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Improving the FilmArray® System's Ability to Process Soil and Powder Matrices for Surveillance Purposes

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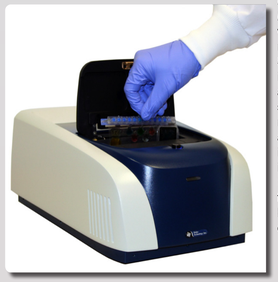
ABSTRACT
Environmental samples encountered during biothreat surveillance testing can consist of matrices with varying complexity. The complexity of the matrix can require pre-processing steps such as filtration, low speed centrifugation, dilution, etc. to occur outside of the system before a sample can be analyzed. Additionally, the pre-processing steps for one matrix may deviate from other matrices which can unfavorably create multiple sample pre-processing protocols for a single system. Idaho Technology Incorporated's next generation multiplex PCR system, the FilmArray®, has an automated sample purification step to remove inhibitors from the sample after the pouch has been loaded. Although the FilmArray has integrated sample purification that purifies both DNA and RNA, some sample matrices with small particulates, such as soils and powders, can exhibit difficulty and inconsistency when loading the sample into the pouch. To prevent sample loading difficulty, and increase system reliability, an integrated filtration system has been developed and incorporated into the FilmArray sample loading process. Because the filter is integrated into the pouch loading process no additional pouch loading steps are required from the user to accomplish the sample pre-processing. The addition of the filter also eliminates the need for multiple sample pre-processing protocols when testing commonly encountered surveillance matrices. The FilmArray biothreat system was challenged with a variety of soils and powders to test the efficacy of particle retention and organism detection. Testing of the filtration system has shown various select agents (*Bacillus anthracis, Francisella tularensis,* Vaccinia virus, and Eastern Equine Encephalitis virus) were detected from soils and powders as well as control samples spiked into buffer only. In addition, use of the filtration system increased the pouch loading success when used with matrices that are known to have a high number of particulates in the sample. The data suggest that this improvement adds no additional pre-processing steps to the already simple FilmArray pouch loading process, increases pouch loading reliability, and does not affect the sensitivity of the system. The integrated filter is also easily applied to alternative panels such as the gastrointestinal panel to allow for clean and quick loading of stool samples.

INTRODUCTION
The critical need for a robust molecular detection tool with a fast turn around time is well recognized by the Biodefense community. In addition, a system capable of detecting NIAID category A and B agents from diverse matrices is desired. In an effort to expedite the detection and identification of NIAID category A and B agents, Idaho Technology Inc. has developed a first generation biothreat panel. The FilmArray BioThreat Panel was designed to detect the following pathogens: *B. anthracis, Y. pestis, F. tularensis, C. burnetii, Rickettsia spp., Brucella spp., Burkholderia spp.,* Ebola, Marburg, equine encephalitis viruses (Eastern, Western, Venezuelan), Variola major, Orthopox, and the genes encoding Ricin toxin, Staphylococcal enterotoxin B, and Botulinum toxin. As illustrated in **Figure 1**, the FilmArray Biothreat Panel is a small footprint instrument that performs an integrated sample preparation processes then uses multiplex nested PCR (**Figure 2**) and LC Green+® chemistry to automatically analyze a sample with minimal operator input. Melt analysis is used for detection which allows increased specificity in organism identification. The BioThreat Panel, in conjunction with the integrated filtration system (**Figure 3**), was tested to detect biothreat agents in two complex matrices known to be capable of inhibiting detection by PCR.



MATERIALS AND METHODS
Inactivated Organisms tested were: *Bacillus anthracis* Ames (washed endospore preparation), *Francisella tularensis* Schu 4, Vaccinia virus Elstree, and Eastern Equine Encephalitis virus PE6. Organisms were selected as a representative of the full panel of organisms on the FilmArray BioThreat system (gram positive spore-forming bacteria, gram negative bacteria, DNA virus, and RNA virus).

SAMPLE PREPARATION
The test matrices used were clay soil and wheat flour, both were selected for their prior inhibitory results in the FilmArray system. The amount of soil or powder used in each sample was determined by the maximum amount a wet IT surveillance swab was able to collect (10-20mg of clay, 10-15mg of wheat flour). The loaded swabs were added to 500µl of FilmArray sample buffer. For the PBS control, 250µl PBS buffer was added to 500µl of sample buffer. Both sample/buffer mixtures were spiked with each organism at ~5X the limit of detection. Two template positive controls were tested filtered and unfiltered.

RESULTS AND DISCUSSION
Table 1 shows the FilmArray positive organism calls for each of the 4 organisms and the sample matrix. The filtered samples all show equal or better performance than the unfiltered samples with the same sample matrices. Both clay unfiltered runs were unsuccessful resulting in no positive calls for any of the assays. Filtered clay samples showed lower detection of EEE than the other sample types, however, the detection is improved over the unfiltered clay samples. All other sample types showed 100% positive detection for all organisms. The negative controls exhibited no false positives.

Table 2 shows the correlation between sample matrix and pouch loading failures or pouch internal control failures. The unfiltered wheat flour exhibited loading difficulty with both pouches. The unfiltered clay sample had one sample with loading difficulty and the other sample with a clogged sample port resulting in failure to load. The single unfiltered clay sample that loaded successfully showed a failure of the pouch internal controls giving a failed pouch call by the FilmArray software. All of the filtered sample types loaded easily and the pouch runs completed successfully.

The melt profiles for *B. anthracis* filtered and unfiltered clay sample types can be seen in **Figure 4**. The melt profiles are not observable in the unfiltered sample, indicating a failure of PCR in the pouch. The filtered samples melt profile is equivalent to that seen in the PBS sample types.

Table 1. Detections of spiked organism in soil and powder matrices.

FilmArray Calls	Clay Filtered	Clay Not Filtered	Wheat Flour Filtered	Wheat Flour Not Filtered	PBS Filtered	PBS Not Filtered	No Organism
***Bacillus* spp.**	3/3	N/A	3/3	2/2	5/5	5/5	0/5
Francisella tularensis	3/3	N/A	3/3	2/2	5/5	5/5	0/5
Orthopox genus virus	3/3	N/A	3/3	2/2	5/5	5/5	0/5
EEE virus	1/3	N/A	3/3	2/2	5/5	5/5	0/5

Table 2. Analysis of pouch loading difficulties and pouch failures compared against sample matrices

	Clay Filtered	Clay Not Filtered	Wheat Flour Filtered	Wheat Flour Not Filtered	PBS Filtered	PBS Not Filtered	No Organism
Control Failure	0/3	2/2	0/3	0/2	0/5	0/5	0/5
Difficulty loading	0/3	1/2	0/3	2/2	0/5	0/5	0/5
Failure to load	0/3	1/2	0/3	0/2	0/5	0/5	0/5

Figure 1. The FilmArray Instrument and Pouch

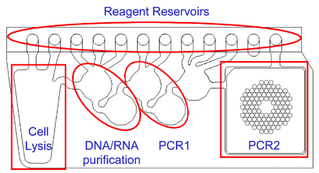
ITI has developed a lab-in-a-pouch system called "FilmArray". It is a medium-scale fluid manipulation system performed in a self-contained, disposable, thin-film plastic pouch. The FilmArray platform processes a single sample, from nucleic acid purification to result, in a fully automated fashion. These system characteristics are ideal for the multiplex testing of pathogens in various sample matrices.

The FilmArray Test System
A FilmArray test is initiated by injecting rehydration solution and a sample into the FilmArray pouch then placing it in the FilmArray instrument. The user enters the sample and pouch type (using a barcode reader) into the software and initiates a run. Results are provided in ~ 1 hour.

The FilmArray pouch has a fitment (see label A) containing all needed freeze-dried reagents.

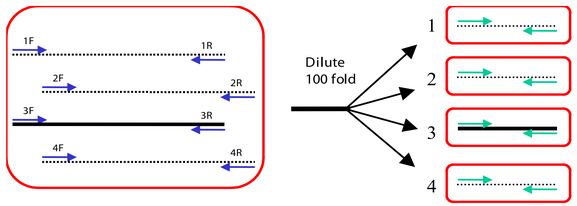
The film portion of the pouch has stations for:
1. Cell lysis (Blister C)
2. Magnetic-bead based nucleic acid purification (D & E)
3. First-stage multiplex PCR (F & G)
4. Array of 102, second-stage nested PCRs (I)

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 real-time using a fluorescent-double-stranded DNA binding dye, LC Green+.



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

Figure 2. Schematic of Multiplex Nested PCR



A large volume multiplex PCR (shown here as 4-plex on the left side of figure) is run for a limited number of cycles (26). The reaction is diluted and distributed to individual small PCR reactions that contain primers (green) nested inside the primers (blue) of the first PCR reaction. A template amplified in the first reaction (by the #3 primers) is further amplified in only one of the second reactions.

Figure 3. FilmArray Integrated Sample Filtration System Concept

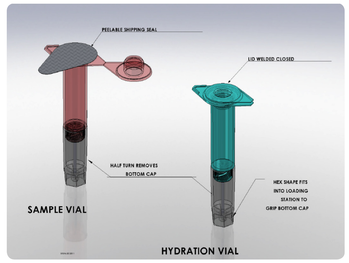
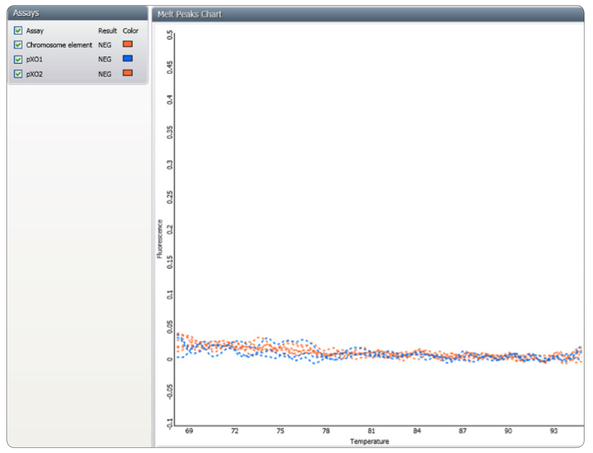
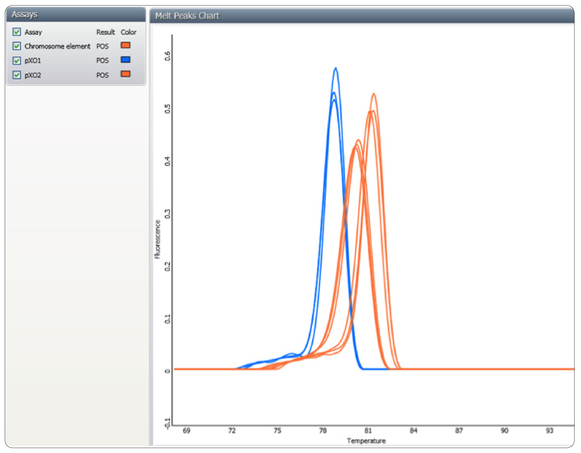


Figure 4. *B. anthracis* melt profile comparison of the filtered and unfiltered clay soil matrix.

Clay Unfiltered



Clay Filtered



CONCLUSIONS
Filtration is a necessary step when testing powder/soil samples for PCR analysis. We have shown that filtration of samples prior to injection into the FilmArray pouch reduces sample-matrix dependent inhibition with no significant reduction in sensitivity or specificity. Integration of a sample filtration device to the workflow alleviates this problem and increases the range of samples that can be tested without increasing user requirements. The integrated filter is also easily applied to alternative panels such as the Gastrointestinal Panel to allow for clean and quick loading of stool samples.

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