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Analytical Performance of the FilmArray® Global Fever Panel

ABSTRACT

Acute Febrile Illness (AFI) can be caused by a large number of pathogens that include bacteria, viruses and parasites. BioFire Defense is developing the Global Fever (GF) Panel to be used on the FilmArray System in collaboration with the Department of Defense^a and NIAID^b. 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-toanswer 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 donovani complex, 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. Estimated LoD studies demonstrate clinically relevant detection levels and exclusivity testing shows high specificity. For example, estimated LoD levels for the following organisms: dengue virus New Guinea C at 360 copies/mL, Marburgvirus Musoke at 50 copies/mL, Zika virus at 130 copies/mL, Leishmania donovani at 10 copies/mL, Plasmodium at 10 copies/mL, Bacillus anthracis at 64 copies/mL, and Yersinia pestis at 15 copies/mL.° Preliminary off-panel exclusivity studies assessing specificity with closely related organisms or organisms that may be found in whole blood show no significant cross-reactivity. A multiplex FilmArray panel

a. MCS-JPEO and USAMMDA Contract No. W911QY-13-D-0080, under the NGDS program.

could aid in rapid and actionable AFI diagnosis.

- b. NIAID Contract No. HHSN272201600002C, "Advanced Development of Multiplex Diagnostic Platforms for Infectious Diseases (Global Fever Panel)".
- c. Estimated LoD levels updated to reflect the most recent

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).

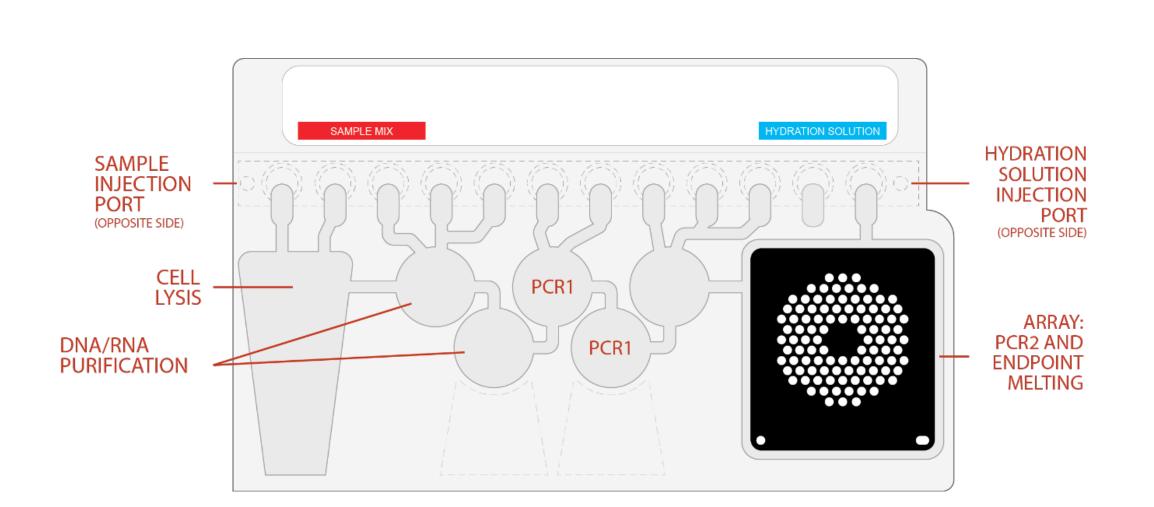


Figure 1. FilmArray Global Fever Pouch

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.

During a run, the FilmArray system:

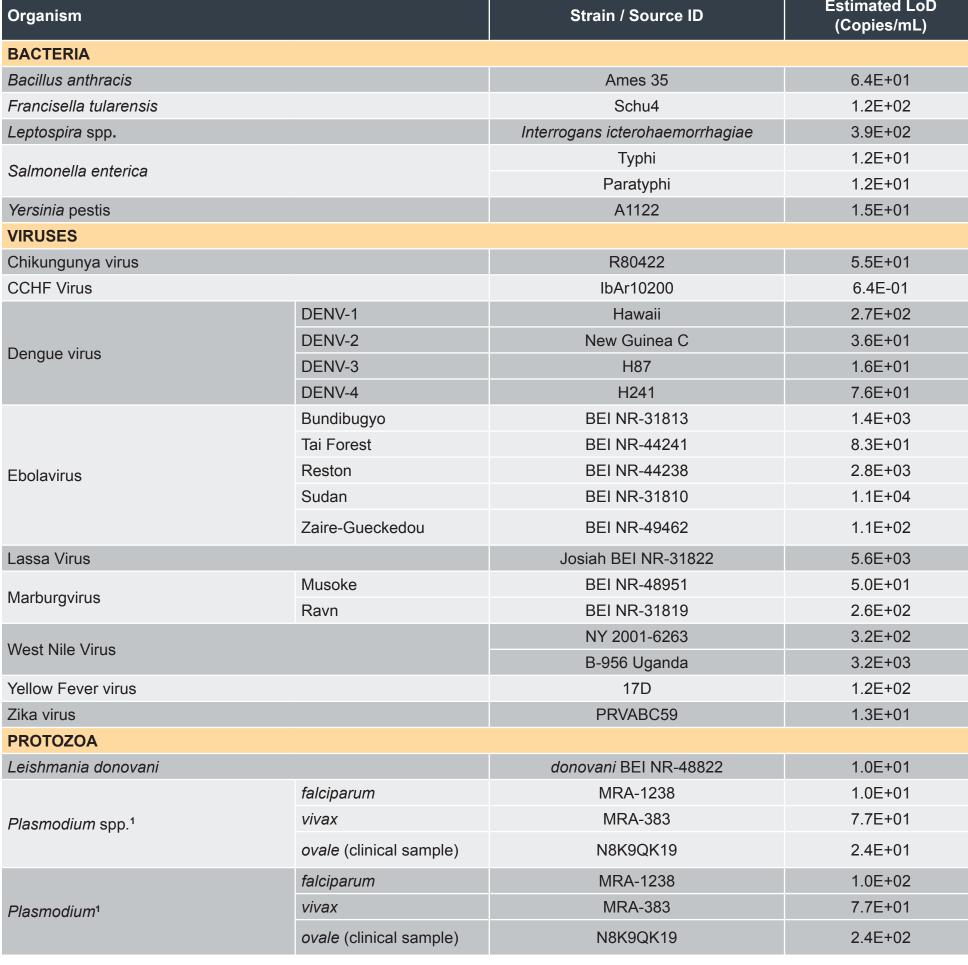
- Lyses the sample by agitation (bead beating).
- Extracts and purifies all nucleic acids from the sample using magnetic bead technology.
- Performs nested multiplex PCR by:
- First performing a single, large volume, highly multiplexed first-stage PCR reaction
- Then performing multiple, singleplex second-stage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

ESTIMATED LIMIT OF DETECTION

The purpose of this study is to determine the estimated Limit of Detection (LoD) for the FilmArray GF Panel using a collection of representative organisms covering each test result. LoD_{o5} 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). An initial Estimated LoD (Table 1) is established using serial 10-fold dilutions. The estimated LoD is the lowest concentration at which 3/3 replicates returned a detected result. Samples are prepared in whole blood obtained from a repository, Bioreclamation IVT.

The estimated LoD is determined for the FilmArray GF Panel test results using 'primary' analytes.

TABLE 1. ESTIMATED LIMIT OF DETECTION VALUES

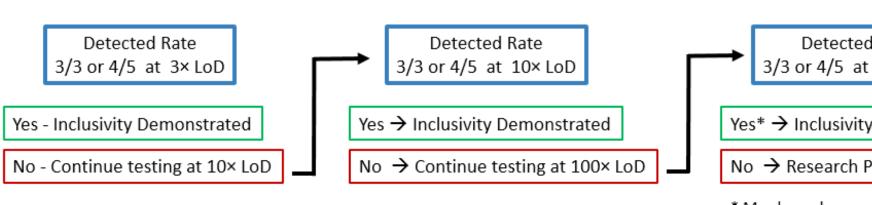


¹Plasmodium spp. refers to the pan assay whereas Plasmodium refers to the specific organism assays.

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. Here we present a subset of inclusive organisms tested.

Samples are prepared by spiking analytes into whole blood from healthy donors (obtained from Bioreclamation IVT repository) at a concentration near 3× estimated LoD. Figure 2 depicts the protocol followed to establish inclusivity of the isolates tested. An initial subset of analytes tested on the GF Panel are shown in Table 4.



Detected Rate 3/3 or 4/5 at 100× LoD Yes* → Inclusivity Demonstrated No → Research Potential Cause(s)

investigation may be needed.

TABLE 4. INCLUSIVITY

FilmArray Global Fever Panel Analyte	# Isolates Detected/Tested	Tested Isolate	Concentration Detected (copies/mL)	Fold Est. LoD	Test Result (Detected/ Tes
BACTERIA		Ames	6.4E+01	1	3/3
		Ames35 (genomic)	0.4⊑+01	3	3/3
Bacillus anthracis	4/4	Sterne (-pXO2)	9.0E+01	3	3/3
		UM23-1 (genomic)	9.001	3	3/3
ancisella tularensis	1/1	Schu4	1.2E+02	1	3/3
riancisena tularensis	1/1	interrogans (icterohaemorrhagiae)	3.9E+02	1	3/3
		kirschneri (bogvere) noguchi (LPT1E) borgpetersenii (LPT1D)			
eptospira spp.	8/8	kmetyi Bejo-Iso9T weilii 6712	1.2E+03	3	3/3
		weilii A102 weilii Topaz			
Salmonella enterica serovar Paratyphi A	2/2	serovar Paratyphi-A Paratyphi A (ATCC-12176)	1.2E+01 3.6E+01	1 3	3/3 3/3
31		Subsp. Enterica Ty2	1.2E+01	1	3/3
almonella enterica se- ovar Typhi	4/4	ATCC 19430 ATCC 19937	3.6E+01	3	5/5 3/3
(avainia naatia	4/4	ATCC 33458	4.55.04	1	3/3
ersinia pestis	1/1	A1122	1.5E+01	'	3/3
Chikungunya virus	1/1	R80422	5.5E+02	1	3/3
CCHF	1/1	IbAr10200	6.4E+00	1	3/3
		Hawaii (DENV-1)	2.7E+01	1	3/3
	6/6	VN/BID-V1792/2007 276RK1	8.1E+01	3	3/3 3/3
	G/O	strain 12150 strain BC89/94	2.7E+03	100	3/3 3/3
		228690 New guinea C (DENV-2)	3.6E+01	1	3/3 3/3
		VN/BID-V1002/2006 (2-1)		3	3/3
	5/5	DakArA1247 (2-1)	1.1E+02	3	3/3
Dengue virus		BC102/94 (2-1)		3	3/3 3/3
		strain 429557 (2-2) H87 (DENV-3)	1.6E+03	1	3/3
	3/3	VN/BID-V1329/2006			3/3
		strain C0360/94	4.8E+03	3	3/3
		H241 (DENV-4)	7.6E-01	1	3/3
		BC258/97	2.3E+00	3	4/5
	5/5	strain 703	7.6E+00	10	3/3
		BC13/97	7.6E+01	100	3/3
		BC287/97 Bundibugyo	1.4E+04	1	3/3 3/3
	1/1 each species	Tai Forest	8.3E+01	1	3/3
		Reston	2.8E+03	1	3/3
Ebolavirus		Sudan	1.1E+04	1	3/3
	2/2	Zaire, Mayinga	1.1E+03	1	3/3
		Zaire, Makona-Guéckédou	3.3E+03	3	3/3
assa virus	1/1	Josiah	5.6E+03	1	3/3
lorburoviruo	3/3	RAVN Ci67	2.6E+01 5.0E+01	1	3/3 3/3
larburgvirus	3/3	Musoke	1.5E+02	3	3/3
	1/1	B-956 Uganda (WNV2)	3.2E+03	1	3/3
Vest Nile virus		NY2001 (WNV 1)			3/3
	2/2	1986 (WNV1)	9.6E+02	0.1	3/3
ellow fever virus	1/1	strain 17D	1.2E+02	1	3/3
		PRVABC59 Ibh 30656	1.3E+01	1	3/3
		MR 766			
Zika virus	7/7	H/PAN/2016/BEI-259634	3.9E+01	3	3/3
		FLR PLCal			
DD070704		R103451			
PROTOZOA		donovani	1.1E+01	1	3/3
		donovani, 1S	3.3E+01	3	3/3
<i>Leishmania</i> spp.	4/4	braziliensis Vianna	1:2.6*10 ^{5a}	N/A	3/3
		infantum Nicolle	1.1E+02	10	3/3
		falciparum, Pursat	1.0E+01 (spp.)	1×	3/3 ^d
		vivax, 11 Strain Chesson	1.0E+02 (falc.) 7.7E+01	1×	3/3°
		ovale, (CDC N8K9QKI9)	2.4E+01 (spp.) 2.4E+02 (ovale)	1×	3/3°
		brasillianum cynomolgi	2.05.04	24	3/3 ^b 3/3 ^c
		fieldi	3.0E+01	3×	2/3°
		Inui			3/3 ^b
		berghei			3/3 ^b
Plasmodium spp	18/18	fragile			3/3 ^c
Plasmodium spp.	18/18				3/3 ^b 2/3 ^b
Plasmodium spp.	18/18	knowlesi	0.00001×a	3×	
Plasmodium spp.	18/18		0.00001×a	3×	3/3°
Plasmodium spp.	18/18	knowlesi malariae	0.00001×a	3×	
Plasmodium spp.	18/18	knowlesi malariae simiovale	0.00001×a 3.0E+01	3× 3×	3/3°
Plasmodium spp.	18/18	knowlesi malariae simiovale vivax Panama falciparum SenTh021.09 falciparum St. Lucia	3.0E+01 3.0E+02		3/3° 3/3° 3/3 ^d 3/3 ^d
Plasmodium spp.	18/18	knowlesi malariae simiovale vivax Panama falciparum SenTh021.09 falciparum St. Lucia falciparum Tanzania	3.0E+01 3.0E+02 3.0E+02	3× 30× 30×	3/3° 3/3° 3/3 ^d 3/3 ^d
Plasmodium spp.	18/18	knowlesi malariae simiovale vivax Panama falciparum SenTh021.09 falciparum St. Lucia	3.0E+01 3.0E+02	3× 30×	3/3° 3/3° 3/3 ^d 3/3 ^d

EXCLUSIVITY

To determine whether the FilmArray GF Panel assays cross-react with sequences from various microorganisms/viruses that may be present in clinical 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: 107-108 copies/mL for protists. Both on-panel and off-panel organisms were evaluated to test inter-assay specificity and overall assay/panel specificity, respectively. Here we report a subset of off-panel testing.

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

TABLE 2: ON-PANEL EXCLUSIVITY

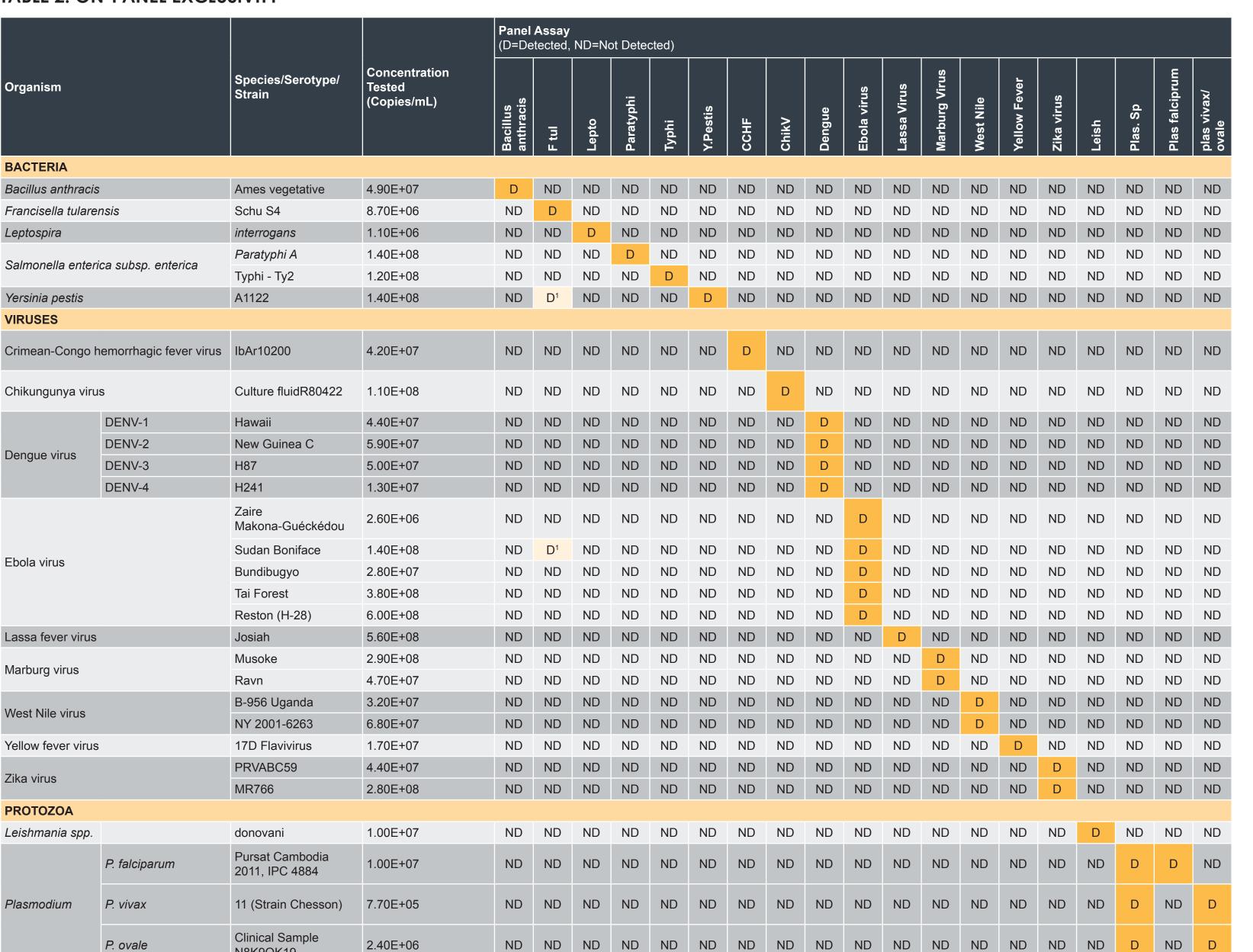


TABLE 3: SUBSET OF OFF-PANEL EXCLUSIVITY ORGANISMS

Organism		Species/Serotype/ Strain	Concentration Tested	Bacillus anthracis	сснғ	ChikV	Dengue	Ebola virus	F tul	Leish	Lepto	ГУ	Marburg Virus	Plas. Sp	Plas. Falciprum	plas vivax/ovale	Paratyphi	Typhi	West Nile	Y. Pestis	Yellow Fever	Zika
BACTERIA																						
Borrelia burgdorfe	ri	B31 Clone 5A1	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Babesia microti		strain GI	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dabesia Microti		Nan-Hs-2011	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Clostridium botulism VPI440		VPI4404	1.5 μg/mL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	meyeri (group III)	serovar Hardjo strain went 5	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	kmetyi (group III)	strain Bejo-Iso9T	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	biflexa (group III)	Patoc 1	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Leptospira	Wolbachii (III)	Serovar Codice starin CDC	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	alstonii (group I)	Sichuan 79601	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	licerasiae (group II)	VAR 010	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella enterica subsp. enterica	Serovar Typhimurium	Outbreak 2004	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		LT2	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		isolate 4	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Serovar Newport	S11975	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		SL317	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		SL254	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
VIRUSES																						
Guanarito virus INH-95551 (Venezuaela prot		INH-95551 (Venezuaela prototype)	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Handra Virus		strain 9409-30-1800 (Australia prototype)	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hepatitis A virus HM 175/18f		HM 175/18f	2.8E+06 TCID50/mL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Influenza A virus A/WS/33 (H1N1)		7.4E+05 TCID50/mL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Machupo virus strain Carvallo		1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Measles virus Edmonston Kamtek		1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Rift Valley fever virus strain ZH501		4.5E+05 Copies/mL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
PROTOZOA																						
	inui	Taiwan I	4.6E+05 Copies/mL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	D	ND	ND	ND	ND	ND	ND	ND	ND
	berghei	Nk 65	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	D	ND	ND	ND	ND	ND	ND	ND	ND
	simiovale	30140	1:10 Dilution	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	D	ND	D	ND	ND	ND	ND	ND	ND
Trypanosoma	brucei	gambiense STIB 386	8.7E+05 Cells/mL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	cruzi	TcVT-1 axenic epimastigote	1.0E+07 Cells/mL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Detected due to suspected contamination of organism stocks

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.

- Estimated LoD values show sensitivity levels at or below clinically relevant concentrations.
- On- and off-panel exclusivity show no evidence of cross-reactivity.
- Preliminary inclusivity maintains expected coverage of relevant target pathogens.