University of California, Davis Health System
Department of Pathology and Laboratory Medicine
Hematology/Hemostasis

BioFire Biothreat E-test Procedure #_1246__
(for the presumptive identification of Ebola Zaire virus)

Background

FilmArray Biothreat-E test from BioFire Diagnostics LLD (Salt Lake City, UT) is a multiplexed nucleic acid test used for the presumptive detection of Ebola Zaire virus in whole blood. Per manufacturer, this method has Emergency Use Authorization (EUA) from the FDA. The diagnosis of Ebola virus infection should be based on clinical signs and symptoms, exposure likelihood and other laboratory tests, and not wholly predicated on the outcome of this test.

The presumptive detection and identification of Ebola Zaire virus nucleic acids from individuals exhibiting signs and symptoms of a respiratory infection aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other management decisions

Principle of Operation

The FilmArray RP pouch is a closed system disposable that houses all the chemistry required to isolate, amplify and detect nucleic acid from multiple respiratory pathogens within a single NPS specimen. The rigid plastic component (fitment) of the FilmArray RP pouch contains reagents in freeze-dried form. The flexible plastic portion of the pouch is divided into discrete segments (blisters) which, through interactions with actuators and sensors in the FilmArray Instrument, are where the required chemical processes are carried out. The user of the FilmArray RP system loads the sample into the FilmArray RP pouch, places the pouch into the FilmArray Instrument, and starts the run. All other operations are automated.

Materials and supplies

BioFire BioThreat-E [RFIT-ASY-0122] contains sufficient reagents to test 6 specimens:

- Individually packaged FilmArray BioThreat-E pouches
- Single-use Sample Injection vials (red cap)
- Single-use Hydration Injection Vials (blue cap)
- Individually packaged Transfer Pipettes
- Individual Sample Buffer Ampoules
- Individually packaged freeze-dried Protease vials

Kits are maintained at room temperature

Open pouches must be used within 30 minutes

Do not use pouches if the outer packaging has been damaged or if the vacuum is not intact.

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 work space must be decontaminated as described in the FilmArray Operator's Manual.

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

FilmArray System including:

- FilmArray Instrument, related PC and software
- FilmArray Pouch Loading Station

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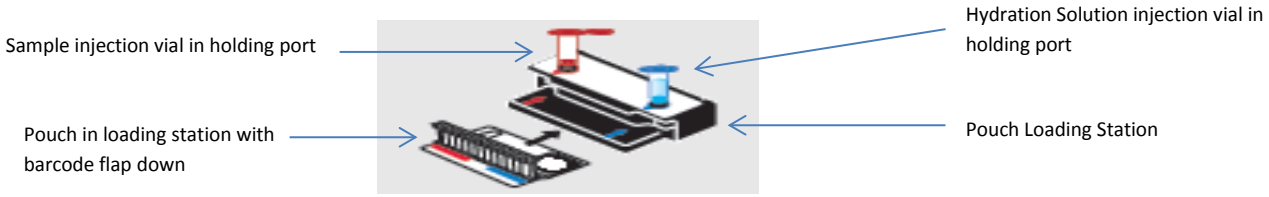
Specimen requirements

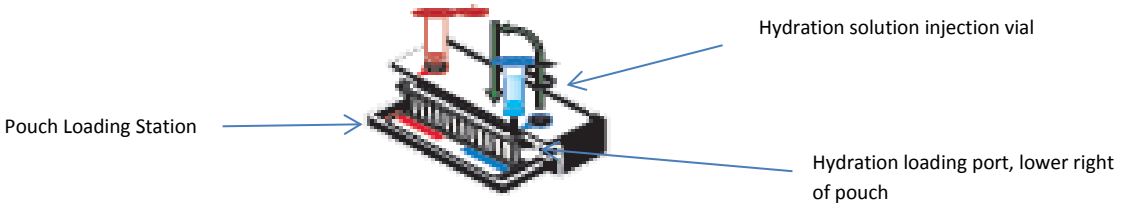
EDTA anticoagulated whole blood is specimen of choice. Heparin anticoagulated whole cannot be used for this method. Minimum volume is 200µL.

Procedure:

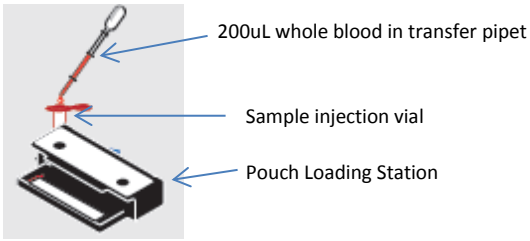
1. Turn on power to instrument and PC. From PC desktop, select FilmArray Instrument Control icon.
2. Testing personnel must be in appropriate PPE with all sample loading procedures must be performed within appropriate biosafety cabinet.
3. Vortex sample to assure adequate suspension.
4. Assure work area is clean to avoid contamination of sample: clean loading station and surrounding work space using 10% bleach, especially the sample loading area around red port, followed by an alcohol wipe.
5. Remove the FilmArray pouch from its vacuum-sealed package. Since solutions are drawn into the FilmArray RP pouch by vacuum, it is important to keep pouches in their protective packaging until the time of use. Place the FilmArray RP pouch into the FilmArray Pouch Loading Station, label facing operator. Flip the pouch label downward to observe pouch sections containing tablets.



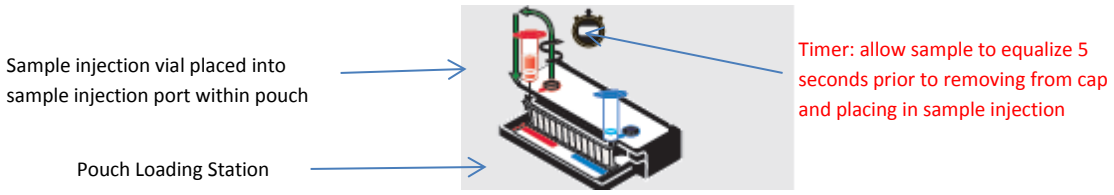
6. Place the Hydration Solution injection vial (blue cap vial) in right (blue) holder and the Sample injection vial (red cap vial) in the left (red) sample holder.
7. Place the Hydration injection vial (blue) tip into the pouch hydration port and press firmly to hydrate the tablets within the pouch.



8. Remove the Protease from vacuum sealed pouch. Remove cap. Add sample buffer from ampoule by pressing gently, but firmly. Avoid touching cap of ampoule to Protease vial. Avoid excess bubbles. Bending the upper portion of the ampoule slowly over the protease vial may yield best results for dispensing sample buffer solution into Protease vial.
9. Recap Protease vial, mix by inversion 3 times and dispense into the sample injection vial.
10. Add 200µL (second line of sample transfer pipet) of well mixed EDTA whole blood into the Protease/Buffer mixture within the sample injection vial.

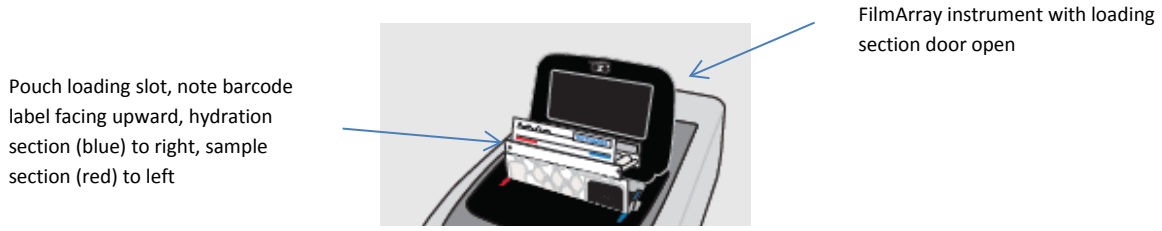


11. Return the protease/buffer/sample injection vial to the pouch loading station. Wait 5 seconds before proceeding (equalizes sample within the injection vial to avoid blood from dropping once the cap is removed.
12. Using a counter-clockwise motion, remove the sample injection vial from the cap and hold over cap to allow any blood drops to remain in the cap and not on work space.
13. Place the blunt end of the injection syringe into the sample port located on the left side of the pouch. Holding the pouch and the syringe body firmly, press down on syringe to puncture the sample port to load pouch with sample.



14. Remove injection vials from pouch and transfer the pouch to the instrument and initiate a run.

    a. With pouch barcode label facing upwards and holding the hard plastic edges of the pouch, glide the soft place portion of the pouch into the loading area (be sure that door is all the way open to allow access to pouch slot).



    b. Gently press the hard plastic portion of the pouch into place (will hear a snap when properly loaded)
    c. Using barcode reader, scan pouch lot (move cursor to pouch lot number area if necessary).
    d. Using barcode reader or PC keyboard, enter sample ID and/or patient name
    e. Using mouse, select “Blood” protocol
    f. Close loading area door.
    g. Using mouse, move cursor to Operator ID, enter ID and appropriate password
    h. Using mouse, locate cursor over “Start run” icon and right click or hit enter.

NOTE: If the pouch does not slide into the instrument easily, gently push the lid of the instrument back to be sure that it is completely open.

NOTE: The barcode cannot be scanned prior to placing the pouch in the instrument.
A “Cannot scan now” message will be displayed.

15. To aid in proper insertion of the pouch in the instrument, the FilmArray Instrument Control application provides on-screen animations illustrating the steps needed to start the run. The clock on the right side

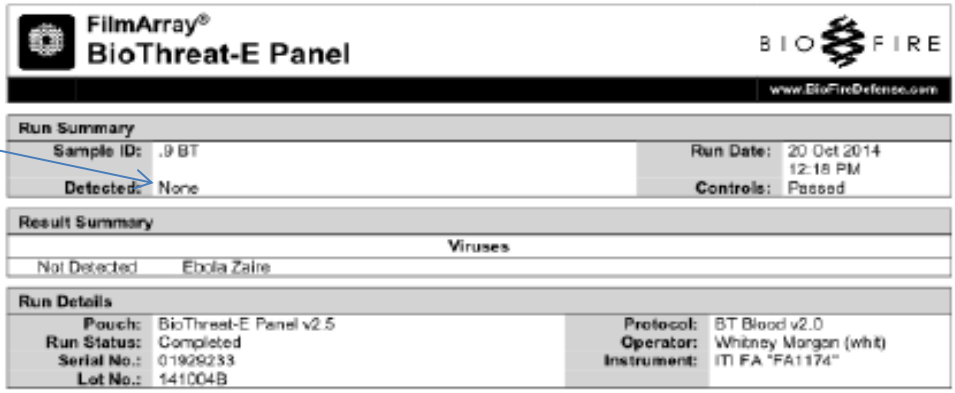
of PC screen will provide estimated time of completion as both a count-down timer and time of day completion

16. View results on the test report at the completion of the run.
17. Once testing is complete, a visual cartoon will illustrate the removal of pouch from loading section.
    a. A pop-up save menu will appear, select Cancel
    b. Using PC mouse, move cursor to folder tab located on right side of screen to open up View Report.
    c. Select "Print All" icon located atop the result page.
    d. Assure that internal pouch control processes (PCR2 control and RNA Process Control) have passed and record results under E-Threat Sample QC in the Daily Log binder (see instructions below under QC). If any of the two control processes do not pass, then the test failed and needs to be repeated.
18. Record appropriate actions in BioFire Daily Log and report results in LIS.
19. Discard all syringes into red biohazard sharps containers. Discard all vials and transfer pipettes into biohazard waste containers.
20. Clean loading station and surrounding work space using 10% bleach, followed by an alcohol wipe.
21. If no additional testing is anticipated (e.g. Respiratory Viral Panel), then power off instrument and PC.

FilmArray RP Test Report

The FilmArray BioThreat-E assay 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 PDF or printed. If no organism is detected, then "None" will display. If organism is detected, then "Ebola Zaire" will be displayed.

Detected Test Report: results of test are reported here



Control Field

The Control field on the test report will display "Passed", "Failed", or "Invalid". The Control field will display "Passed" only if the run completed successfully (no instrument or software errors) and the pouch control assay (RNA Process Control) was successful. The Control field will display "Failed" if the run was completed successfully (no instrument or software errors) but the pouch control assay failed. The Controls field will display "Invalid" if the run did not complete (typically indicates a software or hardware error). If the control result is "Failed" or "Invalid", then the result for Ebola Zaire is displayed as "Invalid" and the sample will need to be retested with a new pouch. See table below for troubleshooting.

NOTE: QC raw data will be recorded as a mean to continuous monitor instrument function (see below)

Control Result	Explanation	Action Required	Outcome
Passed	The run was successfully completed AND The pouch control was successful.	None	Report the results provided on the test report.
Failed	The run was successfully completed BUT The pouch control (RNA Process Control) failed.	Repeat the test using a new pouch.	Accept the results of the repeat testing. If the error persists, contact Technical Support for further instruction.
Invalid	The control is invalid because the run did not complete. (Typically this indicates a software or hardware error).	Note any error codes displayed during the run and the Run Status field in the Run Details section of the report. Refer to the FilmArray Operator's Manual or contact Technical Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another instrument.	Accept the valid results of the repeat testing. If the error persists, contact Technical Support for further instruction.

Assay Interpretation

The Results Summary – Interpretations section lists the Ebola Zaire result. Possible results include "Detected", Not "Detected", and "Invalid".

Result	Explanation	Action
Detected	The run was successfully completed AND The pouch control was successful (Passed) AND The assay(s) associated with the interpretation were positive based on the following requirements for at least 2 of the 3 assay replicates: • a positive melt curve, and • the Tm for the melt data were within the assay specific limits, and • the Tm for the melt data were within 1°C of each other.	None. Report results.
Not Detected	The run was successfully completed AND The pouch control was successful (Passed) AND The assay(s) associated with the interpretation were negative (did not meet the requirements for a positive assay described in Detected).	None. Report results.
Invalid	The run did not complete successfully (Aborted, Incomplete, Instrument Communication Error, Instrument Error, or Software Error) OR The pouch control was not successful (Failed)	See Table 1, *Interpretation of Controls Field on FilmArray Report*, for instruction.

Laboratory Precautions

This test is for *in vitro* diagnostic use under the Emergency Use Authorization only. Local, state, and national public health agencies (for example, county and state health departments or the U.S. Centers for Disease Control and Prevention (CDC)) should be notified of any patient suspected to have Ebola Virus Disease (EVD). Confirmatory testing at the state/local public health laboratory or at CDC is necessary for positive detection results and may be necessary for negative detection results. Laboratories should consult with local, state or national public health officials on any positive detection OR no detection (negative) EVD test result on the need for additional testing and appropriate transportation of specimens.

Preventing amplicon contamination:

A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicon. Because the FilmArray BioThreat-E pouch is a closed system, the risk of amplicon

contamination is low provided that pouches remain intact after the test is completed. Adhere to the following guidelines to prevent amplicon contamination:

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

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Quality and Process Controls

A. **Internal pouch RNA Process Control**
   a. The RNA Process Control assay targets an RNA transcript 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, 1st stage PCR, dilution, 2nd stage PCR and DNA melting. A positive control result indicates that all steps carried out in the FilmArray BT pouch were successful. The process control assay must be positive for the test run to pass. If the control fails, the Control field of the test report (upper right hand corner) will display "Failed" and all results will be listed as "Invalid". If the control fails, the sample should be retested using a new pouch.
   b. Record raw data for each QC process control
      i. From Browse run tab, select Quick Search to load last 100 samples
      ii. Using mouse, place cursor over desired sample, and right click mouse
         1. Select View Control Results
         2. Record TM (melting temperature) for the sample on QC log sheet

B. External Quality Control
   a. Two levels of commercially available control material will be run on the first non-holiday Monday of each month.
   b. Two level commercial controls are available from Maine Molecular Quality Controls, Inc (MMQCI) product: FilmArray® Ebola Control Panel M251. Material is stored at 2 - 8°C. To run QC:
      i. Allow the control to be tested to come to room temperature (18° – 25°C).
      ii. Prepare and hydrate a pouch according to FilmArray® Instructions.
      iii. Immediately before use, vortex the control.
      iv. Analyze the FilmArray® RP Control M211 v1.1 and FilmArray® Ebola Control Panel M251 as you would (including the Protease step) a patient sample using procedure steps previously noted.
      v. Discard after completion of test.
      vi. Record results onto QC worksheet located in PM/QC binder. An example of a package insert for two level MMQCI QC expected results:

Lot Performance Verification

Prior to implementing a new lot or new shipment of same lot material, the material must be tested using the external QC material as described above. The new lot or new shipment pouch QC must be within acceptable limits prior to clinical use.

Limitations of procedure

- Negative test results do not preclude infection with Ebola virus and should not be the sole basis of patient treatment/management decisions.
- This test should not be used to test specimens from asymptomatic individuals.
- This product can only be used with the FilmArray Instrument.
- This test is a qualitative test and does not provide a quantitative value for the virus in the sample.
- This test has been evaluated for use with human whole blood and urine material only.
- All results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms.
- Interpretation of results from the FilmArray BioThreat-E test must account for the possibility of false-positive and false negative results.
- False positive results may occur from cross-contamination by target organism, their nucleic acids, or from PCR amplicon.
- Failure to follow assay procedures may lead to false-negative results.
- Inhibitors present in the samples may lead to false-negative results.
- Specimens from patients who have received therapeutics or vaccines based on nucleic acid sequences derived from Ebola Zaire virus may exhibit false positive or other confounding test results.

Validation of performance characteristics

NOTE: The FilmArray BioThreat-E test (v2.5) 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 (v2.2-2.4).

Analytical Sensitivity/Limit of Detection (LoD)

An estimated Limit of Detection of 6.00 E+05 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. Confirmation of the Limit of Detection was done by confirming the 95% detection rate with 20 replicates.

Imprecision: Imprecision performed for within-day (N=1) and day-to-day for (N=3) QC material demonstrated 100% agreement for Ebola Zaire and negative samples. Imprecision performed on QC material for between testing personnel demonstrated 100% agreement for Ebola Zaire and negative samples.

Accuracy**:** Ebola negative samples (N=3) from shed EDTA blood in Hematology lab, positive and negative Quality Control material (two levels, run three consecutive days) obtained from MMCQI and shed blood EDTA whole blood enriched with positive control material at different concentrations (N=3) were tested. There was 100% agreement between negative samples, negative control, positive control, and positive control-enriched whole blood.

References

BioFire Diagnostics, LLC FilmArray Respiratory Panel (RP) CE-IVD Instruction Booklet 0088 BioFire Diagnostics, LLC | 390 Wakara Way, Salt Lake City, Utah 84108, USA | 1-801-736-6354

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Procedure History

Date	Written/ Revised By	Revision	Approval Date	Approved By
12/2014	B. Gosselin	New procedure	12/12/2014	L Howell, MD
1/2015	B. Gosselin	Monthly QC and Daily log revision		