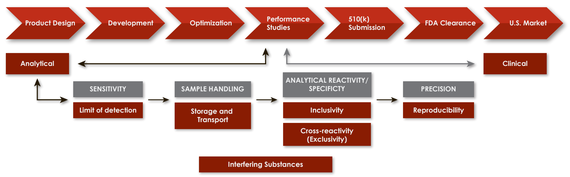
Analytical Studies for FilmArray®: A Rapid and Easy-to-Use Platform for Molecular Detection of Respiratory, Blood, and Gastrointestinal Pathogens

Maggie Buccambuso, Corrin Cook, Jennifer Hoge, Brent Bishop, Cyndi Andjelic, Beth Amiott, Kristen Holmberg, and Beth Lingenfelter

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 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".



The FilmArray System

The FilmArray integrates sample preparation, amplification, detection, and analysis all into one complete process that delivers results in about an hour with only 2 minutes of hands-on time required.

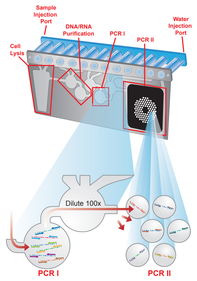
How the FilmArray works…

Preparation

Cell lysis occurs by bead beating and agitation. Nucleic acid purification is performed next by magnetic bead technology that captures, washes, and elutes RNA/DNA in the second and third blisters.

Amplification

Reverse transcription converts RNA into cDNA. Then nested (two sets of primers used in successive runs of PCR) multiplexed (multiple primers sets within a single PCR mixture) PCR is performed in two steps. The first stage enriches the target nucleic acid (PCR I). The second stage takes the PCR I product and mixes it with a fluorescent DNA dye prior to amplification (PCR II).



Detection

After amplification of the nested multiplexed PCR, product is confirmed by high-resolution melt profiling, which delivers a final interpretation. Software displays a positive or negative result for each organism identified.

The Pouch - Stores all necessary reagents for nucleic acid extraction and PCR in a closed system.

THE FILMARRAY PANELS

Respiratory (RP) Panel
- *17 viral pathogens*
- *3 bacterial pathogens*

Blood Culture Identification (BCID) Panel
- *19 bacterial pathogens*
- *5 fungal pathogens*
- *3 antibiotic resistance genes*

Gastrointestinal (GI) Panel
- *8 bacterial pathogens and 6 diarrheagenic E. coli / Shigella pathogens*
- *4 parasitic pathogens*
- *5 viral pathogens*

Limit of Detection

Purpose: The Limit of Detection (LoD), or the lowest concentration at which an analyte is consistently detected (≥95% of all samples tested), is established to determine the analytical sensitivity of the product assay(s). 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 are made by evaluating replicates of ten-fold serial dilutions of at least one organism/isolate detected 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 for 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 highest concentrations. Detection begins to diminish (3/4, 75%) at the next concentration and is eliminated (0/4, 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).

Figure 1. The LoD estimation results for Enteroaggregative *E. coli* (EAEC) as tested by the FilmArray GI Panel

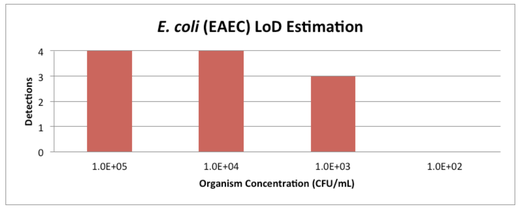


Table 1. The LoD confirmation results for Enteroaggregative *E. coli* (EAEC) as tested by the FilmArray GI Panel

Organism	Test Concentration	Number Detected	Percent Detection
Enteroaggregative *E.coli* (EAEC)	1.0E+04 CFU/mL	20/20	100%

Analytical Reactivity (Inclusivity)

Purpose: A diagnostic test for the detection of pathogens should be capable of reacting with clinically relevant variants of the organism. The FDA requests that manufacturers evaluate "… analytical reactivity to account for potential genetic variation among the pathogens ..."

Approach: To assess inclusivity of FilmArray panel assays, a collection of organisms representing relevant temporal, geographical, and genetic variations at or near the LoD concentration were tested. Samples were prepared by adding organism into sample matrix to demonstrate that different strains are (or are not) detected. 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 PR/8/34 and H3N2 Aichi/2/68, from 1934 and 1968 respectively) were not detected until tested at higher concentration (10xLoD) while all other strains tested were detected at LoD. The FilmArray RP panel was evaluated using a total of 108 strains/isolates to establish analytical reactivity.

Table 2. Inclusivity results for Influenza A isolates as tested by the FilmArray RP Panel

Species	Strain	Multiple of LoD Detected
Influenza A (H1N1)	A/Brisbane/59/07	1x
	A/Solomon Islands/3/2006	1x
	A/Hawaii/15/01	n/a*
	CDC#2001701117	
	A/New Caledonia/20/99	1x
	A1/Denver/1/57	1x
	ATCC VR-546	
	A/Mal/302/54	1x
	ATCC VR-98	
	A1/FM/1/47	1x
	ATCC VR-97	
	A/Weiss/43	1x
	ATCC VR-96	
	A/PR/8/34	10x
	ATCC VR-95	
	A/NWS/33	1x
	ATCC VR-219	
Influenza A (H1N1-2009)	Swine NY/01/2009	1x
	Swine NY/02/2009	1x
	Swine NY/03/2009	1x
Influenza A (H3N2)	A/Brisbane/10/07	1x
	A/Wisconsin/67/2005	1x
	A/NewYork/55/2005	n/a*
	CDC#2005705561	
	A/Victoria/3/75	1x
	ATCC VR-822	
	A/Port Chalmers/1/73	1x
	ATCC VR-810	
	A/Aichi/2/68	10x
	ATCC VR-547	
	A/Hong Kong/8/68	1x
	ATCC VR-544	
	Alice (vaccine) A/England/42/72	1x
	ATCC VR-776	
	MRC-2 Recombinant strain	1x
	ATCC VR-777	

*Unknown multiple of LoD due to lack of quantification in the same units as the LoD strain.

Interfering Substances

Table 3. Examples of Interfering Substances tested for FilmArray Panels

Substance		RP	BCID	GI
Biological	Human blood			
	Tryglycerides			
	Mucin			
	Human genomic DNA			
	Rhinovirus			
Chemical	Bleach			
	Ethanol			
	Ceftriaxone			
	Naproxen			
Media	Viral Transport Media			
	BACTEC Blood culture bottles			
	BacT/ALERT Blood culture bottles			
	Enteric transport media			
	Formalin containing transport media			

substance tested and did not show interference

substance not tested

substance tested and showed interference

Purpose: Interference testing is performed to demonstrate that an "… assay can specifically detect the target organism in the presence of relevant interferents" that could be present in clinical samples or introduced during sample handling.

Approach: Substances such that have the potential to interfere with the accuracy of the test (i.e., inhibit PCR) were added to a sample matrix containing organisms. Results from samples containing the interferent were compared with positive controls to determine whether the tested substance lead to any false results.

Results: The three FilmArray panels have been found to be resistant to common interfering substances. Only one example of interference leading to false negative results has been observed in these studies (Table 3). By identifying the effects of interfering substances, it is possible to inform users.

Analytical Specificity (Exclusivity)

Purpose: This study evaluates the FDA's recommendation to assess "… the potential for 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 assays are tested at very high concentrations to show that they do not cross-react with other assays in the panel. Organisms known to be present in the sample type that are not meant 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:

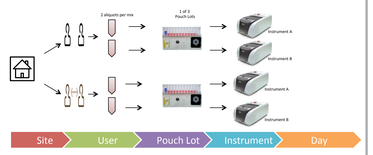
- Relatedness to species detected by panel (genetic or phylogenetic near neighbor)
- Clinical relevance (is it found in humans or in the sample type being tested?)
- Genetic similarity to assay primers (determined by BLAST search)
- Antimicrobial resistance genotype and phenotype (for BCID resistance gene assays)

Results: Nearly 500 different pathogen species and isolates were tested to evaluate 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 different concentrations. Positive (detected) results were expected for organisms present in the mix; 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 tested, and reproducible detection (≥95%) was observed, as well as excellent agreement with the expected negative results (no or few false positive results observed). Equivalent results have been observed between all testing sites, demonstrating that variables such as site, test day, user, instrument, and pouch lot will have no effect on the accuracy of results reported by the system.

CONCLUSION

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

Sensitive, as established by:

- The Limit of Detection study which confirmed consistent detection of pathogens (bacteria, viruses, and fungi) at appropriately low levels.
- Analytical reactivity testing which demonstrated that variants of each pathogen can be detected at low, clinically relevant levels.

Specific, as established by:

- Analytical specificity testing which demonstrated that panel assays cross-react with very few off-panel organisms .

Robust, as established by:

- 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 variables such as site, operator, instrument, and pouch lot.

ACKNOWLEDGMENTS

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Development of the FilmArray GI Panel was supported by grants 5R01 AI089489 and 1R01 AI104593 from the NIAID, NIH.

This poster contains information regarding assays that have not been cleared by the FDA for in vitro diagnostic use.

REFERENCES

Draft Guidance for Industry and Food and Drug Administration Staff "Highly Multiplexed Microbiological/Medical Countermeasure *In Vitro* Nucleic Acid Based Diagnostic Devices" issued Nov 9, 2012.

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CONTACT INFORMATION
Maggie Buccambuso
BioFire Diagnostics
Maggie.Buccambuso@BioFireDX.com