Use of the FilmArray® BioThreat-E Assay for the Presumptive Identification of Ebola Zaire Virus

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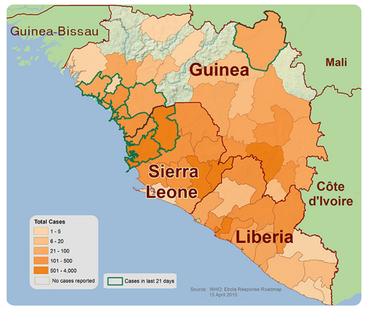
SUMMARY

The 2014 outbreak of Ebola Zaire virus in West Africa is the largest to date. As of April 18, 2015, over 25,000 cases were reported in the three most affected countries (Guinea, Liberia, and Sierra Leone) (http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/case-counts.html), resulting in more than 10,000 deaths. As a result of this outbreak, a public health emergency was declared by the United States Secretary of Health and Human Services on August 5, 2014. There are currently no FDA-cleared diagnostics for detection of Ebola virus, yet there is a need for rapid and accurate detection in persons suspected of infection. The FilmArray® (BioFire Diagnostics, Salt Lake City, UT) is an in vitro diagnostic test platform that combines nucleic acid purification and nested multiplex PCR for the identification of infectious agents in approximately one hour using a closed, sample-to-answer system. BioFire Defense has developed a commercially available FilmArray test (BioThreat-E) capable of detecting Ebola Zaire virus in human specimens. The FilmArray BioThreat-E test is used on the FilmArray Instrument for the presumptive detection of Ebola Zaire virus (detected in the West Africa outbreak in 2014) in individuals with signs and symptoms of Ebola virus infection in conjunction with epidemiological risk factors.

The goal of these studies was to perform preliminary characterization experiments and evaluate the performance of this assay for the detection of Ebola Zaire virus from whole blood and urine. These data were used to support an Emergency Use Authorization application to the US FDA that was granted on October 25, 2014, making the FilmArray BioThreat-E the first commercially available assay for detecting Ebola Zaire.

Figure 1. Total cases of Ebola virus disease in Guinea, Liberia, and Sierra Leone



http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/distribution-map.html
Retrieved April 20, 2015

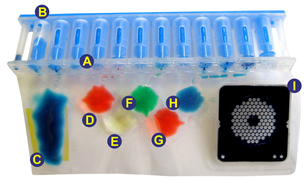
Figure 2. Overview of FilmArray System

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 loaded into the FilmArray pouch using a filter-injection vial. The user enters the sample and pouch type (using a barcode reader) into the software and initiates a run.

The FilmArray 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

ASSAY DEVELOPMENT AND OPTIMIZATION

The FilmArray BioThreat-E test has been incrementally optimized (by adding primer sequence degeneracy) to increase analyte detection efficiency and improve detection of the Ebola Zaire virus currently in circulation in the 2014 West African outbreak. A subset of the performance data presented below was collected using previous versions of the test.

Limit of Detection

An estimated Limit of Detection of 6.0 x 10^5 plaque-forming units (PFU)/mL was determined using gamma-irradiated Ebola Zaire virus in whole blood. Testing was performed on 200 µL aliquots in quadruplicate.

Table 1. Estimation of LoD in whole blood.

Concentration (PFU/mL)	Detected
1.0 x 10^6	4/4
6.0 x 10^5	4/4
6.0 x 10^4	4/4
1.2 x 10^4	0/4

Although 4/4 were detected at the 6.0 x 10^4 level, the median Cp values in 3/4 runs was outside the range of typical values at LoD, which indicated that the concentration did not accurately represent the assay LoD for inactivated Ebola Zaire virus. Because 4/4 were detected at 6.0 x 10^5 PFU/mL and the median Cp values were comparable to typical LoD Cp values in FilmArray assays, this concentration was considered the estimated LoD for this study. Confirmation of the Limit of Detection was done by verifying the 95% detection rate with 20 replicates.

It should be noted that gamma-irradiation can reduce the available target nucleic acid by up to 100-fold as compared to live virus. Based upon comparison to live virus (using a genomic target different from the BioThreat-E Ebola Zaire assay target), the lot of inactivated virus used in this study is approximately 30 – 100 fold lower than live virus in functionally-equivalent target RNA. This results in an estimated LoD with live virus of between 6.0 x 10^3 and 2.0 x 10^4 PFU/mL.

Inclusivity/Reactivity

Due to limitations in acquiring inactivated stocks of Ebola Zaire virus, reactivity of FilmArray BioThreat-E test was not evaluated with any non-Mayinga strains of Ebola Zaire. However, in silico analysis of the primer sequences and the available corresponding target gene sequences (n=30 for non-outbreak strain gene targets, n=99 for current 2014 outbreak strain target gene sequences) was performed. For all 30 non-outbreak strain target sequences, the primer sequences and annealing regions were found to be identical. For the 99 2014 outbreak strain target sequences, all 99 were identical to the primer sequences.

In addition, the ability of FilmArray BioThreat-E test to detect the Ebola Zaire strain currently circulating in West Africa was evaluated using a synthetic template (gBlock, IDT, Coralville, IA, USA) derived from the ZEBOV.Guinea.2014 sequence (Accession: KJ660346), which was used to generate Ebola Zaire L-gene RNA. In vitro transcribed RNA was quantified and spiked into TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) at concentrations varying from 1.00E+00 to 1.00E+05 genomic equivalents (GE) per reaction in duplicate. Detection was observed for all samples tested.

Exclusivity

Throughout development and optimization of this assay, a wide variety of non-Ebola organisms were tested to evaluate the BioThreat-E assay specificity/cross-reactivity. Although not shown in detail here, these organisms included commonly isolated bacteria and viruses, as well as bacteria, viruses, and protozoa that cause similar clinical symptoms as infection with Ebola. A detailed list of the organisms tested including concentration tested can be found in the FilmArray BioThreat-E package insert available from the FDA or on the BioFire Defense website: http://biofiredefense.com/biosurveillance-systems/biothreat-e/.

CONTRIVED CLINICAL SPECIMEN TESTING

Study Design

Surrogate clinical specimens were prepared using inactivated Ebola Zaire Mayinga virus and freshly collected whole blood and urine from healthy donors. Spiking was performed at two different factors of the LoD, as shown in Table 2.

Table 2. Ebola Zaire spiking scheme.

Spike Level	Number
Matrix only	25
2x LoD	20
Max Concentration (1.5 x 10^8 PFU/mL)	5
Total	50

Due to the limitations associated with acquiring inactivated Ebola virus, a high titer stock of inactivated Ebola Zaire was unavailable for this study. The organism stock used in this study was provided at a titer of 1.5 x 10^7 PFU/mL. As a result, the maximum achievable concentration in the contrived specimens (with < 10% total volume of analyte) was 1.5 x 10^6 PFU/mL. Contrived specimens were blinded and randomized prior to testing such that personnel performing testing were blinded to the expected results. All testing was conducted at BioFire Diagnostics.

The goal of the contrived specimen study was to confirm the estimated limit of detection and determine the clinical sensitivity and specificity (positive percent agreement or PPA and negative percent agreement or NPA) of the FilmArray BioThreat-E assay when testing surrogate clinical whole blood and urine specimens. As shown in Table 3.

Table 3. Example 2x2 performance table.

		Surrogate Specimen			
		Spiked	Un-spiked	FilmArray Performance	Two-sided 95%CI[a]
FilmArray	Positive	TP	FP	Sensitivity/PPA =100% x (TP / (TP + FN))	95% Confidence Interval of Sensitivity Proportion
	Negative	FN	TN	Specificity/NPA = 100% x (TN / (TN + FP))	95% Confidence Interval of Specificity Proportion
Total		TP + FN	TN + FP		

The PPA was calculated as 100% × (TP / (TP + FN)) and NPA was calculated as 100% × (TN / (TN + FP)). The two-sided 95% confidence interval was calculated for both performance measures according to the method of Wilson (1). The performance goals were 95% PPA and NPA.

Analyte Detection in contrived specimens

Whole blood was spiked with inactivated Ebola Zaire at two different factors of the estimated LoD (see Table 1) and analyzed using the FilmArray BioThreat-E assay. The contrived specimen set was randomized with 25 negative specimens (matrix only). A breakdown of these results is shown below in Table 4. The observed positive percent agreement for whole blood specimens was 96% and the negative percent agreement was 100%. The single false negative specimen was spiked near the assay's estimated LoD.

Analysis of Instrument and Control Performance

A total of 100 contrived clinical specimens were tested in this evaluation. All 100 initial tests were completed (100% instrument/software performance). Additionally, no internal/process control failures occurred (100% control success).

Table 4. FilmArray BioThreat-E Data Summary for Contrived Whole Blood Specimens.

	Sensitivity/PPA			Specificity/NPA		
	TP/(TP + FN)	%	95% CI[a]	TN/(TN + FP)	%	95% CI[a]
Ebola Zaire in Whole Blood	24/25	96.0%	80.5-99.3%	25/25	100	86.7-100%

[a]The 95% confidence intervals were calculated using the method of Wilson.[1]

Similarly, urine specimens were spiked with inactivated Ebola Zaire at two different factors of the estimated LoD (see Table 1) and analyzed using the FilmArray BioThreat-E assay. A breakdown of these results is shown below in Table 5. The contrived specimen set was randomized with 25 negative specimens (matrix only). The observed positive percent agreement for urine specimens was 96% and the negative percent agreement was 100%. The single false negative specimen was spiked near the assay's estimated LoD.

Table 5. FilmArray BioThreat-E Data Summary for Contrived Urine Specimens.

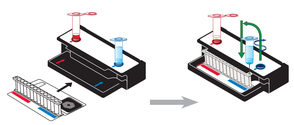
	Sensitivity/PPA			Specificity/NPA		
	TP/(TP + FN)	%	95% CI[a]	TN/(TN + FP)	%	95% CI[a]
Ebola Zaire In Urine	24/25	96.0%	80.5-99.3%	25/25	100	86.7-100%

[a]The 95% confidence intervals were calculated using the method of Wilson.[1]

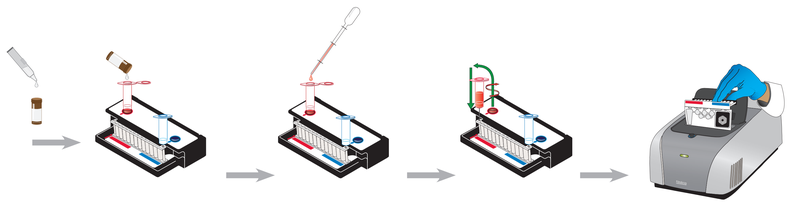
Figure 3. Testing on the FilmArray system requires minimal pre-processing of specimens.

For Whole Blood Samples

Step 1



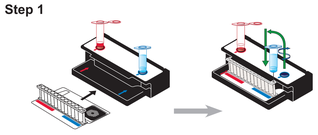
Step 2



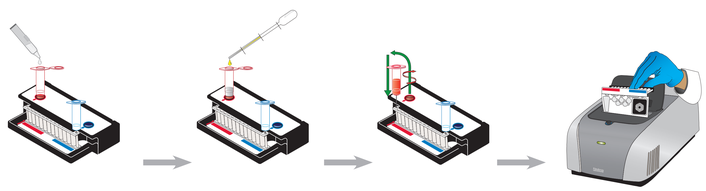
After pouch hydration using the blue hydration vial (Step 1), FilmArray Sample Buffer is added to the Protease Vial and mixed. The protease mixture and the patient specimen (whole blood) is then added to the red sample injection vial and loaded into the pouch (Step 2).

For Urine Samples

Step 1



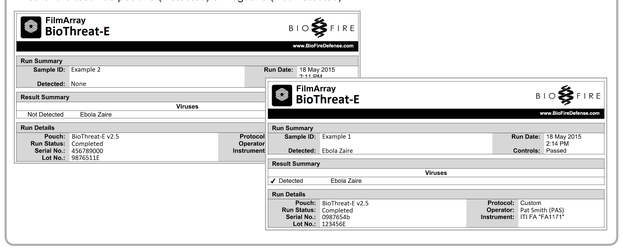
Step 2



After pouch hydration using the blue hydration vial (Step 1), FilmArray Sample Buffer is added directly to the red sample injection vial where it is mixed with the patient urine sample and loaded into the pouch (Step 2).

Figure 4. Example test reports of a negative (top) and positive (bottom) BioThreat-E test.

The BioThreat-E test provides an automated test report that is displayed at the end of a FilmArray run and clearly indicates whether the test was positive (Detected) or negative (Not Detected).



CONCLUSIONS

When combined with appropriate clinical interpretation and used in the appropriate setting, the BioThreat-E assay should be an effective emergency use diagnostic tool for detecting Ebola Zaire virus in in whole blood and urine from patients presenting with Ebola virus disease symptoms.

Disclaimer:
- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an Emergency Use Authorization for use by CLIA Moderate and High Complexity Laboratories
- This test has been authorized only for the detection of Ebola Zaire virus (detected in the West Africa outbreak in 2014) and not for any other viruses or pathogens;
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of the emergency use of in vitro diagnostics for detection of Ebola Zaire virus under section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

BIOFIRE DEFENSE

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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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