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Clinical evaluation of the BioFire Global Fever Panel for the identification of malaria, leptospirosis, chikungunya, and dengue from whole blood: a prospective, multicentre, cross-sectional diagnostic accuracy study

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Conflicts of Interest

OJ, BWJ, CLP, and DSR are employees of the Study Sponsor. All others have no conflicts of interest to declare.

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Abstract

Background: Acute febrile illness (AFI) is a common presentation for patients at hospitals globally. Assays that can diagnose a variety of common pathogens in blood could help to establish a diagnosis for targeted disease management.

Methods: Consenting adults and children ≥6 months presenting with fever in the last 2 days were enrolled consecutively in sub-Saharan Africa (Ghana, Kenya, Tanzania, Uganda), Southeast Asia (Cambodia, Thailand), Central and South America (Honduras, Peru), and the United States (District of Columbia, Saint Louis). We assessed the performance of 6 analytes (chikungunya virus, dengue virus (serotypes 1–4), *Leptospira spp., Plasmodium* spp., *P. falciparum*, and *P. ovale/vivax*) on the BioFire Global Fever Panel (GF Panel), a multiplex nucleic acid amplification test performed on whole blood specimens run on the BioFire FilmArray System. The performance of the GF Panel was assessed using comparator PCR assays with different primers followed by bidirectional sequencing on nucleic acid extracts from the same specimen.

Findings: From March 2018 through September 2019, 1,965 participants were enrolled at 10 sites worldwide. Of the 1,875 with analyzable results, 52.3% (980/1875) were female and the median age (range) was 22 (0,100) years. At least one analyte was detected in 35.0% (657/1875) of specimens. The GF Panel had a positive percent agreement (95% confidence interval, CI) for the 6 analytes evaluated: (chikungunya virus 100%(86.3–100%), dengue virus 94.0%(90.6–96.5%), *Leptospira* spp. 93.8%(69.8–99.8%), *Plasmodium* spp. 98.3%(96.3–99.4%), *P. falciparum* 92.7%(88.8–95.6%), and *P. vivax/ovale 92.7%(*86.7–96.6%)) and negative percent agreement higher than 99.2%(98.6–99.6%) for all analytes.

Interpretation: This one-hour, sample-to-answer, molecular device can detect common etiologic agents of AFI with excellent sensitivity and specificity directly on whole blood. The assay targets are prevalent in tropical and subtropical regions globally and could help to provide both public health surveillance as well as individual diagnoses.

Introduction

Infectious diseases remain an important cause of global mortality, and acute febrile illness (AFI) is a common reason to seek healthcare in low and middle-income countries.[3] The advent of rapid diagnostic tests for malaria has allowed more targeted use of antimalarials. However, with declining malaria transmission, expanding the ability to diagnose the etiology of hospitalized fever could inform empiric algorithmic care and would benefit individuals.[4] Blood cultures are resource-intensive and have a turnaround time of usually >48 hours. Some bacterial infections like leptospirosis cannot be easily cultured and may require early administration of an antimicrobial that is not part of the usual empiric treatment. In addition, the rapid identification of arthropod-borne viral pathogens also protects public health[5] and can limit inappropriate antimicrobial use that accelerates the selection of antimicrobial resistant organisms.

Syndromic multiplex panels have been rapidly adopted by clinical microbiology laboratories for respiratory, central nervous system, blood, and gastrointestinal pathogens.[6] For bloodborne pathogens, most multiplex panels have been developed for use on blood culture bottle broth after 12–48 hours of incubation and positivity.[7] Such panels allow rapid bacterial identification including a limited number of antimicrobial susceptibility genotypic markers. Assays that can detect a large number of pathogens from whole blood are limited. The TaqMan array was developed as a customizable approach to potentially replace blood culture. It requires nucleic acid extraction followed by single plex PCR of 24 to 348 targets simultaneously using microfluidic technology. Evaluation of several array configurations, each of which has to be re-evaluated for performance with each printing, have been published.[8–10] The limit of detection is 10^3 organisms/mL which makes the detection of low burden organisms more challenging.[11–13]

The Biofire Global Fever Panel (GF Panel), developed by BioFire Defense, uses an automated system to extract nucleic acid from whole blood samples and perform multiplex RT-PCR and simultaneous detection for 19 pathogens, including agents of viral hemorrhagic fevers, in under an hour; the detection of ebolavirus has been previously optimized on this platform.[14–16] It is the first AFI sample-to-answer multiplex, *in vitro*, diagnostic assay that

can be performed directly on whole blood. We sought to determine the performance of 6 analytes on this panel in 10 sites across 4 geographically distinct regions (North America, Sub-Saharan Africa, Southeast (SE) Asia, and Central and South America).

Methods

Study Population:

We performed a prospective, multicenter, cross-sectional diagnostic study in which the accuracy of an investigational in vitro multiplex molecular diagnostic test (GF Panel) was compared to at least two PCR assays for each analyte using gene targets distinct from those in the GF Panel. Consenting adults and children were consecutively recruited from 2 sites in the United States (District of Columbia, Saint Louis), 4 countries in sub-Saharan Africa (Ghana, Kenya, Tanzania, Uganda), 2 countries in SE Asia (Cambodia, Thailand), and 2 countries in Central and South America (Honduras, Peru) (See Supplemental Table 1). Participants who provided informed consent/assent with a recorded or self-reported fever within the past 2 days who had not previously enrolled in the study in the last 30 days were consecutively enrolled.

At the time of specimen collection, the study enroller recorded the following information on a Case Report Form: basic demographic information, current medications, disease symptoms, days since onset of symptoms, and any recent vaccinations. This information was collected from a medical record when possible, or from the subjects themselves. Results of standard malaria testing were also recorded when available from a clinical laboratory; results of smear microscopy and rapid lateral flow assay were available for 596 (31.8%) and 11(0.6%) of 1,875 samples, respectively; 6 (0.3%) of 1,875 samples had results from both standard care methods.

The study was approved by local internal review boards at each clinical study site, by investigators' primary Institutional Review Boards, the US Army Medical Research and Development Command Human Research Protection Office, and the University of Utah in compliance with all applicable Federal regulations governing the protection of human subjects.

Specimen collection and testing:

Whole blood (≥ 2.5mL) from participants was collected by venipuncture in EDTA tubes. Specimens were included if they were stored at room temperature for less than 24 hours or refrigerated for less than seven days before testing. For a minority of specimens, samples were frozen at ≤ −70°C if testing on the GF Panel and nucleic acid extractions for comparator testing could not be performed within the refrigerated storage time window. Residual whole blood specimen was stored at ≤−70°C within 7 days of collection in the event additional nucleic acid extraction or repeat GF Panel testing were required. Clinical sites tested whole blood specimens in EDTA on the GF Panel. For comparator testing, sites extracted nucleic acid (DNA and RNA) from the whole blood specimens using the MagNA Pure Compact System (Roche Molecular Systems, Inc., Mannheim, Germany) according to the manufacturer's instructions. Frozen aliquots of this nucleic acid were shipped to

BioFire Defense for comparator testing. All sites tested an external control material (ECM) at the start of each day of specimen testing using the BIOFIRE SHIELD Control Kit, which contains positive and negative external controls using a mix of nucleic acid sequences that are detected by the GF Panel assays. A valid Positive or Negative External Control run was required to accept the results of specimen testing on that day. To monitor for contamination, laboratory personnel performed weekly surveillance by swabbing laboratory areas including the biosafety cabinet where specimens were aliquoted, laboratory benches, MagNA Pure Compact instrument, and FilmArray systems, mixing the swab with hydration buffer, and testing the solution on the GF Panel. Contamination was detected in 5 (1.0%) of 509 of weekly surveillance tests and, when present, was immediately eliminated through area cleaning. On those few occasions, testing was not initiated until surveillance swabs were repeated and negative for contamination.

Comparator testing

Comparator testing consisted of at least two PCR assays for each analyte run as nested PCR multiplexes followed by melt analysis and confirmed by bi-directional sequencing. The comparator PCR assays targeted different genes (or different regions of the same gene) than those targeted by the GF Panel. The comparator assays were independently validated by BioFire Defense for inclusivity and exclusivity and had a limit of detection comparable to the associated GF Panel assays. (Supplemental Table 2) A specimen was only considered positive when a bi-directional sequencing result matched a published GenBank sequence for the expected analyte and, in general, had a Phred quality score of at least 20 and an E-value lower than 1.0E-30. A subset of 6 sites also did quantitative malaria thick and thin smears and/or lateral flow rapid malaria tests using site-specific methods. These data were provided for information purposes only, and were not used as comparators.

Results reporting:

All GF Panel test results were masked at the study sites. Run files were uploaded to BioFire Defense for analysis. Because the GF Panel targets some common and many rare pathogens, the performance of the GF Panel was presented to the US Food and Drug Administration (FDA) in two regulatory submissions. The targets reported herein are those that were part of the De Novo (DEN200043) submission to the US FDA (https://www.accessdata.fda.gov/cdrh_docs/pdf20/DEN200043.pdf): chikungunya virus, dengue virus (serotypes 1–4), *Leptospira spp., Plasmodium* spp., *P. falciparum*, and *P. ovale/vivax. Plasmodium malariae* and *Plasmodium knowlesi* may cross-react with the *P. ovale/vivax* assay. A *P. ovale/vivax* detected result should be confirmed as infection due to *P. ovale* or *P. vivax*. The US FDA has not completed review of the performance of the remaining targets on the GF Panel, so these data are not reported herein.

Sample size estimation:

The enrollment goal of at least 1,500 participants was determined through guidance by and in consultation with the US FDA.[17] Individual enrollment goals for each study site were determined based on expected analyte prevalence.

Statistical methods:

A GF Panel result (Detected or Not Detected) was considered True Positive (TP) or True Negative (TN) only when it agreed with the comparator result. False Negative (FN) indicates that the GF Panel result was Not Detected, while the comparator result was positive. False Positive (FP) indicates that the GF Panel result was Detected, but the comparator result was negative. The positive percent agreement (PPA) or sensitivity calculated as 100 × (TP / (TP + FN)) and negative percent agreement (NPA) or specificity calculated as 100 × (TN / (TN + FP)). The exact binomial two-sided 95% confidence interval was calculated for both performance measures according to the method of Newcombe, et al.[18] The performance goals were at least 90% PPA with at least an 80% lower bound of the 95% confidence interval, and at least 95% NPA with at least a 90% lower bound of the 95% confidence interval.

Samples for which FP and/or FN results were obtained (i.e., discrepant results when comparing the GF Panel results to the comparator method results) were further investigated. The discrepancy investigations were typically performed as follows: 1) Discrepancies between the GF Panel and comparator assays were examined and additional testing, on GF Panel or other PCR-based assays, was performed to determine whether the analyte was initially reported as ‘Negative’ or ‘Not Detected’ because it was near or below the detection threshold; 2) FP and FN were evaluated by at least one additional PCR test that used different primers than the GF Panel assay and the comparator assays; 3) When possible, unresolved discrepancies were evaluated with additional PCR testing that could be verified by sequence analysis. This discrepant analysis was not used to determine the PPA or NPA.

Role of the funding source:

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Results:

From March 2018 through September 2019, 1,965 participants were enrolled at 10 sites worldwide, of whom 1,875 (95.4%) were included in the final analysis (Figure 1). Thirty-two samples were excluded before testing was attempted and an additional 27 sample results were excluded due inability to obtain complete testing data.

The clinical characteristics of the participants enrolled are shown in Table 1. Overall, 980 of 1,875 (52.3%) were female and the median age was 22 years (range 0,100). Headache was the most common symptom in 1,108 (59.1%) of 1,875 subjects, followed by other

non-localizing symptoms of chills (34.7%), fatigue (31.8%), anorexia (31.7%), malaise (31.3%), and nausea (27.3%).

Of the total number of specimens collected, 406 (21.7%) of 1,875 were frozen at ≤−70°C before testing. A fresh-frozen whole blood study demonstrated equivalence for a set of representative analytes. Fifty dual-spiked contrived (near the LOD) and 62 clinical whole blood specimens were evaluated before and after a freeze/thaw cycle. The PPA and NPA for most representative analytes were 100%. Reported differences in Cp and melt temperature values between fresh and frozen specimen testing were within the variations expected of the system and did not systematically favor the fresh over the frozen condition (see supplemental data). Based on this data, all data for fresh and frozen specimens were analyzed in aggregate. At least one analyte was detected in 35.0% (657/1875) of specimens. The analytes detected by region and overall are shown in Table 2. The PPA and NPA are shown for six GF Panel targets in Table 3.

Dengue virus was detected in 266 (14.2%) of 1,875 participants by the GF Panel, and in 283 (15.1%) of 1,875 participants by the comparator assay. Of those detected by GF Panel, 121 were from Central/South America (Honduras (n=101) and Peruvian Amazon (n=20)), 144 from SE Asia (Cambodia (n=90) and Thailand (n=54)), and one from Tanzania. Two of the participants with dengue were co-infected with *Plasmodium*: one *P. falciparum* and one *P. vivax/ovale*.

Of the 27 (1.4%) of 1,875 participants with chikungunya identified on the GF Panel, all were from Thailand and contemporaneous with a reported outbreak during the study enrollment period; 25 (92.6%) of 27 were confirmed by the comparator assay. Two participants with chikungunya detected were also dengue-positive on both GF Panel and comparator PCR; one of these was chikungunya-positive only by GF Panel (and negative by comparator PCR). There were 19 (1.0%) of 1,875 *Leptospira* detections by GF Panel at 5 sites (Peru (n=9), Thailand (n=4), Cambodia (n=4), Ghana (n=1), and US (n=1)). Of these, 15 (78.9%) of 19 were confirmed by comparator. The four unconfirmed cases included one from Ghana and Cambodia, and two from Thailand. One participant was positive by comparator PCR only. The GF Panel identified two participants coinfected with *Plasmodium spp.*, only one of which was comparator PCR-positive (for *Leptospira* co-infection).

Of participant specimens, 351 (18.7%) of 1,875 were identified by the GF Panel to contain *Plasmodium spp.*, 339 (96.6%) were confirmed by comparator PCR. Of those detected by GF Panel, the majority (72.9%; 256/351) were from Africa (Uganda (n=141), Ghana (n=50), Kenya (n=49), and Tanzania (n=16)) with the remainder from Peru (n=84) and SE Asia (Thailand (n=4) and Cambodia (n=7)). The GF Panel showed a strong correlation with site-specific methods (either rapid lateral flow test or smear microscopy) when the site was able to detect malaria (203 matched versus 5 where GF Panel was negative and the site-specific method was positive; 203 (97.6%) of 208). Importantly, for these 5, the PCR comparator method agreed with GF Panel (i.e., they were true negatives). The GF Panel was also able to detect *Plasmodium* in many specimens that the sites determined as negative (20.7%, 81/392). For 71/81, the PCR comparator method agreed with GF Panel. For the 6 sites that did a quantitative assessment of parasitemia, the majority of the discordant samples

were from participants who were GF Panel-positive and smear-negative. (Supplemental Figure 1)

Specimen retesting for discrepancy investigation found evidence of target analytes in 17 (80.9%) of 21 false positive specimens and 39 (76.5%) of 51 false negative specimens. Additional analysis of amplification curves indicated that all false positives and false negatives were the result of analyte level near the limit of detection of the comparator or GF Panel assays.

The GF Panel analytes detected by site are shown in Supplemental Table 1. The clinical characteristics of the participants by analyte detected are shown in Supplemental Table 3. Additional information is available in the GF Panel instructions for use.[19]

Overall, 28 (1.5%) of 1,875 specimens had multiple analytes detected; including 28 (4.3%) of 657 positive specimens. Two specimens had both chikungunya and dengue detected. Two other specimens had both dengue and *Plasmodium* (one *P. falciparum*, one *P. vivax/ovale*) detected. Two *Leptospira*-positive specimens had co-detection of *Plasmodium* (one *Plasmodium spp*, and the other *P. vivax/ovale* and *Plasmodium spp*). Twenty-two specimens had *Plasmodium spp, P. falciparum*, and *P. vivax/ovale*; one was from the South America, and the rest were from the Africa region.

The GF Panel contains two internal controls that must pass for the run result to be valid. The overall proportion of initial specimen tests that produced valid results was 1,868 (96.9%) of 1,928 and includes all tests in participants who met the inclusion criteria; 5 tests did not complete (2 due to loss of power, 2 instrument errors, and 1 software error). Overall, 25 (1.3%) of 1,893 of the tests had pouch internal control failures (either the PCR2 or the yeast RNA process control failed). Of the 30 unsuccessful initial tests, all were retested once, and valid results were produced for 25 of the 30 retested specimens.

Overall, 149 (7.9%) of 1,875 participants had received antimicrobials by the time of enrollment. Antimicrobial use was most common in North America at 35 (18.6%) of 188, followed by 13.7% (82/599; 134 had missing data) in Africa, 23 (5.3%) of 433 (79 had missing data) in Central/South America, and 9 (1.4%) of 655 in SE Asia. African sites had the highest prevalence of antimalarial treatment at 63 (10.5%) of 599, with all other sites <0.7%. Non-steroidal anti-inflammatory treatment was universally high at 43.6% overall, with 31.1% in Africa, 37.8% in North America, 72.7% in Central/South America, and 37.4% in SE Asia, respectively. The prevalence of anti-inflammatory use is expected to reduce the number and severity of recorded fevers in this study.

Discussion:

In this evaluation of the BioFire GF Panel in nine countries, mostly at tropical and subtropical sites, 35% (657/1875) of the specimens tested had at least one of the 6 analytes detected. The Global Fever Panel showed sensitive (PPA ≥92.7%) and specific (NPA≥99.2%) detection of 2 viruses (dengue, chikungunya), leptospirosis, and malaria (*Plasmodium* species, and *P. falciparum* and *P. vivax/ovale* specifically). The

geographic distribution of the positive specimens by organism was consistent with previous surveillance.[20–22]

AFI patients routinely present with general symptoms, so it is difficult to diagnose presumptively based on clinical features. Symptoms of AFI were recorded at the time of enrollment for each subject in this study, and ten different symptoms were present in at least 10% (187/1875) of subjects. Some symptoms were reported with much higher frequency in some regions compared to others. For example, malaise was reported with very high frequency in Central and South America (93.8%; 406/433) but was less common in Africa (1.0%; 6/599) and North America (2.7%; 5/188); in contrast, nausea was common in all regions except Africa (3.3%; 20/599). These differences may reflect uneven geographic disease distribution (Supplemental Table 4), or cultural or linguistic differences in how the patient populations define symptomology.

The majority of detected *Plasmodium* infections were at sites in Africa (mostly *P. falciparum),* with the remainder in Central and South America where *P. vivax/ovale* was the most prevalent. A small number were detected in SE Asia as well. GF Panel showed a strong malaria detection correlation with site-specific methods, although an additional 71 specimens were positive using a nucleic acid amplification test compared to the rapid lateral flow antigen test or smear microscopy. Although in some cases this could represent residual nucleic acid following a cleared infection, a molecular amplification test is likely to be more sensitive for detecting low concentrations of organism. The specimens in which *Plasmodium* was detected were collected in regions where asymptomatic and pre-patent malaria are common.[23] The higher sensitivity of the GF Panel and PCR tests, in general, will detect patients with circulating parasites that may not be the etiology of their fever. Clinicians need to consider this when interpreting the results of PCR tests; PCR will over-diagnose malaria as the cause of the current illness and may encourage clinicians to focus on that result to the exclusion of other causes that may be missed by PCR tests. Conversely, when malaria is rapidly detected with rapid antigen tests, bacterial coinfections may go undetected and untreated because the standard of care diagnosis for malaria is readily available, whereas tests for other pathogens are always not available or infrequently employed (e.g., two cases of malaria leptospirosis coinfection).

Dengue was detected in Central and South America and SE Asia. Interestingly, despite systematic reviews showing dengue RNA detection in 7.1% of febrile participants in 21 studies (n=15,322) in Africa,[24] only one case was detected in our study at the Tanzanian site and reflects the heterogeneity of disease in Africa.[25] In sub-Saharan Africa, dengue rapid test usage is sparse and with the increasing urbanization of the tropics and subtropics favoring the dengue vectors,[26] this may change in the future. Together with the overlap of signs and symptoms of dengue with many other pathogens, having this pathogen included in the multiplex panel may prove useful in all regions including Africa.

Chikungunya virus detections were limited to Thailand where there was a documented outbreak during the enrollment period (February-September 2019).[27] Although chikungunya virus was first described in Tanzania,[28] it has now spread globally with cases in the Pacific in 2011, and then in Central and South American since 2013. Presumably due to overlapping

mosquito vectors, two co-detections of dengue and chikungunya virus were found in our study.

One-half (n=9) of the leptospirosis cases was detected in Iquitos, Peru, although 8 additional cases were detected in both SE Asian sites. Leptospirosis is difficult to detect as rapid tests have poor performance[29], culture is insensitive, and PCR tests are most sensitive in the acute bacteremic phase and lack sensitivity in the immune phase of illness. Although microscopic agglutination tests are the most sensitive, they are cumbersome and require acute and convalescent sera. However, the specificity of PCR and the potential for rapid sample-to-answer is promising for increasing awareness and initiation of appropriate antibiotics.

Very few sites had access to rapid diagnostic tests other than for malaria and were forced to employ empiric algorithmic care which can lead to overtreatment for viral infections and undertreatment of bacterial infections that require specific antibacterials such as doxycycline. Although information on the initial treatment regimen was collected, data on antibiotic treatment prior to and after presentation were not collected nor was the response to treatment. With US FDA De Novo granting for the 6 analytes herein reported, the impact of diagnosis and targeted treatment upon use of the panel would be important to study.

In this study, sites ranged from rural to urban in tropical and sub-tropical settings with uninterrupted electricity as the device is not battery operated (turnaround time < 1hour). The Biofire platform is pouch-based, easy to operate at point-of-need, and does not require skilled technicians to operate. Therefore, the use case could penetrate relatively low levels of the health system—currently, the intended use of the GF Panel on this platform is for clinical laboratories with trained lab technicians. One caveat with all molecular assays is the possibility of false-negative results if the assay is performed outside the window when the pathogen is circulating in blood. The detection window in blood may be relatively short for some pathogens (e.g., arboviruses). For some bacteria such as leptospirosis, the most severe clinical presentations often occur after bacteremia, and as a result of the host immune response which may still require serologic confirmation.

Our study had other limitations. The reference standard for comparator testing relied on similar technology (i.e., detection by RT-PCR), and we cannot demonstrate clinical correlation as there was no additional clinical follow-up of the participants. Some of the samples had to be frozen for future testing due to supply chain or site-related barriers to testing, but the detection difference between fresh and frozen specimens was evaluated and was determined to be not statistically significant. Cross-reactivity of the *P. vivax/ovale* panel with *P. knowlesi* and to a lesser extent *P. malariae* are important to considerations to clinicians depending on the clinical presentation. Some analytes had a low number of positive detections and, as a result, had a wide confidence interval. Many of the panel assays have not been evaluated by the US FDA and therefore are not reported here. Finally, some sites did not record the number of participants screened to enroll this sample, potentially limiting the external validity of this evaluation.

In this geographically diverse evaluation of the BioFire GF Panel, this rapid sample-to-answer platform showed excellent performance for the 6 of 19 analytes presented and contains targets that are epidemiologically important in tropical and subtropical sites.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Evidence before this study

We searched PubMed for articles on blood diagnostics for infectious etiologies of acute febrile illness using the following search strategy ("Blood Specimen Collection"[Mesh] OR "Blood"[Mesh] OR "blood"[tiab]) AND ("acute febrile"[tiab] OR "acute fever"[tiab]) AND ("assay*"[tiab] OR "multiplex"[tiab] OR "panel*"[tiab] OR "device*"[tiab]). The search was done on January 26, 2022 with no date or language restrictions. The search returned 96 results of which only 3 reported on a whole blood based automated test for the detection of multiple infectious etiologies of acute febrile illness. Only 2 papers were found that described a multiplex panel both of which were customized for the evaluations (Feverdisk[1] and the Taqman array card[2]). The majority of papers described lab developed tests for the detection of a single pathogen and were not whole blood, automated tests. We did a multisite, prospective study to assess the diagnostic performance of the BioFire Global Fever Panel in globally diverse populations.

Added value of this study

To our knowledge, this is the first automated, now FDA-cleared, device for the sample-to-answer multiplex detection of commonly encountered infectious etiologies of acute febrile illness in tropical and sub-tropical settings on an existing platform. The study was performed in 10 sites providing generalizability. The epidemiology of pathogens detected varied by region (Central/South America, Africa, Southeast Asia, and US) was consistent with past surveillance. We report here on the first 6 analytes. For the detection of *Plasmodium,* the platform detected more infections than standard smear or rapid diagnostic lateral flow assays.

Implications of all the available evidence

This is the first sample-to-answer, multiplex, nucleic acid amplification platform for acute febrile illness that assays whole blood and does not require pre-extraction. It is easy to perform in diverse global settings where laboratory infrastructure may be limited and empiric algorithmic treatment is the standard of care. Individuals could benefit from a specific diagnosis whilst contributing to public health surveillance.

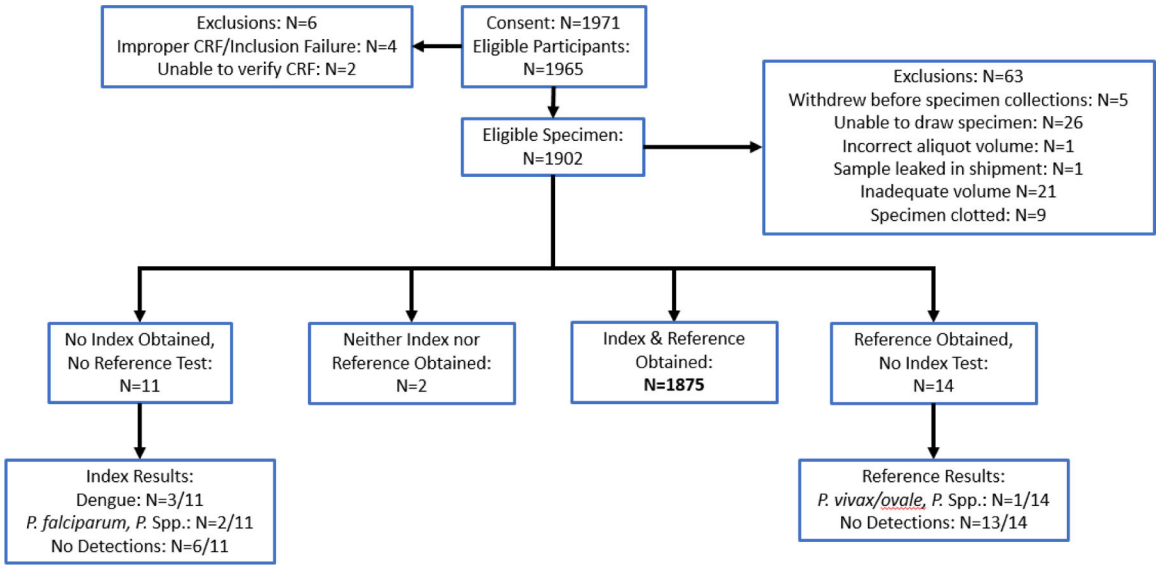

Figure 1:

Standards for Reporting of Diagnostic Accuracy (STARD[30]) Diagram of the Participants and Specimen Testing.

Table 1:

Baseline demographic characteristics of participants for whom index and reference results were obtained

	All N (%)	**Africa N (%)**	**North America N (%)**	**Central/South America N (%)**	**Southeast Asia N (%)**
Total Number	1875	599	188	433	655
Age <5 years 5–21 years 22–50 years >50 years	 163 (8.7%) 765 (40.8%) 672 (35.8%) 275 (14.7%)	 130 (21.7%) 25 (42.9%) 153 (25.5%) 59 (9.8%)	 NA 14 (7.4%) 110 (58.5%) 64 (34.0%)	 8 (1.8%) 163 (37.6%) 207 (47.8%) 55 (12.7%)	 25 (3.8%) 331 (50.5%) 202 (30.8%) 97 (14.8%)
Female	980 (52.3%)	337 (56.3%)	118 (62.8%)	212 (49.0%)	313 (47.8%)
Self-reported fever	1271 (67.8%)	575 (96.0%)	177 (94.1%)	432 (99.8%)	87 (13.3%)
Clinic-assessed fever	1605 (85.6%)	505 (84.3%)	186 (98.9%)	340 (78.5%)	574 (87.6%)
Headache	1108 (59.1%)	279 (46.6%)	59 (31.4%)	411 (94.9%)	359 (54.8%)
Chills	650 (34.7%)	73 (12.2%)	92 (48.9%)	398 (91.9%)	87 (13.3%)
Fatigue	596 (31.8%)	0 (0.0%)	20 (10.6%)	406 (93.8%)	170 (26.0%)
Anorexia	594 (31.7%)	55 (9.2%)	4 (2.1%)	380 (87.8%)	155 (23.7%)
Malaise	587 (31.3%)	6 (1.0%)	5 (2.7%)	406 (93.8%)	170 (26.0%)
Nausea	511 (27.3%)	20 (3.3%)	81 (43.1%)	308 (71.1%)	102 (15.6%)
Cough	447 (23.8%)	139 (23.2%)	38 (20.2%)	118 (27.3%)	152 (23.2%)
Vomiting	435 (23.2%)	130 (21.7%)	50 (26.6%)	195 (45.0%)	60 (9.2%)
Diarrhea	214 (11.4%)	67 (11.2%)	40 (21.3%)	101 (23.3%)	6 (0.9%)
Sore throat	256 (13.7%)	11 (1.8%)	12 (6.4%)	120 (27.7%)	113 (17.3%)
Rash	78 (4.2%)	22 (3.7%)	3 (1.6%)	28 (6.5%)	25 (3.8%)
Dyspnea	33 (1.8%)	7 (1.2%)	25 (13.3%)	1 (0.2%)	0 (0.0%)

N, number

Table 2:

Analytes Detected by Global Fever Panel by Region and Overall*

Region	Number	Chikungunya virus N (%)	Dengue virus N (%)	Leptospira spp.* N (%)	Plasmodium spp. N (%)	Plasmodium falciparum N (%)	Plasmodium vivax/ovale N (%)
Africa	599	0 (0.0%)	1 (0.2%)	1 (0.2%)	256 (42.7%)	225 (37.6%)	27 (4.5%)
North America	188	0 (0.0%)	0 (0.0%)	1 (0.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Central/South America	433	0 (0.0%)	121 (27.9%)	9 (2.1%)	84 (19.4%)	5 (1.2%)	80 (18.5%)
Southeast Asia	655	27 (4.1%)	144 (22.0%)	8 (1.2%)	11 (1.7%)	3 (0.5%)	8 (1.2%)
Overall Total	1875	27 (1.4%)	266 (14.2%)	19 (1.0%)	351 (18.7%)	233 (12.4%)	115 (6.1%)

The *Leptospira* genus is divided into three groups, with pathogenic species classified in Group I. The GF Panel contains an assay for genus-level detection of all *Leptospira* Group 1 species.

N, number; spp., species

Table 3:

Global Fever Panel clinical performance summary overall and stratified by fresh or frozen sample analysis

Analyte		PPA			NPA		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Chikungunya virus[a]	Fresh	25/25	100.0%	86.3–100%	1442/1444	99.9%	99.5–100%
	Frozen	0/0	-	-	406/406	100.0%	99.1–100%
	Overall	**25/25**	**100.0%**	**86.3–100%**	**1848/1850**	**99.9%**	**99.6–100%**
Dengue virus (serotypes 1, 2, 3, and 4)[b]	Fresh	249/263	94.7%	91.2–97.1%	1206/1206	100.0%	99.7–100%
	Frozen	17/20	85.0%	62.1–96.8%	386/386	100.0%	99–100%
	Overall	**266/283**	**94.0%**	**90.6–96.5%**	**1592/1592**	**100.0%**	**99.8–100%**
Leptospira spp.[c]	Fresh	9/9	100.0%	66.4–100%	1456/1460	99.7%	99.3–99.9%
	Frozen	6/7	85.7%	42.1–99.6%	399/399	100.0%	99.1–100%
	Overall	**15/16**	**93.8%**	**69.8–99.8%**	**1855/1859**	**99.8%**	**99.5–99.9%**
Plasmodium spp.[d,e]	Fresh	207/210	98.6%	95.9–99.7 %	1251/1259	99.4%	98.8–99.7%
	Frozen	132/135	97.8%	93.6–99.5%	267/271	98.5%	96.3–99.6%
	Overall	**339/345**	**98.3%**	**96.3–99.4%**	**1518/1530**	**99.2%**	**98.6–99.6%**
Plasmodium falciparum[f]	Fresh	148/158	93.7%	88.7–96.9%	1309/1311	99.8%	99.5–100%
	Frozen	82/90	91.1%	83.2–96.1%	315/316	99.7%	98.2–100%
	Overall	**230/248**	**92.7%**	**88.8–95.6%**	**1624/1627**	**99.8%**	**99.5–100%**
Plasmodium vivax/ovale[g]	Fresh	64/69	92.8%	83.9–97.6%	1400/1400	100.0%	99.7–100%
	Frozen	51/55	92.7%	82.4–98%	351/351	100.0%	99.0–100%
	Overall	**115/124**	**92.7%**	**86.7–96.6%**	**1751/1751**	**100.0%**	**99.8–100%**

PPA, positive percent agreement; NPA, negative percent agreement; TP, true positive; FN, false negative; CI, confidence interval; TN, true negative; FP, false positive; spp., species

[a]Evidence of chikungunya virus was found in 2/2 FP specimens by additional PCR.

[b]Evidence of dengue virus was found in 15/17 FN specimens: five specimens were positive upon GF Panel retest and by additional PCR, two were positive only upon GF Panel retest, and eight were detected only by additional PCR.

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

[e] Evidence of *Plasmodium* spp. was found in 3/6 FN specimens: two specimens were positive upon GF Panel retest and by additional PCR, and one was positive only upon GF Panel retest. Evidence of *Plasmodium* spp. was found in 9/12 FP specimens by additional PCR.

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

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