Analytical Performance of the BioFire® FilmArray® Global Fever Panel

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ABSTRACT

A large number of pathogens that include bacteria, viruses, and parasites can cause Acute Febrile Illness (AFI). BioFire Defense is developing the Global Fever (GF) Panel to be used on the FilmArray® System in collaboration with the U.S. Department of Defense[1] and NIAID[2]. The FilmArray is an in vitro diagnostic test platform that combines nucleic acid purification and nested multiplex PCR for the simultaneous identification of many infectious agents in under an hour using a closed, sample-to-answer system. The FilmArray GF Panel detects and identifies nucleic acid from chikungunya virus, CCHF virus, dengue virus (serotypes 1-4), Ebolavirus, Lassa virus, Marburgvirus, West Nile virus, Yellow fever virus, Zika virus, *Bacillus anthracis*, *Francisella tularensis*, *Leptospira* spp., *Salmonella enterica* serovar Typhi and Paratyphi A, *Yersinia pestis*, *Leishmania* spp., and *Plasmodium* spp. in venous blood specimens from individuals with signs and/or symptoms of AFI or recent AFI and with known or suspected exposure to target pathogens. The LoD studies demonstrate that the GF Panel is a sensitive system that can accurately detect multiple pathogens, including Category A biothreat pathogens, and is appropriate to use in testing samples that may contain multi-analytes. Analytical specificity (exclusivity) testing demonstrates that the GF Panel is highly specific for the pathogens it is designed to detect in blood specimens. Assessment of the analytical reactivity and efficacy (inclusivity) show that the assays possess a high level of reactivity for their intended targets. A multiplex FilmArray panel could aid in rapid and actionable AFI diagnosis.

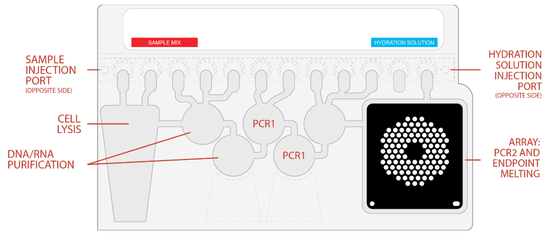
1. MCS-JPEO and USAMMDA Contract No. W911QY-13-D-0080, under the NGDS program.
2. NIAID Contract No. HHSN272201600002C, "Advanced Development of Multiplex Diagnostic Platforms for Infectious Diseases (Global Fever Panel)".

INTRODUCTION

The FilmArray Global Fever (GF) Panel is currently under development as a qualitative, multiplexed, nucleic acid-based test intended for use with the FilmArray 2.0 system. The FilmArray GF Panel detects and identifies bacterial, viral, and protozoan nucleic acids directly from human whole blood (EDTA) collected from individuals with signs and/or symptoms of acute febrile illness or recent acute febrile illness and with known or suspected exposure to target pathogens. The following organisms are identified using the FilmArray GF Panel: *Bacillus anthracis*, *Francisella tularensis*, *Leptospira* spp., *Salmonella enterica* serovar Paratyphi, *Salmonella enterica* serovar Typhi, *Yersinia pestis*, Chikungunya virus, Crimean-Congo hemorrhagic fever virus, Dengue virus, Ebola virus, Lassa virus, Marburg virus, West Nile virus, Yellow fever virus, Zika virus, *Leishmania* spp., and *Plasmodium* spp. (including species differentiation of *Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium ovale*).

Figure 1. FilmArray Global Fever Pouch

The FilmArray GF 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 clinical whole blood specimen. After sample collection, the user injects hydration solution on one side of the pouch and sample combined with sample buffer into the other side of the pouch, places the pouch into a FilmArray instrument, and starts a run. Loading the pouch takes about 2 minutes, and the entire run process takes about an hour.



LIMIT OF DETECTION

The purpose of this study is to determine the Limit of Detection (LoD) for the FilmArray GF Panel using a collection of representative organisms covering each test result. LoD_{95} is defined as the lowest concentration of organism that can be consistently detected by the panel; analyte is detected in at least 19/20 samples (≥ 95% detected). Confirmation of the LoD is performed by testing 20 individual samples at the estimated LoD concentration.

TABLE 1. LIMIT OF DETECTION VALUES

Organism		Strain / Source ID	Specimen	Limit of Detection (Copies/mL)
BACTERIA				
Bacillus anthracis		Ames 35	Live	**6.4E+01**
		Ames (UCC BACI008)[2]	Live	**6.4E+01**
Francisella tularensis		Schu4	Inactivated	**1.2E+03**
		Schu4 (UCC FRAN016)[2]	Live	**1.2E+01**
***Leptospira* spp.**		*Interrogans icterohaemorrhagiae*	Live	**3.9E+02**
Salmonella enterica		Typhi	Live	**1.2E+01**
		Paratyphi	Live	**6.0E+01**
Yersinia pestis		A1122	Live	**1.5E+02**
		CO92 (UCC YERS023)[2]	Live	**1.5E+02**
VIRUSES				
Chikungunya virus		R80422	Inactivated	**5.5E+02**
		B8635 (UCC Alpha031)[2]	Live	**5.5E+02**
		Indo23574 (UCC Alpha008)[2]	Live	**5.5E+02**
CCHF Virus		IbAr10200	Inactivated	**6.4E+00**
		IbAr10200 (UCC Nairo001)[2]	Live	**6.4E+02**
Dengue virus	DENV-1	Hawaii	Live	**2.7E+02**
	DENV-2-1	New Guinea C	Live	**3.6E+02**
	DENV-2-2	Dak AR A1247	Live	**3.6E+03**
	DENV-3	H87	Live	**1.6E+02**
	DENV-4	H241	Live	**7.6E+01**
Ebolavirus	Bundibugyo	811250 (BEI)	Inactivated	**7.0E+04**
		811250 (UCC Ebola005)[2]	Live	**7.0E+02**
	Tai Forest	Ivory Coast (BEI)	Inactivated	**8.3E+03**
		Ivory Coast (UCC Ebola004)[2]	Live	**1.8E+02**
	Reston	119810 RIID (BEI)	Inactivated	**2.7E+04**
		H-28(UCC Ebola003)[2]	Live	**2.7E+03**
	Sudan	Boniface (BEI)	Inactivated	**1.1E+04**
		Boniface(UCC Ebola002)[2]	Live	**1.1E+02**
	Zaire	Guéckédou/Guinea C07 (BEI)	Inactivated	**1.1E+02**
		Makona(UCC Ebola027)[2]	Live	**1.1E+03**
Lassa Virus		Josiah (BEI)	Inactivated	**5.6E+04**
		Josiah (UCC Arena002)[2]	Live	**5.6E+03**
Marburgvirus		Musoke (BEI)	Inactivated	**5.0E+02**
		Ci67 (UCC Marbrg003)[2]	Live	**5.0E+02**
		Ravn (BEI)	Inactivated	**2.6E+02**
		Ravn (UCC Marbrg002)[2]	Live	**2.6E+02**
West Nile Virus		NY 2001-6263 (BEI)	Inactivated	**1.6E+03**
		Bz NY99 (UCC Flavi022)[2]	Live	**1.6E+02**
		B-956 Uganda (BEI)	Inactivated	**3.2E+04**
Yellow Fever virus		17D (BEI)	Live attenuated	**1.2E+02**
		Asibi (UCC Flavi005)[2]	Live	**1.2E+01**
Zika virus		PRVABC59 (BEI)	Live	**1.3E+02**
PROTOZOA				
Leishmania donovani		*donovani* (BEI)	Live	**1.0E+01**
***Plasmodium* spp.**[1]	*falciparum*	IPC 4884 (BEI)	Live	**1.0E+02**
	knowlesi,	Strain H (BEI)	gDNA	**2.2E+01**
	malariae (clinical sample)	DLS17-026015	Live	**1.9E+02**
	vivax	Chesson (BEI)	Live	**7.7E+01**
	ovale (clinical sample)	N8K9QK19	Live	**2.4E+02**
***Plasmodium*[1]**	*falciparum*	IPC 4884 (BEI)	Live	**1.0E+02**
	vivax	Chesson (BEI)	Live	**7.7E+01**
	ovale (clinical sample)	N8K9QK19	Live	**2.4E+02**

1. *Plasmodium* spp. refers to the pan assay whereas *Plasmodium* refers to the specific organism assays.
2. UCC – DoD Unified Culture Collection (USAMRIID)

EXCLUSIVITY

To determine whether the FilmArray GF Panel assays cross-react with sequences from various microorganisms/viruses that may be present in clinical specimens, the analytical specificity of the panel was assessed by *in silico* analysis and by testing a broad spectrum of organisms/viruses at high concentrations. Typical stock concentrations for on-panel analytes tested are: 10^7-10^{10} copies/mL for bacteria, 10^7-10^9 copies/mL for virus, and 10^6-10^8 copies/mL for protists. Both on-panel and off-panel organisms were evaluated to test inter-assay specificity and overall assay/panel specificity, respectively.

- On-panel testing consists of contrived samples spiked into sterile saline with the highest concentration of on-panel analytes that is possible based on the concentration of the organism stock (up to 10% of the total sample volume). On-panel isolates are the same as those evaluated for the LoD study (See Table 1).
- Off-panel organisms are selected based on 1) phylogenetic and/or genetic similarity to the panel analytes and assays, and 2) the possibility that the organism(s) could be present as normal flora, contaminants associated with sample collection, or pathogens in whole blood. (Organisms in red to be performed at The Center)

List of Off-Panel Organisms Tested

BACTERIA

Acinetobacter baumannii
Bacillus brevis (Migula)
Bacillus (12 strains)
Bacteroides fragilis
Bordetella bronchiseptica
Borrelia burgdorferi
Brucella melitensis
Burkholderia (3 strains)
Chlamydophila pneumoniae
Chlamydophila psittaci TBD
Clostridium (4 strains)
Coxiella burnetii
Enterobacter aerogenes
Enterococcus faecalis
Enterococcus faecium
Francisella (4 strains)
hispaniensis
Klebsiella oxytoca
Legionella pneumophila
Leptospira (6 strains)
Listeria monocytogenes
Mycobacterium tuberculosis
Mycoplasma pneumoniae
Neisseria meningitidis
Orientia chuto TBD
Proteus mirabilis
Pseudomonas aeruginosa
Rickettsia prowazekii TBD
Rickettsia ricketsii TBD
Rickettsia typhi TBD
Salmonella bongori
Salmonella enterica (18 Strains)
serovar **Dublin (Paratyphi)**
serovar **Manchester (Typhi)**
Serratia marcescens
Staphylococcus aureus
Streptococcus (3 strains)
Treponema pallidum pallidum
Vibrio cholerae
Yersinia (11 Strains)

VIRUSES

Adenovirus 1, 3, 5
Aura virus
Avalon virus (in silico)
Barmah Forest virus
Bas-Congo (in silico)
Bunyamwera virus
Coronavirus
Cytomegalovirus
Dugbe virus TBD
Eastern equine encephalitis virus
Enterovirus, HEV-71
Epstein Barr virus
Flexal virus
Guanarito virus
Hantaan virus
Hazara virus
Hendra Virus
Hepatitis A, B, C virus
Herpes Simplex Virus Type 2
HPIV-1, 3
Hughes virus
Human Herpesvirus 6B
Human Immunodeficiency Virus Type 1, 2
Human T-Lymphotropic Virus Type 1, 2
Influenza A H1N1-2009
Influenza A H3N2
Influenza B virus
Japanese encephalitis virus
Junin virus
Lymphocytic choriomeningitis virus
Machupo virus
Mayaro virus
Measles virus
Metapneumovirus
Middelburg virus TBD
Mopeia virus TBD
Mumps virus
Murray Valley encephalitis virus TBD
Omsk hemorrhagic fever
O'Nyong Nyong virus (CHIKV)
Parvovirus
Pirital virus (In Silico)
Powassan virus TBD
Rabies virus
Rift Valley Fever Virus
Ross River virus
Human respiratory syncytial virus
Rubella virus
Saint Louis encephalitis virus
Sabia virus (in silico)
Semliki Forest virus TBD
Sindbis virus
Spondweni virus
Tickborne encephalitis virus
Tonate virus TBD
Una Virus
Usutu virus
Vaccinia Virus
Varicella Zoster virus
Variola majora (In Silico)
Venezuelan Equine Encephalomyelitis virus
Western Equine Encephalomyelitis virus

FUNGI AND PROTISTS

Babesia microti
Crithidia fasciculata (Leish)
Cyclospora cayetanensis
Leptomonas seymouri (Leish)
***P. berghei* (plas vivax/ovale)**
***P. brasilianum* (plas vivax/ovale)**
***P. cynomolgi* (plas vivax/ovale)**
***P. fieldi* (plas vivax/ovale)**
***P. fragile* (plas vivax/ovale)**
***P. inui* (plas vivax/ovale)**
***P. simiovale* (plas vivax/ovale)**
Schistosoma mansoni
Toxoplasma gondii
Trypanosoma (3 strains)
Aspergillus fumigatus
Cryptococcus neoformans var. *grubii*

INCLUSIVITY

To ensure the FilmArray GF Panel is inclusive for the genetic variation expected for each analyte, the analytical reactivity of the assays was evaluated by testing multiple isolates per analyte. Isolates are selected based on the availability of live and inactivated stocks, genetic, temporal and geographic diversity, and clinical relevance of the various species, strains, subspecies, serotypes, genotypes and genetic variants available for testing. In addition, *in silico* data (sequence searches and alignments to assay primers) are also used to support the inclusivity of the FilmArray GF Panel assays.

Samples are prepared by spiking analytes into whole blood from healthy donors (obtained from Bioreclamation IVT repository) at a concentration near 3× LoD.

TABLE 2. INCLUSIVITY

FilmArray Global Fever Panel Analyte		# Isolates Detected/ Isolates Tested
BACTERIA		
Bacillus anthracis		4/4
Francisella tularensis		5/5
Leptospira spp.		16/16
Salmonella enterica serovar Paratyphi A		**3/4**
Salmonella enterica serovar Typhi		5/5
VIRUSES		
Chikungunya virus		3/3
Dengue virus	Denv1	7/7
	Denv2	**6/7**
	Denv3	5/5
	Denv4	7/7
Ebolavirus		Zaire 2/2
Marburgvirus		3/3
West Nile virus		3/3
		2/2
Zika virus		7/7
PROTOZOA		
Leishmania spp.		9/9
Plasmodium spp.		17/17

REPRODUCIBILITY

This study evaluated the reproducibility of the FilmArray GF Panel test results. The study was designed to evaluate the potential for run-to-run or day-to-day variation in panel performance, as well as potential variation linked to test location (site), reagent lot, operator, or instrument for samples containing analytes at and near their LoD.

Three contrived whole blood samples were spiked with different mixtures of six representative panel analytes, two bacteria (***Leptospira interrogans*** and ***Salmonella enterica enterica*** serovar Typhi), two viruses (dengue virus and Zika virus), and two protozoa (*Leishmania donovani* and *Plasmodium falciparum*). One sample was spiked at a Moderate Positive (3× LoD) level, and another sample at a Low Positive (1× LoD) level; the third sample was not spiked. The three samples were aliquoted and distributed between three test locations. Six replicates of each sample were tested at each location on five different days, with two operators and two instruments, providing a total of 90 replicate test results per sample. In total, valid results were obtained from testing 270 sample aliquots to demonstrate the reproducibility of the FilmArray GF Panel.

The overall test results for six analytes showed a 97.5% agreement of the observed test results with the expected Detected or Not Detected results (1843/1890), with a 95% confidence interval of 96.7-98.1% (Table 3).

Table 3. Results for Reproducibility testing on the Global Fever Panel

Moderate Positive (3X)	626/630 (99.4%)	**Overall Agreement** (All analytes, all levels)	**1843/1890 97.5%** [96.7-98.1%]
Low Positive (1X)	587/630 (93.2%)		
Negative	630/630 (100%)		

INTERFERING SUBSTANCES

This study was designed to evaluate the potential for certain substances to interfere with the performance and accuracy of FilmArray GF Panel test results. These substances included; (i) endogenous substances, (ii) exogenous substances, (iii) microorganisms such as viral and bacterial species, and (iv) technique specific substances that may be introduced into a sample (intentionally or not) during routine laboratory handling. Interference was not detected under any conditions tested (Table 4).

Table 4. Results for Potentially Interfering Substances Tested on the Global Fever Panel.

Potentially Interfering Substances						
EXOGENOUS SUBSTANCES (EX)			**ENDOGENOUS SUBSTANCES (EN)**	**COMPETITIVE MICROORGANISMS (MO)**	**BLOOD COLLECTION TUBES (BT)**	**TECHNIQUE SPECIFIC SUBSTANCES (TS)**
Artemether Lumefantrine	Clindamycin	Oseltamivir	Albumin	Corynebacterium diphtheriae	Citrate (sodium)	Bleach
Acetaminophen	Cortisol	Pentamidine	Bilirubin (Conjugated)	Cytomegalovirus (CMV) AD-169	Acid-citrate-dextrose (ACD)	Acetone
Amoxicillin	Cycloserine	Prednisolone	Bilirubin (Unconjugated)	Epstein-Barr virus	EDTA in excess (5x)	Chloroform
Amphotericin B	Doxycycline	Prednisone	Cholesterol (total)	Escherichia coli	Heparin	DMSO
Artesunate	Fluconazole	Proguanil	Glucose	Haemophilus influenzae	Sodium polyanethe-nole sulfonate (SPS)	Ethanol
Aspirin (Acetylsalicylic Acid)	Gentamicin	Ribavirin	Hemoglobin	Herpes Simplex virus		Methanol
Atovaquone	Ibuprofen	Sulfamethoxazole	Immunoglobulins	Human Immunodefi-ciency virus (HIV)		Povidone-iodine
Azithromycin	Isoniazid	Tenofovir	Triglycerides	Klebsiella pneumoniae		Saline
Ceftriaxone	Mefloquine	Vancomycin	White Blood Cells	Plasmodium vivax		TRIzol
Ciprofloxacin	Meropenem			Staphylococcus epidermidis		

SAMPLE STORAGE AND TRANSPORT

The purpose of this study was to validate that accurate FilmArray GF Panel test results can be obtained when testing human whole blood (WB) specimens that have been stored at ambient room temperature for one day, at 4°C for up to seven days, or in an ultra-low temperature freezer. The aim of the study was not to establish the limits of analyte stability and sample storage, but to confirm that analytes are stable and detected by the panel when stored under the selected conditions.

Testing was conducted using samples composed of human WB, each contrived with three representative GF Panel analytes at a concentration equal to 3× their LoD. To evaluate the impact of storage, 10 replicates were tested at each storage condition.

All analytes were Detected in ≥9/10 replicates at each storage condition. The robust detection of representative mixes observed in this study supports the conclusion that accurate FilmArray GF Panel test results can be obtained from human WB specimens stored in the conditions outlined.

SUMMARY

The development of a multiplex FilmArray panel would aid in rapid and actionable AFI diagnosis. The FilmArray GF Panel provides a broad spectrum analysis of target pathogens in a sensitive and specific manner.

- LoD values show sensitivity levels at or below clinically relevant concentrations.
- On- and off-panel exclusivity show very limited evidence of cross-reactivity.
- Inclusivity maintains expected coverage of relevant target pathogens.
- Reproducibility study ensures consistent test results.
- No interference from substances tested show panel robustness.
- The GF Panel provides accurate results for Whole Blood samples stored under several standard conditions.

Data presented are from assays that are Investigational Use Only (IUO) and have not been cleared or approved for diagnostic use.

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