EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR FILMARRAY GLOBAL FEVER PANEL DECISION SUMMARY

A. De Novo Number:

DEN200043

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the FilmArray Global Fever Panel.

C. Measurand:

DNA and RNA sequences from the following organisms: *Leptospira* spp., dengue virus serotypes 1-4, chikungunya virus, and *Plasmodium* spp.

D. Type of Test:

Multiplex Nucleic Acid Amplification Test

E. Applicant:

BioFire Defense, LLC

F. Proprietary and Established Names:

FilmArray Global Fever Panel

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3966

2. Classification:

   Class II

3. Product code(s):

   QMV

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):
   The FilmArray Global Fever Panel is a qualitative, multiplexed, nucleic acid-based in vitro diagnostic test intended for use with the FilmArray 2.0 system. The FilmArray 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: *Leptospira* spp., chikungunya virus, dengue virus (serotypes 1, 2, 3 and 4), 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 FilmArray 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. Patient travel history and consultation of the CDC Yellow Book should be considered prior to use of the FilmArray Global Fever Panel as some pathogens are more common in certain geographical locations.

2. Indication(s) for use:

   Same as intended use.

3. Special conditions for use statement(s):

   For prescription use only.

   For *in vitro* diagnostic use only.

4. Special instrument requirements:

   The FilmArray Global Fever Panel is performed on the FilmArray 2.0 system.

I. Device Description:

The FilmArray Global Fever Panel is a multiplex nucleic acid-based test designed to be used with the FilmArray 2.0 system ("FilmArray system" or "FilmArray instrument"). The FilmArray Global Fever Panel includes a FilmArray Global Fever Panel pouch (pouch) which contains freeze-dried reagents to perform nucleic acid purification and nested, multiplex polymerase chain reaction (PCR) with DNA melt analysis. The FilmArray Global Fever Panel simultaneously conducts six tests for the identification of bacterial, viral, and protozoan organisms from whole blood specimens collected in EDTA tubes. Results from the FilmArray Global Fever Panel are available within about one hour.

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

The FilmArray instruments contain a coordinated system of inflatable bladders and seal points, which act on the pouch to control the movement of liquid between the pouch blisters. When a bladder is inflated over a reagent blister, it forces liquid from the blister into connecting channels. Alternatively, when a seal is placed over a connecting channel it acts as a valve to open or close a channel. In addition, electronically controlled pneumatic pistons are positioned over multiple plungers in order to deliver the rehydrated reagents into the blisters at the appropriate times. Two Peltier devices control heating and cooling of the pouch to drive the PCR reactions and the melt curve analysis.

Nucleic acid extraction occurs within the FilmArray pouch using mechanical and chemical lysis followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, a nested multiplex PCR is executed in two stages. During the first stage, a single, large volume, highly multiplexed reverse transcription PCR (rt-PCR) reaction is performed. The products from first stage PCR are then diluted and combined with a fresh, primer-free master mix and a fluorescent double stranded DNA binding dye (LC Green Plus, BioFire Defense, LLC). The solution is then distributed to each well of the array. Array wells contain sets of primers designed specifically to amplify sequences internal to the PCR products generated during the first stage PCR reaction. The 2nd stage PCR, or nested PCR, is performed in each well of the array. At the conclusion of the 2nd stage PCR, the array is interrogated by melt curve analysis for the detection of signature amplicons denoting the presence of specific targets. A digital camera placed in front of the array captures fluorescent images of the PCR2 reactions and software interprets the data.

The FilmArray software automatically interprets the results of each DNA melt curve analysis and combines the data with the results of the internal pouch controls to provide a test result

for each organism on the panel. A description of the individual assays and their result interpretation is included below:

- Chikungunya Virus: The Global Fever Panel contains two assays for species-level detection of all chikungunya virus strains (CHIKV1 and CHIKV2). The FilmArray software will interpret any single positive chikungunya assay as a Chikungunya Virus Detected result.

- Dengue Virus: The Global Fever Panel contains five individual assays for the detection of dengue virus serotypes 1, 2, 3, and 4 with two assays specifically dedicated to detecting dengue virus serotype 2 (DENV1, DENV2_1, DENV2_2, DENV3, and DENV4). The FilmArray software will interpret any single positive dengue virus assay as a Dengue Virus Detected result.

- *Leptospira*: The Global Fever Panel contains a single pan assay for the genus-level detection of all *Leptospira* Group 1 species (LEPTO1). A positive pan-Leptospira assay will result in a *Leptospira* spp. Detected call.

- *Plasmodium*: The Global Fever Panel contains three *Plasmodium* assays, one genus-level assay and two species-level assays. The genus-level assay (*Plasmodium* spp.) detects all 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 both *Plasmodium vivax* and *Plasmodium ovale*. Each individual assay is reported as a Detected or Not Detected result separately based on the results of the specific Global Fever Panel assay.

Materials provided in each FilmArray Global Fever Panel kit:

- Individually packaged FilmArray Global Fever Panel pouches (6)
- Individually-packaged Transfer Pipettes (7)
- Single-use (1.0 mL) Sample Buffer Tubes (7)
- Single-use pre-filled (1.5 mL) Hydration Injection Vials (Blue) (7)
- Single-use Sample Injection Vials (Red) (7)
- Instructions and Documents
  - FilmArray Global Fever Panel Instructions for Use
  - FilmArray Global Fever Panel Quick Guide

Materials required but not provided:

- 10% bleach solution

FilmArray system including:

- FilmArray 2.0 instrument, computer, and software
- FilmArray Pouch Loading Station

J. Standard/Guidance Document Referenced (if applicable):

14971:2007/(R)2010 (Corrected 4 October 2007), 'Medical Devices – Applications of Risk Management to Medical Devices'

Guidance for Industry and Food and Drug Administration Staff – De Novo Classification Process (Evaluation of Automatic Class III Designation) (October 30, 2017)

Guidance for Industry and Food and Drug Administration Staff – Highly Multiplexed Microbiological/Medical Countermeasure In Vitro Nucleic Acid Based Diagnostic Devices (August 27, 2014)

Guideline for Industry and Food and Drug Administration Staff – Class II Special Controls Guideline: Dengue Virus Nucleic Acid Amplification Test Reagents

Guideline for Industry and Food and Drug Administration Staff – Class II Special Controls Guidance Document: Plasmodium Species Antigen Detection Assays

Guidance for Industry and FDA Staff – Assayed and Unassayed Quality Control Material

Guidance for Industry and FDA Staff – Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests (March 13, 2007)

MM03-Ed3, Molecular Diagnostic Methods for Infectious Diseases, CLSI Approved Guideline—Third Edition

EP07-Ed3, Interference Testing in Clinical Chemistry; Approved Guideline—Third Edition

Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, FDA Guidance Document

Guidance for Industry, FDA Reviewers and Compliance on Off-The-Shelf Software Use in Medical Devices

Guidance for Industry, FDA Reviewers and Compliance on Off-The-Shelf Software Use in Medical Devices

ISO 62304:2006, 'Medical Device Software – Software Life Cycle Processes' – IEC 62304:2006, November 27, 2008

ISO 62304:2006, 'Medical Device Software – Software Life Cycle Processes' – IEC 62304:2006, November 27, 2008

ISO 15223-1:2012, 'Medical Devices – Symbols to be used with medical device labels, labeling and information to be supplied – Part 1: General requirements'

Guidance for Industry and FDA on Alternative to Certain Prescription Device Labeling Requirements

K. Test Principle:

The FilmArray Global Fever Panel pouch is a closed system disposable that houses all the chemistry required to isolate, amplify, and detect nucleic acid from multiple biothreat pathogens within whole blood and positive blood culture. The rigid plastic component (fitment) of the pouch contains reagents in freeze-dried form. The flexible plastic portion of the pouch is divided into discrete segments (blisters) where the required chemical processes are carried out. The user of the FilmArray Global Fever Panel loads the sample into the pouch, places the pouch into the FilmArray instrument, and starts the run. All other operations are automated. Operations and processes that occur during a FilmArray run include the following:

- **Nucleic Acid Purification**- Nucleic acid purification occurs in the (b)(4) of the pouch. The sample is lysed by agitation (bead beating) and the liberated nucleic acid is captured, washed, and eluted using magnetic bead technology. These steps require about ten minutes and the bead-beater apparatus can be heard as a high-pitched whine during the first minute of operation.

- **Reverse Transcription and 1st Stage Multiplex PCR** - Some pathogens identified by the FilmArray Global Fever pouch are RNA viruses, and a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a preheated master mix to initiate the RT step and subsequent thermocycling for multiplex PCR. The effect of 1st stage PCR is to enrich for the target nucleic acids present in the sample.

- **2nd Stage PCR** - The products of 1st stage PCR are diluted and mixed with fresh PCR reagents containing an intercalating fluorescent DNA dye (LCGreen Plus, BioFire Defense, LLC). This solution is distributed over the 2nd stage PCR array. The individual wells of the array contain primers for different assays (each present in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material. These primers are "nested" or internal to the specific products of the 1st stage multiplex reaction, which enhances both the sensitivity and specificity of the reactions.

- **DNA Melting Analysis** – After 2nd stage PCR, the temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melt curve. The temperature at which a specific PCR product melts (melting temperature or Tm) is consistent and predictable and the FilmArray software automatically evaluates the data from replicate wells for each assay to report results.

The FilmArray software controls the operation of the instrument, collects and analyzes data and automatically generates a test report at the end of the run.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   To evaluate the reproducibility of the FilmArray Global Fever Panel, three contrived whole blood samples were prepared with different mixtures of representative panel analytes. For each analyte, one sample was spiked at a Moderate Positive concentration (3 x LoD), another sample at a Low Positive (1 x LoD) and a third sample that was negative (unspiked) for the given analyte. For *P. falciparum*, a dilution error occurred that resulted in lower than expected analyte concentration being evaluated for all replicates. Six replicates of each sample were tested across 3 different sites on five different days, providing a total of 90 replicate test results per sample. On each test day at each site, two different operators used three FilmArray instruments; GF Panel pouch lot was rotated daily. In total, 270 valid test results were obtained for the reproducibility evaluation of the FilmArray GF Panel.

Table 1. Contrived Whole Blood Samples for Reproducibility Testing

Organism (Isolate)	Sample 1	Sample 2	Sample B3
Leptospira interrogans **serovar icterohaemorrhagiae**	Low Positive 3.3E+02 copies/mL 1 x LoD	Moderate Positive 1.0E+03 copies/mL 3 x LoD	Negative
Dengue Virus (DENV-2) New Guinea C	Low Positive 3.3E+02 copies/mL 1 x LoD	Moderate Positive 1.0E+03 copies/mL 3 x LoD	Negative
Plasmodium falciparum IPC 4884	Moderate Positive 5.4E+02 copies/mL 1.5 x LoD	Negative	Low Positive 9.0E+01 copies/mL 0.5 x LoD

Combined reproducibility results are shown below in **Table 2**.

Table 2: Reproducibility of the FilmArray Global Fever Panel Qualitative Results

	Test Analyte Isolate		Concentration	Expected Test Result	% Agreement with Expected Results				
					Site 1	Site 2	Site 3	All Sites	95% Confidence Interval
BACTERIA	***Leptospira interrogans* serovar *icterohaemorrhagiae***		**Moderate Positive 3× LoD** (1.2E+03 copies/mL)	**Detected**	30/30 100%	30/30 100%	30/30 100%	**90/90 100%**	96.0-100%
			Low Positive 1× LoD (3.9E+02 copies/mL)	**Detected**	27/30 90.0%	28/30 93.4%	26/30 86.7%	**81/90 90.0%**	81.9-95.3%
			Negative (No Analyte)	**Not Detected**	30/30 100%	30/30 100%	30/30 100%	**90/90 100%**	96.0-100%
VIRUSES	**Dengue virus DENV-2** New Guinea C		**Moderate Positive 3× LoD** (1.1E+03 copies/mL)	**Detected**	29/30 96.7%	30/30 100%	30/30 100%	**89/90 98.9%**	94.0-100%
			Low Positive 1× LoD (3.6E+02 copies/mL)	**Detected**	30/30 100%	30/30 100%	30/30 100%	**90/90 100%**	96.0-100%
			Negative (No Analyte)	**Not Detected**	30/30 100%	30/30 100%	30/30 100%	**90/90 100%**	96.0-100%
PROTOZOA	***Plasmodium falciparum*** IPC 4884	***Plasmodium* spp. Detection Results**	**Moderate Positive 1.5× LoD[1]** (2.7E+02 copies/mL)	**Detected**	30/30 100%	30/30 100%	30/30 100%	**90/90 100%**	96.0-100%
			Low Positive 0.5× LoD[1] (9.0E+01 copies/mL)	**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%**	96.0-100%
		***Plasmodium falciparum* Detection Results**	**Moderate Positive 1.5× LoD[1]** (2.7E+02 copies/mL)	**Detected**	29/30 96.7%	30/30 100%	28/30 93.4%	**87/90 96.7%**	90.6-99.3%
			Low Positive 0.5× LoD[1] (9.0E+01 copies/mL)	**Detected**	18/30 60%	24/30 80%	21/30 70%	**63/90 70.0%**	59.4-79.2%
			Negative (No Analyte)	**Not Detected**	30/30 100%	30/30 100%	30/30 100%	**90/90 100%**	96.0-100%
		Overall Agreement with the Expected Test Result All Analytes and All Test Levels (95% Confidence Interval)			**1,037/1,080 96.0%** (94.7-97.1%)				

[1]Due to a correction in the stock concentration, *P. falciparum* was evaluated at 1.5x LoD and 0.5x LoD.

Detected results were as expected for all analytes except for the Low Positive *Leptospira interrogans* sample which exhibited 90% agreement. The observed negative specimens were distributed across all runs, sites, testing days, and reagent lots and likely reflect underspiking with *Leptospira interrogans*.

A secondary assessment of reproducibility is based on variability in the melt temperature (Tm) of the amplification products (measured as standard deviation). Melt temperature mean and standard deviations are shown in **Table 3.** for control and organism assays for the three test sites and overall. Variability in the melt temperatures for the assays evaluated was within the expected range (≤0.5°C) for each assay at each site and overall, with a standard deviation of 0.2-0.3°C.

Table 3: Summary of Tm (°C) Analyses for FilmArray Global Fever Panel Assays

Analyte (Isolate tested)	Assay	Observed Tm (°C)							
		Site 1		Site 2		Site 3		All Sites	
		Mean	±StDev	Mean	±StDev	Mean	±StDev	Mean	±StDev
CONTROLS									
RNA Process Control	yeast RNA	(b)(4)							
PCR2 Control	PCR2								
BACTERIA									
Leptospira interrogans	Lepto1	(b)(4)							
VIRUSES									
Dengue virus Type 2	DENV2_1	(b)(4)							
PROTOZOA									
Plasmodium falciparum	Plas spp.	(b)(4)							
	Plas falciparum								

Variability in the melt temperatures for the assays evaluated was within the expected range (≤0.5°C) for each assay at each site and overall, with a standard deviation of (b)(4) °C.

Cumulatively, the results suggest that there are no significant differences between variables evaluated in the reproducibility study. Therefore, the reproducibility studies for the FilmArray Global Fever Panel are acceptable.

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Internal Controls:

Two internal controls are included in each FilmArray Global Fever Panel pouch:

- 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, 1st stage PCR, dilution, 2nd stage PCR, and DNA melting. A positive control result indicates that all steps carried out in the FilmArray Global Fever Panel pouch were successful.

- 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 result indicates that 2nd stage PCR was successful.

Both control assays must be positive for the test run to pass. If either control fails, the Controls field of the test report will display "Failed" and all results will be listed as "Invalid". If the controls fail, the sample should be retested using a new pouch.

Recommended External Controls:

External controls are not provided with the FilmArray Global Fever Panel but are recommended in the package insert. External controls should be used in accordance with laboratory protocols and the appropriate accrediting organization requirements, as applicable. Molecular grade water or saline can be used as an external negative control. Previously characterized positive samples or negative samples spiked with well-characterized organisms can be used as external positive controls.

BioFire Defense provides an external positive and negative assayed quality control kit to monitor the performance of *in vitro* laboratory nucleic acid testing procedures for the qualitative detection of the FilmArray Global Fever Panel performed on FilmArray 2.0 systems. The positive external control is a surrogate control material comprised of dried synthetic DNA (positive only) in buffer and stabilizer, supplied in a FilmArray Injection Vial that is used directly with the FilmArray Global Fever Panel. The FilmArray Global Fever Panel Control Kit is composed of two controls: FilmArray Global Fever Positive External Control Material (Positive ECM) and FilmArray Global Fever Negative External Control Material (Negative ECM). The DNA in the Positive ECM includes DNA segments to assess the presence of each individual assay in the FilmArray Global Fever Panel listed above. There is no DNA in the Negative ECM. The Global Fever Panel Control Kit contains no biological hazards and is 100% non-infectious. To use the product, the operator opens and uses the FilmArray Injection Vial in place of the Sample Injection Vial, and otherwise runs the test according to protocol. This control is shipped and stored at 18-28 °C.

The external control kit is available for purchase directly from BioFire Defense.

Quantification of nucleic acid derived from live or inactivated viral and bacterial cultures

Quantification using the genesig qPCR kits was performed at two different locations, 1) (b)(4) – live chikungunya virus stocks, 2) BioFire Defense – live and inactivated bacteria, viral, and protozoan stocks.

Organisms evaluated at BioFire Defense were obtained from (b)(4) (b)(4) (b)(4) Verification of the stocks, whenever available, was provided with a Certificate of Analysis (CofA) from the source. The majority of organisms obtained did not come quantified or came without any enumeration value.

Nucleic acids from the bacterial and viral stocks were extracted using (b)(4) (b)(4) (b)(4) All virus stocks were treated with (b)(4) reagent prior to extraction. The nucleic acid concentration of each extract was then determined using the commercially available genesig qPCR assay kits from (b)(4) (b)(4) and the (b)(4) were used to perform the qPCR assays at BioFire Diagnostics/BioFire Defense and (b)(4) respectively. Cycling conditions were as recommended by (b)(4) and are shown below in Table 4. (b)(4).

Table 4. Amplification Cycling Conditions for the (b)(4) g qPCR Kits

(b)(4)

Both DNA and RNA target nucleic acid from organism stocks was extracted using the (b)(4) The nucleic acid concentration of the extract (Unknown) was then determined using commercially available quantitative real-time PCR assay Standard Kits from (b)(4)

Table 5. Quantified Organisms and the (b)(4) qPCR Assay Kits Targets and the FilmArray Global Fever Panel Targets

FilmArray Global Fever Panel Analyte	Quantified Species	Strain	(b)(4) qPCR Kit Gene Target	FilmArray Global Fever Panel Target
Bacteria				
Leptospira spp.	*kirschneri*	200701401	(b)(4)	(b)(4)
		3522 C (Cynopteri)		
	interrogans	Serovar icterohaemorrhagiae		
		HAI0156 (Copenhagen)		
		L495 (Manilae)		
	alexanderi	L 60 (Manhao 3)		
	santarosai	LT 821 (Shermani)		
	kmetyi	Bejo-Iso9T (Malaysia)		
	noguchii	CZ 214T (Panama)		
	borgpetersenii	Veldrat Bataviae 46		
	weilii	Celledoni 20160426		
		A 102 (Mengrun)		
		6712 (Hainan)		
		H 27 (Hekou)		
		LT 89-68 (Vughia)		
		94-79970/3 (Topaz)		

	(b)(4)	(b)(4)		
Viruses				
Chikungunya Virus	R80422		(b)(4)	(b)(4)
	St. Martin 2013			
	DHS4263			
	(b)(4)			
	(b)(4)			
Dengue Virus	Serotype 1	Hawaii	(b)(4)	(b)(4)
		276RK1		
		Strain 12150		
		BC89/94		
		228690		
		VN/BID-V1792/2007		
		SL-6-6-04		
	Serotype 2	New Guinea C		
		VN/BID-V1002/2006		
		DakArA1247		
		BC102/94		
		429557		
		1349		
		ArA6894		
		(b)(4)		
	Serotype 3	H87		
		VN/BID-V1329/2006		
		(b)(4)		
		271242		
		C0360/94		
	Serotype 4	H241		
		703		
		(b)(4)		
		BC13/97		
		BC287/97		
		BC258/97		
		PR 06-65-740		
Plasmodium spp.	*P.falciparum*	IPC 4884 Pursat Cambodia 2011	(b)(4)	(b)(4)
		SenTh021.09		
		St. Lucia		
		Tanaznia, 02000708		
	P. vivax	Chesson		
		Panama		
	P. ovale	Wallikeri		
		Curtisi		
	P. knowlesi	Strain H		
	P. malariae	Unknown		

d. Detection limit:

A study to establish the detection limit of the FilmArray Global Fever panel was conducted utilizing whole blood samples contrived with isolates of viruses, bacteria, and protozoa detected by the Global Fever panel. (b)(4) (b)(4) (b)(4), analytical testing was performed simultaneously for all analytes. Specifically, testing utilized a combination of live and inactivated organisms prepared in multi-spiked mixes that contain (b)(4) individual target organisms as described below:

Table 6. Composition of Organism Mixes used in GF Panel LoD Testing

Mix	Organism	Mix	Organism
(b)(4)			

The LoD was first estimated by testing dilutions of contrived whole blood samples containing known dilutions of organisms bracketing an initial LoD concentration based on early development testing. Confirmation of LoD was achieved by testing (b) replicates of a contrived sample containing analyte at the estimated LoD. LoD was successfully confirmed when the organism was detected in at least (b) of the (b) of the replicates tested over (b)(4) days using (b)(4) pouch lots. Complete results for individual analytes included on the Global Fever Panel are included in Table 7 below.

Table 7. Limit of Detection for FilmArray Global Fever Panel Analytes

Global Fever Panel Test Result	Species/Strain Tested	LoD	
		Based on qPCR (copies/mL)	Based on Provided Stock Concentration (units/mL)
Leptospira interrogans	Icterohaemorrhagiae Budapest	(b)(4)	
Chikungunya virus	R80422 (heat inactivated)		
	B8635 (live)		
	Indo23574 (live)		
Dengue Virus 1	Hawaii		
Dengue Virus 2	New Guinea C		
	DakArA1247		
Dengue Virus 3	H87		
Dengue Virus 4	H241		
Plasmodium spp.	*P. falciparum* IPC4884 Pursat Cambodia 2011		
	P. knowlesi H Strain		
	P. malariae clinical specimen		
	P. ovale Wallikeri		
	P. vivax Chesson		
Plasmodium falciparum	IPC4884 Pursat Cambodia 2011		
Plasmodium vivax / Plasmodium ovale	*P. ovale* Wallikeri		
	P. vivax Chesson		

[1] Quantification of the organism stock material was not available.
[2]LoD for live chikungunya virus strains was only estimated by identifying the lowest concentration of analyte for which (b)(4) replicates were positive.

e. Analytical Reactivity (Inclusivity):

The analytical reactivity (inclusivity) of the FilmArray Global Fever Panel was evaluated with a collection of isolates to represent relevant species, subspecies, or serotypes. Testing was performed by evaluating (b)(4) replicates. If there was one undetected result, (b)(4) additional replicates were tested and if (b)(4) replicates were detected the isolate was considered inclusive. Most isolates were detected by the FilmArray Global Fever Panel at spiked concentrations within (b)(4) × LoD of testing either inactivated or live organisms (based on molecular quantification of nucleic acids for each isolate) in whole blood. Several isolates with reduced assay reactivity are described in more detail below.

When possible, in silico analysis of sequence data was used to make predictions of assay reactivity for less common strains or serotypes that were not tested.

Table 8 includes a summary of FilmArray Global Fever Panel reactivity based on empirical data.

Table 8. Summary of FilmArray Global Fever Panel Analytical Reactivity (Inclusivity)

FilmArray Global Fever Panel Analyte	# Isolates Detected / Tested	Isolates Tested		Concentration Detected [1]	x LoD	FilmArray Global Fever Panel Result (Replicates Detected/Tested)
BACTERIA						
Leptospira spp.	(b)/19	**Species**	**Strain**	(b)(4)		
		interrogans	Serovar (Budapest)			Detected ((b)(4))
			HAI0156 (Copenhageni)			Detected ((b)(4))
			L495 (Manilae)			
		alexanderi	L60 (Manhao 3)			
		alstonii	Sichuan 79601			Detected ((b)(4))
		borgpetersenii	Castellon 3 (Castellonis)			Detected ((b)(4))
			Veldrat Bataviae 46 (Javanica)			
		kirschneri	200701401 (Bogvere)			
			3522 C (Cynopteri)			
		kmetyi	Bejo-Iso9T (Malaysia)			
		mayottensis	200901116			
		noguchii	CZ 214T (Panama)			
		santarosai	LT 821 (Shermani)			
		weilii	6712			
			94-79970/3 Topaz			
			A 102 (Mengrun)			
			Celledoni 20160426			
			H 27 (Hekoou)			
			LT89-68 (Vughia)			
VIRUSES						
Chikungunya Virus	(b)/3	**Strain**		(b)(4)		
		R80422				Detected ((b)(4))
		DHS4263				Detected ((b)(4))
		St. Martin 2013				
Dengue virus	(b)/28	**Serotype**	**Strain**	(b)(4)		
		Serotype 1	Hawaii			Detected ((b)(4))
			Strain 12150			Detected ((b)(4))
			228690			
			276RK1			
			BC89/94			
			SL-6-6-04			
			UIS 1162			
			VN/BID-V1792/2007			
		Serotype 2	New Guinea C (DENV2_1)			Detected ((b)(4))

FilmArray Global Fever Panel Analyte	# Isolates Detected / Tested	Isolates Tested		Concentration Detected [1]	x LoD	FilmArray Global Fever Panel Result (Replicates Detected/Tested)
			DakArA1247 (DENV2_2)	(b)(4)		
			1349			Detected ((b)(
			429557			
			ArA6894			
			BC102/94			
			DKA 811			Detected ((b)(
			VN/BID-V1002/2006			Detected ((
		Serotype 3	H87			Detected ((b)(4)
			271242			Detected ((b)(
			BC188/97			
			C0360/94			
			VN/BID-V1329/2006			
		Serotype 4	H241			Detected ((b)(4)
			703			Detected ((b)(
			BC13/97			
			BC287/97			
			BC258/97			
			D85-019			
			PR 06-65-740			
PROTOZOA						
Plasmodium spp.	10/10	**Species**	**Strain**			
		falciparum	IPC 4884	(b)(4)		Detected ((b)(4)
			SenTh021.09			Detected ((b)(
			St. Lucia			
			Tanzania, 02000708			Detected ((b)(
		vivax	Chesson			Detected ((b)(4)
			Panama			Detected ((b)(
		ovale	Wallikeri			Detected ((b)(4)
			Curtisi			Detected ((b)(
		knowlesi	Strain H			Detected ((b)(4)
		malariae	Clinical specimen			
Plasmodium falciparum	4/4	*falciparum*	IPC4884			Detected ((b)(
			SenTh021.09			Detected ((b)(
			St. Lucia			
			Tanzania, 02000708			Detected ((b)(
Plasmodium vivax/ovale	4/4	*vivax*	Chesson			Detected ((b)(4)
			Panama			Detected ((b)(
		ovale	Wallikeri			Detected ((b)(4)
			Curtisi			Detected ((b)(

Organisms which exhibited reduced or no assay reactivity have limitations included in the labeling and are described specifically in the table below.

Observed Result	Detection Level	Analyte	Serotype/Strain/Isolate
Detected (may be underreported)	10 x LoD	*Plasmodium falciparum*[1]	SenTh021.09
	~100 x LoD	Dengue virus[1]	Serotype 3 BC188/97
			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 strains were not available in public databases.
[2]*In silico* analysis predicted reduced sensitivity or missed detection of this isolate due to sequence variation. Wet testing of this rare sylvatic strain at 10,000 x LoD confirmed detection was significantly impaired.

f. Microbial Interference Studies:

Potentially interfering microorganisms were evaluated for their effect on FilmArray Global Fever Panel performance. To evaluate the potential for interference, FilmArray Global Fever Panel test results from a control blood sample containing representative panel analytes (*Leptospira interrogans*, *P. falciparum*, dengue virus type 3) at concentrations near 3×LoD were compared to results from a sample with the same composition plus the potentially interfering microorganism, as well as a negative sample (no analytes) containing only the potentially interfering microorganism. Each potentially competing microorganism was tested at the highest concentration possible (1:10 dilution of the stock). The samples containing the potentially interfering microorganism were evaluated for their effects on the FilmArray Global Fever Panel internal control assays and analyte detection. Reproducible internal control failures or loss of analyte detection associated with the presence of a particular potentially interfering microorganism would be recognized as interference by that microorganism.

Table 9. Organisms Evaluated for Potential Microbial Interference

Microorganisms	Concentration Tested	Results
Corynebacterium diphtheriae	1:10 dilution of stock	No interference observed
Staphylococcus epidermidis	3.8E+06 CFU/mL	No interference observed
Escherichia coli	1:10 dilution of stock	No interference observed
Klebsiella pneumoniae	5.5E+04 CFU/mL	No interference observed
Haemophilus influenzae	1.0E+08 CFU/mL	No interference observed
Herpes Simplex virus	1.2E+05 $TCID_{50}$/mL	No interference observed
Epstein-Barr virus	3.3E+07 copies/mL	No interference observed
Cytomegalovirus	1:10 dilution of stock	No interference observed
Human Immunodeficiency virus (HIV) 1/2	HIV-1: 1.3E+05 U/mL HIV-2: 2.2E+05 U/mL	No interference observed
Plasmodium vivax	1.5E+06 copies/mL	No interference observed

None of the ten microorganisms tested showed interference with the pouch controls or specific Global Fever Panel assay targets.

g. Analytical specificity/Cross-reactivity:

The potential for non-specific amplification and detection by the FilmArray Global Fever Panel assays (cross-reactivity) was evaluated by testing high concentrations of on-panel (identified by the FilmArray Global Fever Panel assays) and off-panel (not intended to be identified by the FilmArray Global Fever Panel assays) organisms or purified nucleic acids, as well as by *in silico* analysis. All on-panel organisms were tested live at high concentration (> 10^6 copies/mL). As can be seen in Table 10 below, testing of *P. knowlesi* demonstrated cross-reactivity with the *P. vivax/ovale* assay at concentrations above 2.2E+03 copies/mL.

Table 10. FilmArray Global Fever Panel Results for On-Panel Organism Testing Assessing Potential Cross-reactivity

Pathogen			Live or Inactivated		Results
			Live	Inactivated	
Leptospira interrogans (Schu S4)			√	-	As expected (only *Leptospira spp.* Detected)
Chikungunya virus (R80422 culture fluid)			√	-	As expected (only Chikungunya virus Detected)
Dengue virus	DENV-1 (Hawaii)		√	-	As expected (only Dengue virus Detected)
	DENV-2 (New Guinea C)		√	-	As expected (only Dengue virus Detected)
	DENV-3 (H87)		√	-	As expected (only Dengue virus Detected)
	DENV-4 (H241)		√	-	As expected (only Dengue virus Detected)
Plasmodium		*falciparum* (Pursat Cambodia 2011)	√	-	As expected (*Plasmodium* spp. Detected and *P. falciparum* Detected)
		knowlesi (Strain H)	√	-	*Plasmodium* spp Detected as expected. *Plasmodium* vivax/ovale detected at concentrations above (b)(4) copies/mL.
		malariae (DLS17-026015)	√	-	As expected (only *Plasmodium* spp. Detected)
		vivax (11 Chesson)	√	-	As expected (*Plasmodium* spp. Detected and *P. vivax/ovale* Detected)
		ovale (Wallikeri)	√	-	As expected (*Plasmodium* spp. Detected and *P. vivax/ovale* Detected)

Although no cross-reactivity with *P. malariae* was observed in this study, in silico analysis shows (b)(4) mismatches between *P. malariae* and the *P. vivax/ovale* assay primers. Wet testing with *P. brasilianum* (a monkey *Plasmodium* thought to have descended from *P. malariae* and which has identical sequence as *P. malariae*) showed reactivity for the *P. vivax/ovale* assay at (b)(4) copies/mL.

Off-panel organisms were selected for testing based on a combination of several factors including:

- Relatedness to the species detected by the Global Fever Panel (near-neighbors)
- Clinical relevance (causing symptoms similar to the panel pathogens)
- likelihood of being present in blood as a co-infection based on a geographical region or specific population to which a panel pathogen is endemic
- genetic similarity to Global Fever Panel assay primers as determined by *in silico* analysis

BSL3/4 organisms and viruses that could not be obtained as inactivated stocks or purified nucleic acids were tested live in an appropriate facility (b)(4) and (b)(4) (b)(4) to assess potential cross-reactivity.

Table 11 below lists the off-panel organisms that were tested at high concentrations (> 10^6 copies/mL) for potential cross-reactivity with the FilmArray Global Fever Panel.

Table 11. Off-Panel Organisms Tested by the FilmArray Global Fever Panel

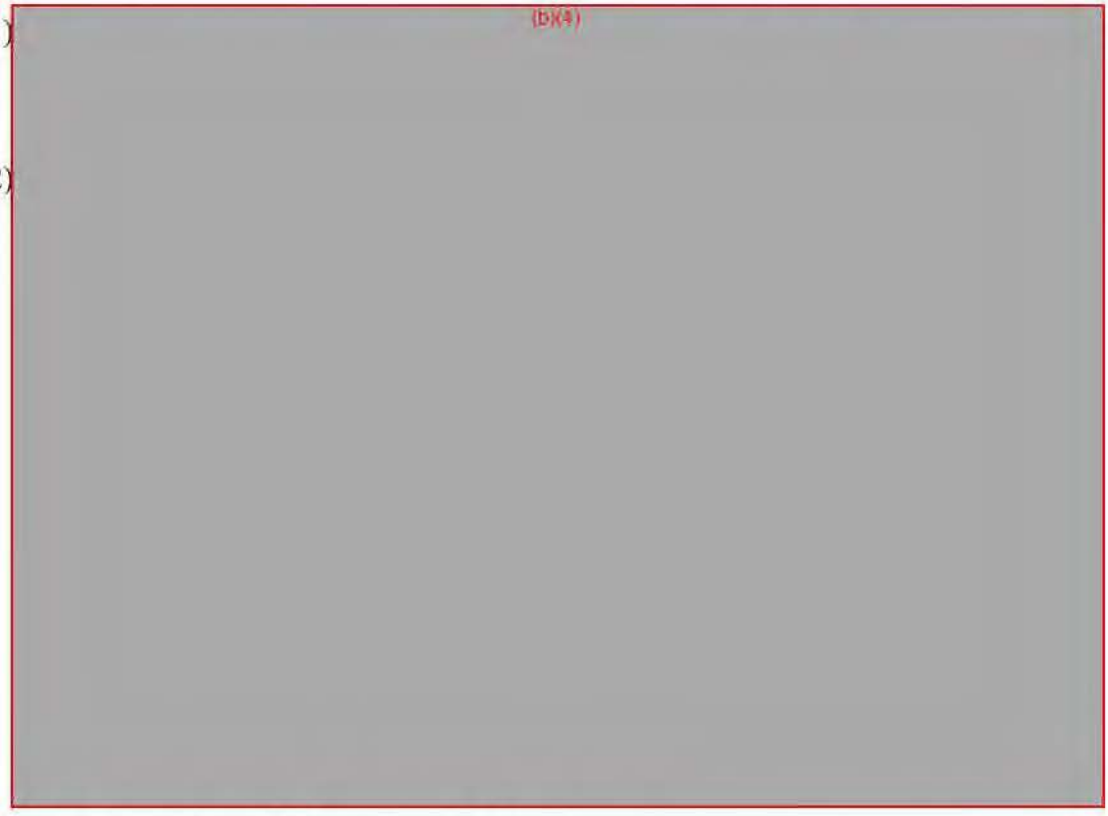
Bacteria		Viruses	
Acinetobacter baumannii	*Mycoplasma pneumoniae*	Adenovirus 1	Omsk hemorrhagic fever virus
Bacillus anthracis	*Neisseria meningitidis*	Adenovirus 3	**O'nyong-nyong virus**[1]
Bacillus brevis	*Proteus mirabilis*	Adenovirus 5	Parvo virus
Bacillus cereus	*Pseudomonas aeruginosa*	Aura virus	Powassan virus
Bacillus circulans	*Rickettsia typhi*	Barmah Forest virus	Rabies virus
Bacillus coagulans Hammer	*Salmonella enterica* subsp. *arizonae*	Bunyamwera virus	Rift Valley Fever Virus
Bacillus halodurans	*Salmonella enterica* subsp. *bongori*	Coronavirus NL63	Ross River virus
Bacillluus licheniformis	*Salmonella enterica* subsp. *diarizoniae*	Crimean-Congo Hemorrahagic Fever Virus	Human respiratory syncytial virus
Bacillus megaterium	*Salmonella enterica* subsp. *enterica* serovar Dublin	Dugbe virus	Rubella virus
Bacillus mycoides	*Salmonella enterica* subsp. *enterica* serovar Enteritidis	Eastern Equine Encephalitis Virus	Saint Louis encephalitis virus
Bacillus pumilus	*Salmonella enterica* subsp. *enterica* serovar Javiana	Ebolavirus (Zaire, Sudan, Bundibugyo, Tai Forest, Reston)	Sindbis virus
Bacillus subtilis	*Salmonella enterica* subsp. *enterica* serovar Manchester	Enterovirus, HEV-71	Spondweni virus
Bacillus thuringiensis	*Salmonella enterica* subsp. *enterica* serovar Montevideo	Epstein Barr virus	Tickborne encephalitis virus
Bacteroides fragilis	*Salmonella enterica* subsp. *enterica* serovar Muenchen	Flexal virus	Una virus
Bordetella bronchiseptica	*Salmonella enterica* subsp.	Guanarito virus	Usutu virus

	enterica serovar Newport		
Borrelia burgdorferi	*Salmonella enterica* subsp. *enterica* serovar Paratyphi	Hantaan virus	Vaccinia virus
Brucella melitensis	*Salmonella enterica* subsp. *enterica* serovar Rubislaw	Hazara virus	Varicella zoster virus
Burkholderia cepacia	*Salmonella enterica* subsp. *enterica* serovar Saintpaul	Hendra virus	Venezuelan Equine Encephalomyelitis (VEE) virus
Burkholderia mallei	*Salmonella enterica* subsp. *enterica* serovar Tennessee	Hepatitis A Virus (HAV)	Western Equine Encephalitis (WEE) virus
Burkholderia pseudomallei	*Salmonella enterica* subsp. *enterica* serovar Thompson	Hepatitis B Virus (HBV)	West Nile virus Lineage 1
Chlamydophila pneumoniae	*Salmonella enterica* subsp. *enterica* serovar Typhi	Hepatitis C Virus (HCV)	West Nile virus Lineage 2
Clostridium bifermentans	*Salmonella enterica* subsp. *enterica* serovar Typhimurium	Herpes Simplex Virus 2 (HSV-2)	Yellow Fever virus
Clostridium perfringens	*Salmonella enterica* subsp. *houtenae*	Human Parainfluenza Virus 1	Zika virus
Clostridum sordelli	*Salmonella enterica* subsp. *indica*	Human Parainfluenza Virus 3	**Fungi**
Clostridium sporogenes	*Salmonella enterica* subsp. *salamae*	Hughes virus	*Aspergillus fumigatus*
Clostridium perfringens	*Serratia marcescens*	Human herpesvirus 6B	*Cryptococcus neoformans*
Coxiella burnetii	*Staphylococcus aureus*	Human Immunodeficiency Virus 1	**Pathogenic Protozoa**
Enterobacter aerogenes	*Streptococcus agalactiae*	Human Immunodeficiency Virus 2	*Babesia microti*
Enterococcus faecalis	*Streptococcus pneumoniae*	Human T-lymphotropic virus type 1	*Crithidia fasciculata*
Enterococcus faecium	*Streptococcus pyogenes*	Human T-lymphotropic virus type 2	*Cyclospora cayetanensis*
Francisella hispaniensis	*Vibrio cholerae*	Influenza A H1N1-2009	*Leishmania donovani*
Francisella tularensis subsp. *tularensis*	*Yersinia aldovae*	Influenza A H3N2	*Leptomonas seymouri*
Francisella persica	*Yersinia bercovieri*	Influenza B virus	*Schistsoma mansoni*
Francisella philomiragia	*Yersinia entericolitica*	Japanese Encephalitis Virus (JEV)	*Toxoplasma gondii*
Klebsiella oxytoca	*Yersinia fredericksenii*	Junin virus	*Trypanosoma brucei*
Legionella pneumophila	*Yersinia intermedia*	Lassa virus	*Trypansoma cruzi*
Leptospira biflexa	*Yersinia kristensenii*	Machupo virus	*Trypansomsa rangeli*
Leptospira meyeri	*Yersinia mollarettii*	Marburg Marburgvirus variant Musoke	***Plasmodium berghei***[2]
Leptospira terpstrae	*Yersinia pestis*	Marburg Marburgvirus (RAVN)	***Plasmodium brasilianum***[3]
Leptospira vanthielii	*Yersinia pseudotuberculosis*	Mayaro virus	***Plasmodium cynomolgi***[4]
Leptospira wolbachii	*Yersinia rohdei*	Measles virus	***Plasmodium fieldi***[5]
Leptospira yanagawae		Metapneumovirus	***Plasmodium fragile***[6]
Listeria monocytogenes		Mopeia Virus	***Plasmodium inui***[7]
Mycobacterium tuberculosis (avirulent)		Mumps Virus	***Plasmodium simiovale***[8]

There were two instances of cross-reactivity observed during testing:

1)

2)



No other cross-reactivity was observed in wet testing.

In addition to the experimental testing, *in silico* analyses directed toward the potential to cross-react with specific pathogens that were unavailable for testing was also performed. This included Avalon virus, Bas-Congo virus, *Bacillus luciferensis*, *Chalmydophila psittaci*, *Francisella mediasiatica.* Lymphocytic choriomeningitis virus, Middleburg virus, Murray Valley encephalitis virus, *Orientia chuto (tsutsugamushi)*, Pirital virus, *Rickettsia prowazekii*, *Rickettsia ricketsii*, Sabia virus,

Semliki Forest virus, Tonate virus, and Variola major. No expected cross-reactivity with off-panel pathogens were predicted by *in silico* analysis.

h. Interfering Substances:

Potentially interfering substances that could be present in whole blood or introduced during specimen collection and testing were evaluated for their effect on the FilmArray Global Fever Panel performance. Each substance was added to contrived samples containing representative FilmArray Global Fever Panel organisms (*Leptospira interrogans*, dengue virus Type 3, and *P. falciparum*) at concentrations equivalent to approximately 3×LoD. The concentrations of endogenous and exogenous substances tested were based on a reference concentration of "normal" to "high" levels expected to be present in clinical specimens.

The following two tables show the results for tested endogenous and exogenous substances, and for technique specific substances and anticoagulants, respectively.

Table 12. Results for Potentially Interfering Substances Tested on the FilmArray Global Fever Panel (Endogenous and Exogenous Substances)

Endogenous Substances	Concentration Tested [a]	Results
Albumin	60.0 mg/mL	No interference observed
Bilirubin (Conjugated)	0.41 mg/mL	No interference observed
Bilirubin (Unconjugated)	0.41 mg/mL	No interference observed
Cholesterol (total)	4.2 mg/mL	No interference observed
Glucose	10.1 mg/mL	No interference observed
Hemoglobin	137.0 mg/mL	No interference observed
Immunoglobulins	60 mg/mL	No interference observed
Triglycerides	15.1 mg/mL	No interference observed
Human cells (K-562 Human Leukemia Cells)	6.1E+06 cells/mL	No interference observed
Exogenous Substances	**Concentration Tested [a]**	**Results**
Artemether-Lumefantrine	0.0004 mg/mL	No interference observed
Atovaquone	0.005 mg/mL	No interference observed
Proguanil	0.001 mg/mL	No interference observed
Mefloquine	0.0017 mg/mL	No interference observed
Amphotericin B	0.002 mg/mL	No interference observed
Pentamidine	0.0015 mg/mL	No interference observed
Fluconazole	0.026 mg/mL	No interference observed
Amoxicillin	0.062 mg/mL	No interference observed
Azithromycin	0.011mg/mL	No interference observed
Cetriazone	1.0 mg/mL	No interference observed
Ciprofloxacin	0.012 mg/mL	No interference observed
Clindamycin	0.055 mg/mL	No interference observed
Doxycycline	0.02 mg/mL	No interference observed

Gentamicin	0.036 mg/mL	No interference observed
Meropenem	0.39 mg/mL	No interference observed
Sulfamethoxazole	0.38 mg/mL	No interference observed
Vancomycin	0.12 mg/mL	No interference observed
Cycloserine	75.0 mg/mL	No interference observed
Isoniazid	0.06 mg/mL	No interference observed
Oseltamivir	0.0005 mg/mL	No interference observed
Ribavirin	0.011 mg/mL	No interference observed
Tenofovir	0.001 mg/mL	No interference observed
Acetaminophen	0.16 mg/mL	No interference observed
Aspirin	0.03 mg/mL	No interference observed
Ibuprofen	0.22 mg/mL	No interference observed
Prednisone	0.0001 mg/mL	No interference observed
Prednisolone	1.2 mg/mL	No interference observed
Cortisone	0.001 mg/mL	No interference observed
Artesunate	0.1 mg/mL	No interference observed

[a]Concentrations of interfering substances were based on guidelines contained in CLSI guidelines for interference testing (EP07 and EP37) when available.

Table 13. Results for Potentially Interfering Substances Tested on the FilmArray Global Fever Panel (Disinfectants, Solvents, and Anticoagulants)

Technique Specific Substances	Concentration Tested	Results
Bleach	1% (v/v) or 525 ppm NaOCl	No interference observed
Povidone-iodine	1% (v/v)	No interference observed
Ethanol	2% (v/v)	No interference observed
TRIzol	2-3% (v/v)	Potentially Interfering
DMSO[1]	2% (v/v)	No interference observed
Methanol[1]	2% (v/v)	No interference observed
Saline[1]	2% (v/v)	No interference observed
Chloroform[1]	2% (v/v)	No interference observed
Acetone[1]	2% (v/v)	No interference observed
Hydrochloric Acid[1]	0.0005 N	No interference observed
Anticoagulant	**Concentration Tested**	**Results**
Citrate (sodium)	~0.32 % (10.9 mmol/L)	No interference observed
K·EDTA in excess (5x)[2]	~9 mg/mL	No interference observed
Na·Heparin	~19 USP/mL	Potentially Interfering
Acid-citrate-dextrose (ACD)	~2.2 g/L citrate 0.8 g/L citric acid 2.5 g/L dextrose	No interference observed
Sodium polyanethenole sulfonate (SPS)	0.72 mg/mL	No interference observed
Serum separation tubes	N/A	No interference observed

[1]Testing of these specific substances was performed to account for their use as solvents for resuspension of other potential interferants.

[2]Testing was performed to evaluate potential interference caused by a short blood draw and a collection tube that thus contained higher anticoagulant concentration

dengue virus, *Leptospira* spp., and *Plasmodium* spp. vaccines were not evaluated in this study but are predicted to be reactive with the corresponding Global Fever Panel assay targets.

All evaluated exogenous substances exhibited no interference effect. Among technique specific substances, initial testing of TRIzol exhibited an interference effect wherein (b)(4) *P. falciparum* replicates were incorrectly reported as a Not Detected result. Subsequent retesting at the same interferent level and additionally with samples containing (b)(4)% TRIzol (v/v) did not produce unexpected assay results but may result in delayed Cp values. Similarly, initial testing with Heparin resulted in two unexpected Not Detected results for a single analyte with only (b)(4) replicates positive. However, repeat testing at the same heparin concentration successfully met acceptance criteria with (b)(4) replicates positive. These data demonstrate that Heparin levels near the evaluated concentration may be inhibitory for analytes near the LoD.

i. Assay cut-off:

The FilmArray Global Fever Panel Melt Detector software determines whether a FilmArray Global Fever Panel assay result is positive or negative using a predefined algorithm that includes Tm values, fluorescence values, and analysis of melting curves.

Initial melt ranges for each analyte-specific FilmArray Global Fever Panel assay were determined based on a combination of mathematical modeling using known sequence variations of different strains/isolates/variants of targeted organisms as well as data from testing of clinical specimens and known isolates.

After completion of the analytical and clinical studies on the FilmArray Global Fever Panel, a final validation of the melt ranges was performed and included review of data from the Inclusivity study and clinical studies. The observed sensitivity and specificity rates for the individual melt curves and assay calls as compared to expert annotation was greater than 99.8% and 99.9% respectively. The sensitivity, specificity, and accuracy for the validation data were determined to be well above the acceptance criteria.

j. Specimen Transport and Storage (Specimen Stability)

Stability for whole blood specimens was evaluated to support labeling recommendations for storage of samples at room temperature for up to 24 hours, or at 2-8°C for up to seven days, or in an ultra-low temperature freezer (≤-70°C). These storage conditions are consistent with or exceed standard storage and transport conditions and times for most laboratory testing of clinical human whole blood specimens. Testing was conducted using samples composed of human WB contrived with (b)(4) representative GF Panel analytes in (b)(4) mixes at a concentration of (b)(4) their limit of detection (LoD). Samples were tested immediately (b)(4) replicates) after sample preparation as a no storage control or stored at the appropriate condition for

various amounts of time (one day, three days, or seven days) prior to testing of additional (b)(4) replicates per sample per storage condition.

As shown in Table 14 below, study results demonstrated 10/10 replicates positive for each evaluated storage condition for all analytes. All *P. falciparum* contrived specimens were reactive by both the *Plasmodium* spp. and *Plasmodium falciparum* assays. Mean Cp values for all analytes were also consistent between the control and stored samples thereby further supporting the storage claims.

Table 14. Summary of Organism Detection in Whole Blood for All Storage Conditions Tested

Organism/Analyte (Strain)	Concentration Tested	# Detected / # Tested						
		No Storage (Control)	Ambient Storage		Refrigerated Storage			Ultra-low freezer
			(b)(4) °C for 24 h	(b) °C for 24 h	Day 1	Day 3	Day 7	
BACTERIA								
Leptospira interrogans	(b)(4)							
VIRUSES								
Dengue virus	(b)(4)							
PROTOZOA								
Plasmodium falciparum	(b)(4)							

Fresh vs. Frozen Study

Frozen specimens were evaluated as part of the clinical validation of the Global Fever panel due to circumstances that did not permit immediate testing. To determine whether specimen freezing adversely affects analyte detection by the Global Fever Panel, a total of (b)(4) contrived specimens and (b)(4) previously tested prospective clinical specimens were evaluated both fresh and after freezing at ≤ -70°C for at least five days. Contrived specimens were prepared with multiple analytes spiked at concentrations near (b)(4) in clinical matrix. Contrived specimens were retested after having been frozen for 6-29 days. Clinical specimens were retested after having been frozen for 111-406 days. Overall agreement of the BioFire Global Fever panel results between fresh and frozen specimens were evaluated as PPA and NPA and results are shown in the tables below.

Table 15. Performance Comparison of Fresh vs Frozen Contrived Specimens

Organism/Analyte (Strain)	PPA			NPA		
	TP / (TP+ FN)	%	95% CI	TN / (TN + FP)	%	95% CI
BACTERIA						
Leptospira spp.	(b)(4)	100%	81.6-100%	(b)(4)	100%	92.0-100%
VIRUSES						
Dengue virus	(b)(4)	100%	81.6-100%	(b)(4)	100%	91.8-100%
PROTOZOA						
Plasmodium falciparum	(b)(4)	100%	51.0-100%	(b)(4)	100%	93.6-100%
P. vivax/ovale	(b)(4)	100%	64.6-100%	(b)(4)	100%	93.2-100%
Plasmodium spp.	(b)(4)	100%	64.6-100%	(b)(4)	100%	91.8-100%

Table 16. Performance Comparison of Fresh vs. Frozen Clinical Specimens

Organism/Analyte (Strain)	PPA			NPA		
	TP / (TP+ FN)	%	95% CI	TN / (TN + FP)	%	95% CI
BACTERIA						
Leptospira spp.	(b)(4)	100%	56.6-100%	(b)(4)	100%	90.8-100%
VIRUSES						
Dengue virus (Serotypes 1, 2, 3, 4)	(b)(4)	100%	81.6-100%	(b)(4)	100%	87.1-100%
Chikungunya Virus	(b)(4)	100%	56.6-100%	(b)(4)	100%	90.8-100%
PROTOZOA						
Plasmodium falciparum	(b)(4)	100%	70.1-100%	(b)(4)	97.1%	85.1-99.5%
P. vivax/ovale	(b)(4)	100%	72.2-100%	(b)(4)	97.0%	84.7-99.5%
Plasmodium spp.	(b)(4)	100%	80.6-100%	(b)(4)	100%	87.5-100%

[a]Three of the 16 *Plasmodium* spp.-positive fresh specimens were positive for both *P. falciparum* and *P. vivax/ovale*
[b]One specimen negative for *P. falciparum* assay when tested fresh was positive for *P. falciparum* when tested after freezing.
[c]One specimen that was negative for the *P. vivax/ovale* assay when tested fresh was positive for *P. vivax/ovale* when tested after freezing

2. Comparison studies:

   a. *Method comparison with predicate device:*

   Not applicable

b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity:

Prospective Clinical Study
The clinical performance of the Global Fever Panel was established during a multi-center study conducted at ten geographically distinct study sites, including two in the United States.

Prospective whole blood specimens were collected from subjects meeting the following eligibility criteria:

Inclusion criteria

- Subject has a recorded or self-reported fever within the past two days
- Subject provides informed consent prior to enrollment and specimen collection
- Subject has not participated in the study within the last 30 days

Exclusion criteria

- Subject does not have or did not self-report having a fever within the past two days
- Subject was unable to provide informed consent
- Subject has participated in the study within the last 30 days.

As recorded in site-specific study protocols, some sites applied additional eligibility criteria such as age limits, citizenship within the host country, and/or suspicion in the opinion of the physician that the subject's fever could be due to a pathogen on the Global Fever Panel.

A total of 1,971 collected specimens were generated across all study sites. The most common reason for exclusion was difficulty drawing blood ([redacted]96). [redacted] ([redacted]96) were excluded due to errors by study personnel. [redacted] specimens ([redacted]96) were excluded due to inability to obtain a Global Fever Panel or comparator result and [redacted] ([redacted]96) were excluded because of the subject withdrawing before the specimen was collected. In total, 1875 eligible whole blood specimens, including 1469 (78.3%) Category I prospective fresh and 406 (21.7%) Category II prospective frozen specimens, were collected over eighteen months (March 2018 – September 2019).

Table 17 below shows the participating study sites and the number of prospective specimens enrolled at each study site.

Table 17. Prospectively Collected Samples Across Sites in the Global Fever Panel Clinical Study

Study Site (Location)	Study Site #	Enrolled Population	Total Subjects Enrolled	Total Valid WB Specimens Enrolled (excluded)	Study Started – Completed (2016)
USA	Site 7	Emergency Department (ED)	(b)(4)	179 (21)	August 2018- November 2018
	Site 14	Inpatient and ED		9 (3)	August 2019- September 2019
Africa	Site 1	Inpatient		134 (11)	March 2018- May 2019
	Site 2	Inpatient and Outpatient		108 (5)	June 2019= September 2019
	Site 5	Inpatient and Outpatient		199 (4)	January 2019- June 2019
	Site 11	Inpatient and Outpatient		158 (5)	July 2019- September 20109
Southeast Asia	Site 8	Inpatient and Outpatient		249 (31)	November 2018 – September 2019
	Site 9	Outpatient		406 (9)	February 2019 – September 2019
Central & South America	Site 12	Outpatient		297 (3)	November 2018 – July 2019
	Site 13	Inpatient, Outpatient, and ED		136 (4)	August 2019 – September 2019
		Total	1971	1875 (96)	

Table 18 below shows breakdown of gender and age in clinical study.

Table 18. Overall and Per Site Demographic Analysis for the Global Fever Panel Clinical Study

		Overall	Site #1	Site #2	Site #5	Site #7	Site #8	Site #9	Site 11	Site #12	Site #13	Site #14
Sex	Male	895 (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%)
	Female	980 (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 years	163 (8.7%)	44 (32.8%)	20 (18.5%)	0 (0%)	0 (0%)[a]	25 (10.0%)	0 (0%)[b]	66 (41.8%)	3 (1.0%)	5 (3.7%)	0 (0%)[a]
	5 – 21 years	765 (40.8%)	21 (15.7%)	38 (35.2%)	128 (64.3%)	14 (7.8%)[a]	127 (51%)	204 (50.2)[b]	70 (44.3%)	102 (34.3%)	61 (44.9%)	0 (0%)[a]
	22 – 50 years	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+ years	275 (14.6%)	27 (20.1%)	19 (17.6%)	12 (6%)	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

In the laboratory, specimens were thoroughly mixed by vortexing or inverting and pipetted into a 700-µL aliquot for FilmArray testing, a 1300-µL aliquot for nucleic acid extraction, and the remaining volume (typically ≥500 µL) into an aliquot for archiving. Extracted nucleic acid was stored at ≤-70 ˚C and shipped on dry ice to BioFire Defense for molecular comparator testing.

0.3% (5/1902) of eligible specimens were ultimately excluded due to inability to obtain a Global Fever Panel test result. (Four specimens were initially eligible and tested on the Global Fever Panel, but were later excluded, for a total of 1898 specimens included in the analysis of System Performance.) The overall success rate for initial specimen tests on the Global Fever Panel was 98.7% (1868/1898); five tests did not complete (two due to loss of power, two instrument errors, and one software error) and 25 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.

The incomplete runs and instrument errors accounted for 0.3% (5/1898) of initial runs, resulting in an instrument success rate of 99.7% (1893/1898). Of the five unsuccessful initial tests due to instrument performance, all were retested once, and three produced valid results. The two specimens that failed after retesting were excluded from further analysis.

Of the 1893 initial tests that completed, 1.3% (25/1893) did not produce valid internal controls, resulting in a pouch control success rate of 98.7% (1868/1893). Of the 25 tests with internal control failures, all were retested once, and 22 produced valid control results. Internal controls failed a second time for three specimens upon retesting, so these three specimens were excluded from further analysis.

All specimens were evaluated with the FilmArray Global Fever Panel at the clinical study sites. Frozen nucleic acid extracts were sent to BioFire Defense for comparator testing with well-validated nested PCR assays followed by bi-directional Sanger sequencing. Two comparator assays were utilized for each assay target with positive result from both assays considered positive. If the results of the two comparator assays for a particular analyte disagreed, the samples were subjected to repeat comparator testing with samples determined as positive if at least 2/3 replicates were positive for a single comparator assay.

PPA for each analyte was calculated as 100% x (TP/(TP + FN)). True positive (TP) indicates that both the FilmArray Global Fever Panel and the comparator method had a positive result for this specific analyte, and false negative (FN) indicates that the FilmArray Global Fever Panel result was negative while the comparator result was positive. NPA was calculated as 100% x (TN/(TN + FP)). True negative (TN) indicates that both the FilmArray Global Fever Panel and the comparator method had negative results and a false positive (FP) indicates that the FilmArray Global Fever Panel result was positive, but the comparator result was negative. The exact binomial two-sided 95% confidence interval was calculated. Samples for which false positive

and/or false negative results (i.e., discrepant results) were obtained when comparing the FilmArray Global Fever Panel results to the comparator method results were further investigated. The discrepancy investigations were typically performed as follows: 1) All FP and FN were examined to determine whether additional testing on Global Fever Panel or with comparator assays could detect the analyte, but initial testing reported as not detected or negative because analyte was near or below the detection threshold; 2) FP and FN were evaluated by at least one additional PCR test that used different primers than the Global Fever Panel assay or the comparator assays; 3) When possible, unresolved discrepancies were evaluated with additional PCR testing that could be verified by sequence analysis. Discrepancy results are footnoted below in Table 19.

Table 19. FilmArray Global Fever Panel Clinical Performance: Summary – Whole Blood (EDTA)

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95% CI
VIRUSES							
Chikungunya virus[a]	Fresh	25/25	100%	86.7-100%	1442/1444	99.9%	99.5-100%
	Frozen	0/0	-	-	406/406	100%	99.1-100%
	Overall	25/25	100%	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%	99.7-100%
	Frozen	17/20	85.0%	64.0-94.8	386/386	100%	99-100%
	Overall	266/283	94.0%	90.6-96.2%	1592/1592	100%	99.8-100%
BACTERIA							
Leptospira spp.[c]	Fresh	9/9	100%	70.1-100%	1456/1460	99.7%	99.3-99.9%
	Frozen	6/7	85.7%	48.7-97.4%	399/399	100%	99-100%
	Overall	15/16	93.8%	71.7-98.9%	1855/1859	99.8%	99.4-99.9%
PROTOZOA							
Plasmodium spp.[d,e]	Fresh	207/210	98.6%	95.99-99.5%	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%	92.7-97.1%	351/351	100.0%	98.9-100%
	Overall	115/124	92.7%	86.8-96.1%	1751/1751	100.0%	99.8-100%

[a]2/2 FP Chikungunya virus specimens were positive by additional PCR

[b]Evidence of Dengue virus was found in 15/17 FN specimens; five were positive upon retesting with Global Fever Panel and additional PCR, two were positive upon Retest with Global Fever Panel, and eight were detected only by additional PCR.

[c]Evidence of Leptospira spp. was found in 1/1 FN by GF 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 1/6 was *P. vivax/ovale.* Three (3/12) *Plasmodium* spp. FP specimens were also *P. falciparum* FP

[e]*Plasmodium* spp. were detected in 3/6 FN Specimens: two were positive upon GF Panel retest and additional PCR, one was positive only upon GF Panel retest. *Plasmodium* spp. were also detected in 10/12 FP specimens by additional PCR.

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

[g]*P. vivax/ovale* was detected in 7/9 FN specimens: two of which were positive both upon GF Panel retest and by additional PCR, two were positive only by GF Panel retest, and three were positive only by additional PCR.

Twenty-five specimens had a positive *Plasmodium* spp. Detected result with a concomitant Not Detected result for either *P. falciparum* or *P. vivax/ovale*. For 19 of these specimens, Cp value of the *Plasmodium* spp. assay was near or later than the LoD range and/or comparator testing identified *P. falciparum* or *P. vivax/ovale* infection. A further 3/25 of these specimens had robust *Plasmodium* spp. amplification but no further evidence of *P. falciparum* or *P. vivax/ovale* infection. These cases likely reflect infection with a different malaria species (*P. knowlesi* or *P. malariae*) not differentiated by the Global Fever Panel malaria assays.

When possible, performance of malaria testing as part of Standard of Care was compared to the Global Fever Panel result. Because most sites did not attempt to speciate malaria, only the *Plasmodium* spp. assay result was used in this analysis. Each clinical site followed its own standard procedure for malaria testing with significant differences between sites. Data is listed in Table 20 below as Site-specific PPA and NPA with site-specific microscopic results.

Table 20. Performance Comparison Between Global Fever Panel and Site-Specific Malaria Testing.

Site (Method)	Site PPA		Site NPA	
	Site Positive Agreement	95% CI	Site Negative Agreement	95% CI
(b)(4)				

Overall, the Global Fever Panel showed strong correlation with site-specific methods when the site was able to detect malaria (b)(4) true positives versus only [redacted] false negatives). Importantly, for all [redacted] of the false negative results, the comparator method agreed with Global Fever Panel. The Global Fever Panel was also able to detect *Plasmodium* in many specimens that were determined to be negative by site-specific methods ((b)(4)). For (b)(4) of these Detections, the comparator method agreed with the Global Fever Panel result. While it cannot be ruled out that the Global Fever Panel detected residual nucleic acid following a cleared infection, these results could also reflect that the Global Fever Panel is more sensitive than the other methods utilized.

Assay performance among positive specimens that were quantified by site-specific methods is included in the table below stratified by overall parasitemia.

Table 21. Global Fever Panel Positive Percent Agreement with Site-Specific Malaria Testing Stratified by Parasitemia

Parasitemia (Parasites/µL)	PPA	95% CI
(b)(4)		

The Global Fever Panel reported a total of (b)(4) specimens with multiple analyte detections ((b)(4)% of all specimens, (b)(4)% of all positive specimens) as described in Table 22 below. The majority of co-detections contained *Plasmodium* spp. ((b)(4)). No co-detections contained more than two discernible analytes.

Table 22. Global Fever Panel Clinical Study Specimen Co-Detections

Distinct Co-detection Combinations			Total Co-detections
Analyte 1	**Analyte 2**	**Analyte 3**	(b)(4)
Chikungunya virus[a]	Dengue virus	-	
Dengue virus	*Plasmodium* spp.	*P. falciparum*	
Dengue virus	*Plasmodium* spp.	*P. vivax/ovale*	
Leptospira spp.[b]	*Plasmodium* spp.[b]	-	
Leptospira spp.	*Plasmodium* spp.	*P. vivax/ovale*	
Plasmodium spp.	*P. falciparum*	*P. vivax/ovale*	

[a]Chikungunya virus was detected by Global Fever Panel but not by comparator testing in one of the two co-detections
[b]Comparator testing did not identify *Leptospira* spp or *Plasmodium* spp. in this specimen.

b. Clinical specificity:

See section L.3a above.

c. Other clinical supportive data (when a. and b. are not applicable):

See section L.3a above.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The prevalence of individual analytes detected by the Global Fever Panel as observed during the clinical study is described in Table 23 below.

Table 23. Prevalence of Detected Analytes Stratified by Region and Clinical Study Site

Analyte	Overall (N=1875)		USA				Africa								Southeast Asia				Central & S. 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=404)		Site 12 (N=297)		Site 13 (N=136)	
	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV
Chikungunya virus	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)	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	13.4%	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%

The Global Fever Panel detected at least one analyte in 657 of the 1875 included specimens (35.1% positivity rate).

Table 24. Expected Values Summary (Detections) as Determined in the Global Fever Panel Clinical Study

FilmArray Result	Number Detected	% of Total (% of Positives)
Detected (at least one result)	657	35.0% (100%)
One analyte result	629	33.5% (95.7%)
Two analyte results[a]	28	1.5% (4.3%)

[a]Data for discernible co-detections only. The *Plasmodium* spp. assay is not considered a unique Global Fever Panel detection when co-detected with a Plasmodium species-level assay. Specimens containing multiple species within a genus are not always discernible (e.g., a specimen containing *P. malariae* and *P. falciparum* would not produce a discernible co-detection.)

Table 25. Expected Values Summary (Analytes) as Determined in the Global Fever Panel Clinical Study

FilmArray Result	Number Detected	% of Positives
Chikungunya virus	27	4.1%
Dengue virus (serotypes 1, 2, 3, and 4)	266	40.5%
Leptospira spp.	19	2.9%
Plasmodium spp.	351	53.4%
Plasmodium falciparum	233	35.5%
Plasmodium vivax/ovale	115	17.5%

The most prevalent analyte result was *Plasmodium* spp. (351/657; 53.4%), of which, 233/351 (66.4%) also had *Plasmodium falciparum* identified, 115/351 (32.8%) also had *Plasmodium vivax/ovale* identified, 25/351 (7.1%) had no species-level identification, and 22/351 (6.3%) had a combination of *P. falciparum* and *P. vivax/ovale*. The second most prevalent analyte was dengue virus (266/657, 40.5%). Chikungunya virus was detected in 4.1% (27/657) of specimens and *Leptospira* spp. was detected in 2.9% (19/657).

M. Instrument Name:

Not applicable. The device does not utilize an instrument for result generation.

N. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ___X___ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes ___X_____ or No ___ _____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X_____ or No _____

   The device does not contain any software or instrument components.

3. Specimen Identification:

   The Sample ID can be entered manually or scanned in by using the FilmArray barcode scanner

4. Specimen Sampling and Handling:

   Not applicable.

5. Calibration:

   Not applicable.

6. Quality Control:

   See Quality Control Section above (L.1.c "Traceability, Stability, Expected Values (controls, calibrators, or methods)")

O. Other Supportive Instrument Performance Characteristics Data Not Covered in the "Performance Characteristics" Section above:

None.

P. Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

Q. Identified Risks to Health and Identified Mitigations

Identified Risks to Health	Mitigation Measures
Risk of an inaccurate test result (false positive or false negative result) leading to improper patient management	Certain labeling information, including certain limiting statements and performance information. Certain design verification and validation, including certain analytical studies and clinical studies. Use of certain specimen collection devices.
Misinterpretation of test results leading to misdiagnosis and associated risk of false test results	Certain labeling information, including certain limiting statements and performance information. Certain design verification and validation, including certain analytical studies and clinical studies.
Failure to correctly operate the device leading to inaccurate test results	Certain labeling information, including certain limiting statements and performance information. Certain design verification and validation, including certain analytical studies and clinical studies. Use of certain specimen collection devices.

R. Benefit/Risk Analysis:

Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

Summary of the Assessment of Benefit

Fever is a host response to infection that is not pathognomonic of a specific disease. The benefit of the assay is aiding the accurate diagnosis of infections that cause acute febrile illness in the specific population of patients who have been potentially exposed to pathogens detected by the Global Fever Panel. When guided by appropriate risk factors and epidemiological information, the Global Fever Panel result can be helpful to identify

the specific cause of fever and initiate appropriate treatment, including, but not limited to, antimicrobial therapy. Earlier identification of fever causing pathogens and an appropriate course of treatment may improve patient outcomes. Additionally, the Global Fever Panel fills an unmet need for in vitro diagnostics as no currently approved or cleared tests exist for many of the pathogens on the Global Fever Panel.

While the performance of the device in the clinical and analytical studies suggests that patients will benefit from the assay, expected and acceptable sources of uncertainty are the wide confidence intervals around point estimates of performance. The special controls, including the interpretation of results and the limiting statements in device labeling will help to ensure that errors will be uncommon and will facilitate accurate assay implementation and interpretation of results.

Summary of the Assessment of Risk

The risks associated with the device, when used as intended, are those related to the risk of inaccurate test results, the failure to correctly interpret test results, and failure to correctly operate the device.

The risk of a false positive test result is improper patient management, including potentially inappropriate administration of unnecessary antibiotics or anti-malarial medications. Inappropriate administration of prolonged courses of antibiotics is associated with toxicity, allergic reactions, and other adverse outcomes, including secondary infections such as *C. difficile* colitis. Inappropriate administration of anti-malarial medications may also have adverse effects that depend upon the drug administered. No specific treatments exist for dengue and chikungunya virus infections, although a vaccine was recently developed for dengue virus. This vaccine is only recommended in individuals who had previously had confirmed dengue infection and individuals who have not been infected are at increased risk of severe dengue if they are infected after being vaccinated. In the broader population, individual false positive results could lead to increased burden on the CDC to perform confirmatory testing since all targets detected by the Global Fever Panel are nationally notifiable.

During the analytical evaluation of exclusivity, some pathogen cross-reactivity was identified that could pose risks of patient harm. Specifically, *P. knowlesi* and *P. malariae* may cross-react with the *P. vivax/ovale* assay on the Global Fever Panel. *P. vivax/ovale* infections require a specific course of treatment to clear hypnozoite liver stage of infection and prevent recrudescence. A false positive *P. vivax/ovale* result could therefore lead to patients receiving unnecessary and potentially toxic treatment.

The risk of a false negative test result is delayed identification of the cause of the disease in the patient, which could lead to improper patient management, including administration of unnecessary treatment and/or discontinuation of appropriate treatment. An undiagnosed infection or delayed diagnosis, particularly with *P. falciparum* or *Leptospira* can result in increased morbidity and mortality.

Failure to correctly operate the device can lead to false test results. Failure to correctly interpret test results can lead to treatment of a clinically positive patient in the same manner as a false negative test result and a clinically negative patient in the same manner as a false positive test result, with the corresponding implications discussed above.

Summary of the Assessment of Benefit-Risk

General controls are insufficient to mitigate the risks associated with the device. However, the probable clinical benefits outweigh the probable risks for the proposed assay, considering the mitigations of the risks provided for in the listed special controls established for this device, as well as general controls. The required special controls will help ensure that errors will be uncommon and will facilitate accurate assay implementation and interpretation of results.

The risk of inaccurate test results (both positive and negative) is mitigated by the intended use clearly stating that the assay results are intended to be used with other clinical, epidemiologic, and laboratory data. The risk of false results is also mitigated by the inclusion of performance characteristics from analytical and clinical studies in the labeling. Risks from cross-reactivity are mitigated by appropriate limitations in the labeling that indicate potential cross-reactivity of these malaria strains and further state that a *P. vivax/ovale* result should be further confirmed. Additionally, a separate limitation states that all *Plasmodium* spp. detected results from patients exposed in Southeast Asia should be further investigated for possible *P. knowlesi* infection.

Risks of failure to correctly interpret the test results are mitigated through the inclusion in the labeling of a detailed description of what the device detects, the specimen type for which testing is indicated, the type of results provided to the user in the intended use statement, as well as a detailed explanation of the interpretation of results. Finally, the risk of failure to correctly operate the device is mitigated by the inclusion of detailed directions for use in the package insert, such that the operator can successfully use the instrument.

The clinical performance observed in the clinical trial suggests that errors will be uncommon and that the assay will provide substantial benefits to patients in the diagnosis of acute febrile illness and when used in conjunction with other clinical and diagnostic findings.

Given the combination of the device's indications for use, labeling, the required general controls, and the special controls established for this device, the probable benefits would outweigh the probable risks.

S. Conclusion

The De Novo request is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code: QMV

Device Type: Device to detect and identify selected microbial agents that cause acute febrile illness
Class: II
Regulation: 21 CFR 866.3966