

Clinical Evaluation of the BioFire® FilmArray® Global Fever Special Pathogens Panel

(Currently under FDA review)

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BioFire Defense has developed the BioFire® Global Fever Panel, in collaboration with the U.S. Department of Defense and NIAID (contract Nos. W911QY-13-D-0080 and HHSN272201600002C), as an in vitro diagnostic test for the simultaneous identification of viral, bacterial, and protozoan pathogens directly from whole blood in under an hour using the BioFire® FilmArray® platform. The BioFire Global Fever Panel has been granted De Novo classification by the US FDA for the detection of chikungunya virus, dengue virus (serotypes 1-4), *Leptospira* spp., and *Plasmodium* spp. from individuals with signs and/or symptoms of AFI and known or suspected exposure to target pathogens.

The BioFire Global Fever Special Pathogens Panel is a modified version of the Global Fever Panel, which, in addition to the pathogens described above, contains assays for the detection of 10 additional pathogens: Crimean-Congo hemorrhagic fever virus (CCHFV), *Ebolavirus* spp., Lassa virus, Marburg virus, West Nile virus (WNV), yellow fever virus (YFV), *Bacillus anthracis*, *Francisella tularensis*, *Yersinia pestis*, and *Leishmania* spp.

BioFire Defense performed a prospective clinical evaluation for the Global Fever Special Pathogens Panel at eleven geographically distinct sites across the globe. A total of 2139 prospective whole blood specimens were tested between March 2018 and March 2021. The Global Fever Special Pathogens Panel detected at least one analyte in 34.1% of specimens (730/2139). The most prevalent analyte was *Plasmodium*, followed by dengue virus. Chikungunya virus, *Leptospira* spp., *Leishmania* spp., CCHFV, and WNV were detected in fewer than 5% of specimens. All other analytes were not detected by the Global Fever Special Pathogens Panel. Positive percent agreement between the Global Fever Special Pathogens Panel and comparator testing ranged between 94.0-100%, and the negative percent agreement ranged between 99.9-100%. The results show that the BioFire Global Fever Special Pathogens Panel could aid in rapid and actionable AFI diagnosis caused by multiple, sometimes co-occurring, pathogens.

GLOBAL FEVER SPECIAL PATHOGENS PANEL ANALYTES

Bacteria	Protozoa	Viruses
<i>Bacillus anthracis</i>	<i>Leishmania</i> spp.	Chikungunya virus
<i>Francisella tularensis</i>	<i>Plasmodium</i> spp.	CCHF virus
<i>Leptospira</i> spp.	<i>Plasmodium falciparum</i>	Dengue virus (serotypes 1-4)
<i>Yersinia pestis</i>	<i>Plasmodium vivax/ovale</i>	Ebola virus
		Lassa virus
		Marburg virus
		West Nile virus
		Yellow fever virus

ARCHIVED

Certain analytes on the FilmArray Global Fever Special Pathogens Panel were either not observed or were not encountered in large enough numbers to adequately demonstrate system performance during the clinical evaluation. Archived specimens with known analyte content and/or archived specimens with a high likelihood of containing a given analyte were tested using the Global Fever Special Pathogens Panel to supplement the prospective clinical evaluation data. Wherever possible, archived WB specimens were tested. Where no WB specimens could be obtained blood plasma and blood serum were tested instead (only West Nile virus samples required blood plasma and serum). Although plasma and serum are not the intended specimen type for the Global Fever Special Pathogens Panel, these blood components are expected to provide similar results to WB specimens.

Specimens were coded and either tested at the clinical site or shipped to BioFire Defense for testing on the Global Fever Special Pathogens Panel. Nucleic acid was extracted from each specimen and tested using plate-based PCR comparator methods. The selection of comparator assays used to test specimens from each site was based on suspected and/or potential analyte content.

TABLE 2. BIOFIRE GF SP PANEL ARCHIVED SPECIMEN PERFORMANCE SUMMARY

Pathogen ^a	PPA			NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
Crimean-Congo hemorrhagic fever virus	0/0	-	-	281/281	100%	98.7-100%
Ebola virus	0/0	-	-	279/279	100%	98.6-100%
Lassa virus ^b	10/12	83.3%	55.2-95.3	265/267	99.2%	97.3-99.8%
Marburg virus	0/0	-	-	279/279	100%	98.6-100%
West Nile virus ^{c,d}	59/65	90.8%	81.3-95.7%	345/347	99.4%	97.9-99.8%
Yellow fever virus	0/0	-	-	279/279	100%	98.6-100%
Bacteria						
<i>Bacillus anthracis</i>	0/0	-	-	281/281	100%	98.7-100%
<i>Francisella tularensis</i>	0/0	-	-	281/281	100%	98.7-100%
<i>Yersinia pestis</i>	0/0	-	-	281/281	100%	98.7-100%
Protozoa						
<i>Leishmania</i> spp.	0/0	-	-	283/283	283/283	98.7-100%

a. Results are not shown for assays cleared in DEN200043 (i.e., chikungunya virus, dengue virus, *Leptospira* spp., and *Plasmodium* spp.).

b. Evidence of Lassa virus was found in 1/2 FN specimens and 1/2 FP specimens by additional PCR.

c. Archived specimens included blood serum and blood plasma.

d. Evidence of West Nile virus was found in 6/6 FN specimens: four specimens were positive upon GF SP Panel retesting and three of these were also positive by additional PCR. The other two FN specimens were positive only by additional PCR testing. Evidence of West Nile virus was detected in 1/2 FP specimens by additional PCR testing.

CLINICAL PROSPECTIVE SITES

The clinical performance of the Global Fever Special Pathogens Panel was established during a multi-center study conducted at eleven geographically distinct study sites, including two in the United States. The Global Fever Special Pathogens Panel detected at least one analyte in 730 of the 2139 included specimens (34.1% positivity rate).

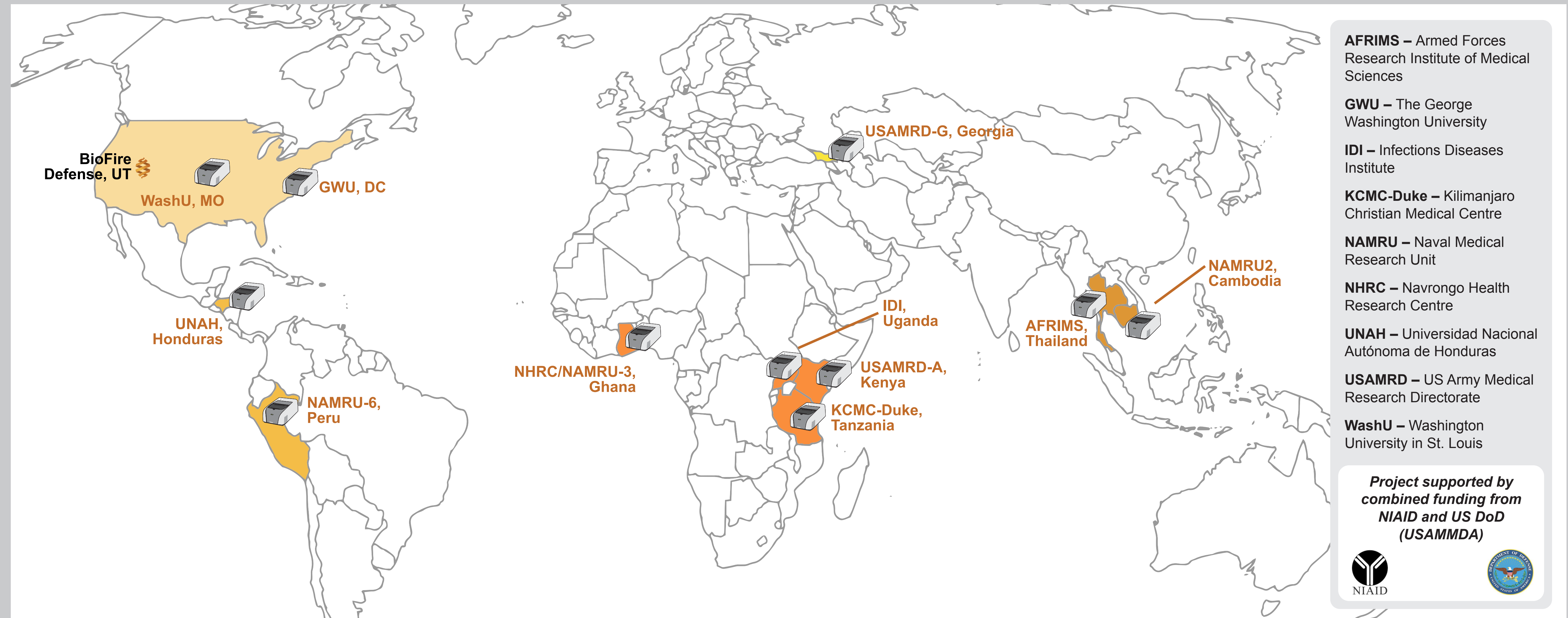


TABLE 1. EXPECTED VALUE (AS DETERMINED BY THE GLOBAL FEVER SPECIAL PATHOGENS PANEL) SUMMARY BY REGION # = NUMBER; EV= EXPECTED VALUE

Analyte	Overall n=2139		USA n=258		Africa n=680		Southeast Asia n=655		Europe n=113		Central & South America n=433	
	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV
Chikungunya virus	27	1.30%	0	0.00%	0	0.00%	27	4.10%	0	0.00%	0	0.00%
Crimean-Congo hemorrhagic fever virus	1	0.00%	0	0.00%	1	0.10%	0	0.00%	0	0.00%	0	0.00%
Dengue virus (serotypes 1, 2, 3, 4)	266	12.40%	0	0.00%	1	0.10%	144	22.00%	0	0.00%	121	27.90%
West Nile virus	1	0.00%	1	0.40%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
<i>Leptospira</i> spp.	19	0.90%	1	0.40%	1	0.10%	8	1.20%	0	0.00%	9	2.10%
<i>Leishmania</i> spp.	10	0.50%	0	0.00%	0	0.00%	0	0.00%	10	8.80%	0	0.00%
<i>Plasmodium</i> spp.	407	19.00%	0	0.00%	311	45.70%	11	1.70%	1	0.90%	84	19.40%
<i>P. falciparum</i>	283	13.20%	0	0.00%	275	40.40%	3	0.50%	0	0.00%	5	1.20%
<i>P. vivax/ovale</i>	118	5.50%	0	0.00%	30	4.40%	8	1.20%	0	0.00%	80	18.50%

CONTRIVED

For analytes for which no archived specimens were available, or for which there were an insufficient number of archived specimens, testing was performed using contrived specimens. Contrived specimens were prepared using residual human WB specimens from patients with signs/symptoms of AFI. For each analyte, fifty (50) or more replicates were contrived using quantified isolates at a range of concentrations relative to the LoD. This study evaluated Global Fever Special Pathogens Panel sensitivity and specificity when testing whole blood specimens contrived with *Bacillus anthracis*, Crimean-Congo hemorrhagic fever virus, *Ebolavirus*, *Francisella tularensis*, Lassa fever virus, *Leishmania* spp., *Marburgvirus*, West Nile virus, yellow fever virus, and *Yersinia pestis*.

TABLE 4. SUMMARY OF FILMARRAY GF SP PANEL CONTRIVED SPECIMEN PERFORMANCE DATA

Analyte	PPA			NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
<i>Bacillus anthracis</i>	50/50	100	92.9-100	332/332	100	98.9-100
CCHF virus ^a	98/100 ^a	98	93.0-99.5	282/282	100	98.7-100
<i>Ebolavirus</i>	50/50	100	92.9-100	332/332	100	98.9-100
<i>Francisella tularensis</i>	50/50	100	92.9-100	332/332	100	98.9-100
Lassa virus	50/50	100	92.9-100	332/332	100	98.9-100
<i>Leishmania</i> spp.	50/50	100	92.9-100	332/332	100	98.9-100
<i>Marburgvirus</i> ^b	99/100 ^c	99	94.6-99.8	282/282	100	98.7-100
West Nile virus	50/50	100	92.9-100	331/332 ^a	99.7	98.3-100
Yellow fever virus	49/50 ^d	98	89.5-99.7	332/332	100	98.9-100
<i>Yersinia pestis</i>	50/50	100	92.9-100	332/332	100	98.9-100

a. Tested at additional concentrations to better represent clinically relevant range. d. Discrepancy testing showed near LoD levels of WNV in a single WB sample.

b. Tested at additional concentrations to better represent clinically relevant range. e. Single false negative Yellow fever virus at 5× LoD.

c. Single false negative Marburgvirus at 10× LoD.

f. Two false negative CCHF virus at 2× LoD.

TABLE 3. CONTRIVED ORGANISM SPIKE LEVELS

Testing Concentration	Number of Specimens Tested
2× LoD	25
5× LoD	10
10× LoD	5
100× LoD	5
1,000× LoD ^a	5
Total	50

a. If unachievable, the highest level possible was tested.

CONCLUSIONS

- The BioFire Global Fever Special Pathogens Panel is a specific in vitro diagnostic for the detection of bacteria, viruses, and parasites from whole blood specimens compared to a well-validated molecular method (NPA of 99.2% or greater).
- The BioFire Global Fever Special Pathogens Panel is a sensitive in vitro diagnostic for the detection of bacteria, viruses, and parasites from whole blood specimens compared to a well-validated molecular method (PPA of 92.7% or greater for analytes present in at least 10 specimens).
- The recommendations for contamination prevention along with the nature of a closed and disposable PCR testing pouch greatly reduce the chances of false positive results due to analyte or amplicon contamination.