Pursuit of a CLIA-waived FilmArray® System for Detection of Multiple Respiratory Pathogens from a Single Specimen

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ABSTRACT

Acute respiratory infections are a significant public health issue, and the ability to rapidly diagnose specific respiratory pathogens can greatly influence patient care. Testing for respiratory pathogens is currently limited or non-existent in locations without moderate/high-complexity diagnostic laboratories (e.g. physician offices, urgent care clinics, and forward deployed military settings). In collaboration with the Defense Threat Reduction Agency of the Department of Defense and BioFire Defense, BioFire Diagnostics has developed a simplified version of the FilmArray Respiratory Panel (the FilmArray RP EZ), which detects 13 common viral and bacterial respiratory pathogens in about one hour. BioFire is pursuing Clinical Laboratory Improvement Amendments (CLIA)-waived classification for the FilmArray RP EZ, which would allow the device to be used with minimal training by non-laboratory personnel. Subsequent expansion of the FilmArray CLIA-waived classification to additional disease panels, including biothreat panels, would enhance the utility of the platform in a deployed setting, ultimately protecting the warfighter via rapid diagnosis for correct treatment and timely prevention and control measures.

A prospective method comparison study began in November 2014 at seven different CLIA-waived sites across the US. Ultimately, at least 1000 NPS specimens from subjects with suspected respiratory illness will be enrolled and tested at these sites through the remainder of the 2015 respiratory illness season. An aliquot of each specimen tested at the CLIA-waved sites is frozen for later testing at a central, moderate-complexity laboratory using the FDA-cleared FilmArray RP as a comparative method. Archived and contrived specimens will be used to supplement the testing of prospectively collected specimens for rare analytes. In addition to contributing to the sensitivity and specificity assessment of the CLIA-waived system, testing of the contrived specimens by CLIA-waived-type users will allow for evaluation of the ability of these users to achieve accurate results for analytes near the assay limits of detection. The combined prospective, archived, and contrived specimen testing will provide information on accuracy of analyte detection, system reliability, patient demographics, and geographic distribution of respiratory pathogens.

As of April 27, 2015, 636 specimens have been enrolled and tested in the study. Of these, 299 were positive for at least one respiratory pathogen (61.8% positivity), and 25 of those 299 were positive for multiple pathogens (8.4% co-infection rate). Testing on both systems has resulted in 96.4% positive and 99.5% negative agreement. These preliminary data support the concept that the simplified FilmArray system is accurate and easy-to-use in the CLIA-waived setting.

SUMMARY

The FilmArray Respiratory Panel (RP) EZ detects 14 viral and bacterial pathogens associated with upper respiratory infection (Table 1) from a single nasopharyngeal swab (NPS) specimen. The RP EZ is a simplified version of the FDA-cleared FilmArray RP that is designed for use in a CLIA-waived setting. A prospective method comparison study began in November 2014 at eight (8) different CLIA-waived sites across the US. At least 1000 nasopharyngeal swab (NPS) specimens from subjects with suspected respiratory illness will be enrolled and tested at these sites through the remainder of the 2015 respiratory illness season. This poster contains data collected for the first 636 specimens enrolled in the study. An aliquot of each specimen was tested at the CLIA-waved sites (by CLIA-waived type operators) using the FilmArray RP EZ, and an aliquot of each specimen was frozen for later testing at a central, moderate-complexity laboratory using the FDA-cleared FilmArray RP as a comparative method The performance goal for the RP EZ is 95% positive and negative percent agreement (PPA and NPA) with the FDA-cleared FilmArray RP. specimen testing will provide information on accuracy of analyte detection, system reliability, patient demographics, and geographic distribution of respiratory pathogens.

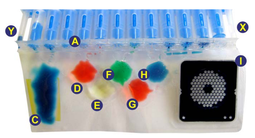
Figure 1. Overview of FilmArray RP EZ System

The FilmArray is a lab-in-a-pouch medium-scale fluid manipulation system performed in a self-contained, disposable, thin-film plastic pouch. The FilmArray platform processes a single sample, from nucleic acid purification to result, in a fully automated fashion.

Testing requires minimal pre-processing of specimens. The sample is filtered by gravity-flow through a coarse strainer, and loaded into the FilmArray RP EZ pouch using a novel filter-injection vial. The user enters the sample and pouch type (using a barcode reader) into the software and initiates a run.

The FilmArray RP EZ pouch has a fitment containing all needed freeze-dried reagents and plungers that plunge liquids to the film portion of the pouch. This portion consists of stations for cell lysis (blister C), magnetic-bead based nucleic acid purification (D & E), first-stage multiplex PCR (F & G) and an array of 102, second-stage nested PCRs (I).

PCR primers are dried into the wells of the array and each primer set amplifies a unique product of the first-stage multiplex PCR. The second-stage PCR product is detected in a melting analysis using a fluorescent-double-stranded DNA binding dye, LCGreen® Plus+.



A. Fitment with freeze-dried reagents
B. Plungers- deliver reagents to blisters
C. Sample lysis and bead collection
D. Wash station
E. Magnetic bead collection blister
F. Elution Station
G. Multiplex Outer PCR blister
H. Dilution blister
I. Inner Nested PCR array

Table 1. Analytes detected by the FilmArray RP EZ system.

Viral Pathogens	Bacterial Pathogens
Influenza A H1 subtype, H3 subtype, 2009 H1 subtype Influenza B Adenovirus Coronavirus Human Metapneumovirus Parainfluenza Virus Respiratory Syncytial Virus Rhinovirus and Enterovirus	*Bordetella pertussis* *Chlamydophila pneumoniae* *Mycoplasma pneumoniae*

Table 2. Age and Sex Distribution of Enrolled Patients as of April 27, 2015

NPS specimens were collected from each participant and tested fresh on the RP EZ system by an operator in a CLIA-waived setting. The number of RP EZ detections for each analyte is shown in Figure 4.

		Overall
Sex	Male	263 (41%)
	Female	373 (59%)
Age	≤ 5 Year	252 (40%)
	6 - 21 Years	125 (20%)
	22 - 49 Years	173 (27%)
	50+ Years	86 (14%)
	Total	**636**

Figure 3. Geographic location and enrollment of RP EZ testing sites as of April 27, 2015

Clinical Evaluation in a CLIA-waived Setting
The accuracy and ease-of-use of the RP EZ system was established during a multi-center study conducted at eight (8) CLIA-waived and one CLIA moderate complexity locations across the US (Figure 3). Sites include public health/community, primary care, pediatric, and family practice clinics.

Site	Location	Type of Setting	Start Date	Enrollment
University of Utah South Main Clinic University of Utah Clinic 6	Salt Lake City,UT Salt Lake City,UT	Community Health Pediatric	Jan. 2015	198
Naval Hospital San Diego Naval Branch Health Clinic Kearny Mesa Naval Hospital Camp Pendleton	San Diego,CA San Diego,CA San Diego,CA	Primary Care Primary Care Primary Care	Jan. 2015	159
NCH Healthcare	Naples,FL	Family Practice	Jan. 2015	24
UTMB Primary Care Pavilion UTMB Pediatrics Primary Care	Galveston,TX Galveston,TX	Primary Care Pediatric	Nov. 2014	255

Adult and pediatric subjects with signs or symptoms of respiratory infection (e.g. cough, runny nose, fever) were eligible to participate in the study. Informed consent/assent/parental permission was obtained as appropriate.

Table 3. Percentage of RP EZ Analytes by Age Group as of April 27, 2015

Analyte	Age ≤ 5	6-21	22-49	50+
Adenovirus	12 (5.9%)	1 (0.9%)	0 (0%)	0 (0%)
Coronavirus	25 (12.3%)	7 (6.5%)	13 (9.4%)	7 (10.4%)
Human Metapneumovirus	20 (9.8%)	6 (5.6%)	3 (2.2%)	0 (0%)
Human Rhinovirus/Enterovirus	59 (28.9%)	22 (20.6%)	24 (17.3%)	12 (17.9%)
Influenza A	10 (4.9%)	15 (14%)	7 (5%)	5 (7.5%)
Influenza A H1	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Influenza A H1-2009	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Influenza A H3	10 (4.9%)	15 (14%)	7 (5%)	5 (7.5%)
Influenza B	5 (2.5%)	3 (2.8%)	2 (1.4%)	4 (6%)
Parainfluenza Virus	13 (6.4%)	4 (3.7%)	3 (2.2%)	1 (1.5%)
Respiratory Syncytial Virus	44 (21.6%)	12 (11.2%)	10 (7.2%)	5 (7.5%)
Bordetella pertussis	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Chlamydophila pneumoniae	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Mycroplasma pneumoniae	0 (0%)	0 (0%)	0 (0%)	0 (0%)

In addition to testing on the RP EZ system, a frozen aliquot of each NPS specimen was tested on the FDA-cleared FilmArray RP system. Performance of the RP EZ was compared to that of the RP. The positive percent agreement (PPA) between FilmArray RP EZ and the RP (comparative method) was calculated as 100% x (TP / (TP + FN)). Negative percent agreement (NPA) was calculated as 100% x (TN / (TN + FP)). The two-sided 95% confidence interval (95% CI) was calculated using the Wilson score method1. Examples of possible results and interpretations are shown in Table 4 and results of the RP EZ and RP comparison are shown in Table 5.

Figure 4. Breakdown of FilmArray RP EZ detections in the CLIA-waived testing setting as of April 27, 2015

NPS specimens were collected from each participant and tested fresh on the RP EZ system by an operator in a CLIA-waived setting. The number of RP EZ detections for each analyte is shown in Figure 4.

Analyte	EZ
Adenovirus	21
Coronavirus HKU1	57
Human Metapneumovirus	40
Human Rhinovirus/ Enterovirus	141
Influenza A/H1	0
Influenza A/H1-2009	0
Influenza A/H3	39
Influenza B	18
Parainfluenza Virus	38
Respiratory Syncytial Virus	76
Bordetella pertussis	0
Chlamydophila pneumoniae	0
Mycoplasma pneumoniae	1
Total FA Runs	**636**

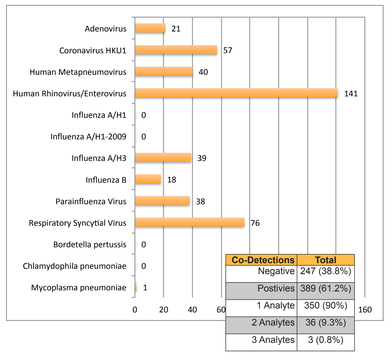


Table 5. Performance of the RP EZ system with prospectively collected NPS specimens as of April 27, 2015

Analyte	Sensitivity/PPA TP/(TP + FN)	%	95% CI*	Specificity/NPA TN/(TN + FP)	%	95% CI*
Viruses						
Adenovirus	11/12	91.7	64.6-98.5	503/505	99.6	98.6-99.9
Coronavirus	45/46	97.8	88.7-99.6	464/471	98.5	97-99.3
Human Metapneumovirus	27/27	100	87.5-100	488/490	99.6	98.5-99.9
Human Rhinovirus/ Enterovirus	101/103	98.1	93.2-99.5	398/414	96.1	93.8-97.6
Influenza A	37/37	100	90.6-100	480/480	100	99.2-100
Influenza A H1	0/0	-	-	517/517	100	99.3-100
Influenza A H1-2009	0/0	-	-	517/517	100	99.3-100
Influenza A H3	35/35	100	90.1-100	480/482	99.6	98.5-99.9
Influenza B	12/12	100	75.8-100	503/505	99.6	98.6-99.9
Parainfluenza Virus	20/20	100	83.9-100	496/497	99.8	98.9-100
Respiratory Syncytial Virus	69/75	92	83.6-96.3	440/442	99.5	98.4-99.9
Bacteria						
Bordetella pertussis	0/0	-	-	517/517	100	99.3-100
Chlamydophila pneumoniae	0/0	-	-	517/517	100	99.3-100
Mycoplasma pneumoniae	0/0	-	-	517/517	100	99.3-100
Overall						
Overall	357/367	97.3	95.1-98.5	6837/6871	99.5	99.3-99.6

*Calculated using the Score method[1]

Table 4. Example of result comparison.

Comparison Result	Assay Result RP EZ	RP
True Positive (TP)	+	+
False Positive (FP)	+	-
True Negative (TN)	-	-
False Negative (FN)	-	+

Acknowledgements

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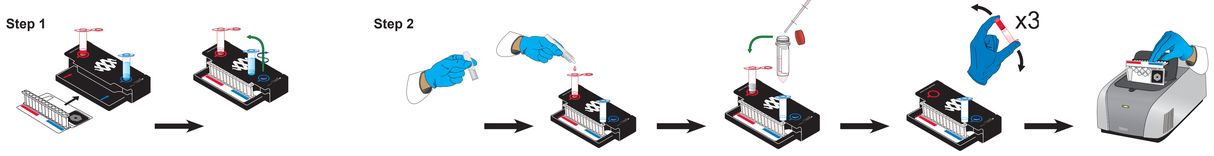
This poster contains information regarding an assay that has not been cleared by the FDA for in vitro diagnostic use. BioFire acknowledges the dedicated clinical staff and study coordinators at each of the enrollment and comparative method testing sites.

Figure 2. Sample Processing and Pouch Loading Instruction



Testing on the RP EZ system requires minimal pre-processing of specimens. After pouch hydration using the blue hydration vial (Step 1), FilmArray Sample Buffer and the NPS specimen in viral transport media are combined in the red Sample vial and loaded into the pouch (Step 2).

ONGOING AND FUTURE EXPERIMENTS

Discordant Result Resolution

Discordant results observed between RP EZ and RP tests will undergo further analysis. An aliquot of each NPS specimen will be tested with well-characterized independent PCR assays. Results will be used to resolve discordant results and determine the true state (i.e. true positive or true negative) of the specimen.

Archived Specimen Testing

As many of the analytes detected by the RP EZ system are low prevalence, archived specimens with previously verified analyte content will be used to supplement the prospective arm of the study. Archived specimens will be selected based on reported content alone, and analyte levels will not be determined prior to use. Each archived specimen will be used to generate two de-identified aliquots, which will be randomized among negative specimens and tested on the RP EZ and RP systems. Performance of the RP EZ system will be compared to RP as described for the prospective specimen testing.

Contrived Specimen Testing

For analytes that are exceedingly rare (e.g. C. pneumoniae or non-2009 Influenza A H1 strains) contrived specimens will be generated using stock organism(s). At least 50 positive specimens will be tested for each contrived analyte. Specimens will be blinded and randomized with negative contrived specimens (matrix only). Performance of the RP EZ system will be compared to RP as described for the prospective and archived testing parts of the study.

Near LoD Contrived Testing

In addition to the contrived specimens that will supplement the prospective study, specimens that contain mixtures of each RP EZ analyte at 2× the established LoD will be prepared for testing in the CLIA-waived setting. Each analyte will be tested at least 50 times, and specimens will be randomized with 50 negative (matrix only) specimens. These contrived specimens will help evaluate the ability of the users in a CLIA-waived setting to detect analytes near the assays' limit of detection. Performance of the RP EZ system will be compared to the known analyte content of the contrived specimens.

The combined results from the prospective, archived, and contrived arms of the clinical study will be used to support a 510(k) submission and CLIA-waiver application for the FilmArray RP EZ.

References

1. Edwin Wilson. Probable inference, the law of succession, and statistical inference. J. Am. Stat. Assoc. 22, 209–212 (1927).

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