

REF

Kit Part No: RFIT-ASY-0094



FilmArray®

BioThreat Panel


Instructions for Use



The Symbols Glossary is provided on Page 23 of this booklet.

**For Environmental Surveillance and Research Use Only.
Not for use in diagnostic procedures.**

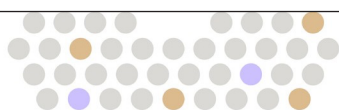
Manufactured by

 **BioFire Defense, LLC**

79 West 4500 South, Suite 14
Salt Lake City, UT 84107 USA

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INTENDED USE

The FilmArray® BioThreat Panel is a qualitative, multiplexed, nucleic acid-based test intended for use with the BioFire® FilmArray® 2.0 instrument. The FilmArray BioThreat Panel detects bacterial and viral pathogens, and toxin-encoding genes, directly from environmental samples, including liquid, powder, and surface swabs. The pathogens and toxin-encoding genes identified by the FilmArray BioThreat Panel are listed in **Table 1**. Results from the FilmArray BioThreat Panel are not intended to be used for diagnosis, treatment, or other patient management decisions.

For Environmental Surveillance and Research Use Only. Not for use in diagnostic procedures.

SUMMARY AND EXPLANATION OF THE TEST

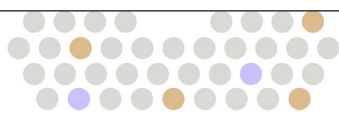
The FilmArray BioThreat Panel tests for sixteen (16) bacterial, viral, and toxin-encoding biothreats with a total of 26 assay targets (**Table 1**). Results for the FilmArray BioThreat Panel are available in about one hour.

Table 1. Targets Detected by the FilmArray BioThreat Panel

Bacteria
<i>Bacillus anthracis</i> / <i>Bacillus</i> spp.
<i>Brucella melitensis</i> / <i>Brucella</i> spp.
<i>Burkholderia mallei</i> / <i>psuedomallei</i> ¹
<i>Coxiella burnetii</i>
<i>Francisella tularensis</i>
<i>Rickettsia prowazekii</i> / <i>Rickettsia</i> spp.
<i>Yersinia pestis</i>
Viruses
<i>Zaire ebolavirus</i>
Eastern equine encephalitis (EEE) virus
<i>Marburg marburgvirus</i>
Orthopox viruses
Variola virus
Venezuelan equine encephalitis (VEE) virus
Western equine encephalitis (WEE) virus
Toxin-Encoding Genes²
Botulinum toxin-encoding genes (<i>Clostridium botulinum</i>)
Ricin toxin-encoding genes (<i>Ricinus communis</i>)

¹ The *Burkholderia mallei/psuedomallei* assay may be cross-reactive with other *Burkholderia* species.

² The FilmArray BioThreat Panel does not detect toxins directly. The panel only detects genes that encode these toxins.



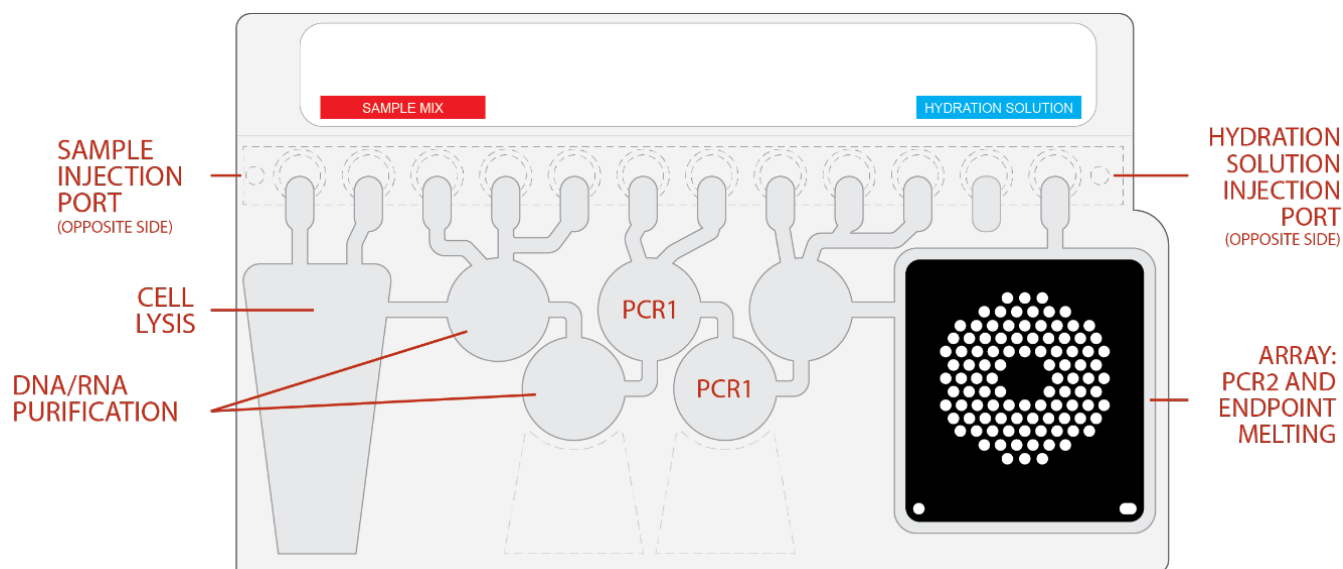
PRINCIPLE OF THE PROCEDURE

The FilmArray BioThreat Panel is a closed-system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acids from multiple pathogens within a single sample. A variety of environmental samples can be tested by the FilmArray BioThreat Panel. After sample collection and preparation, the user injects FilmArray® Hydration Solution and sample combined with FilmArray® Sample Buffer into the pouch, places the pouch into a BioFire® FilmArray® 2.0 Instrument, and starts a run.

The entire run process takes about an hour. Additional details can be found in the BioFire FilmArray 2.0 Operator's Manual.

During a run, the FilmArray system:

- Lyses the sample by agitation (bead beating) in addition to chemical lysis mediated by the Sample Buffer.
- Extracts and purifies all nucleic acids from the sample using magnetic bead technology.
- Performs nested multiplex PCR by:
 - First performing reverse transcription and a single, large volume, highly-multiplexed reaction (PCR1).
 - Then performing multiple singleplex second-stage PCR reactions (PCR2) to amplify sequences within the PCR1 products.
- Uses endpoint melting curve data to detect and generate a result for each target on the FilmArray BioThreat Panel array.



MATERIALS PROVIDED

Each FilmArray BioThreat Panel kit contains sufficient reagents to test 6 samples (Part No. RFIT-ASY-0094). Materials include:

- Individually packaged FilmArray BioThreat Panel pouches
- Single-use (1.0 mL) FilmArray® Sample Buffer tubes
- Single-use pre-filled (1.5 mL) FilmArray® Hydration Injection Vials (blue)
- Individually packaged FilmArray® Sample Injection Vials (red)
- Individually packaged Transfer Pipettes
- Instructions available online at <https://www.biofiredefense.com/product-support/filmarray-support/>
 - *FilmArray BioThreat Panel – Instructions for Use*
 - *FilmArray BioThreat Panel – Quick Guide*

NOTE: Additional documentation is available online at www.biofiredefense.com

MATERIALS REQUIRED BUT NOT PROVIDED

- BioFire® FilmArray® 2.0 System including:
 - BioFire® FilmArray® 2.0 instrument and computer with accompanying software
 - BioFire® FilmArray® Pouch Loading Station
 - FilmArray® BioThreat Panel Pouch Module Software is required to run the FilmArray BioThreat Panel and is available by request at www.biofiredefense.com if not already installed on the instrument system
- 10% bleach solution or a similar disinfectant

OPTIONAL MATERIALS

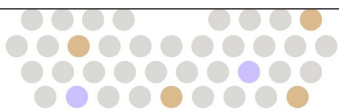
- For dry air filter samples:
 - 50 mL conical tube
 - PBS with 0.1% Triton X-100
- For powder or surface swab samples:

Option 1

- Sterile, single-use synthetic sample swab (dacron, rayon, macrofoam, polyester, etc)
- Sterile scissors, to cut handles of swabs without molded breakpoint

Option 2 (Non-sterile)

- BioFire® FilmArray® Powder or Surface Swab Accessory Kit (Part No. NGDS-ASY-0012)



WARNINGS AND PRECAUTIONS

General Precautions

1. The FilmArray BioThreat Panel is for Environmental Surveillance and Research Use Only. This product is not intended for use in diagnostic procedures.
2. This test is intended only for use in biological surveillance activities as described in the Intended Use.
3. FilmArray BioThreat Panel pouches are only for use with BioFire FilmArray 2.0 System.
4. The FilmArray BioThreat Panel is a qualitative test and does not provide quantitative values for the targets in the sample.
5. Always check the expiration date on the pouch. Do not use a pouch after its expiration date.
6. BioFire FilmArray pouches are stored under vacuum in individually wrapped canisters. To preserve the integrity of the pouch vacuum for proper operation, be sure that a BioFire FilmArray 2.0 instrument will be available and operational before unwrapping any pouches for loading.
7. Components from this kit should not be stored or used with any other kit. Discard any extra components from the kit after all pouches have been used.
8. Bleach introduced in a sample may damage nucleic acids in the sample, which may inhibit detection of the targets in the sample.

Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable clean powder-free gloves and lab coats. Protect skin, eyes, and mucus membranes. Change gloves often when handling reagents or samples.
2. Handle all samples and waste materials as if they were capable of transmitting infectious agents. Observe safety guidelines such as those outlined in:
 - CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*¹
 - CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*²
3. Follow your organization's safety procedures for handling biological samples.
4. Dispose of materials used in this test (including reagents, samples, and used buffer tubes) according to federal, state, and local regulations.
5. Sample Buffer is assigned the following classifications:

The following statements apply:

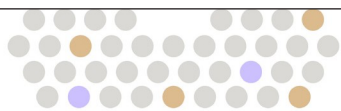
- Acute toxicity (Category 4),
- Serious eye damage (Category 1), and
- Skin irritation (Category 2).

Please refer to the appropriate Safety Data Sheet (SDS) for more information:

<https://www.biofiredefense.com/product-support/safety-data-sheets/>

6. Sample Buffer will form hazardous compounds and fumes when mixed with bleach or other disinfectants.

**WARNING: To avoid generating chlorine gas,
never add bleach to Sample Buffer or sample waste.**



7. Bleach, a recommended disinfectant, is corrosive and may cause severe irritation or damage to eyes and skin. Vapor or mist may irritate the respiratory tract. Bleach is harmful if swallowed or inhaled.
 - Eye contact: Hold eye open and rinse with water for 15-20 minutes. Remove contact lenses after the first 5 minutes and continue rinsing eye. Seek medical attention.
 - Skin contact: Immediately flush skin with plenty of water for at least 15 minutes. If irritation develops, seek medical attention.
 - Ingestion: Do not induce vomiting. Drink a glassful of water. If irritation develops, seek medical attention.
 - Please refer to the appropriate Safety Data Sheet (SDS) for more information.

Laboratory Precautions

1. Preventing Sample Contamination

Due to the sensitive nature of the FilmArray BioThreat Panel, it is important to guard against contamination of the sample and work area by carefully following the testing process outlined in this instruction document, including these guidelines:

- To avoid potential contamination, samples should be processed in a biosafety cabinet. If a biosafety cabinet is not available, a dead air box or protective shield should be used when preparing sample for testing.
- Do not handle samples or pouches in a biosafety cabinet which is used for manipulating pathogen culture.
- Prior to processing samples, thoroughly clean both the work area and the BioFire FilmArray Pouch Loading Station using a suitable cleaner such as freshly prepared 10% bleach or a similar disinfectant. To avoid residue buildup and potential damage to the sample or interference from disinfectants, wipe disinfected surfaces with water.
- Samples and pouches should be handled and/or tested one-at-a-time. Always change gloves and clean the work area between each pouch and sample.
- Use clean gloves to remove materials from bulk packaging bags and reseal bulk-packaging bags when not in use.

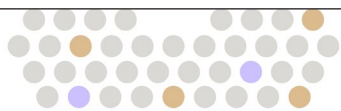
2. Preventing Amplicon Contamination

A common concern with PCR-based assays is contamination of the work area with PCR amplicon. Because the FilmArray BioThreat Panel pouch is a closed system, the risk of amplicon contamination is low, provided that the recommended procedures are followed, and pouches remain intact after the test is completed. Adhere to the following guidelines, in addition to those above, to prevent amplicon contamination:

- Discard used pouches in a biohazard container immediately after the run has completed.
- Avoid excessive handling of pouches after test runs.
- Change gloves after handling a used pouch.
- Avoid exposing pouches to sharp edges or anything that might cause a puncture.

WARNING: If liquid is observed on the exterior of a pouch, the liquid and pouch should be immediately contained and discarded in a biohazard container. The instrument and workspace must be decontaminated as described below and in the appropriate BioFire FilmArray Operator's Manual.

DO NOT PERFORM ADDITIONAL TESTING UNTIL THE AREA HAS BEEN DECONTAMINATED.



REAGENT STORAGE, HANDLING, AND STABILITY

1. Store the test kit, including reagent pouches and buffers, at room temperature (18-25°C). **DO NOT REFRIGERATE.**
2. Avoid storage of any materials near heating or cooling vents, or in direct sunlight.
3. All kit components should be stored and used together. Do not use components from one kit with those of another kit. Discard any extra components from the kit after all pouches have been consumed.
4. Do not remove pouches from their packaging until a sample is ready to be tested. Once the pouch packaging has been opened, the pouch should be loaded as soon as possible (within approximately 30 minutes). A pouch may be run when the vacuum seal is broken if hydration is successful. If hydration is not successful, the pouch must be discarded.
5. Once a pouch has been loaded, the test run should be started as soon as possible (within approximately 60 minutes). Do not expose a loaded pouch to temperatures above 40°C (104°F) prior to testing.
6. Always check the kit expiration date and do not use reagents beyond the expiration date printed on the pouch or kit.

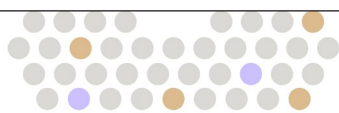
SAMPLE AND SAMPLE COLLECTION

The following table describes recommendations for sample collection, preparation, and handling that will help ensure accurate test results. Samples should be tested with the FilmArray BioThreat Panel as soon as possible. Users collecting suspicious powder and swab samples for on-site assessment should follow industry best practices in accordance with ASTM E2458-17 and their organization's procedures.

Table 2. Recommendations for Sample Collection.

	Potential Sample Types			
	Liquid Samples (including Air-into- PBS / Dry Filter / Liquid)	Powder Samples	Surface Swab Samples	ASTM E2458-17 Method B Swab
Recommended Sample Collection	Collect ~300 µL of liquid sample.	Wet a sample swab with sample buffer. Touch wetted swab to a powder pile or source, then add to Sample Injection Vial.	Drag a dry sample swab across the surface suspected of contamination, then add to the Sample Injection Vial.	Add the collected swab to the Sample Injection Vial, cutting off the tip so it remains in the vial.

NOTE: Bleach can damage organisms/nucleic acids within the sample, potentially causing interference or failure to detect the intended targets. Contact between bleach and samples during collection, disinfection, and testing procedures should be avoided.



The sample collecting instructions below are recommendations. Follow your organization's guidelines for sample collection as requirements may vary. For questions about compatibility of sample collection methods contact BioFire Defense Technical Support.

Dry Air Filter

1. Place dry air filter into a 50 mL conical tube with collection side facing away from the side of the tube.
2. Add 10 mL PBS with 0.1% Triton X-100 to the conical tube.
3. Cap tube and shake by hand for 2 minutes.

For Powder Samples:

Wet the tip of the swab. Touch the tip of sample swab to powder sample.

For Surface Swabs Samples:

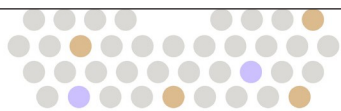
Wet the tip of the swab. Wipe the swab over sampling area using horizontal S-strokes or Z-strokes over the surface. Recommended maximum surface area should be 20 by 20 cm (8 by 8 in.).

PROCEDURE

Samples should be collected prior to opening a pouch. Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one FilmArray BioThreat pouch at a time and change gloves between samples and pouches. Particular attention should be given to the laboratory precautions noted under the *Warnings and Precautions* section to avoid potential contamination of the sample or testing area.

Once sample is added to the pouch, promptly transfer to the instrument to start the run. After the run is complete, discard the pouch in a biohazard container.

Refer to the appropriate *BioFire FilmArray 2.0 Operator's Manual* for more details.

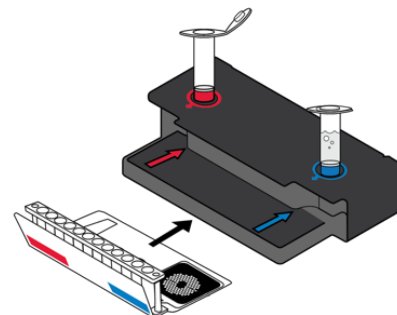


Step 1: Prepare Pouch

1. Thoroughly clean the work area and the BioFire FilmArray Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.
2. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.

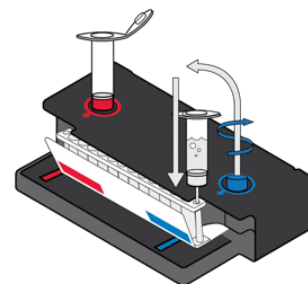
NOTE: *The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.*

3. Check the expiration date on the pouch. Do not use expired product.
4. Insert the pouch into the Pouch Loading Station, aligning the **red** and **blue** labels on the pouch with the **red** and **blue** arrows on the BioFire FilmArray Pouch Loading Station.
5. Remove the **Sample Injection Vial** from its package by tearing or cutting the notched outer packaging. Remove the clear cap from the end of the **Sample Injection Vial** (red cover). Place a **Sample Injection Vial** (with red cover) into the **red well** of the Pouch Loading Station.
6. Place a **Hydration Injection Vial** (with blue cover) into the **blue well** of the Pouch Loading Station.



Step 2: Hydrate Pouch

1. Unscrew the **Hydration Injection Vial** from the **blue cover**.
2. Remove the **Hydration Injection Vial**, leaving the **blue cover** in the Pouch Loading Station.
3. Insert the **Hydration Injection Vial** cannula into the **pouch hydration port** located directly below the **blue arrow** of the Pouch Loading Station.
4. Forcefully push down in a firm and quick motion to puncture seal until a faint “pop” is heard and there is an ease in resistance. Wait as the correct volume of Hydration Solution is pulled into the pouch by vacuum.
 - If the hydration solution is not automatically drawn into the pouch, re-insert **Hydration Injection Vial** to ensure that the seal of the **pouch hydration port** was broken. If Hydration Solution is again not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.
5. Verify that the pouch has been hydrated.
 - Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen.
 - If the pouch fails to hydrate (dry reagents appear as white pellets), re-insert **Hydration Injection Vial** to ensure that the deal of the **pouch hydration port** was broken. If Hydration Solution is still not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.



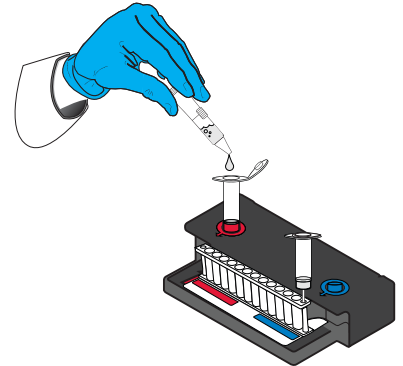
Step 3: Prepare Sample Mix

Add Sample Buffer:

1. Hold the Sample Buffer Tube with the tip facing up.
NOTE: Avoid touching the tube tip during handling, as this may introduce contamination.
2. Firmly pinch at textured plastic tab on the side of the tube until the seal snaps.
3. Invert the tube over the **Sample Injection Vial** and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

NOTE: Avoid squeezing the tube additional times. This will generate foaming, which should be avoided.

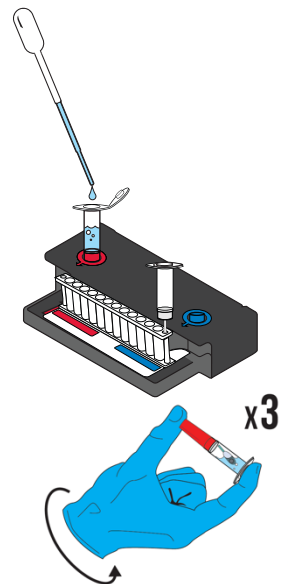
WARNING: The Sample Buffer is harmful if swallowed and can cause serious eye damage and skin irritation.



For Liquid Samples (including Air-into-PBS / Dry Filter / Liquid):

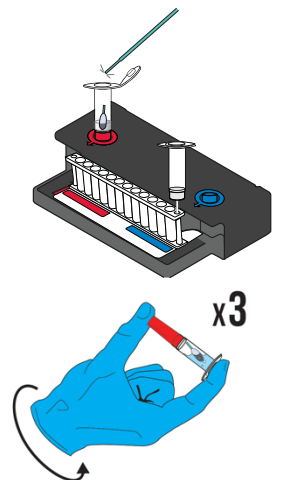
NOTE: Gently invert sample container until thoroughly mixed

1. After collecting the liquid sample, use Transfer Pipette to draw filter wash or liquid to the 3rd line (approx. 300 uL) and add to **Sample Injection Vial**.
2. Tightly close the lid of the **Sample Injection Vial** and mix sample by gently inverting **3 times**.
3. Return the **Sample Injection Vial** to the red well of the Pouch Loading Station.



For Swab Samples (including Powder / Surface):

1. After collecting the sample, place the swab into the **Sample Injection Vial** and swirl **for 5 seconds**.
2. Break the swab handle at the molded break point, leaving the tip of the swab in the **Sample Injection Vial**.
NOTE: If the swab handle does not have a molded break point, cut the tip of the swab with clean scissors, leaving the tip of the swab in the **Sample Injection Vial**.
3. Discard the handle in an appropriate waste container.
4. Tightly close lid of **Sample Injection Vial** and mix sample by gently inverting **3 times**.
5. Return **Sample Injection Vial** to red well of the Pouch Loading Station.



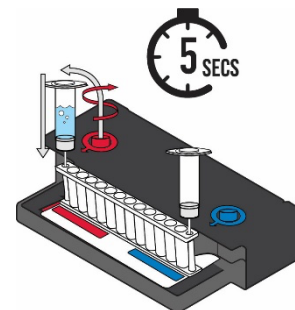
Step 4: Load Sample Mix

1. Slowly twist to unscrew the **Sample Injection Vial** from the red cover and wait for 5 seconds with the vial resting in the cover.

NOTE: Waiting **5 seconds** decreases the risk of dripping and contamination from the sample.

2. Lift the **Sample Injection Vial**, leaving the **red cover** in the well of the Pouch Loading Station, and insert the **Sample Injection Vial** into the **pouch sample port** located directly below the red arrow of the Pouch Loading Station.
3. Forcefully push down in a firm and quick motion to puncture seal (a faint “pop” is heard) and sample is pulled into the pouch by vacuum.
4. Verify that the sample has been loaded.
 - Flip the barcode label down and check to see that fluid has entered the reagent well next to the **sample loading port**.
 - If the pouch fails to pull sample from the **Sample Injection Vial**, the pouch should be discarded. Discard the **Sample Injection Vial** and the **Hydration Injection Vial** in a biohazard container. Retrieve a new pouch and repeat from *Step 1: Prepare Pouch*.
5. Screw the injection vials back into their plastic covers in the Pouch Loading Station before disposing of them in a biohazard container.
6. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the Pouch Loading Station.

NOTE: Optional added operator protection: Before removal from biosafety cabinet, run a bleach wipe, a paper towel with 10% bleach (one part bleach to nine parts water), across the top of the pouch from the **pouch hydration port** to the **pouch sample port**, and follow with a water wipe. This reduces the potential for contact with small amounts of sample mixed with Sample Buffer that may be retained at the **pouch sample port**.



Step 5: Run Pouch

The BioFire FilmArray Software includes step-by-step on-screen instructions that guide the operator through performing a run. Brief instructions for BioFire FilmArray 2.0 are given below. Refer to the BioFire FilmArray 2.0 operator's manual for more detailed instructions.

BioFire FilmArray 2.0

1. Ensure that the BioFire FilmArray 2.0 System (instrument and computer) is powered on and the software is launched.
2. Follow on-screen instructions and procedures described in the appropriate BioFire FilmArray 2.0 operator's manual to place the pouch in an instrument and enter pouch, sample, and operator information.
3. Pouch identification (Lot Number and Serial Number) and Pouch Type information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number and Pouch Type can be manually entered from the information provided on the pouch label into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

NOTE: *When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the FilmArray BioThreat Panel pouch.*

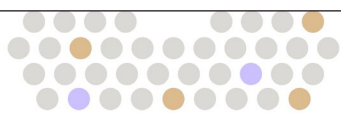
4. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.
5. Select the BT PBS v3.0 protocol.
6. Enter a username and password in the Name and Password fields.

NOTE: *The font color of the username is red until the username is recognized by the software.*

7. Review the entered run information on the screen. If correct, select Start Run. Once the run has started, the screen displays a list of the steps being performed by the instrument and the number of minutes remaining in the run.

NOTE: *The bead-beater apparatus makes an audible, high-pitched noise during the first minute of operation.*

8. When the run is finished, follow the on-screen instructions to remove the pouch, then immediately discard it in a biohazard waste container.
9. The run file is automatically saved in the BioFire FilmArray Software database, and the test report can be viewed and/or saved as a PDF file.
10. To view run data, double click on a run file, select the interpretation tab and click on an analyte for a specific assay.



QUALITY CONTROL

Process Controls

Three internal process controls are included in each pouch:

1. RNA and DNA Process Controls

The RNA and DNA Process Control assays target an RNA transcript and genomic region, respectively, from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, PCR1, dilution, PCR2, and DNA melting. Positive control results indicate that all steps carried out in the FilmArray BioThreat Panel pouch were successful.

2. PCR2 Control

The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that PCR2 was successful.

The RNA Process control assay must be positive for the FilmArray run to pass. If the RNA Process control fails, all results will be listed as 'Invalid' and the sample should be retested with a new pouch. DNA process control and PCR2 controls are included for troubleshooting purposes.

INTERPRETATION OF RESULTS

Assay Interpretation

When PCR2 is complete, the FilmArray instrument performs a DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well (for more information see the FilmArray 2.0 Operator's Manual). The FilmArray Software then performs several analyses and assigns a final assay result for every well. The steps in the analyses are described below.

Analysis of Melt Curves. The FilmArray Software evaluates the DNA melt curve for each well of the PCR2 array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (T_m) of the curve and compares it against the expected T_m range for the assay. If the software determines that the T_m falls inside the assay-specific T_m range, the melt curve is called positive. If the software determines that the melt curve is not in the appropriate T_m range, the melt curve is called negative.

Analysis of Replicates. Once positive melt curves have been identified, the software evaluates the replicates for each assay to determine the assay result. For an assay to be called positive, at least two associated melt curves must be called positive, and both T_m values must be similar. Assays that do not meet these criteria are called negative.

The FilmArray BioThreat Panel automatically interprets and returns results (Detected or Not Detected) for each pathogen. The interpretation is based on the results of one or more assays for each pathogen as shown in **Table 3**. In cases where the RNA Process control assay has failed all analyte results are reported as Invalid (**Figure 3**).

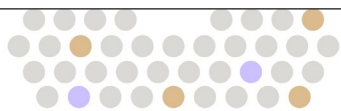


Table 3. Assay Number and Interpretation Rules for the FilmArray BioThreat Panel

Organism	No. of Assays	Assay Interpretation Rules
BACTERIA		
<i>Bacillus anthracis</i> / <i>Bacillus</i> spp.	3	See Table 4
<i>Brucella melitensis</i> / <i>Brucella</i> spp.	2	See Table 5
<i>Burkholderia mallei</i> / <i>pseudomallei</i> ¹	2	Any Positive = Detected
<i>Coxiella burnetii</i>	2	Any Positive = Detected
<i>Francisella tularensis</i>	2	Any Positive = Detected
<i>Rickettsia prowazekii</i> / <i>Rickettsia</i> spp.	2	See Table 6
<i>Yersinia pestis</i>	2	Any Positive = Detected
VIRUSES		
Ebola Zaire	1	Positive = Detected
Marburg virus	2	Any Positive = Detected
Orthopox genus virus	2	Any Positive = Detected
Variola virus	2	Both Assays Positive AND Both Orthopox Genus Virus Assays Positive = Detected
EEE Virus	1	Positive = Detected
VEE Virus	2	Any Positive = Detected
WEE Virus	1	Positive = Detected
TOXIN-ENCODING GENES		
<i>Clostridium botulinum</i>	1	Positive = Detected
<i>Ricinus communis</i>	1	Positive = Detected

¹ Cross-reactivity with other *Burkholderia* spp. may be observed.

Table 4. *Bacillus anthracis* / *Bacillus* species Calling Scheme

Assays			Results	
pXO1	pXO2	Chromosome Element	Interpretation	Call
Pos	Pos	Pos	<i>Bacillus anthracis</i>	Detected
Neg	Neg	Neg	<i>Bacillus anthracis</i>	Not Detected
Neg	Neg	Pos	Bacillus species	Detected
Neg	Pos	Neg	Bacillus species	Detected
Neg	Pos	Pos	Bacillus species	Detected
Pos	Neg	Neg	Bacillus species	Detected
Pos	Neg	Pos	Bacillus species	Detected
Pos	Pos	Neg	Bacillus species	Detected

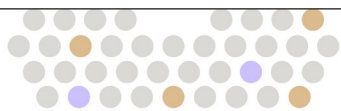


Table 5. *Brucella melitensis* / *Brucella* species Calling Scheme

Assays		Results	
Genus	Species-Specific	Interpretation	Call
Pos	Neg	<i>Brucella</i> species	Detected
Neg	Pos	<i>Brucella melitensis</i>	Detected
Pos	Pos	<i>Brucella melitensis</i>	Detected
Neg	Neg	<i>Brucella melitensis</i>	Not Detected

Table 6. *Rickettsia prowazekii* / *Rickettsia* species Calling Scheme

Assays		Results	
Genus	Species-Specific	Interpretation	Call
Pos	Neg	<i>Rickettsia</i> species	Detected
Neg	Pos	<i>Rickettsia prowazekii</i>	Detected
Pos	Pos	<i>Rickettsia prowazekii</i>	Detected
Neg	Neg	<i>Rickettsia prowazekii</i>	Not Detected

Bacteria Reporting

***Bacillus anthracis* / *Bacillus* species**

The FilmArray BioThreat Panel contains three (3) assays for the detection of *Bacillus* spp. Two (2) assays target regions on the pXO1 and pXO2 virulence plasmids, respectively, and the third targets a chromosomal region of the *Bacillus* genus. If at least one of the assays is positive, but not all three, the result is reported as *Bacillus* species Detected. If all three (3) assays are positive the result is reported as *Bacillus anthracis* Detected. See **Table 4**.

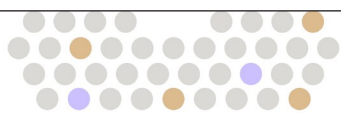
***Brucella melitensis* / *Brucella* species**

The FilmArray BioThreat Panel contains two (2) assays for the detection of *Brucella melitensis*/*Brucella* spp. One *Brucella* genus level assay and one assay specific to *Brucella melitensis*. If the *B. melitensis* assay is positive, the result is reported as *Brucella melitensis* Detected. If only the genus level assay is positive, the result is reported as *Brucella* species Detected. See **Table 5**.

Burkholderia mallei* / *pseudomallei

The FilmArray BioThreat Panel contains two (2) assays for the detection of *Burkholderia mallei* and *Burkholderia pseudomallei*. If either assay is positive the result is reported as *Burkholderia mallei/pseudomallei* Detected.

NOTE: This assay may be cross-reactive with other species in the *Burkholderia* genus.



Coxiella burnetii

The FilmArray BioThreat Panel contains two (2) assays for the detection of *Coxiella burnetii*. If either assay is positive the result is reported as *Coxiella burnetii* Detected.

Francisella tularensis

The FilmArray BioThreat Panel contains two (2) assays for the detection of *Francisella tularensis*. If either assay is positive the result is reported as *Francisella tularensis* Detected.

***Rickettsia prowazekii* / *Rickettsia* species**

The FilmArray BioThreat Panel contains two (2) assays for the detection of *Rickettsia prowazekii*. One *Rickettsia* genus level assay and one assay specific to *Rickettsia prowazekii*. If the *R. prowazekii* assay is positive, the result is reported as *Rickettsia prowazekii* Detected. If only the genus level assay is positive, the result is reported as *Rickettsia* species Detected. See **Table 6**.

Yersinia pestis

The FilmArray BioThreat Panel contains two (2) assays for the detection of *Yersinia pestis*. If either assay is positive the result is reported as *Yersinia pestis* Detected.

Virus Reporting**Ebola Zaire**

The FilmArray BioThreat Panel contains one (1) assay for the detection of Ebola Zaire. If the assay is positive the result is reported as Ebola Zaire Detected.

Marburg virus

The FilmArray BioThreat Panel contains two (2) assays for the detection of Marburg viruses. If either assay is positive the result is reported as Marburg virus Detected.

Orthopox virus genus

The FilmArray BioThreat Panel contains two (2) assays for the detection of Orthopox genus viruses. If either assay is positive the result is reported as Orthopox genus virus Detected.

Variola virus

The FilmArray BioThreat Panel contains two (2) assays for the detection of variola virus. Both variola virus assays AND both Orthopox virus genus assays must be positive for the result to be reported as Variola virus Detected.

EEE Virus

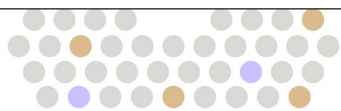
The FilmArray BioThreat Panel contains one (1) assay for the detection of EEE Virus. If the assay is positive the result is reported as EEE Virus Detected.

VEE Virus

The FilmArray BioThreat Panel contains two (2) assays for the detection of VEE Virus. If either assay is positive the result is reported as VEE virus Detected.

WEE Virus

The FilmArray BioThreat Panel contains one (1) assay for the detection of WEE Virus. If the assay is positive the result is reported as WEE virus Detected.



Toxin-Encoding Genes

Clostridium botulinum

The FilmArray BioThreat Panel contains one (1) assay for the detection of botulinum toxin-encoding genes. If the assay is positive the result is reported as *Clostridium botulinum* Detected.

NOTE: The FilmArray BioThreat Panel does not detect botulinum toxins directly.

Ricinus communis

The FilmArray BioThreat Panel contains one (1) assay for the detection of ricin toxin-encoding genes. If the assay is positive the result is reported as *Ricinus communis* Detected.

NOTE: The FilmArray BioThreat Panel does not detect ricin toxins directly.

FilmArray BioThreat Panel Test Report

The FilmArray BioThreat Panel test report is automatically displayed upon completion of a run and contains three sections: the Run Summary, the Result Summary, and the Run Details. The test report can be saved as a file or printed (see **Figure 1**).

FilmArray BioThreat Panel		BIO FIRE [®] A BIOMÉRIEUX COMPANY www.BioFireDefense.com	
Run Summary			
Sample ID:	32638-EN	Run Date:	01 Sep 2022 12:04 PM
Detected:	Orthopox genus virus	Controls:	Passed
Result Summary			
Bacteria			
Not Detected	<i>Bacillus anthracis</i>		
Not Detected	<i>Brucella melitensis</i>		
Not Detected	<i>Burkholderia mallei/pseudomallei</i>		
Not Detected	<i>Coxiella burnetii</i>		
Not Detected	<i>Francisella tularensis</i>		
Not Detected	<i>Rickettsia prowazekii</i>		
Not Detected	<i>Yersinia pestis</i>		
Viruses			
Not Detected	Ebola Zaire		
Not Detected	Marburg virus		
✓ Detected	Orthopox genus virus		
Not Detected	Variola virus		
Not Detected	EEE virus		
Not Detected	VEE virus		
Not Detected	WEE virus		
Toxins			
Not Detected	<i>Clostridium botulinum</i>		
Not Detected	<i>Ricinus communis</i>		
Run Details			
Pouch:	BioThreat Panel v2.5	Protocol:	BT PBS v3.0
Run Status:	Completed	Operator:	MJ - RktMn
Serial No.:	D07654321	Instrument:	2FA00045
Lot No.:	1234567		

Figure 1. Example of FilmArray BioThreat Panel Report

The **Run Summary** section of the test report (**Figure 1**) is displayed at the top of the page. It provides information about the run including: the Sample ID, date and time of the run, the target(s) detected, and the control results. Control results are listed as Passed, Failed, or Invalid. The Control field will display Passed only if the run completed successfully (no instrument or software errors) and the RNA Process Control assay was successful. The Control field will display Failed if the run was completed successfully (no instrument or software errors) but the RNA Process Control assay failed. If the internal control result is Failed, then the result for all of the targets on the panel are displayed as Invalid and the sample will need to be retested with a new pouch. If repeated internal control failures are observed, consider any potential inhibitory effects the sample input may have or contact BioFire Defense Technical Support for assistance. **Table 7** provides additional information for each of the possible control field results. See **Table 8** for complete result interpretation and required actions.

Table 7. Interpretation of Internal Control Field on the BioThreat Panel Test Report

RNA Process Control Result	Explanation
Pass	The run was successfully completed AND RNA Process control was successful.
Fail	The run was successfully completed BUT RNA Process Control failed.
Invalid	The RNA Process control is invalid because the run failed (typically, a software or hardware error).

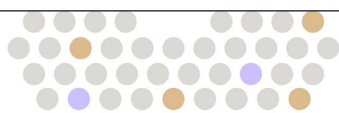
The **Results Summary** section of the test report lists each target tested as either Detected, Not Detected, or Invalid. See the Results Explanation section below for detailed information about interpretation of test results and appropriate follow-up for Invalid results.

The **Run Details** section provides additional information about the pouch including the pouch type, serial number, and lot number.

Once a run has completed, it is possible to edit the Sample ID. If this information has been changed, an additional section called **Change Summary** will be added to the test report. This Change Summary section lists the field that was changed, the original entry, the revised entry, the operator that made the change and the date that the change was made (**Figure 2**). Sample ID is the only field of the report that can be changed.

Change Summary				
Field	Changed To	Changed From	Operator	Date
¹ Sample ID	New Example Id	Old Example Id	Anonymous	24 Mar 2021

Figure 2. Change Summary Field



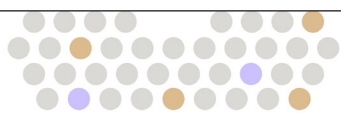
Results Explanation and Required Actions

The Results Summary section provides a complete list of all test results. Possible results include Detected, Not Detected, and Invalid. **Table 8** provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

Table 8. Interpretation of Results on the FilmArray BioThreat Panel Test Report

Results	Explanation	Report
Not Detected	<p>The run was successfully completed</p> <p>AND</p> <p>RNA Process control was successful (Pass)</p> <p>AND</p> <p>The assay(s) for the organism were NEGATIVE</p>	Results are valid.
Detected	<p>The run was successfully completed</p> <p>AND</p> <p>RNA Process control was successful (Pass)</p> <p>AND</p> <p>The assay(s) for the organism were POSITIVE</p>	Results are valid.
Fail	<p>Run completed and control failed</p> <p>OR</p> <p>Run did not complete</p>	<p>All results are invalid because the run failed.</p> <p>NOTE: <i>If any error codes are displayed, refer to the BioFire FilmArray Operator's Manual for more information. If the error persists, contact Technical Support for further instruction.</i></p> <p>Retest the sample.</p>

WARNING: Detection of three or more pathogens may indicate a possible contamination event. Therefore, it is recommended to retest a sample that has three or more pathogen detected to confirm the result. If the three or more positive results are not duplicated, contact BioFire Technical Support and discontinue testing until the test area has been decontaminated.





FilmArray BioThreat Panel



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Run Summary

Sample ID: VAL028976_0034_R19

Run Date: 13 Jun 2023

Detected: ▲ Invalid

8:42 AM

Controls: ▲ Failed

Result Summary

Bacteria

▲ Invalid	<i>Bacillus anthracis</i>
▲ Invalid	<i>Brucella melitensis</i>
▲ Invalid	<i>Burkholderia mallei/pseudomallei</i>
▲ Invalid	<i>Coxiella burnetii</i>
▲ Invalid	<i>Francisella tularensis</i>
▲ Invalid	<i>Rickettsia prowazekii</i>
▲ Invalid	<i>Yersinia pestis</i>

Viruses

▲ Invalid	Ebola Zaire
▲ Invalid	Marburg virus
▲ Invalid	Orthopox genus virus
▲ Invalid	Variola virus
▲ Invalid	EEE virus
▲ Invalid	VEE virus
▲ Invalid	WEE virus

Toxins

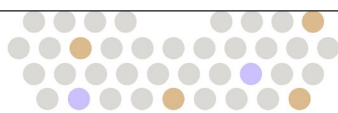
▲ Invalid	<i>Clostridium botulinum</i>
▲ Invalid	<i>Ricinus communis</i>

Run Details

Pouch: BioThreat Panel v2.5
Run Status: Completed
Serial No.: 0034_R19
Lot No.: VAL028976

Protocol: BT Blood v3.0
Operator: Im CnstVig
Instrument: 2FA00074

Figure 3. FilmArray BioThreat Panel Report with failed Internal Control and Invalid results.



CLEANING MATERIALS

This list provides items that are necessary in a laboratory to keep contamination to a minimum.

1. 10% bleach solution in a squeeze or spray bottle (1 part bleach to 9 parts water)
2. Distilled, de-ionized, sterile, or molecular grade water in a squeeze or spray bottle
3. DNAZap™ or equivalent DNA degrading system
4. Paper towels
5. Bleach wipes

DECONTAMINATION PROCEDURES

The decontamination and cleaning procedures listed are intended to limit spread of contaminants as a result of a BioThreat Panel Detected pathogen, a suspected positive pouch, or a broken or leaked pouch. A suspected positive sample is one that the user strongly suspects may be positive for an analyte on the FilmArray BioThreat Panel.

If a pouch leak or breakage occurs, change gloves and other potentially contaminated personal protective equipment (PPE). Change gloves often during the decontamination process, especially during the first steps of decontamination and before touching any clean surface. All PPE should be disposed of after decontamination.

CAUTION: It is important that contamination from leaking and/or punctured pouches be contained and cleaned immediately. Pouches that break after PCR contain amplified nucleic acid material that can contaminate future pouch runs. This material, although noninfectious, is easily spread if precautions are not taken. Very small (molecular) quantities can be amplified by PCR in future runs, which can result in false positives. Treat all broken pouches as capable of contaminating the work area.

BIOLOGICAL RISKS: If the pouch contains potentially infectious material, the risk of biohazard contamination exists in addition to sample contamination.

NOTE: Use of distilled water is recommended for cleaning.

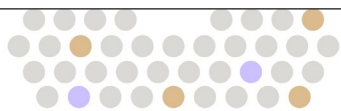
BioFire FilmArray Pouch Loading Station Decontamination

Routine cleaning of the BioFire FilmArray Pouch Loading Station includes a 10% bleach wipe followed by two water wipes before each new pouch is loaded.

In the event of work with a BioThreat Panel Detected pathogen, a suspected positive sample, or contamination from a sample spill, or a pouch leak, perform the following decontamination procedures:

1. Put on clean PPE, such as lab coat and gloves.
2. Fill a sink or bin with water and add bleach to create a 10% bleach solution.
3. Submerge the Pouch Loading Station until completely covered with bleach solution. Soak for 15 minutes.
4. Remove Pouch Loading Station from sink or bin. Replace bleach solution with distilled water.
5. Rinse the Pouch Loading Station by completely submerging in water two additional times.

Contact BioFire Defense Technical Support to obtain a replacement Pouch Loading Station, if necessary.



Decontamination Related to Detected Pathogen or Toxin-Encoding Gene, or Pouch Leakage

If a pouch was loaded with a FilmArray BioThreat Panel detected pathogen, a suspected positive sample, or if the pouch leaks, take the following precautions to avoid contamination:

1. Put on clean PPE, such as a lab coat, gloves, and eye protection.
2. Ensure no one uses the instrument or potentially contaminated areas until the decontamination is complete.
3. Decontaminate the instrument and work area and dispose of the pouch using the following steps:
 - a. Dispose of potentially contaminated gloves and put on clean gloves.
 - b. Dispose of the potentially contaminated lab coat and put on a clean lab coat.
 - c. Discard leaking pouch in biohazard container.
 - d. Change gloves.
 - e. Clean the instrument and affected work areas per the guidelines below.

CAUTION: Use only 10% bleach solution, water, and/or DNAZap to decontaminate the instrument and Pouch Loading Station.

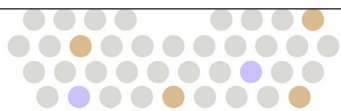
BioFire FilmArray 2.0 Instrument Decontamination

Pouch Loading Chamber Decontamination

1. Put on clean PPE, such as a lab coat and gloves.
2. Remove pouch from instrument and discard in biohazard waste container. Change gloves and any contaminated PPE.
3. Wet a paper towel with 10% bleach (one part bleach to nine parts water) and wipe the inner chamber and under the lid. Let it stand for at least 3 minutes to allow the bleach solution to react with any contaminants. Discard paper towel in biohazard waste. Change gloves.
4. Repeat Step 3 twice with fresh paper towels for a total of three bleach wipes.
5. Wet a paper towel with water and wipe the inner chamber.
6. Repeat Step 5 with fresh gloves and paper towel.

Instrument Exterior Decontamination

1. Put on clean PPE, such as a lab coat and gloves.
2. Wet a paper towel with the 10% bleach solution and wipe all exterior surfaces of the instrument, including the bottom and the bench top where the instrument had contact. Let it stand for at least 3 minutes to allow the bleach solution to react with any contaminants. Discard paper towel in biohazard waste. Change gloves.
3. Repeat Step 2 twice with fresh paper towels and clean gloves, for a total of three bleach wipes.
4. Change gloves, then wet a new paper towel with water and wipe all surfaces of the exterior of the instrument, including the bottom and the bench top where the instrument had contact.
5. Repeat Step 4 with fresh gloves.



Decontamination of Bench Tops and Other Areas

1. Put on clean PPE, such as a lab coat and gloves.
2. Spray the 10% bleach solution on the area that may have been contaminated. Let it stand for at least three minutes to allow the bleach solution to react with any contaminants on the surface.
3. Wipe the area with a clean paper towel. Change gloves.
4. Repeat Steps 2 and 3 twice, for a total of three wipes.
5. Change gloves. Spray the area with distilled water.
6. Wipe the area dry with a new paper towel. Change gloves.
7. Spray the area with DNAZap™ or an equivalent product. Follow the product's instructions for correct use. Change gloves.
8. Rinse the area by spraying it with distilled water and wiping it dry.

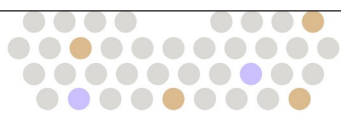
Check Function of Decontaminated Instrument

1. Test a negative sample by preparing a pouch, using water as the sample. Use distilled, sterile, or molecular grade water for the test.
2. If run is successful and all results are negative, continue using the instrument as normal.
3. If unexpected positive results are obtained or the run fails, please contact BioFire Defense Technical Support for further instructions.

Check for Environmental Contamination

After decontaminating instrument as described above, use environmental swabs to check for contamination by following the protocol below:

















1. Prepare four aliquots of 0.2 mL of molecular grade water.
2. Place one environmental swab in each aliquot and let soak for five minutes.
3. Thoroughly swab exterior of instrument and accessories, including laptop, especially areas of operator contact.
4. Return each swab to its original aliquot and mix the sample well.
5. Dispose of swabs and combine the four aliquots into one.
6. Load pouch as described in Procedure section of this document.
 - a. Load 0.2 mL of combined swabbing aliquot as the sample using Transfer Pipette, by drawing liquid up to the 2nd line.
 - b. Add sample to Sample Injection Vial.
 - c. Proceed with normal pouch loading procedure.
7. Run pouch using the BT PBS v3.0 protocol.
8. If positive result is found, repeat decontamination step, and contamination testing until no contamination is detected.
9. If problems persist, contact BioFire Defense Technical Support for further instructions.



APPENDIX A

Symbols Glossary

The following symbols can be found on labeling for the BioFire FilmArray 2.0, FilmArray BioThreat Panel kit, kit components, and throughout accompanying packaging.

ISO 7000 Graphical symbols for use on equipment – Registered Symbols					
3082 	Manufacturer	2607 	Use by date (YYYY-MM-DD)	2492 	Batch Code (Lot Number)
2493 	Catalogue Number	2498 	Serial Number	2606 	Do Not Use if Package Is Damaged
0624 	Keep Away from Sunlight	0632 	Temperature Limit	1051 	Do not re-use
1641 	Operator's Manual	0518 	Counting (Quantity)		
United Nations Globally Harmonized System of Classification and Labeling of chemicals (GHS) (ST/SG/AC.10/30)					
	Serious eye damage Category 1		Acute aquatic hazard Category 1 Long-term aquatic hazard Category 1		Acute toxicity Oral, Category 4 Skin corrosion/irritation, Category 2
Manufacture Symbols (BioFire Defense, LLC)					
	BioFire Defense Logo		FilmArray BioThreat Panel		

APPENDIX B

Contact and Legal Information

Customer and Technical Support	
Contact Us on the Web http://www.BioFireDefense.com	Contact Us by E-mail support@BioFireDefense.com
Contact Us by Mail 79 West 4500 South, Suite 14 Salt Lake City, Utah USA 84107	Contact Us by Phone 1-801-262-3592 – US and Canada 1-801-262-3592 – International
	Contact Us by Fax 1-801-447-6907



BioFire Defense, LLC
79 West 4500 South, Suite 14
Salt Lake City, UT 84107 USA

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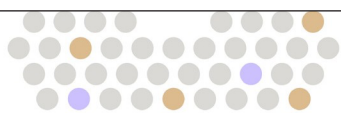
DFA2-PRT-0220-01, August 2023

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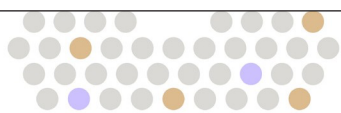
APPENDIX C

References

1. *Biosafety in Microbiological and Biomedical Laboratories*. June 2020. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf. Accessed July 27, 2023.
2. *CLSI. Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline-Fourth Edition*. 2014. CLSI document M29-A4. Wayne, PA: Clinical and Laboratory Standards Institute;
3. *Standard Practices for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biological Agents and Toxins from Nonporous Surfaces*. April 2019. ASTM Int'l,

Revision History

Version	Revision Date	Description of Revision(s)
01	August 2023	Initial Release





*For additional information regarding our products and applications,
contact BioFire Defense Customer Support.*