Analytical Studies for FilmArray®: A Rapid and Easy-to-Use Platform for Molecular Detection of Respiratory, Blood, and Gastrointestinal Pathogens

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INTRODUCTION

Before a medical device may enter the US market, an application to the FDA known as a 510(k) must be submitted. Industry is provided with guidance documents which outline recommendations to establish the performance of the medical device. Although guidance documents provide recommendations for analytical studies, it is to the manufacturer to design the studies so that the sensitivity, specificity, and precision of the device may be established. Within the purpose section of each study described below, recommendations from FDA guidance documents are presented in "quotation marks.

Limit of Detection

| Purpose | The Limit of Detection (LoD) of the assay and the concentration of the target pathogen as determined by amplification and confirmation testing. The LoD is established to test the analytical sensitivity of the assay.

| Approach | Initial estimates of LoD for each assay were calculated by evaluating replicates of ten-fold serial dilutions of at least one organism isolated/identified by each assay. The lowest concentration where detection is observed in all replicates is selected and confirmed by additional testing of 20 individual samples. If detection is achieved at least 90% (90%) of the replicates, the LoD is confirmed at the level indicated.

| Results | An example of LoD estimate and confirmation testing is presented for the detection of Enteroaggregative E. coli (EAEC) by the FilmArray GI Panel. The LoD estimation dilution series (Figure 1) achieved 100% (44) detection at the two concentration levels. Detection began to diminish at 10^4 CFU/mL at the next concentration and eliminated (0%) of the lowest concentration. The 10^5 CFU/mL concentration was selected and confirmed by testing 20 replicates, 100% of which were detected (Table 1).

Analytical Reactivity (Inclusivity)

| Purpose | A diagnostic test for the detection of pathogens should be capable of reacting with all relevant variants of the organism. The FDA requires that manufacturers evaluate “…reactivity to account for potential genetic variation among the pathogens…”

| Approach | To assess inclusivity of FilmArray panel assays, a collection (or set) of organisms representing relevant, temporal, geographical, and genetic variations as well as the LoD concentration were tested. Samples were prepared by adding organisms into sample matrix to demonstrate that different strains are not unrecognized in addition to laboratory testing, clinical data and in silico (sequence alignments performed via computer) analyses were used to predict reactivity.

| Result | The table below shows examples from inclusivity testing for influenza A and demonstrates that strains isolated from various years and parts of the world were detected. Two strains of influenza A (H1N1 and H3N2) from 1908 and 1968 were not detected until tested at higher concentration (10^5 CFU/mL) while all other strains were detected at LoD. The FilmArray GI panel was evaluated using a total of 106 strains/isolates to establish analytical inclusivity.

Analytical Specificity (Exclusivity)

| Purpose | Analytical specificity of the assay is based on the target pathogen, with the exception of the BioFire FilmArray panels which are designed to detect a broad range of pathogens. The FDA requires that manufacturers test the specificity of the assay on three different FilmArray panels. Only small number of organisms were found to cross-react with FilmArray assays, typically organisms closely related to the pathogens detected by the assay at high concentrations or organisms not expected to be encountered in clinical specimens. This type of analytical specificity testing allows a manufacturer to identify and inform users of the potential for false positive results caused by cross-reactivity.

Reproducibility

| Purpose | According to the FDA, “the site-to-site reproducibility study should include an evaluation of the major sources of variability,…” in the FilmArray system introduced by multiple test sites, days, users, pouch lots, and instruments.

| Approach | For each panel three testing sites were given pre-made samples containing organisms at different concentrations. Results (detections) were expected for organisms present in the panel, all other results were expected to be negative (not detected). Each sample was tested on multiple days at each test site by different users, on different instruments and with different pouch lots (see figure on right). The results are compiled and reviewed for reproducibility and performance (detection) that may be associated with one of the variables being evaluated.

CONCLUSION

Analytical studies for all three FilmArray pathogen detection panels have demonstrated the system to be:

Reproducible, as established by:
- The Limit of Detection study which confirmed consistent detection of pathogens at low, clinically relevant levels.
- One of the main advantages of the FilmArray system is the ability to perform all of these assays on the same instrument, with the exception of the BioFire FilmArray panels which are designed to detect a broad range of pathogens.
- The Analytical Specificity testing which demonstrated that variants of each pathogen can be detected at low, clinically relevant levels.
- Interference studies which demonstrated that even in the presence of potentially interfering substances, accurate results are obtained.
- Reproducibility studies which showed that the system can tolerate various conditions such as site, operator, instrument, and pouch lot.

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REFERENCES