The FilmArray® Global Fever Panel is currently under development as a qualitative, multiplex, nucleic acid-based test intended for use with the FilmArray® 2.0 system. The FilmArray Global Fever Panel detects and identifies bacterial, viral, and protozoan nucleic acids directly from human whole blood (EDTA) collected from individuals with signs and symptoms of acute febrile illness or recent acute febrile illness and with known or suspected exposure to target pathogens. The following organisms are detected using the FilmArray® GP Panel: Bacillus anthracis, Francisella tularensis, Leptospira spp., 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 from Plasmodium vivax and Plasmodium ovale).

ESTIMATED LIMIT OF DETECTION

The purpose of this study is to determine the estimated Limit of Detection (LoD) for the FilmArray® Global Fever Panel using a collection of representative organisms covering each test result. LoD is defined as the lowest concentration of organism that can be detected at least 95% of the time for 3 trials. The estimated LoD (Table 1) is established using serial 10-fold dilutions. The Estimated LoD is the lowest concentration at which 3/3 replicates failed a detection result. Samples are prepared in whole blood obtained from a repository, BioFire Global Disease Surveillance (NGDS) program.

The estimated LoD is determined for the FilmArray® Global Fever Panel using results from 'primary' analyses.

INCLUSIVITY

To ensure the FilmArray Global Fever Panel is inclusive for the genetic variation expected for each analyte, the genetic diversity of the genomes were 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, INH data (sequence searches and alignments to assays primary) are also used to support the inclusivity of the FilmArray Global Fever Panel.

Samples are prepared by spiking analytes into whole blood from healthy donors (obtained from BioFire Global Disease Surveillance repository) at concentrations near 1× estimated LoD. Figure 2 depicts the protocol followed to establish inclusivity of the isolates tested. An initial subset of analyses tested on the GP Panel are shown in Table 4.

INCLUSIVITY

<table>
<thead>
<tr>
<th>Organism</th>
<th>Analyte</th>
<th>PPD</th>
<th>Phytopath</th>
<th>Serogroup/Strain/Genotype</th>
<th>Species</th>
<th>Strain/Isolate/Genotype</th>
<th>Estimated Limit of Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella enterica</td>
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<td></td>
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<td></td>
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<tr>
<td>Yersinia pestis</td>
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<tr>
<td>Chikungunya virus</td>
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<tr>
<td>Ebola virus</td>
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<td></td>
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<tr>
<td>Yellow fever virus</td>
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</tr>
</tbody>
</table>

TABLE 1: ESTIMATED LIMIT OF DETECTION VALUES

The FilmArray Global Fever test 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 white blood cell. After sample collection, the user mixes the hydrating solution into 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. 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 beading).
- Extracts and purifies all nucleic acid from the sample using magnetic bead technology.
- Performs nested multiplex PCR to:
  - Final performing single, single-volume, highly multiplexed endpoint PCR reaction (PCR).
  - Then performing multiple, singleplex second-stage PCR reactions (PCR) to amplify sequences within the PCR products.
- Uses sample melting curve data to detect and generate a result for each target on the FilmArray® Global Fever array.

The FilmArray Global Fever panel can aid in rapid and actionable AFI diagnosis:

- 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 20% 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 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.

TABLE 2: OFF-Panel EXCLUSIVITY

EXCLUSIVITY

To determine whether the FilmArray Global Fever Panel assay 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: 10⁻¹⁰ copies/ml, for bacteria, 10⁻¹⁰ copies/ml, for virus, and 10⁻¹¹ copies/ml, for protozoa. 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.

- Off-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 20% 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 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.

TABLE 3: SUBSET OF OFF-Panel EXCLUSIVITY ORGANISMS

SUMMARY

The development of a multiple FilmArray panel would aid in rapid and actionable AFI diagnosis. The GP Panel provides a broad spectrum of targets 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.
- Positive results are correlated with known or suspected target pathogens.

Preparation for full analytical and clinical studies for the FilmArray® GP Panel.

Presented at the American Society of Tropical Medicine and Hygiene (ASTMH) Annual Meeting, November 2017 Poster 1445

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