

Evaluation of the Idaho Technology FilmArray® system for detection of *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis*

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Pacific Northwest
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Idaho Technology's FilmArray®



Fully automated instrument platform that incorporates cellular lysis, nucleic acid purification, and two-stage multiplexed PCR for detection of a panel of pathogens.

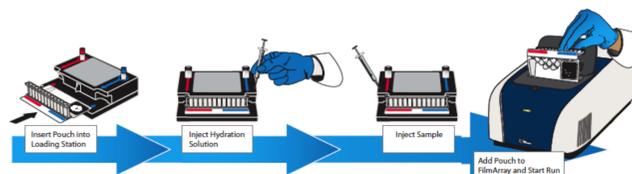
Biothreat pouch tests for 17 biothreat targets simultaneously in one hour from sample to answer.

Project Goal

To evaluate a multiplex biodetection unit, the FilmArray®, for the selective and specific identification of three potential biothreat agents:

- Francisella tularensis*
- Bacillus anthracis*
- Yersinia pestis*

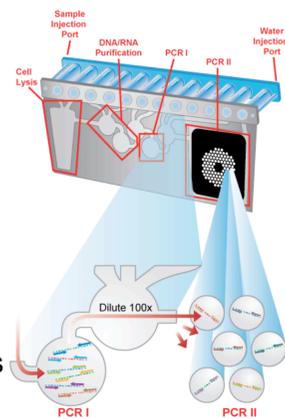
Running a Pouch



- To run a pouch, user injects 1000 µL of hydration solution (provided).
- Sample is mixed with 500 µL of sample buffer (provided), and approximately 250 µL of this solution is aspirated into the sample port.
- Pouch is placed into the FilmArray®, scanned, and run is initiated.

Inside the Pouch

- Cell lysis by ceramic beads
- Magnetic bead hybridization for nucleic acid purification
- Initial multiplex PCR followed by 100 fold dilution into separate wells for a second, single-plex PCR.



Genomic DNA Samples Tested

	Target Strains	Near Neighbor Strains
<i>B. anthracis</i>	<i>B. anthracis</i> Ames <i>B. anthracis</i> BA1035 <i>B. anthracis</i> Canadian bison	<i>B. cereus</i> E33L <i>B. cereus</i> G9241 <i>B. thuringiensis</i> 97-27
<i>F. tularensis</i>	<i>F. tularensis</i> subsp. holarctica 425 <i>F. tularensis</i> subsp. holarctica LVS <i>F. tularensis</i> subsp. tularensis SCHU S4	<i>F. novicida</i> U112 <i>F. philomiragia</i> Jensen ATCC 25016 <i>F. philomiragia</i> Jensen ATCC 25017
<i>Y. pestis</i>	<i>Y. pestis</i> Antiqua <i>Y. pestis</i> Java9 <i>Y. pestis</i> Harbin35	<i>Y. enterocolitica</i> WA <i>Y. pseudotuberculosis</i> YPIII p+ <i>Y. pseudotuberculosis</i> YPIII p- <i>Y. ruckeri</i> YERS012

Table 1. Target and near neighbor strains tested on the FilmArray

- Target strains were tested with 50 µL aliquots containing 25000, 2500, 250, 25, and 12.5 genome equivalents (GEs).
- Near neighbor strains were tested with 50 µL aliquots containing 250000, 25000, and 2500 GEs.
- The runs were randomized with a blank control (buffer) every five tests.

Total G.E. DNA in 550 µL sample	G.E. DNA aspirated into pouch
250,000	113,000
25,000	11,300
2,500	1,130
250	113
25	11
12.5	6

Table 2. Sample loss during loading of the Biothreat pouch

- Our initial 50 µL samples are diluted into the sample buffer, and less than half of this mixture is added to the pouch, which reduces the DNA present for detection as calculated above.

Results: Genomic DNA Testing

Limit of Detection Testing

For all three pathogens, the FilmArray® was able to detect consistently at 25000, 2500, and 250 GEs (data not shown), and was able to detect with some exceptions at 25 and 12.5 GEs across the nine target strains, as shown in the Table 3. Our results indicate that the FilmArray limit of detection is less than 250 GEs, and that in many cases lower levels of target can also be detected.

B. anthracis	DNA (GEs)	Probe			F. tularensis	DNA (GEs)	Probe			Y. pestis	DNA (GEs)	Probe		
		Chro m	pX01	pX02			FTT2	FTT3	Ypt 1			Ypt3		
1035	25				Hol. 425	25				Antiqua	25			
1035	25				Hol. 425	25				Antiqua	25			
1035	25				Hol. 425	25				Antiqua	25			
1035	12.5				Hol. 425	12.5				Antiqua	12.5			
1035	12.5				Hol. 425	12.5				Antiqua	12.5			
1035	12.5				Hol. 425	12.5				Antiqua	12.5			
1035	12.5				Hol. 425	12.5				Antiqua	12.5			
Ames	25				Hol. LVS	25				H. 35	25			
Ames	25				Hol. LVS	25				H. 35	25			
Ames	25				Hol. LVS	25				H. 35	25			
Ames	12.5				Hol. LVS	12.5				H. 35	12.5			
Ames	12.5				Hol. LVS	12.5				H. 35	12.5			
Ames	12.5				Hol. LVS	12.5				H. 35	12.5			
Ames	12.5				Hol. LVS	12.5				H. 35	12.5			
C. bison	25				SCHU S4	25				Java 9	25			
C. bison	25				SCHU S4	25				Java 9	25			
C. bison	25				SCHU S4	25				Java 9	25			
C. bison	25				SCHU S4	12.5				Java 9	12.5			
C. bison	12.5				SCHU S4	12.5				Java 9	12.5			
C. bison	12.5				SCHU S4	12.5				Java 9	12.5			
C. bison	12.5				SCHU S4	12.5				Java 9	12.5			
C. bison	12.5				SCHU S4	12.5				Java 9	12.5			

Table 3. FilmArray testing of 25 and 12.5 GEs of target strains. Red indicates a negative result, green is a positive, and yellow is an incomplete run (software crash). We expect all "green" signatures except the Yp Java 9 strain is expected to be positive only for the Ypt1 signature).

Summary

- The FilmArray® was both sensitive and selective for the detection of *B. anthracis*, *F. tularensis*, and *Y. pestis* genomic DNA samples.
- Our limited spore testing showed similar results to our extensive DNA testing.
- The FilmArray® offers a highly multiplexed bioagent detection system that meets many of the essential needs of first responders.

Specificity Testing

- The system was very specific in our evaluation using target and near neighbor DNA strains (224 total runs at up to 250000 GE).
- We saw no pathogen positives in our near neighbor testing from 2500-250000 GE.
- We observed only 1 unexpected pathogen positive in a pathogen sample; this was an SEB signature in an *Ft holarctica* 425 sample at 25000 GE. The system correctly detected the presence of *Ft* in this sample.
- We had 4 samples in which one *Ba* signature was positive when not expected; however 3 *Ba* signatures are required for a *Ba* positive sample.
- The FilmArray cannot distinguish *F. novicida* from *F. tularensis*, as *F. novicida* was positive for both *Ft* assays across all replicates.
- Of the 38 total blank control samples, 37 were blank. One blank reported five false positive assays (CbT2, FTT2, FCN1, Var1, and YpT1). We hypothesize this was due to reagent pouch failure.

Results: Spore Testing

B. anthracis Sterne spores	Ba Chrom	Ba pX01
25 CFU		

Table 4. *B. anthracis* Sterne spore testing results

- We tested six replicates of 25 CFU *B. anthracis* Sterne spores.
- The FilmArray® was positive 4/6 for *Ba* Chrom assay, and positive 5/6 for the pX01 assay. Note: *Sterne* does not contain the pX02 plasmid.

Acknowledgements

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Science and Technology



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