INTRODUCTION

Before a medical device may enter the US market, an application to the FDA as a 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 up 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) is defined as the lowest concentration of an organism that can be consistently detected (95% all samples tested, is established to determine the analytical sensitivity of the test system). Proper determination of the LoD is recommended by the FDA: “…since many of the [analytical validation] studies…are based on this target concentration.”

Approach: Initial estimates of LoD for each strain were made by evaluating replicates of ten-fold serial dilutions of at least one organism/isolate detected by each assay. The lowest concentration where detection was observed in all replicates is selected and confirmed by additional testing of 20 individual samples. If detection is achieved at least 19/20 (95%) of the samples, the LoD is confirmed at the level tested.

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% (4/4) detection at the two lowest concentrations. Detection began to diminish (94-75%) at the next concentration and was eliminated (0%, 0%) at the lowest concentration. The 1.0E+04 CFU/mL concentration was selected and confirmed by testing 20 replicates, 100% of which were detected (Table 1).

Analytical Repeatability (Inclusivity)

Purpose: A diagnostic test for the detection of pathogens should be capable of reacting with relevantly selected organisms of the pathogen. The FDA requires that manufacturers evaluate “…analytical sensitivity to account for potential genetic variation among the pathogens…”

Approach: The inclusivity of the FilmArray panel assay, a collection of 103 organisms representing relevant, temperate, geographical, and genetic variations, was assessed to confirm the LoD concentration were detected. Samples were prepared by adding organism into sample matrix to demonstrate that different strains are not detected. In addition to laboratory testing, clinical data and in silico (sequence alignments performed via computer) analyses were used to predict sensitivity.

Result: The table below shows examples from inclusivity testing for different classes of pathogens and demonstrates that strains isolated from different years and parts of the world were detected. Two strains of Influenza A (H1N1/2009 and H3N2 Aichi/2/68, from 1934 and 1968 respectively) were and parts of the world were detected. Two strains of Influenza A (H1N1/2009 and H3N2 Aichi/2/68, from 1934 and 1968 respectively) were detected. Eighteen additional strains of different serotypes and subtypes were detected, including two strains of Influenza B (Yamagata/188, and Singapore/0409/03, from 1977 and 2004 respectively) were detected. Additional strains of other influenza viruses were detected, including the PR/8/34 and the 1918 strains (Table 1). The FilmArray RP panel was evaluated using a total of 50 strains/isolates to demonstrate analytical sensitivity.

Analytical Specificity (Exclusivity)

Purpose: Analytical specificity (Exclusivity) refers to the ability of the test to detect and specifically identify organisms that are not expected to be encountered in the sample. This generally includes non-pathogens, segments of the microbial community including endogenous, non-targeted microorganisms, or off-panel organisms.

Approach: Analytical specificity (Exclusivity) testing allows a manufacturer to identify and inform users of the potential for false positive results caused by cross-reactivity.

Results: Nine different organisms were selected to test the analytical specificity of the assay on three different FilmArray panels. Only a 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 established concentrations. Samples (detected) results were expected for organisms present in the panel, all other results were expected to be negative (not detected). Each sample is 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 differences in performance (detection) that may be associated with one of the variables being evaluated.

Results: In all the reproducibility studies (RP, BCID and GI panels), over 1,800 samples have been observed between all testing sites, demonstrating that variables such as site, test day, user, pouch lot, and instrument. For this study, pathogens that are meant to be detected by the assays are tested at very high concentrations to show that they do not cross-react with other assays in the panel. Organisms are chosen to be present in the panel that are not expected to be detected by the assays are also tested at very high concentrations to demonstrate that false positive results are not observed. Organisms for cross-reactivity testing were selected based on: • Relatedness to species detected by panel (genetic or phylogenetic near neighbor) • Clinical relevance (is it found in humans or in the sample type being tested?) • Genetic similarity to assay primers (determined by BLAST search) • Antimicrobial resistance genotype and phenotype (for antibiotic resistance genes)

Replicates

Reproducibility

Purpose: “The study evaluates the FDA’s recommendation to assess “… the potential of interference from other microorganisms present in the specimen” by measuring cross-reactivity or unexpected results when testing organisms that may be in a sample but are not meant to be detected by the assay(s).”

Approach: For this study, pathogens that are meant to be detected by the assay are tested at very high concentrations to show that they do not cross-react with other assays in the panel. Organisms are chosen to be present in the panel that are not expected to be detected by the assay are also tested at very high concentrations to demonstrate that false positive results are not observed. Organisms for cross-reactivity testing were selected based on: • Relatedness to species detected by panel (genetic or phylogenetic near neighbor) • Clinical relevance (is it found in humans or in the sample type being tested?) • Genetic similarity to assay primers (determined by BLAST search) • Antimicrobial resistance genotype and phenotype (for antibiotic resistance genes)

RESULTS

In the reproducibility studies, all organisms present in the panels were detected by the assay(s). Expected results were observed when testing organisms that may be in a sample but are not meant to be detected by the assay(s).

CONCLUSION

Analytical studies for all three FilmArray panel detection pathways have demonstrated the system to be:

Sensitively, as established by: • The Limit of Detection study which confirmed consistent detection of pathogens (bacteria, viruses, and fungi) at appropriately low levels.
• Analytical sensitivity testing which demonstrated that variants of each pathogen can be detected at low, clinically relevant levels.

Specifically, as established by: • Analytical specificity testing which demonstrated that panel assays cross-react with very few off-panel organisms.
• Reproducibility testing which allowed the system to tolerate variable results, such as site, operator, instrument, and pouch lot.

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REFERENCES

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