Guidelines for Laboratory Verification of Performance of the FilmArray® BioThreat-E Test

TECHNICAL
::: NOTE

Purpose

The Clinical Laboratory Improvement Amendments (CLIA) establishes quality standards for all U.S. laboratory testing to ensure the accuracy and reliability of patient test results, regardless of where the test is performed. The CLIA regulations include a requirement for verifying the performance specifications of unmodified tests cleared or approved by the US FDA. The FilmArray® BioThreat-E Test is for use under an Emergency Use Authorization (EUA). Laboratories should determine the depth of verification needed, in coordination with the Laboratory Director, based on current EUA regulatory guidelines.

This document provides examples of verification procedures to assist your laboratory in developing a protocol for the verification of FilmArray BioThreat-E Test performance on BioFire® FilmArray® 2.0 Systems. Each verification scheme has been designed to provide positive and negative tests for Zaire ebolavirus and may be easily modified or expanded to meet specific criteria. Day-to-day variation is evaluated by testing each control type on two separate days. To evaluate operator-to-operator variability, multiple laboratory technicians may test the same controls. In addition, testing patient samples for verification or to evaluate matrix effects on the performance of the FilmArray BioThreat-E Test should be done under the guidance of the Laboratory Director, but is not described here.

The Laboratory Director is ultimately responsible for ensuring that verification procedures meet the appropriate standards for CLIA and any other applicable laboratory accrediting agencies.

Refer to the *FilmArray BioThreat-E Instructions for Use* for detailed information regarding the FilmArray BioThreat-E Test.

Performance Verification: Overview

Two different examples of performance verification procedures are described (Table 1): a Simple Protocol using the Maine Molecular FilmArray® Ebola Control Panel and a Clinical Matrix Protocol for testing controls in either blood or urine. These protocols are examples of procedures to assist your laboratory in developing a protocol for the

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verification of FilmArray BioThreat-E Test performance on BioFire FilmArray 2.0 Systems.

A BioFire FilmArray System is defined as one or more BioFire FilmArray 2.0 instruments that are connected to and controlled by a single computer system. If the Laboratory Director chooses not to perform the verification protocol on each individual instrument, it is advised that test replicates be evenly distributed among the instruments.

Clinical/patient samples may be used in place of, or in addition to, the verification examples described here to assess clinical sensitivity and sample matrix effects as part of the performance verification. Other commercially available controls may also be used for performance verifications. Ensure the controls contain the entire Zaire ebolavirus genome. Controls which include only partial Zaire ebolavirus genome regions may not be compatible with the FilmArray BioThreat-E Test.

Table 1. Overview of Verification Protocols

Verification Protocol	Required Control Material	Number of Tests	Expected Positive Results	Expected Negative Results	Days of Testing
Simple Protocol	Maine Molecular FilmArray Ebola Control Panel	8	4	4	2
Clinical Matrix Protocol	Irradiated Zaire ebolavirus	8	4	4	2

Performance Verification: Materials

Table 2 lists the materials that may be needed to perform the verification procedures, depending on the chosen protocol.

Table 2. Materials for Verification Procedures

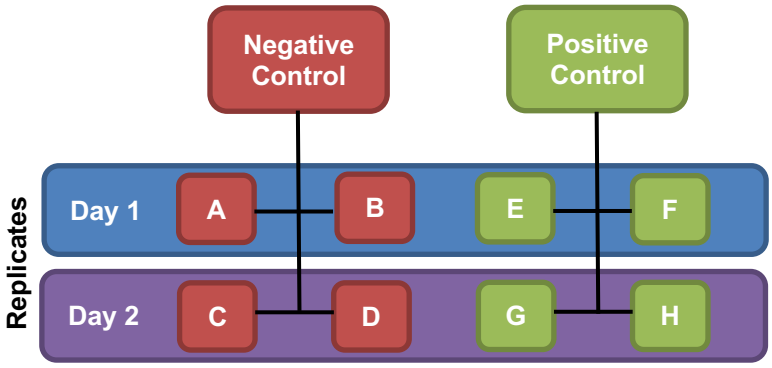
Material	Part Number
FilmArray BioThreat-E Test Kit (6 tests)	RFIT-ASY-0122
Maine Molecular FilmArray Ebola Control Panel	M251
Irradiated Zaire ebolavirus [1, 2]	Multiple sources
Human blood or urine	Various sources are appropriate

[1] When selecting material for use, ensure that the material contains the entire viral genome. Material that does not contain the entire viral genome may not be compatible with the FilmArray BioThreat-E test.
[2] To obtain consistent results, it is important that the material be accurately quantified.

Performance Verification Protocols

Each described protocol can be followed to test a total of eight controls, providing four positive results and four negative results on a single instrument. An overview of the testing scheme is shown in Figure 1. The estimated total time for completion for each verification protocol is two days for a BioFire FilmArray System configured with a single BioFire FilmArray 2.0 instrument. The testing protocol may be modified to run more samples per day based on the number of instruments in the BioFire FilmArray System and should be determined by individual laboratory requirements.

Figure 1. Single Instrument Verification Workflow



Simple Protocol

The Simple Protocol uses the Maine Molecular Ebola Control Panel. The panel includes single use positive and negative controls in a non-infectious matrix and are ready to use directly with the FilmArray BioThreat-E Test. Refer to the *Maine Molecular FilmArray Ebola Control Panel Product Insert* for details regarding the use of the control panel with the FilmArray BioThreat-E Test.

Day 1

1. Obtain two FilmArray Ebola Positive Controls, two FilmArray Ebola Negative Controls, and four FilmArray BioThreat-E Tests.

   NOTE: *The replicates for each day should be run by the same operator to evaluate run-to-run variation or different operators to evaluate operator-to-operator variation.*

   NOTE: *To minimize the risk of cross-contamination, set up only one test at a time.*

2. Follow the instructions on the *FilmArray BioThreat-E Quick Guide* to prepare and run two Negative Controls on the FilmArray BioThreat-E Test using the Whole Blood Sample Mix instructions (replicates A and B in Figure 1).
3. Follow the instructions on the *FilmArray BioThreat-E Quick Guide* to prepare and run two Positive Controls on the FilmArray BioThreat-E Test using the Whole Blood Sample Mix instructions (replicates E and F in Figure 1).

   NOTE: *Controls should be prepared according to the Whole Blood Sample Mix instructions. Do not prepare controls using the Urine Sample Mix instructions.*

Day 2

To evaluate day-to-day variation, test the remaining Negative External Controls (replicates C and D in Figure 1) and Positive External Controls (replicates G and H in Figure 1) by repeating Steps 1-3 of Day 1.

Result Interpretation

The FilmArray BioThreat-E Test Report will automatically be displayed upon completion of each run and can be printed or saved as a PDF file. The test result will be listed in the Result Summary. For Positive Controls, the expected result is 'Ebola Zaire Detected'. For Negative Controls, the expected result is 'Ebola Zaire Not Detected'. Refer to the *FilmArray BioThreat-E Instructions for Use* for additional information on how to interpret the Test Report.

Clinical Matrix Protocol

The Clinical Matrix Protocol is designed to evaluate FilmArray BioThreat-E Test performance with clinical matrix. The external controls for testing are contrived using irradiated Zaire ebolavirus in the laboratory's medium of choice (whole blood or urine). Note that viral genomic RNA should not be used to contrive controls as the RNA may degrade.

Day 1

1. Prepare the Negative Control by aliquoting 1.5mL of the selected medium into a sterile tube.
2. In a new tube, prepare the Positive Control by diluting the irradiated Zaire ebolavirus to a final concentration of 1.0×10^5 copies/mL in 1.5mL of the same selected medium.

   NOTE: *To obtain consistent results, it is important that the material be accurately quantified.*

3. Obtain four FilmArray BioThreat-E Tests.

   NOTE: *The replicates for each day should be run by the same operator to evaluate run-to-run variation or different operators to evaluate operator-to-operator variation.*

   NOTE: *To minimize the risk of cross-contamination set up only one test at a time.*

4. Follow the instructions on the *FilmArray BioThreat-E Quick Guide* to prepare and run two Negative Controls on the FilmArray BioThreat-E Test using the appropriate Sample Mix instructions (replicates A and B in Figure 1).
5. Follow the instructions on the *FilmArray BioThreat-E Quick Guide* to prepare and run two Positive Controls on the FilmArray BioThreat-E Test using the appropriate Sample Mix instructions (replicates E and F in Figure 1).
6. Refrigerate (2-8˚C) remaining volume of controls for up to three days for the evaluation of day-to-day variation.

Day 2

To evaluate day-to-day variation, test two additional negative control replicates (replicates C and D in Figure 1) and two additional positive control replicates (replicates G and H in Figure 1) by repeating Steps 3-5 as in Day 1.

Result Interpretation

The FilmArray BioThreat-E Test Report will automatically be displayed upon completion of each run and can be printed or saved as a PDF file. The test result will be listed in the Result Summary. For Positive Controls, the expected result is 'Ebola Zaire Detected'. For Negative Controls, the expected result is 'Ebola Zaire Not Detected'. Refer to the *FilmArray BioThreat-E Instructions for Use* for additional information on how to interpret the Test Report.

Conditions of Authorization

For all precautions, warnings, and limitations, reference the *FilmArray BioThreat-E Instructions for Use.*

- The FilmArray BioThreat-E has not been FDA cleared or approved
- The FilmArray BioThreat-E has been authorized by FDA under an Emergency Use Authorization for use by CLIA Moderate and High Complexity Laboratories;

- This test has been authorized only for the detection of Ebola Zaire virus (detected in the West Africa outbreak in 2014) and not for any other viruses or pathogens.
- The FilmArray BioThreat-E is only authorized for the duration of the declaration that circumstances exist justifying the authorization of the emergency use of in vitro diagnostics for detection of Ebola Zaire virus under section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

Technical Support Contact Information

BioFire Defense is dedicated to providing the best customer support available. For any questions or concerns, please contact the BioFire Technical Support team for assistance.

General Information
Email: support@biofiredefense.com
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BioFire® FilmArray® Instrument
Verification Record

TECHNICAL NOTE

Instrument Serial #: _____

FilmArray Biothreat-E Kit Part #: _____ Lot #: _____

Organism/Sample Source and Lot #: _____

_____

Organism	Was the Organism Detected?	No. Positive	No. Negative	No. Days Tested	No. Users	Patient Samples Tested?
Ebola Zaire	☐ Yes ☐ No					

Reviewed by:

_____

Signature Date