BIOFIRE SHIELD Control Kit for the BioFire Global Fever 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 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 Global Fever 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 selected module.
   - 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 Sample protocol. The BioFire Global Fever Panel Test uses a single sample protocol for the testing of all clinical sample types.

   NOTE*: Two additional protocols are provided for use with the BIOFIRE SHIELD Control Kit for the BioFire Global Fever Panel. It is necessary to select the appropriate protocol prior to running the test. The Positive External Control and Negative External Control protocols are only for use with the BIOFIRE SHIELD Control Kit and should not be used to test clinical samples or other types of controls. Refer to the BIOFIRE SHIELD Control Kit for the BioFire Global Fever 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 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 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[20,21]. 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. 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 can be used as positive external controls using the GF Blood pouch protocol.

Evaluation of external controls is recommended prior to using a new shipment or new lot of BioFire Global Fever 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 Global Fever 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 Global Fever Panel as part of the laboratory's Quality Control program.

BioFire Defense provides an external quality control kit to monitor the performance of in vitro laboratory nucleic acid testing procedures for the qualitative detection of the BioFire Global Fever Panel performed on BioFire FilmArray Systems. The BIOFIRE® SHIELD™ Control Kit for the BioFire Global Fever 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 in a FilmArray Control Injection Vial that is used directly with the BioFire Global Fever Panel.

The BIOFIRE SHIELD Control Kit for the BioFire Global Fever 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 Panel
Part Number: DFA2-ASY-0006

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 (i.e., the melt curve peak is located within the white area of the melt curve chart). If the software determines that the Tm of the curve is not in the appropriate Tm range, the melt curve is called negative (i.e., the melt curve peak is located in the grey area of the melt curve chart).

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

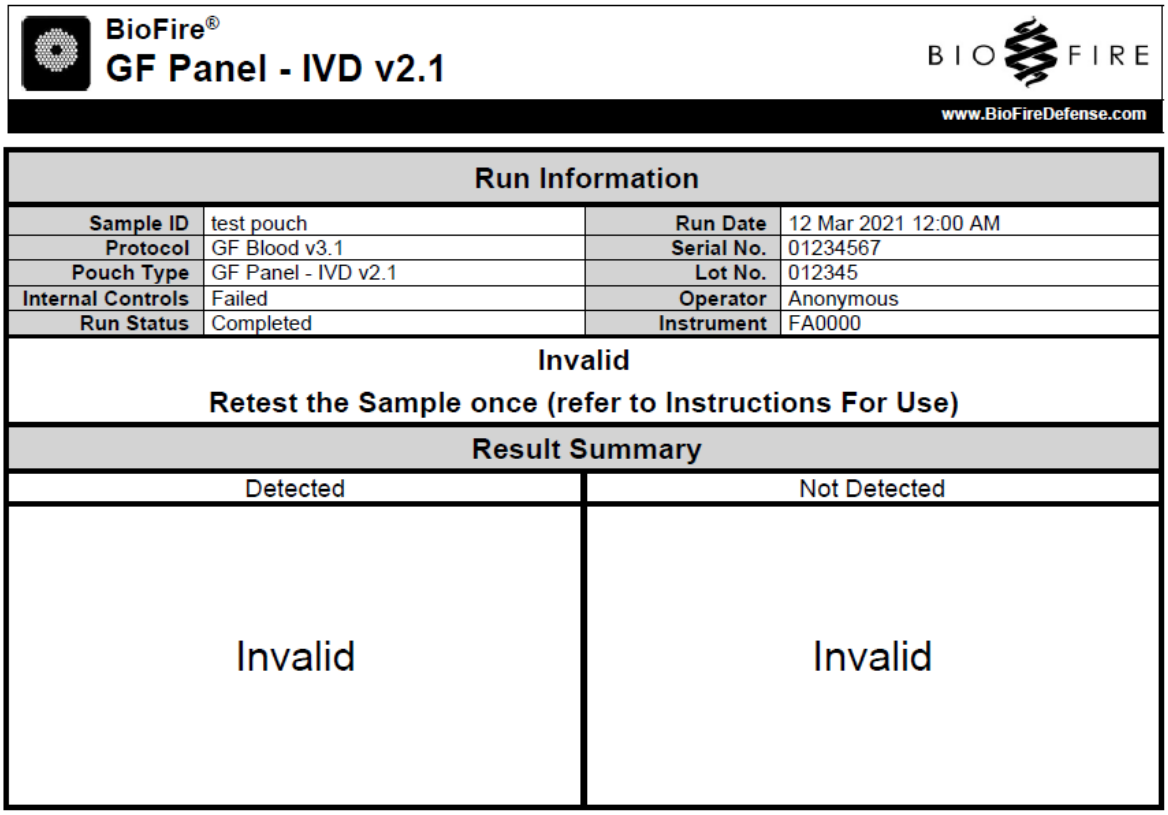
The reported BioFire Global Fever Panel organism interpretation of results (Detected or Not Detected) may be based on the result of a single assay or on results from a combination of multiple assays, as shown in **Table 2**. In cases where either or both of the control assays have failed, all analyte results are reported as Invalid (**Figure 1**). Nationally notifiable results are to be reported to public health authorities in accordance with local, state, and federal law. In the United States, when Detected, all of the pathogens on this panel must be reported to the Centers for Disease Control and Prevention.

Table 2. Assay Number and Interpretation Rules for the BioFire Global Fever Panel

Organism	No. of Assays	Assay Interpretation Rules
BACTERIA		
Leptospira spp.	1	Positive = Detected
VIRUSES		
Chikungunya virus	2	Any Positive = Detected
Dengue virus[a]	5	Any Positive = Detected
PROTOZOAN		
Plasmodium spp.	1	Positive = Detected
Plasmodium falciparum	1	Positive = Detected
Plasmodium vivax/ovale	1[b]	Positive = Detected

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

[b.] The BioFire Global Fever Panel contains one multiplexed assay for the detection of both *Plasmodium vivax* and *Plasmodium ovale*, which is reported with a single interpretation call.



BioFire®
GF Panel - IVD v2.1
BIOFIRE
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Run Information			
Sample ID	test pouch	Run Date	12 Mar 2021 12:00 AM
Protocol	GF Blood v3.1	Serial No.	01234567
Pouch Type	GF Panel - IVD v2.1	Lot No.	012345
Internal Controls	Failed	Operator	Anonymous
Run Status	Completed	Instrument	FA0000

Invalid

Retest the Sample once (refer to Instructions For Use)

Result Summary	
Detected	Not Detected
Invalid	Invalid

Figure 1. BioFire Global Fever Panel Report with failed Internal Controls resulting in an Invalid report

Leptospira spp. Reporting

The BioFire Global Fever 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.

Chikungunya virus Reporting

The BioFire Global Fever 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.

Dengue virus Reporting

The BioFire Global Fever 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.

Plasmodium Reporting

The BioFire Global Fever 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*). 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.

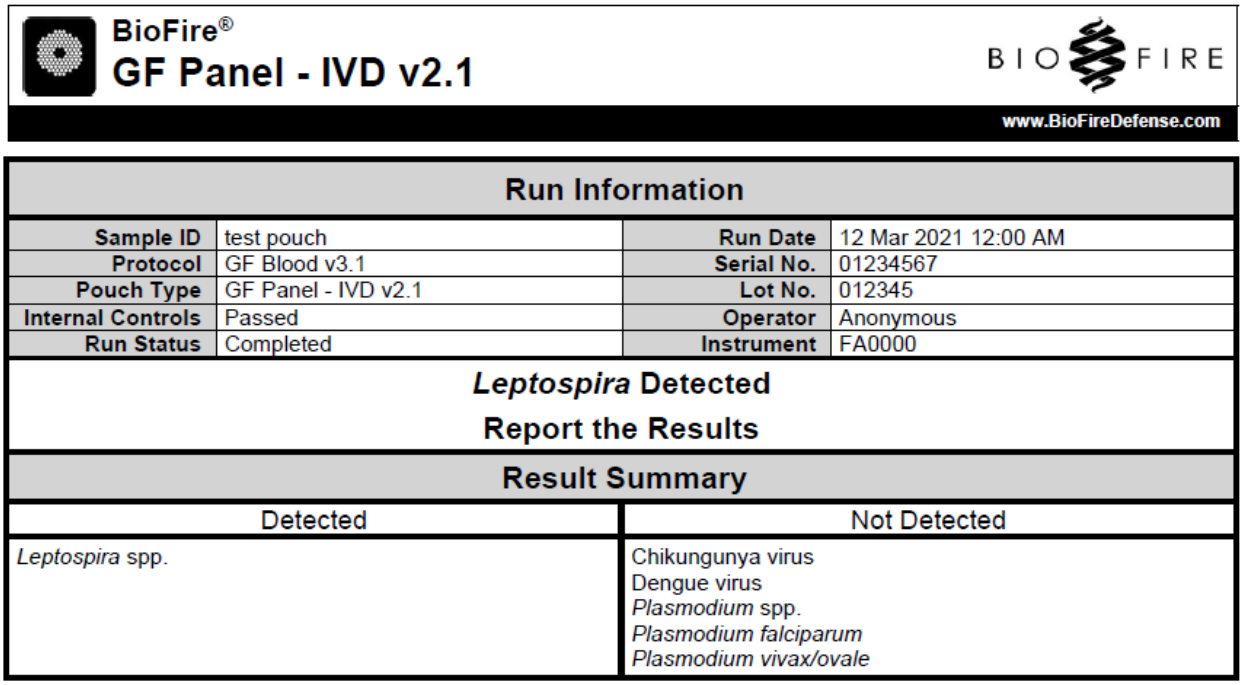
Table 3. *Plasmodium* Report Scheme

Assay Detection Outcomes			Result Banner	Result Summary[a]
***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**

[a] 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 Panel Test Report

The BioFire Global Fever 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®
GF Panel - IVD v2.1

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Run Information

Sample ID	test pouch	**Run Date**	12 Mar 2021 12:00 AM
Protocol	GF Blood v3.1	**Serial No.**	01234567
Pouch Type	GF Panel - IVD v2.1	**Lot No.**	012345
Internal Controls	Passed	**Operator**	Anonymous
Run Status	Completed	**Instrument**	FA0000

***Leptospira* Detected**

Report the Results

Result Summary

Detected	Not Detected
Leptospira spp.	Chikungunya virus Dengue virus *Plasmodium* spp. *Plasmodium falciparum* *Plasmodium vivax/ovale*

Figure 2. Example BioFire Global Fever 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, Incomplete, 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 control field results. See **Table 5** for complete results interpretation and required actions.

Table 4. Interpretation of Internal Controls Field on the BioFire Global Fever Panel Test Report

Internal Control Results	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 Technical Support for further instructions.
Invalid	The controls are invalid because the run did not complete. (Typically this 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 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, Not Detected, or Invalid. See the Results Explanation section below for detailed information about interpretation of test results and appropriate follow-up for Invalid results. 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.

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 include Detected, Not Detected, and Invalid. **Table 5** provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

Table 5. Interpretation of Results on the BioFire Global Fever Panel Test Report

Results	Explanation	Report
Not Detected <Organism Name(s)>	The run was successfully completed AND The pouch controls were successful (Pass) AND The assay(s) for the organism were NEGATIVE	Results are valid. Follow any instructions provided in the Result Banner.
Detected <Organism Name(s)>	The run was successfully completed AND The pouch internal controls were successful (Pass) AND The assay(s) for the organism were POSITIVE	Results are valid. Follow any instructions provided in the Result Banner.
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 Technical Support for further instruction. Retest the sample.

NOTE: *Detection of two or more unique pathogens may indicate a possible contamination event. Follow the instructions in the Result Banner for retesting. If the two or more positive results are not duplicated, contact BioFire Technical Support and discontinue testing until the test area has been decontaminated.*

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

DECONTAMINATION PROCEDURES

The decontamination and cleaning procedures listed are intended to limit spread of contaminants. Decontamination is necessary to prevent false-positive results in subsequent runs.

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.

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 and gloves.
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

If a pouch leaks, take the following precautions to avoid contamination:

1. Put on clean PPE, such as a lab coat and gloves.
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:
   a. Dispose of potentially contaminated gloves and put on clean gloves.
   b. Dispose of the potentially contaminated lab coat and put on a clean lab coat.
   c. Discard leaking pouch in biohazard container.
   d. Change gloves.
   e. 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, such as a lab coat and gloves.
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, such as a lab coat and gloves.
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 Torch in Cleaning Mode. The Cleaning Mode allows 30 seconds for the touch screen to be cleaned. Access this feature form the Settings toolbar (See 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 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.
   a. Load 0.2 mL of combined swabbing aliquot as the sample using Transfer Pipette, by drawing liquid up to the 2nd line.
   b. Add sample to Sample Injection Vial.
   c. 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 the 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 Panel test performance has only been established on the BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems.
3. This test is a qualitative test and does not provide a quantitative value for the analyte(s) in the sample.
4. A false negative BioFire Global Fever Panel result may occur when the concentration of analyte(s) in the sample is below the device limit of detection.
5. 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.
6. The performance of the BioFire Global Fever Panel was evaluated only with human whole blood collected in EDTA tubes.
7. A false negative BioFire Global Fever Panel result may occur in the presence of heparin or TRIzol.
8. Performance of the test has not been established for monitoring treatment of malaria, dengue fever, chikungunya fever, or leptospirosis.
9. A dengue virus Detected result does not provide information on the severity of the disease. The potential for hemorrhagic dengue fever should be considered.
10. A *Plasmodium* spp. Detected result does not provide parasitemia level information. The potential for severe malaria should be considered.
11. 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.
12. *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.
13. 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.
14. This test is not intended for screening asymptomatic individuals.
15. BioFire Global Fever Panel results for *Plasmodium* may not directly correlate with microscopy performed on the same specimen.
16. Recent administration of a vaccine for a BioFire Global Fever Panel pathogen prior to whole blood specimen collection may lead to a false positive result.
17. Dengue virus inclusivity testing and in silico analyses demonstrated that the BioFire Global Fever Panel may have variable detection or reduced sensitivity for some strains detected by the dengue virus assays.

EXPECTED VALUES

In the prospective clinical evaluation of the BioFire Global Fever Panel, 1875 whole blood specimens were collected and tested at ten study sites across the world over approximately eighteen months (March 2018 – September 2019). Expected value summaries (as determined by the BioFire Global Fever Panel), stratified by region and site, are presented in **Table 6**.

Table 6. Expected Value (As Determined by the BioFire Global Fever Panel) Summary by Region and Study Site (Specimens Acquired March 2018 – September 2019); # = Number; EV= Expected Value

Analyte	Overall (n=1875)		USA				Africa								Southeast Asia				Central & South America			
			Site 07 (n=179)		Site 14 (n=9)		Site 01 (n=134)		Site 02 (n=108)		Site 05 (n=199)		Site 11 (n=158)		Site 08 (n=249)		Site 09 (n=406)		Site 12 (n=297)		Site 13 (n=136)	
	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV
Chikungunya virus[a]	27	1.4%	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%
Dengue virus (serotypes 1, 2, 3, and 4)[b]	266	14.2%	0	0.0%	0	0.0%	1	0.7%	0	0.0%	0	0.0%	0	0.0%	90	36.1%	54	13.3%	20	6.7%	101	74.3%
Leptospira spp.	19	1.0%	1	0.6%	0	0.0%	0	0.0%	1	0.9%	0	0.0%	0	0.0%	4	1.6%	4	1.0%	9	3.0%	0	0.0%
Plasmodium spp.	351	18.7%	0	0.0%	0	0.0%	16	11.9%	50	46.3%	141	70.9%	49	31.0%	7	2.8%	4	1.0%	84	28.3%	0	0.0%
P. falciparum	233	12.4%	0	0.0%	0	0.0%	14	10.4%	44	40.7%	125	62.8%	42	26.6%	3	1.2%	0	0.0%	5	1.7%	0	0.0%
P. vivax/ovale	115	6.1%	0	0.0%	0	0.0%	3	2.2%	0	0.0%	12	6.0%	12	7.6%	4	1.6%	4	1.0%	80	26.9%	0	0.0%

[a] Chikungunya virus was detected in specimens collected in Songkhla Province, 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.

Also included are the prevalence of the BioFire Global Fever Panel pathogens in the United States and Territories since the beginning of 2010 (as reported by the Centers for Disease Control and Prevention (CDC)). Total Number of cases in the United States are provided in **Table 7** and in the United States Territories in **Table 8** of this document.

Table 7. Number of Cases of BioFire Global Fever Panel Diseases Reported in the United States since 2010 as Reported by the CDC (MMWR)

Disease	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019 [a]	2020 [b]	2021 [b,c]
Chikungunya virus disease [d]	n/a	n/a	n/a	n/a	n/a	896	247	156	117	192	22	6
Dengue virus infections[e]	700	254	547	843	680	951	953	454	474	1414	332	32
Leptospirosis[f]	n/a	n/a	n/a	n/a	38	40	78	72	91	94	31	23
Malaria	1773	1724	1503	1594	1653	1390	1955	2056	1748	1936	360	569

[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] Leptospirosis data became available in 2014.

Table 8. Number of Cases of BioFire Global Fever Panel Diseases Reported in the United States Territories since 2010 as Reported by the CDC (MMWR)

Disease	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020 [a]	2021 [a,b]
Chikungunya virus disease [c]	n/a	n/a	n/a	n/a	n/a	237	180	39	8	2	0	0
Dengue virus infections [d]	10,911[e]	1,541	6167[e]	9,884[f]	544	61	216	512	157	106	295	287
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

[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.

Clinical Performance

The clinical performance of the BioFire Global Fever Panel was established during a multi-center study conducted at ten geographically distinct study sites, including two in the United States, from March 2018 to September 2019. A total of 1971 subjects were enrolled in the prospective clinical study; 96 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 Global Fever Panel or comparator result. The final data set consisted of 1875 whole blood specimens. **Table 9** 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 Global Fever Panel Study).

Table 9. Demographics: Overall and Per Site Enrollment

		Overall	Site 01	Site 02	Site 05	Site 07	Site 08	Site 09	Site 11	Site 12	Site 13	Site 14
Sex	Female	980 (52.3%)	58 (43.3%)	67 (62.0%)	125 (62.8%)	113 (63.1%)	107 (43.0%)	206 (50.7%)	87 (55.1%)	132 (44.4%)	80 (58.8%)	5 (55.6%)
	Male	895 (47.7%)	76 (56.7%)	41 (38.0%)	74 (37.2%)	66 (36.9%)	142 (57.0%)	200 (49.3%)	71 (44.9%)	165 (55.6%)	56 (41.2%)	4 (44.4%)
Age	<5	163 (8.7%)	44 (32.8%)	20 (18.5%)	0 (0%)	0[a] (0%)	25 (10.0%)	0[b] (0%)	66 (41.8%)	3 (1.0%)	5 (3.7%)	0[a] (0%)
	5 to 21	765 (40.8%)	21 (15.7%)	38 (35.2%)	128 (64.3%)	14[a] (7.8%)	127 (51.0%)	204[b] (50.2%)	70 (44.3%)	102 (34.3%)	61 (44.9%)	0[a] (0%)
	22 to 50	672 (35.8%)	42 (31.3%)	31 (28.7%)	59 (29.6%)	106 (59.2%)	63 (25.3%)	139 (34.2%)	21 (13.3%)	146 (49.2%)	61 (44.9%)	4 (44.4%)
	>50	275 (14.6%)	27 (20.1%)	19 (17.6%)	12 (6.0%)	59 (33.0%)	34 (13.7%)	63 (15.5%)	1 (0.6%)	46 (15.5%)	9 (6.6%)	5 (55.6%)
Total		**1875**	**134**	**108**	**199**	**179**	**249**	**406**	**158**	**297**	**136**	**9**

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

Specimens were evaluated with the BioFire Global Fever Panel at clinical study sites. Nucleic acids were also extracted at clinical study sites and shipped to BioFire Defense for polymerase chain reaction (PCR)/sequencing-based comparator methods. A BioFire Global Fever 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 Global Fever 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 Global Fever 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 Global Fever Panel results to the comparator method results were further investigated. The discrepancy investigations were typically performed as follows: 1) Discrepancies between the BioFire Global Fever 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 Global Fever 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 10.**

The majority of specimens (1469/1875; 78.3%) were tested fresh, while 406 (21.7%) 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 Global Fever Panel performance with frozen compared to fresh specimens. Similarly, a comparison of BioFire Global Fever Panel performance on fresh and frozen specimens tested in this study also demonstrated equivalence (**Table 10**). Thus, all data for fresh and frozen specimens are combined for all analyses.

Table 10. BioFire Global Fever Panel Clinical Performance Summary

Analyte		PPA			NPA		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Chikungunya virus[a]	Fresh	25/25	100.0%	86.7-100%	1442/1444	99.9%	99.5-100%
	Frozen	0/0	-	-	406/406	100.0%	99.1-100%
	Overall	**25/25**	**100.0%**	**86.7-100%**	**1848/1850**	**99.9%**	**99.6-100%**
Dengue virus (serotypes 1, 2, 3, and 4)[b]	Fresh	249/263	94.7%	91.3-96.8%	1206/1206	100.0%	99.7-100%
	Frozen	17/20	85.0%	64.0-94.8%	386/386	100.0%	99.0-100%
	Overall	**266/283**	**94.0%**	**90.6-96.2%**	**1592/1592**	**100.0%**	**99.8-100%**
Leptospira spp.[c]	Fresh	9/9	100.0%	70.1-100%	1456/1460	99.7%	99.3-99.9%
	Frozen	6/7	85.7%	48.7-97.4%	399/399	100.0%	99.0-100%
	Overall	**15/16**	**93.8%**	**71.7-98.9%**	**1855/1859**	**99.8%**	**99.4-99.9%**
Plasmodium spp.[d,e]	Fresh	207/210	98.6%	95.9-99.5%	1251/1259	99.4%	98.7-99.7%
	Frozen	132/135	97.8%	93.7-99.2%	267/271	98.5%	96.3-99.4%
	Overall	**339/345**	**98.3%**	**96.3-99.2%**	**1518/1530**	**99.2%**	**98.6-99.6%**
Plasmodium falciparum[f]	Fresh	148/158	93.7%	88.7-96.5%	1309/1311	99.8%	99.4-100%
	Frozen	82/90	91.1%	83.4-95.4%	315/316	99.7%	98.2-99.9%
	Overall	**230/248**	**92.7%**	**88.8-95.4%**	**1624/1627**	**99.8%**	**99.5-99.9%**
Plasmodium vivax/ovale[g]	Fresh	64/69	92.8%	84.1-96.9%	1400/1400	100.0%	99.7-100%
	Frozen	51/55	92.7%	82.7-97.1%	351/351	100.0%	98.9-100%
	Overall	**115/124**	**92.7%**	**86.8-96.1%**	**1751/1751**	**100.0%**	**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 Panel retest and by additional PCR, two were positive only upon BioFire Global Fever Panel retest, and eight were detected only by additional PCR.

[c] Evidence of *Leptospira* spp. was found in 1/1 FN specimens by BioFire Global Fever Panel retest and by additional PCR, and in 3/4 FP specimens by additional PCR.

[d] Five (5/6) *Plasmodium* spp. FN specimens were *P. falciparum* FN and one (1/6) was *P. vivax/ovale* FN. Three (3/12) *Plasmodium* spp. FP specimens were also *P. falciparum* FP.

[e] Evidence of *Plasmodium* spp. was found in 3/6 FN specimens: two specimens were positive upon BioFire Global Fever Panel retest and by additional PCR, and one was positive only upon BioFire Global Fever Panel retest. Evidence of *Plasmodium* spp. was found in 10/12 FP specimens by additional PCR.

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

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

The prevalence of unique analytes in co-detections is presented in **Table 11.** The BioFire Global Fever Panel reported a total of 28 specimens with discernible multiple analyte detections (1.5% of all specimens, 28/1875; 4.3% of positive specimens, 28/657). The majority of co-detections (26/28; 92.9%) 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* (3.3% of all positive specimens; 22/657).

Table 11. Prevalence of Analytes in Co-Detections as Determined by the BioFire Global Fever Panel

Analyte[a]	Co-Detections in Overall Detections for the Analyte		Prevalence in all Co-Detections	
Chikungunya virus	2/27	7.4%	2/28	7.1%
Dengue virus (serotypes 1, 2, 3, and 4)	4/266	1.5%	4/28	14.3%
Leptospira spp.	2/19	10.5%	2/28	7.1%
Plasmodium spp.[b]	26/351	7.4%	26/28	92.9%
P. falciparum[c]	23/233	9.9%	23/28	82.1%
P. vivax/ovale[c]	24/115	20.9%	24/28	85.7%

[a] Multiple species/strains within a genus are not always discernible as individual detections.
[b] One out of 26 (1/26) *Plasmodium* spp. co-detections did not have a species-level *Plasmodium* assay detection.
[c] Twenty-two (22) co-detections were positive for *P. falciparum* and *P. vivax/ovale*.

The overall success rate for initial specimen tests on the BioFire Global Fever Panel was 98.4% (1868/1898); five tests did not complete (two due to loss of power, two instrument errors, and one software error), and 25 tests had pouch internal control failures. Of the 30 unsuccessful initial tests, all were retested once, and valid results were produced for 25 of the 30 retested specimens. Only 0.3% (5/1902) of eligible specimens were ultimately excluded due to the inability to obtain a BioFire Global Fever Panel test result.

Limit of Detection

The Limit of Detection (LoD) was first estimated by testing dilutions of contrived multi-spiked whole blood samples containing known analyte concentrations. Confirmation of LoD was achieved by testing 20 replicates of a contrived sample 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 LoD was further confirmed by demonstrating a detection rate of ≤18/20 (≤90%) at a concentration below the LoD.

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 12**).

Table 12. Summary of Limit of Detection (LoD) for the BioFire Global Fever Panel

BioFire Global Fever Panel Interpretation	Isolate Species/Strain/Serotype	Source (ID)	Material	LoD Concentration	
				Copies/mL [1]	Units/mL [2]
BACTERIA					
Leptospira spp.	*interrogans* serovar *budapest*[4]	ATCC (VR-23581)	Live	3.4E+02	NA
VIRUSES					
Chikungunya Virus	R80422	ZeptoMetrix (0810105CFHI)	Inactivated	5.5E+02	3.6E-01 units/mL
Dengue virus	Serotype 1 / Hawaii	ZeptoMetrix (0810088CF)	Live	2.2E+02	NA
	Serotype 2 / New Guinea C [3]	ZeptoMetrix (0810089CF)	Live	3.4E+02	NA
	Serotype 2 / Dak AR A1247[3]	BEI (NR-12221)	Live	2.7E+03	1.5E+02 $TCID_{50}$/mL
	Serotype 3 / H87	ZeptoMetrix (0810090CF)	Live	1.3E+02	3.7E+00 units/mL
	Serotype 4 / H241	ZeptoMetrix (0810091CF)	Live	6.4E+01	1.8E+02 units/mL
PROTOZOA					
Plasmodium spp.	*falciparum* IPC 4884	BEI (MRA-1238)	Live	1.8E+02	NA
	knowlesi H strain	BEI (MRA-456G)	genomic DNA	2.4E+01	20 pg/mL
	malariae	Discovery Life Sciences (DLS17-026015)	Live (Clinical Specimen)	1.9E+02	2.3E-01 cells/mL
	ovale Wallikeri	CDC (N8K9QK19)	Live (Clinical Specimen)	2.4E+02	NA
	vivax, Strain Chesson	BEI (MRA-383)	Live	1.5E+02	NA
Plasmodium falciparum	IPC 4884 Pursat Cambodia 2011	BEI (MRA-1238)	Live	1.8E+02	NA
Plasmodium vivax/ovale	*ovale* Wallikeri (Clinical Specimen)	CDC (N8K9QK19)	Live	2.4E+02	NA
	vivax, Strain Chesson (Clinical Specimen)	BEI (MRA-383)	Live (Clinical Specimen)	1.5E+02	NA

[1] Stock concentrations determined using commercially available quantitative real-time PCR assay kits.
[2] Concentration/titer based on values provided by vendor.
[3] Representative strains were used to evaluate each of the two dengue virus 2 assays.
[4] Strain previously known as serovar *icterohaemorrhagiae*.

For live chikungunya virus, LoD was estimated for two additional strains by testing dilutions of whole blood samples contrived with live virus (performed in a BSL3 laboratory). The estimated LoD is defined as the lowest concentration in a serial 10-fold dilution with four out of four (4/4) replicates testing positive (See **Table 13**).

Table 13. Summary of Live Chikungunya Virus Estimated LoD for the BioFire Global Fever Panel

BioFire Global Fever Panel Interpretation	Strain	Source (ID)	Material	LoD Concentration	
				Copies/mL[1]	Units/mL[2]
Chikungunya Virus	B8635 [3]	UCC [4] (Alpha031)	Live	5.5E+02	4.4E+01 pfu/mL
	Indo23574 [3]	UCC [4] (Alpha008)	Live	5.5E+02	9.0E+01 pfu/mL

[1] Stock concentration determined using commercially available quantitative real-time PCR assay kits.
[2] Concentration/titer based on values provided by vendor.
[3] Estimated LoD determined in BSL3 laboratory (lowest concentration with 4/4 detection rate).
[4] UCC – US Department of Defense Unified Culture Collection.

Analytical Reactivity (Inclusivity)

Analytical reactivity of the BioFire Global Fever Panel assays was evaluated for several *Leptospira* species/strains, chikungunya virus strains, dengue virus serotypes/strains, and *Plasmodium* species/strains. Isolates representing relevant species, serotypes, and strains, as well as temporal and geographic diversity for each of the panel analytes were selected.

If an isolate was detected at ≤3.5×LoD, the isolate was considered inclusive. Isolates detected in a range of 10× to 100×LoD are considered to have reduced sensitivity. Any isolates not detected within 100×LoD are considered not inclusive.

Isolates with limitations on assay reactivity (based on wet testing observations) are noted in **Table 14**, 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 **Table 14 – Table 19.**

Table 14. Limitations on Analytical Reactivity of BioFire Global Fever Assays

Observed Result	Detection Level	Analyte	Serotype/Strain/Isolate
Detected (may be underreported)	10×LoD	*Plasmodium falciparum*[1]	SenTh021.09
	~100×LoD	Dengue virus[1]	Serotype 3 BC188/97
		Dengue virus[1]	Serotype 4 D85-019
Not Detected	-	Dengue virus[2]	Serotype 2 DKA 811

[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 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 15. *Leptospira* Isolates Tested and Detected

***Leptospira* spp.**	**Strain (Serovar)**	**Isolate Source/ID**	**Test Level**	**Result**
			×LoD	
L. interrogans	Serovar ((Budapest)	ATCC / 23581	1×	*Leptospira* spp. Detected
	HAI0156 (Copenhageni)	BEI / NR-19891	3.5×	
	L495 (Manilae)	BEI / NR-19816	3.5×	
L. alexanderi	L60 (Manhao 3)	ATCC / 700520	3×	
L. alstonii	Sichuan 79601	ATCC / BAA-2439	3.5×	
L. borgpetersenii	Castellon 3 (Castellonis)	ATCC / 23580	3.5×	
	Veldrat Bataviae 46 (Javanica)	ATCC / 43292	3×	
L. kirschneri	200701401 (Bogvere)	BEI /NR-19942	3.5×	
	3522 C (Cynopteri)	ATCC / 49945	1.6×	
L. kmetyi	Bejo-Iso9T (Malaysia)	BEI / NR-22254	3.5×	
L. mayottensis	200901116 (undesignated)	NRL / KIT0254	3.5×	
L. noguchii	CZ 214T (Panama)	BEI / NR-22283	3.5×	
L. santarosai	LT 821 (Shermani)	ATCC / 43286	2.5×	
L. weilii	6712	NRL / KIT0220	3.5×	
	94-79970/3 (Topaz)	NRL / KIT0237	3.5×	
	A 102 (Mengrun)	NRL / KIT0023	3.5×	
	Celledoni 20160426	ATCC / 43285	3.5×	
	H 27 (Hekou)	NRL / KIT0074	3.5×	
	LT 89-68 (Vughia)	NRL / KIT0127	3.5×	

Table 16. Chikungunya Virus Strains Tested and Detected

Virus	**Material**	**Strain**	**Isolate Source/ID**	**Test Level**	**Result**
				×LoD	
Chikungunya virus[1]	Inactivated	R80422	ZeptoMetrix / 0810105CFHI	1×	Chikungunya Virus Detected
		DHS4263	BEI / NR-50884	3×	
		St. Martin 2013	BEI / NR-50883	2.5×	

[1] Two live chikungunya virus isolates were tested. Reference **Table 13.**

Table 17. Dengue Virus Isolates Tested and Detected

Dengue Serotype	Strain	Isolate Source/ID	Test Level ×LoD	Result
Serotype 1	Hawaii	ZeptoMetrix / 0810088CF	1×	Dengue virus Detected
	Strain 12150	BEI / NR-3785	3×	
	228690	BEI / NR-3786	3×	
	276RK1	BEI / NR-3782	3×	
	BC89/94	BEI / NR-3787	3×	
	SL-6-6-04	BEI / NR-49744	3×	
	UIS 1162	BEI / NR-49707	3×	
	VN/BID-V1792/2007	BEI / NR-44083	3×	
Serotype 2	New Guinea C (DENV 2_1)	ZeptoMetrix / 0810089CF	1×	
	1349	BEI / NR-12219	3.2×	
	429557	BEI / NR-12216	3.2×	
	BC102/94	BEI / NR-3789	2.8×	
	VN/BID-V1002/2006	BEI / NR-44085	3.2×	
	Dak Ar A1247 (DENV 2_2)	BEI / NR-12221	1×	
	ArA6894	BEI / NR-12220	0.4×	
Serotype 3	H87	ZeptoMetrix / 0810090CF	1×	
	271242	BEI / NR-3802	3×	
	C0360/94	BEI / NR-48800	3×	
	VN/BID-V1329/2006	BEI / NR-44087	3×	
Serotype 4	H241	ZeptoMetrix / 0810091CF	1×	
	703	BEI / NR-48801	3×	
	BC13/97	BEI / NR-3805	3×	
	BC287/97	BEI / NR-3806	3×	
	BC258/97	BEI / NR-3807	3×	
	PR 06-65-740	BEI / NR-49757	3.4×	

Table 18. *Plasmodium* spp. Tested and Detected – Genus Level

***Plasmodium* spp.**	**Strain**	**Isolate Source/ID**	**Test Level**	**Result**
			×LoD	
P. falciparum	IPC 4884	BEI / MRA-1238	1×	*Plasmodium* spp. Detected
	SenTh021.09	BEI / MRA-1182	1.7×	
	St. Lucia	BEI / MRA-331	1.7×	
	Tanzania, 02000708	BEI / MRA-1169	1.7×	
P. vivax	Chesson	BEI / MRA-383	1×	
	Panama	ATCC / 30138	2.1×	
P. ovale	Wallikeri	CDC / N8K9QKI9	1×	
	Curtisi	CDC / N8K9QL0S	3×	
P. knowlesi	Strain H	BEI / MRA-456G	1×	
P. malariae	Clinical Sample	DLS / DLS17-026015	1×	

Table 19. Plasmodium Species/Strains Tested and Detected – Species Level

***Plasmodium* spp.**	**Strain**	**Isolate Source/ID**	**Test Level**	**Result**
			×LoD	
P. falciparum	IPC 4884	BEI / MRA-1238	1×	*Plasmodium falciparum* Detected
	St. Lucia	BEI / MRA-331	1.7×	
	Tanzania, 02000708	BEI / MRA-1169	1.7×	
P. vivax	Chesson	BEI / MRA-383	1×	*Plasmodium vivax/ovale* Detected
	Panama	ATCC / 30138	2.1×	
P. ovale	Wallikeri	CDC / N8K9QKI9	1×	
	Curtisi	CDC / N8K9QL0S	3×	

Analytical Reactivity (Cross-Reactivity and Exclusivity)

The potential for non-specific amplification and detection by the BioFire Global Fever Panel assays was evaluated by empirical (wet) testing of high concentrations of organisms/viruses in contrived samples. Organisms/viruses not available for testing were evaluated by in silico analysis. On-panel organisms were tested to assess the potential for intra-panel cross-reactivity (**Table 20**). 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 BioFire Global Fever 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 Global Fever Panel assay primers, as determined by in silico analyses.

On-panel and off-panel testing included more than 210 isolates of bacteria, viruses, fungi, and protozoa evaluated at high concentrations (typically >1E+06 genomic copies/mL). **Table 20** lists the organisms and virus that showed cross-reactivity with BioFire Global Fever Panel assays with results of the in silico analysis shown in the footnotes. **Table 21** and **Table 22** list 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). Erroneous BioFire Global Fever Panel test results due to cross-reactivity with organisms not evaluated or due to new variant sequences is possible.

Table 20. Observed Cross-Reactivity of BioFire Global Fever Panel Assays

Cross-Reactive Organism/Virus	Global Fever Panel Test Result
On-Panel	
Plasmodium knowlesi [1]	*Plasmodium vivax/ovale*
Plasmodium malariae [2]	
Off-Panel	
O'nyong-nyong virus	Chikungunya virus
Plasmodium berghei [3]	*Plasmodium* spp. and *Plasmodium vivax/ovale*
Plasmodium brasilianum [3]	
Plasmodium cynomolgi [3]	
Plasmodium fieldi [3]	
Plasmodium fragile [3]	
Plasmodium inui [3]	
Plasmodium simiovale [3]	

[1] Cross-reactive with the *Plasmodium vivax/ovale assay* at reduced sensitivity (~1,000×LoD).
[2] In silico analysis predicts potential cross-reactivity with the *Plasmodium vivax* assay at concentrations greater than 10^5 copies/mL.
[3] *Plasmodium* spp. that typically infect non-human primates and rodents, but are rarely found in humans.

Table 21. On-Panel Organisms and Viruses with No Cross-Reactivity with BioFire Global Fever Panel Assays (Observed or Predicted by in silico Analysis)

On-Panel		
Bacteria		
Leptospira interrogans		
Viruses		
Chikungunya virus	Dengue virus Serotype 1 Dengue virus Serotype 2	Dengue virus Serotype 3 Dengue virus Serotype 4
Protozoa		
Plasmodium falciparum	*Plasmodium vivax*	*Plasmodium ovale*

Table 22. Off-Panel Organisms and Viruses with No Cross-Reactivity with BioFire Global Fever Panel Assays (Observed or Predicted by in silico Analysis)

Off-Panel		
Bacteria		
Tested		
Acinetobacter baumannii *Bacillus anthracis* *Bacillus brevis* *Bacillus cereus* *Bacillus circulans* *Bacillus coagulans* *Bacillus halodurans* *Bacillus licheniformis* *Bacillus megaterium* *Bacillus mycoides* *Bacillus pumilus* *Bacillus subtilis* *Bacillus thuringiensis* *Bacteroides fragilis* *Bordetella bronchiseptica* *Borrelia burgdorferi* *Brucella melitensis* *Burkholderia cepacia* *Burkholderia mallei* *Burkholderia pseudomallei* *Chlamydophila pneumoniae* *Clostridium bifermentans* *Clostridium botulinum/sporogenes* *Clostridium perfringens* *Clostridium sordellii* *Coxiella burnetii* *Enterobacter aerogenes* *Enterococcus faecalis* *Enterococcus faecium* *Francisella persica* (formerly *Wolbachia persica*) *Francisella philomiragia* (formerly *Yersinia)* *Francisella hispaniensis* *Francisella tularensis* subsp. *tularensis*	*Klebsiella oxytoca* *Legionella pneumophila* *Leptospira biflexa* *Leptospira meyeri* *Leptospira terpstrae* genomospecies 4 *Leptospira vanthielii* genomospecies 3 *Leptospira wolbachii* *Leptospira yanagawae* genomospecies 5 *Listeria monocytogenes* *Mycobacterium tuberculosis* *Mycoplasma pneumoniae* *Neisseria meningitidis* *Proteus mirabilis* *Pseudomonas aeruginosa* *Rickettsia typhi* *Salmonella enterica* subs. *bongori* *Salmonella enterica* subs. *arizonae* *Salmonella enterica* subs. *diarizoniae* *Salmonella enterica* subs. *enterica* serovar Enteritidis *Salmonella enterica* subsp. *enterica* serovar Heidelberg *Salmonella enterica* subsp. *enterica* serovar Javiana *Salmonella enterica* subsp. *enterica* serovar Montevideo *Salmonella enterica* subsp. *enterica* serovar Muenchen *Salmonella enterica* subsp. *enterica* serovar Newport *Salmonella enterica* subsp. *enterica* serovar Paratyphi A *Salmonella enterica* subsp. *enterica* serovar Rubislaw *Salmonella enterica* subsp. *enterica* serovar Saint Paul	*Salmonella enterica* subs. *enterica* serovar Tennessee *Salmonella enterica* subs. *enterica* serovar Thompson *Salmonella enterica* subs. *enterica* Typhi *Salmonella enterica* subs. *enterica* serovar Typhimurium *Salmonella enterica* subs. *enterica* serovar Dublin *Salmonella enterica* subs. *enterica* serovar Manchester *Salmonella enterica* subs. *houtenae* *Salmonella enterica* subs. *indica* *Salmonella enterica* subs. *salamae* *Serratia marcescens* *Staphylococcus aureus* *Streptococcus agalactiae* *Streptococcus pneumoniae* *Streptococcus pyogenes* *Treponema pallidum* subs. *pallidum* *Vibrio cholerae* *Yersinia aldovae* *Yersinia bercovieri* *Yersinia entericolitica* *Yersinia fredericksenii* *Yersinia intermedia* *Yersinia kristensenii* *Yersinia mollaretii* *Yersinia pestis* (A1122 and CO92) *Yersinia pseudotuberculosis* *Yersinia rohdei* *Yersinia ruckeri* *Yersinia similis*
In silico Analysis Only		
Chlamydophila psittac *Bacillus luciferensis*	*Francisella tularensis* subsp. *mediasiatica* *Orientia chuto (tsutsugamushi)*	*Rickettsia prowazekii* *Rickettsia ricketsii*

Viruses		
Tested		
Adenovirus 1 Adenovirus 3 Adenovirus 5 Aura virus Barmah Forest virus Bunyamwera virus Coronavirus NL63 Crimean-Congo hemorrhagic fever virus Cytomegalovirus Dugbe virus Eastern equine encephalitis virus *Ebolavirus* (Zaire, Sudan, Bundibugyo, Tai Forest, Reston) Enterovirus, HEV-71 Epstein Barr virus Flexal virus Guanarito virus Hantaan virus Hazara virus Hendra virus Hepatitis A virus Hepatitis B virus	Hepatitis C virus Herpes simplex virus Type 2 HPIV-1 HPIV-3 Hughes virus Human herpesvirus 6B Human immunodeficiency virus, type 1 Human immunodeficiency virus, type 2 Human T-lymphotropic virus, type 1 Human T-lymphotropic virus, type 2 Influenza A H1N1-2009 Influenza A H3N2 Influenza B virus Japanese encephalitis virus Junin virus (2 strains: XJ and Candid) Lassa virus Machupo virus Marburgvirus (Musoke and RAVN) Mayaro virus Measles virus Metapneumovirus Mopeia virus Mumps virus	Omsk hemorrhagic fever Parvovirus Powassan virus Rabies virus Rift Valley fever virus Ross River virus Human respiratory syncytial virus Rubella virus Saint Louis encephalitis virus Sindbis virus Spondweni virus Tickborne encephalitis virus Una virus Usutu virus Vaccinia virus Varicella zoster virus Venezuelan equine encephalomyelitis virus Western equine encephalomyelitis virus West Nile virus (Lineage 1 and 2) Yellow Fever virus Zika virus
In silico Analysis Only		
Avalon virus Bas-Congo virus Lymphocytic choriomeningitis virus Middelburg virus	Murray Valley encephalitis virus Pirital virus Sabia virus (Brazilian Hemorrhagic Fever)	Semliki Forest virus Tonate virus Variola major
Protozoa		
Tested		
Babesia microti *Crithidia fasciculate* *Cyclospora cayetanensis* *Leptomonas seymouri*	*Leishmania donovani* *Schistosoma mansoni* *Toxoplasma gondii*	*Trypanosoma brucei* *Trypanosoma cruzi* *Trypanosoma rangeli*
Fungus		
Tested		
Aspergillus fumigatus	*Cryptococcus neoformans* var. *grubii*	

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 three representative panel analytes, one bacterium, one virus, and one protozoan. 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 23.**

Table 23. Reproducibility of the BioFire Global Fever Panel Test Results

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 *budapest*[1]** (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 93.4%	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
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 93.4%	**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				**1037/1080 96.0%** [94.7-97.0%]			

[1] Serovar formerly known as *icterohaemorrhagiae.*
[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 Platforms

Performance of the BioFire Global Fever Panel was compared between the BioFire FilmArray 2.0 and BioFire FilmArray Torch platforms 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 180 replicates for each analyte concentration distributed equally over three (3) BioFire FilmArray 2.0 systems and three (3) BioFire FilmArray Torch systems.

A summary of results (percent (%) agreement with the expected Detected or Not Detected result) for the analytes by simulated site is provided in **Table 24.** Reproducibility of Global Fever Panel Test Results on BioFire FilmArray 2.0 and BioFire FilmArray Torch in **Table 24**.

NOTE: All other performance evaluations were performed using the BioFire FilmArray 2.0 system. Performance of the BioFire Global Fever Panel when using the BioFire FilmArray Torch system was comparable to the BioFire FilmArray 2.0 system.

Table 24. Reproducibility of Global Fever 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%** [87.6-97.6I]
		Negative (No Analyte)	Not Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100]
Dengue virus DENV-2 New Guinea C (Zeptometrix / 0810089CF)		Moderate Positive 3×LoD (1.0E+03)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]
		Low Positive 1×LoD (3.4E+02)	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	29/30 96.7%	30/30 100%	**89/90 98.9%** [94.0-99.8%]
		Negative (No Analyte)	Not Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** [95.9-100%]	30/30 100%	30/30 100%	29/30 96.7%	**89/90 98.9%** [94.0-99.8%]
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				**1073/1080 99.4%** [98.7-99.7%]				**1068/1080 98.9%** [98.1-99.4%]			

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 Global Fever 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 25**. 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 Global Fever Panel at the concentration tested.

Table 25. Evaluation of Potentially Interfering Substances on the BioFire Global Fever 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
Sulfamethoxazole	0.38 mg/mL	No interference
Vancomycin	0.12 mg/mL	No interference

Potentially Interfering Substance	Concentration Tested	Results
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 Global Fever Panel kits, kit components, and throughout accompanying packaging.

ISO 15223-1 Medical devices – Symbols to be used with information to be supplied by the manufacturer – Part 1: General requirements					
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 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

Revision History

Version	Revision Date	Description of Revision(s)
01	June 2021	Initial Release
02	June 2021	Minor concentration updates to Reproducibility section (Table 23). Relative LoD levels remain unchanged.
03	December 2022	Revised to include information for use with BioFire FilmArray Torch system and minor clarifications.

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 DFA2-PRT-0026-03, December 2022

APPENDIX C

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