Guidelines for Laboratory Verification of Performance of the BioFire® COVID-19 Test

TECHNICAL NOTE

Purpose

The Clinical Laboratory Improvement Amendments (CLIA) establishes quality standards for all 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 FDA. The BioFire® COVID-19 Test is for use under an Emergency Use Authorization (EUA). Due to the urgency of COVID-19 testing, 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 BioFire® COVID-19 Test performance on FilmArray® 2.0 and FilmArray® Torch Systems. Each verification scheme has been designed to provide positive and negative tests for SARS-CoV-2 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 BioFire® COVID-19 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 *BioFire® COVID-19 Test Instructions for Use* for detailed information regarding the BioFire® COVID-19 Test.

Technical Note

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Performance Verification: Overview

Three different examples of performance verification procedures are described: (1) a Simple Protocol using the BIOFIRE® SHIELD™ Control Kit for the BioFire® COVID-19 Test, (2) an Upper Respiratory Protocol for testing upper respiratory specimen procedures, and (3) a Lower Respiratory Protocol for testing lower respiratory specimen procedures. These protocols are examples of procedures to assist your laboratory in developing a protocol for the verification of BioFire® COVID-19 Test performance on FilmArray® 2.0 and FilmArray® Torch Systems.

A FilmArray® System is defined as one or more FilmArray® instruments or modules 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 schemes 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 SARS-CoV-2 genome. Controls which include only partial SARS-CoV-2 genome regions may not be compatible with the BioFire® COVID-19 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	BIOFIRE® SHIELD™ Control Kit for the BioFire® COVID-19 Test	8	4	4	2
Upper Respiratory Protocol	Inactivated SARS-CoV-2 Virus	8	4	4	2
Lower Respiratory Protocol	Inactivated SARS-CoV-2 Virus	8	4	4	2

Performance Verification: Materials

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

Table 2. Materials for Verification Procedures

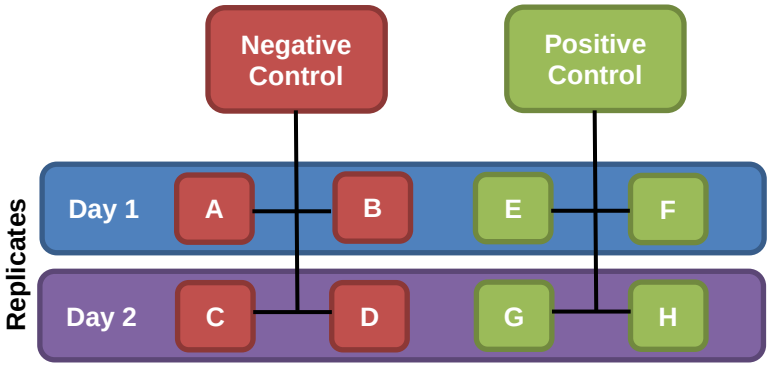
Material	Part Number
BioFire® COVID-19 Test Kit (6 tests)	423745
BioFire® COVID-19 Test Kit (30 tests)	423744
BioFire® COVID-19 Test Sample Swab Kit (for lower respiratory samples)	424063
BIOFIRE® SHIELD™ Control Kit for the BioFire® COVID-19 Test (6 positive controls)	424062
Inactivated SARS-CoV-2 Isolate[a]	BEI Resources NR-52286 or equivalent

[a] BioFire Defense recommends the use of inactivated virus when contriving controls. Viral genomic RNA may degrade and give inaccurate results.

Performance Verification Protocols

All described protocols 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 example is two days for a FilmArray® 2.0 or FilmArray® Torch System configured with a single module. The testing schemes may be modified to run more samples per day based on the number of instruments in the FilmArray® System and should be determined by individual laboratory requirements.

Figure 1. Single Instrument Verification Workflow



Simple Protocol

The Simple Protocol uses the BIOFIRE® SHIELD™ Control Kit for the BioFire® COVID-19 Test. Positive controls included in the BIOFIRE® SHIELD™ Control Kit contain freeze-dried synthetic RNA transcripts designed to be amplified by the BioFire® COVID-19 Test. The amplified products produce melting temperatures distinct from amplified products from SARS-CoV-2, which mitigates the risk of potential contamination. The Positive and Negative External Control pouch protocols are required when performing testing with the BIOFIRE® SHIELD™ Control Kit.

Day 1

1. Obtain two BIOFIRE® SHIELD™ COVID-19 Positive External Control Vials and four BioFire® COVID-19 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 BIOFIRE® SHIELD™ *Control Kit for the BioFire® COVID-19 Test - Quick Guide* to prepare and run two Negative External Controls on the BioFire® COVID-19 Test (replicates A and B in Figure 1).
3. Follow the instructions on the BIOFIRE® SHIELD™ *Control Kit for the BioFire® COVID-19 Test - Quick Guide* to prepare and run two Positive External Controls on the BioFire® COVID-19 Test (replicates E and F in Figure 1).

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 BioFire® COVID-19 Test External Control Report will automatically be displayed upon completion of each run and can be printed or saved as a PDF file. The report will indicate whether the control 'Passed', or 'Failed'. Refer to the *BIOFIRE® SHIELD™ Control Kit for the BioFire® COVID-19 Test – Instructions for Use* for additional information on how to interpret the report.

Upper Respiratory Protocol

The Upper Respiratory Protocol is designed to evaluate BioFire® COVID-19 Test performance with upper respiratory procedures. The external controls for testing are contrived using inactivated SARS-CoV-2 virus in the laboratory's medium of choice. Transport medium, sterile normal saline, or sterile PBS may be used to contrive the controls. Alternatively, known negative upper respiratory clinical specimens may also be used to contrive the controls. Prior to using clinical specimens, confirm they are negative using the BioFire® COVID-19 Test. SARS-CoV-2 genomic RNA should not be used to contrive controls as the RNA may degrade.

Day 1

1. Prepare the negative control by aliquoting 2.0mL of the selected medium (transport medium, sterile normal saline, or sterile PBS) into a sterile tube.
2. In a new tube, prepare the positive control by diluting the SARS-CoV-2 material to a final concentration of 1.6×10^3 genomic equivalent copies/mL in 2.0mL of the same selected medium.

   NOTE: To obtain consistent results, it is important that the SARS-CoV-2 material be accurately quantified.
3. Obtain four BioFire® COVID-19 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 in the *BioFire® COVID-19 Upper Respiratory Quick Guide* to prepare and run two negative control replicates on the BioFire® COVID-19 Test (replicates A and B in Figure 1).
5. Follow the instructions in the *BioFire® COVID-19 Upper Respiratory Quick Guide* to prepare and run two positive control replicates on the BioFire® COVID-19 Test (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 BioFire® COVID-19 Test Report will automatically be displayed upon completion of each run and can be printed or saved as a PDF file. The overall SARS-CoV-2 test result will be listed in the Result Summary. For positive controls, the overall result should return a 'Detected' result. For negative controls, the overall result should return a 'Not Detected' result. Refer to the

BioFire® COVID-19 Test Quick Guide for additional information on how to interpret the report.

Lower Respiratory Protocol

The Lower Respiratory Protocol is designed to evaluate BioFire® COVID-19 Test performance with lower respiratory procedures. The external controls for testing are contrived using inactivated SARS-CoV-2 virus in sterile saline. Alternatively, known negative clinical specimens may also be used to contrive the controls. However, the protocol may need to be modified due to the difficulty in accurately measuring some types of lower respiratory specimens. Prior to using clinical specimens, confirm they are negative using the BioFire® COVID-19 Test. SARS-CoV-2 genomic RNA should not be used to contrive controls as the RNA may degrade.

Day 1

1. Prepare the negative control by aliquoting 2.0mL of sterile normal saline into a sterile tube.
2. In a new tube, prepare the positive control by diluting the SARS-CoV-2 material to a final concentration of 5.0×10^4 genomic equivalent copies/mL in 2.0mL of sterile saline and mix well.

   NOTE: To obtain consistent results, it is important that the SARS-CoV-2 material be accurately quantified.
3. Obtain four BioFire® COVID-19 Tests and four BioFire® COVID-19 Sample Swabs.

   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 in the *BioFire® COVID-19 Lower Respiratory Quick Guide* to prepare and run two negative control replicates on the BioFire® COVID-19 Test (replicates A and B in Figure 1).
5. Follow the instructions in the *BioFire® COVID-19 Lower Respiratory Quick Guide* to prepare and run two positive control replicates on the BioFire® COVID-19 Test (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 BioFire® COVID-19 Test Report will automatically be displayed upon completion of each run and can be printed or saved as a PDF file. The overall SARS-CoV-2 test result and results for each individual assay will be listed in the Result Summary. For positive controls, the overall result should return a

'Detected' result. For negative controls, the overall result should return a 'Not Detected' result. Refer to the *BioFire® COVID-19 Test Quick Guide* for additional information on how to interpret the report.

Expanding the Protocol

The protocols described above can be expanded to include more replicates per day, more replicates per operator, and/or more instruments per BioFire® System.

Verification of Replaced, Repaired or New Instruments

If it becomes necessary to verify the performance of a loaner or repaired instrument, the above protocol may serve as a guideline but should be verified by the Laboratory Director. The Laboratory Director should determine the appropriate number of replicates to test. Two positive replicates and two negative replicates following the Day 1 testing plan may be sufficient.

Conditions of Authorization

For all precautions, warnings, and limitations, reference the BioFire® COVID-19 Test Instructions for Use.

- The BioFire® COVID-19 Test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- The BioFire® COVID-19 Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of the BioFire® COVID-19 Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.

Technical Support Contact Information

BioFire Defense is dedicated to providing the best customer support available. If you have any questions or concerns about this process, please contact the FilmArray Technical Support team for assistance.

General Information
Email: support@biofiredefense.com
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