

REF

423745 (6 pack test)
423744 (30 pack test)
423748 (6 pack (+) control)

BioFire® **COVID-19 Test** Instructions for Use

IVD
Rx Only



The Symbols Glossary is provided on Page 31 of this booklet.

For use under an Emergency Use Authorization (EUA) only

Please visit us at www.biofireshield.com/covid-19test

Manufactured by
 BioFire Defense, LLC

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INTENDED USE

The BioFire® COVID-19 Test is a nested multiplexed real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs in transport media from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to U.S. laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests, and in U.S. laboratories certified under CLIA to perform high complexity tests, or in similarly qualified non-U.S. laboratories.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swabs in transport media during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The BioFire COVID-19 Test is intended for use by laboratory personnel who have received specific training on the use of the FilmArray® 2.0 and/or the FilmArray® Torch Instrument Systems. The BioFire COVID-19 Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

For In Vitro Diagnostic Use.

SUMMARY AND EXPLANATION OF THE TEST

The BioFire® COVID-19 Test is a qualitative test on the FilmArray® 2.0 or FilmArray® Torch systems for the detection of the 2019 coronavirus (SARS-CoV-2) RNA in nasopharyngeal swabs (NPS) in transport media. Internal controls are used to monitor all stages of the test process. The BioFire® COVID-19 Test External Control Kit (+) includes positive external control material and may be used for quality control and laboratory verification.

PRINCIPLE OF THE PROCEDURE

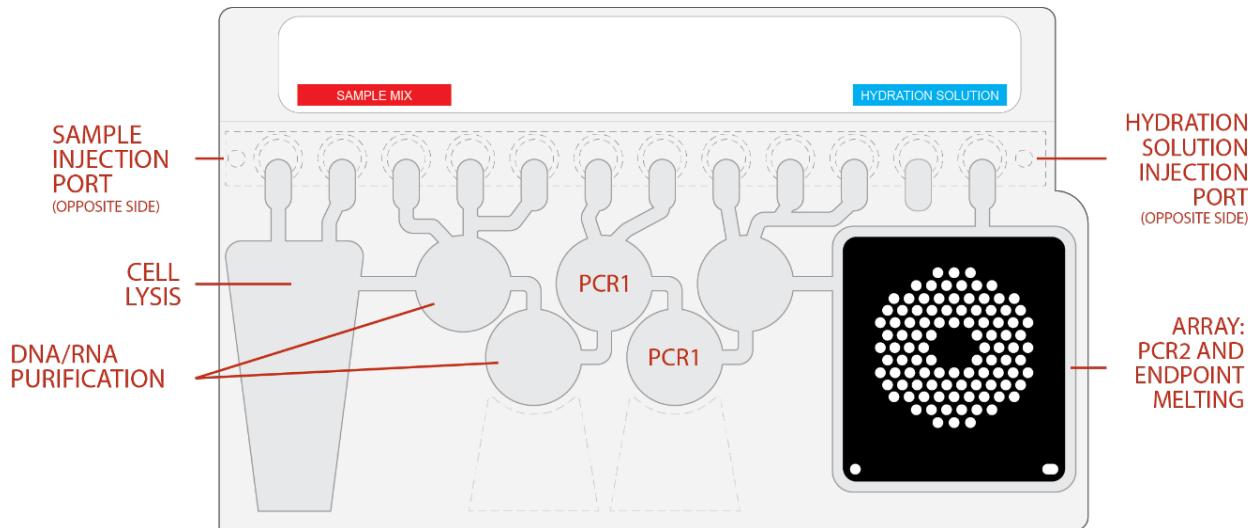
The BioFire COVID-19 Test is a closed system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from the SARS-CoV-2 virus within a single NPS specimen. After sample collection, the user injects hydration solution, and sample combined with sample buffer into the pouch, places the pouch into a FilmArray instrument, and starts a run. The entire run process takes about 50 minutes. Additional details can be found in the appropriate FilmArray operator's manual.

During a run, the FilmArray® system:

- Lyses the sample by agitation (bead beading).
- 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, 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 assay on the BioFire COVID-19 Test.



MATERIALS PROVIDED

Each BioFire COVID-19 Test Kit contains sufficient reagents to test 6 samples (6-test kit; 423745) or 30 samples (30-test kit; 423744):

- Individually-packaged BioFire COVID-19 Test pouches
- Single-use (1.0 mL) Sample Buffer tubes
- Single-use pre-filled (1.5 mL) Hydration Injection Vials (blue)
- Single-use Sample Injection Vials (red)
- Individually-packaged Transfer Pipettes
- Instructions and Documents
 - *BioFire COVID-19 Test – Quick Guide*

Each BioFire COVID-19 Test External Control Kit (+) contains sufficient reagents for six positive control runs (6-control kit; 423748). Negative controls may be run using only the BioFire COVID-19 Test with no additional materials as described in BioFire COVID-19 Test External Control Procedure.

- Individually-packaged BioFire COVID-19 Test External Control (+) Vials
- Instructions and Documents
 - *BioFire COVID-19 Test External Control Kit – Quick Guide*

NOTE: Optional verification protocol (for laboratory verification which may not use External Control Kit) is available online at www.biofiredefense.com/covid-19test



MATERIALS REQUIRED BUT NOT PROVIDED

- FilmArray system including:
 - FilmArray® 2.0/Torch Instrument Systems and accompanying software
 - FilmArray® Pouch Loading Station
- 10% bleach solution or a similar disinfectant
- Transport media or 0.9% saline solution (for External Control Testing)
- BioFire COVID-19 Additional Documentation
 - *BioFire COVID-19 Test – Patient Fact Sheet*
 - *BioFire COVID-19 Test – Healthcare Provider Fact Sheet*

NOTE: Additional labeling documents are available online at www.biofiredefense.com/covid-19test

WARNINGS AND PRECAUTIONS

General Precautions

1. For *in vitro* diagnostic (IVD) use under Emergency Use Authorization only.
2. Positive results are indicative of the presence of SARS-CoV-2 RNA.
3. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
4. BioFire COVID-19 Test pouches are only for use with FilmArray 2.0 and FilmArray Torch systems.
5. BioFire COVID-19 External Control Kit (+) is only for use with FilmArray 2.0 and FilmArray Torch systems.
6. Always check the expiration date on the pouch. Do not use a pouch after its expiration date.
7. 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 FilmArray instrument/module will be available and operational before unwrapping any pouches for loading.
8. Bleach introduced in a sample may damage nucleic acids in the sample, which may lead to a false negative result.
9. If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable clean powder-free gloves. 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
 - Refer to Interim Laboratory Safety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
3. Follow your institution's safety procedures for handling biological samples.



4. Dispose of materials used in this assay, including reagents, samples, and used buffer tubes, according to federal, state, and local regulations.
5. Sample Buffer is assigned the following classifications:
 - Acute toxicity (Category 4),
 - Serious eye damage (Category 1), and
 - Skin irritation (Category 2).

Please refer to the BioFire COVID-19 Test Safety Data Sheet (SDS) for more information.

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

WARNING: 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 Organism Contamination

Due to the sensitive nature of the BioFire COVID-19 Test, 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:

- Do not handle samples or pouches in a biosafety cabinet which is used for SARS-CoV-2 culture or immunofluorescence testing.
- Prior to processing samples, thoroughly clean both the work area and the 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 false positive results caused by contamination of the work area with PCR amplicon. Because the BioFire COVID-19 Test pouch is a closed system, the risk of amplicon contamination is low if 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 or sample injection vials to sharp edges or anything that might cause a puncture.
- Change gloves after loading the External Control (+) material.
- Clean thoroughly after loading the External Control (+) material to avoid contamination with the External Control (+).

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 FilmArray operator's manual.

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

Precation Related to Public Health in the United States

Local, state, and federal regulations for notification of reportable disease are continually updated and include a number of organisms/viruses for surveillance and outbreak investigations.^{3,4} Laboratories are responsible for following their state and/or local regulations and should consult their local and/or state public health laboratories for isolate and/or clinical sample submission guidelines.

REAGENT STORAGE, HANDLING, AND STABILITY

1. Store the test and control kit, including reagent pouches and provided buffers, at room temperature (15-30°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).
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.



SAMPLE REQUIREMENTS

See below for the recommended requirements for sample collection, preparation, and handling that will help ensure accurate test results.

| | |
|------------------------------|---|
| Specimen Type | Nasopharyngeal Swab (NPS) collected according to standard technique and immediately placed in 1-3 mL of transport media. |
| Minimum Sample Volume | 0.3 mL (300 µL) |
| Transport and Storage | <p>Samples should be processed and tested with the BioFire COVID-19 Test as soon as possible.</p> <p>If storage is required, samples can be held:</p> <ul style="list-style-type: none">• At room temperature for up to 4 hours (15-25°C)• Refrigerated for up to 3 days (2-8°C)• Frozen ($\leq -15^{\circ}\text{C}$ or $\leq -70^{\circ}\text{C}$) for up to 30 days |

NOTE: NPS samples should not be centrifuged before testing.

NOTE: Bleach can damage organisms/nucleic acids within the specimen, potentially causing false negative results. Contact between bleach and specimens during collection, disinfection, and testing procedures should be avoided.

BIOFIRE® COVID-19 TEST PROCEDURE

Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one BioFire COVID-19 Test pouch at a time and change gloves between samples and pouches. 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 BioFire COVID-19 Test Quick Guide or the appropriate FilmArray operator's manual for more details.

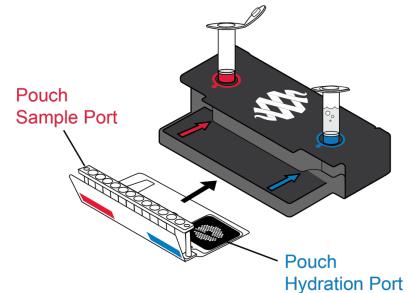
Step 1: Prepare Pouch

1. Thoroughly clean the work area and the 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.

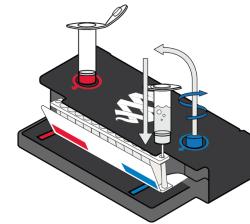


3. Check the expiration date on the pouch. Do not use expired products.
4. Insert the pouch into the FilmArray Pouch Loading Station, aligning the red and blue labels on the pouch with the red and blue arrows on the FilmArray Pouch Loading Station.
5. Place a **Sample Injection Vial** (with red cover) into the **red well** of the FilmArray Pouch Loading Station.
6. Place a **Hydration Injection Vial** (with blue cover) into the **blue well** of the FilmArray 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 FilmArray Pouch Loading Station.
3. Insert the **Hydration Injection Vial** cannula tip into the **pouch hydration port** located directly below the blue arrow of the FilmArray 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, repeat Step 2 to verify 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), repeat Step 2 to verify that the seal 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

1. Thoroughly mix the NPS sample by vortex or inversion.
2. Use the Transfer Pipette provided in the test kit to draw the sample to the third line (approximately 0.3 mL) of the Transfer Pipette.
3. Add the sample to the **Sample Injection Vial**.
4. Discard the Transfer Pipette in a biohazard waste container.

NOTE: DO NOT use the Transfer Pipette to mix the sample once it is loaded into the Sample Injection Vial.

5. Add Sample Buffer to the **Sample Injection Vial**.
 - Hold the Sample Buffer Tube with the tip facing up.



NOTE: Avoid touching the tube tip during handling, as this may introduce contamination.

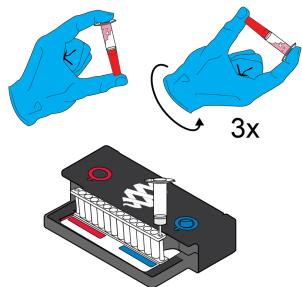
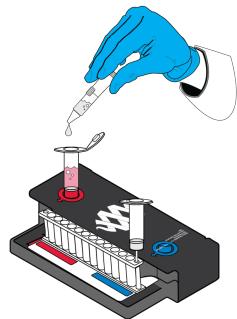


- Firmly pinch at textured plastic tab on the side of the tube until the seal snaps.
- Invert the tube over the red-capped **Sample Injection Vial** and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

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

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

6. Tightly close the lid of the **Sample Injection Vial**.
7. Remove the **Sample Injection Vial** from the FilmArray Pouch Loading Station and invert the vial at least 3 times to mix.
8. Return the **Sample Injection Vial** to the red well of the FilmArray Pouch Loading Station.

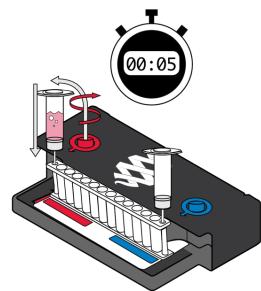


Step 4: Load Sample Mix

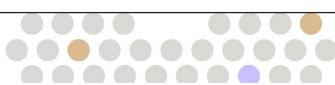
1. Slowly twist to unscrew the **Sample Injection Vial** from the red cover and wait for 5 seconds with the vial resting in the cover.

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 FilmArray Pouch Loading Station, and insert the **Sample Injection Vial** cannula tip into the **pouch sample port** located directly below the red arrow of the FilmArray 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. Retrieve a new pouch and repeat from Step 1: Prepare Pouch.
5. Discard the **Sample Injection Vial** and the **Hydration Injection Vial** in a biohazard sharps 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 FilmArray 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 hydration port to the 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 sample injection port.



Step 5: Run Pouch

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

FilmArray 2.0

1. Ensure that the 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 FilmArray 2.0 operator's manual to place the pouch in an instrument, enter pouch, sample, and operator information.
3. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol, 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 BioFire COVID-19 Test 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 and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The BioFire COVID-19 Test has a single NPS2 protocol available from the drop down list.
6. Enter a user name and password in the Name and Password fields.

NOTE: The font color of the user name is red until the user name 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 can be heard as a 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 FilmArray database, and the test report can be printed, 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 a specific assay result.



FilmArray Torch

1. Ensure that the FilmArray Torch system is powered on.
2. Select an available Module (instrument) on the touch screen or scan the barcode on the FilmArray pouch using the barcode scanner.
3. Pouch identification (Lot Number and Serial Number), Pouch Type, and Protocol 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, Pouch Type, and Protocol 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 BioFire COVID-19 Test 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. Insert the pouch into the available Module (instrument).

Ensure that the pouch fitment label is lying flat on top of pouch and not folded over. As the pouch is inserted, the Module (instrument) will grab onto the pouch and pull it into the chamber.

6. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The BioFire COVID-19 Test has a single NPS2 protocol available from the drop down list.
7. Enter operator user name and password, then select Next.

NOTE: The font color of the user name is red until the user name is recognized by the software.

8. 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 Module (instrument) and the number of minutes remaining in the run.

NOTE: The bead-beater apparatus can be heard as a high-pitched noise during the first minute of operation.

9. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.
10. The run file is automatically saved in the FilmArray database, and the test report can be viewed, printed, and/or saved as a PDF file.



QUALITY CONTROL

Process Controls

Two process controls are included in each pouch:

1. RNA Process Control

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, PCR1, dilution, PCR2, and DNA melting. A positive control result indicates that all steps carried out in the BioFire COVID-19 Test 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.

Both control assays must be positive for the test run to pass. If controls fail, the sample should be retested using a new pouch.

Monitoring Test System Performance

The FilmArray software will automatically fail the run if the melting temperature (Tm) for either the RNA Process Control or the PCR2 Control is outside of an acceptable range (80.3-84.4°C for the RNA Process Control and 73.8-78.2°C for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices.^{5,6} Refer to the appropriate FilmArray operator's manual for instructions on obtaining control assay Tm values. The PCR2 Control is used in several FilmArray pouch types (e.g., RP2, BCID, GI, ME) and can therefore be used to monitor the system when multiple pouch types are used on the same FilmArray system or instrument.



BIOFIRE® COVID-19 TEST EXTERNAL CONTROLS

External Controls

For quality control and laboratory test verification, BioFire Defense provides an optional external positive assayed control kit to monitor the performance of *in vitro* laboratory nucleic acid testing procedures for the qualitative detection of the BioFire COVID-19 Test performed on FilmArray 2.0 and FilmArray Torch systems. Offered separately, the BioFire COVID-19 Test External Control (+) kit is a surrogate control material comprised of dried synthetic RNA in buffer and stabilizer, supplied in an External Control Vial that is used directly with the BioFire COVID-19 Test. The BioFire COVID-19 Test External Control (+) Kit contains six (6) BioFire COVID-19 Test External Control (+) Vials. The RNA in the external control includes RNA segments to monitor whether the PCR primers for each SARS-CoV-2 assay are present for both stages of the nested PCR.

The BioFire COVID-19 Test External Control (+) Kit contains no biological hazards and is 100% non-infectious. This control is stored at 15-30°C. To run a positive external control, reference BioFire COVID-19 Test External Control Procedure (+) below or BioFire COVID-19 External Control Kit (+) Quick Guide. To run a negative external control, use the BioFire COVID-19 Test and reference BioFire COVID-19 External Control Procedure (-) below.

An optional verification protocol may be obtained from the BioFire Defense Product Support webpage www.biofiredefense.com/covid-19test. Customers may also use these BioFire COVID-19 Test External Control Kit (+) or an alternate Quality Control material for verification testing.

Good laboratory practice recommends running positive and negative external controls regularly. Evaluation of external controls is recommended prior to using a new shipment or new lot of BioFire COVID-19 Test Kits. Evaluation of external controls is also recommended when there is a new operator, and following replacement/repair of a FilmArray® 2.0 or FilmArray® Torch system.

External controls may also be used in initial laboratory validations of the FilmArray 2.0 or FilmArray Torch system used with the BioFire COVID-19 Test in accordance with appropriate federal, state, and local guidelines or accreditation requirements, as applicable.

It is ultimately the responsibility of each laboratory to determine the frequency and type of material used for external control testing as part of the laboratory's Quality Control program.

Information on how to obtain optional external control material is posted on the BioFire Defense webpage.

BioFire COVID-19 Test External Control Kit (+)

Part Number: 423748

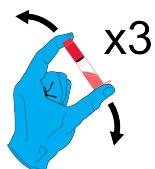
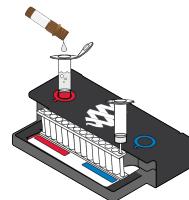
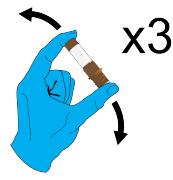


BioFire® COVID-19 Test External Control Procedure (+)

1. Follow Step 1 and Step 2 from the BioFire COVID-19 Test Procedure to prepare and hydrate the pouch.
2. Use the Transfer Pipette provided in the test kit to draw the transport media or saline to the third line (approximately 0.3mL) of the Transfer Pipette. Add to the **Sample Injection Vial**.
3. Using clean gloves, obtain a Sample Buffer Tube from the BioFire COVID-19 Test Kit.
4. Uncap the **External Control Vial (+)** and place the cap on a clean surface (a paper towel may be used).
5. Add Sample Buffer to the **External Control Vial (+)**.
 - Hold the Sample Buffer Tube tip facing up and firmly pinch at textured plastic tab on the side of the tube until the seal snaps.
 - Invert the Sample Buffer tube over the uncapped **External Control Vial (+)** and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

NOTE: Avoid generating excessive foam.

6. Recap the **External Control Vial (+)** and mix by gently inverting three (3) times.
7. Pour the rehydrated **External Control (+)** into the **Sample Injection Vial** and immediately dispose of the **External Control Vial (+)**.
 - Change gloves.
8. Tightly close lid of **Sample Injection Vial** and mix by gently inverting at least three (3) times.
9. Return **Sample Injection Vial** to **red well** of Pouch Loading Station.
10. Continue at Step 4 of the BioFire COVID-19 Test Panel Procedure to load the pouch and run it on the FilmArray.



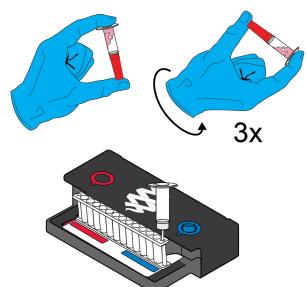
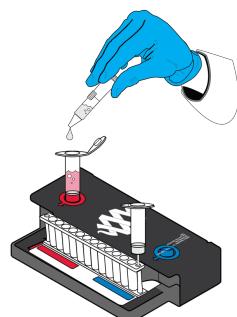
BioFire® COVID-19 Test External Control Procedure (-)

1. Follow Step 1 and Step 2 from the BioFire COVID-19 Test Procedure to prepare and hydrate the pouch.
2. Use the Transfer Pipette provided in the test kit to draw the transport media or saline to the third line (approximately 0.3mL) of the Transfer Pipette. Add to the **Sample Injection Vial**.
3. Using clean gloves, obtain a Sample Buffer Tube from the BioFire COVID-19 Test Kit.
4. Add Sample Buffer to the **Sample Injection Vial**.
 - Hold the Sample Buffer Tube tip facing up and firmly pinch at textured plastic tab on the side of the tube until the seal snaps.
 - Invert the Sample Buffer Tube over the **Sample Injection Vial** and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.



NOTE: Avoid generating excessive foam.

5. Tightly close the lid of the **Sample Injection Vial**.
6. Remove the **Sample Injection Vial** from the FilmArray Pouch Loading Station and invert the vial at least three (3) times to mix.
7. Return the **Sample Injection Vial** to the red well of the FilmArray Pouch Loading Station.
8. Continue at Step 4 of the BioFire COVID-19 Test Procedure to load the pouch and run the FilmArray.



INTERPRETATION OF RESULTS

The BioFire COVID-19 Test consists of three independent and non-overlapping assays targeting two SARS-CoV-2 open reading frame sequences: ORF1ab and ORF8. The target of each assay is shown in Table 1 below. The assays are designed to detect SARS-CoV-2 specifically. Detection of SARS-CoV-2 is based on the combined results of the three assays as described below.

Table 1. Gene targets for assays on the BioFire COVID-19 Test.

| Assay Name | SARS-COV-2 Genomic Region |
|-------------|---------------------------|
| SARS-CoV-2a | ORF1ab |
| SARS-CoV-2d | ORF1ab |
| SARS-CoV-2e | ORF8 |

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 appropriate FilmArray 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 of the curve is within the assay specification T_m range, the melt curve is called positive. If the software determines that the T_m of the 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 three replicates for each assay to determine the assay result. For an assay to be called positive, at least two of the three associated melt curves must be called positive, and the T_m for at least two of the three positive melt curves must be similar (within 1.0°C). Assays that do not meet these criteria are called negative.



Organism Interpretation

SARS-CoV-2

The BioFire COVID-19 Test contains three different assays (SARS-CoV-2a, SARS-CoV-2d, SARS-CoV-2e) for the detection of SARS-CoV-2. The FilmArray Software interprets each of these assays independently (as described above) and the results are combined as a final test result for the virus. For interpretation results, refer to Table 2.

If two or more assays are ‘Detected’, the result on the test report will be SARS-CoV-2 ‘Detected’. If all assays are ‘Not Detected’, the result on the test report will be SARS-CoV-2 ‘Not Detected’. If only one of the assays is ‘Detected’, the test report result will be SARS-CoV-2 ‘Equivocal’. If an ‘Equivocal’ result is obtained, retest the original sample using a new pouch. If the result of the retest is ‘Equivocal’ or ‘Detected’, the overall interpretation will be ‘Detected’. If the retest is ‘Not Detected’, seek confirmatory testing. In cases where either or both of the control assays have failed, all results are reported as ‘Invalid’ and retesting is required.

Table 2. Interpretation Rules for the BioFire COVID-19 Test

| SARS-CoV-2 Interpretation | Assay Results | Action |
|---------------------------|--|---|
| Detected | 3/3 Assays ‘Detected’ 2/3 Assays ‘Detected’ | Report the Results |
| Equivocal | 1/3 Assays ‘Detected’ | Retest the original sample and report the results of the retest. If the result of the retest is ‘Equivocal’ or ‘Detected’, the overall interpretation will be ‘Detected’. If the retest is ‘Not Detected’, seek confirmatory testing. |
| Not Detected | 0/3 Assays ‘Not Detected’ | Report the Results |
| Invalid | Invalid | Retest the original sample. If repeated errors occur, contact the BioFire Defense Customer Support Team. Refer to Table 3 for more information. |



BioFire® COVID-19 Test Report

The BioFire COVID-19 test report is automatically displayed upon completion of a run and can be printed or saved as a PDF file. Each report contains a Run Summary, a Result Summary, and a Run Details section.

| BioFire® COVID-19 Test v1.0 | | BIO FIRE | www.BioFireDefense.com |
|--|--------------------|---------------------------|------------------------|
| Run Summary | | | |
| Sample ID: Example Report | | | |
| Detected: | SARS-CoV-2 | Run Date: | 31 Dec 2019 8:00 AM |
| Equivocal: | None | Internal Controls: | Passed |
| Result Summary | | | |
| Viruses | | | |
| <input checked="" type="checkbox"/> Detected | SARS-CoV-2 | | |
| <input checked="" type="checkbox"/> Detected | SARS-CoV-2a | | |
| <input checked="" type="checkbox"/> Detected | SARS-CoV-2d | | |
| <input checked="" type="checkbox"/> Detected | SARS-CoV-2e | | |
| Run Details | | | |
| Pouch: | COVID-19 Test v1.0 | Protocol: | NPS2 v3.2 |
| Run Status: | Completed | Operator: | Anonymous |
| Serial No.: | 01234567 | Instrument: | FA0000 |
| Lot No.: | 012345 | | |

Run Summary

The **Run Summary** section of the test report provides the Sample ID, time and date of the run, internal control results, and an overall summary of the test results. If the SARS-CoV-2 interpretation is ‘Detected’, it will be listed in the ‘Detected’ field. If all of the assays are ‘Not Detected’ then ‘None’ will be displayed in the Detected field. Internal controls are listed as ‘Passed’, ‘Failed’, or ‘Invalid’. Table 3 provides additional information for each of the possible internal control field results.



Table 3. Interpretation of Internal Controls Field on the BioFire COVID-19 Test Report

| Internal Controls Result | Explanation | Action |
|--------------------------|---|--|
| Passed | The run was successfully completed AND Both pouch controls were successful. | None Report the results provided on the test report |
| Failed | The run was successfully completed BUT At least one of the pouch controls (RNA Process Control and/or PCR2 Control) failed. | Repeat the test using a new pouch. If the error persists, contact BioFire Defense Customer Support for further instruction. |
| Invalid | The controls are 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 appropriate FilmArray operator's manual or contact BioFire Defense Customer Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another instrument. |

Result Summary

The **Result Summary** section of the test report lists the result for the overall target and each individualized assay result. Possible results for each assay are ‘Detected’, ‘Equivocal’, ‘Not Detected’, or ‘Invalid’. Table 4 provides an explanation for each interpretation and any follow-up necessary to obtain a final result. The SARS-CoV-2 target and the three associated assays are listed in the Results Summary section. According to the result for the target, ‘Detected’, ‘Not Detected’, ‘Equivocal’, or ‘Invalid’ will be indicated to the left of the target name. According to the result for each associated assay, ‘Detected’, ‘Not Detected’, or ‘Invalid’ will be indicated to the left of each assay name.



Table 4. Reporting of Results and Required Actions

| SARS-CoV-2 Results | Explanation | Action |
|---------------------|---|---|
| Detected | The run was successfully completed AND The pouch controls were successful (Passed) AND Two or three assays for the virus were 'Detected' (i.e., met the requirements for a positive result described in the Assay Interpretation section above) | Report results. |
| Not Detected | The run was successfully completed AND The pouch controls were successful (Passed) AND The three assays for the virus were 'Not Detected' (i.e., did not meet the requirements for a positive result described in the Assay Interpretation section above) | Report results. |
| Equivocal | The run was successfully completed AND The pouch controls were successful (Passed) AND Only one of three assays was 'Detected' for the virus. The combination of 'Detected' and 'Not Detected' assay results were inconclusive | Retest the original sample using a new pouch and report the results of the retest. If the retest is 'Equivocal' or 'Detected', report the results as 'Detected'. If the result is 'Not Detected' seek confirmatory testing. |
| Invalid | The pouch controls were not successful (Failed) OR The run was not successful (Run Status displayed as: Aborted, Incomplete, Instrument Error or Software Error) | See Table 3, Interpretation of Internal Controls Field on the BioFire Test Report for instruction. |



Run Details

The **Run Details** section provide additional information about the run including: pouch information (type, lot number, and serial number), run status (Completed, Incomplete, Aborted, Instrument Error, Instrument Communication Error, or Software Error), the protocol that was used to perform the test, the identity of the operator that performed the test, and the instrument used to perform the test.

Change Summary

It is possible to edit the Sample ID once a run has completed. 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. 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 | 14 Dec 2019 |

Analysis of BioFire COVID-19 Test External Control (+) Assays

The BioFire COVID-19 External Control (+) passes when all three SARS-CoV-2 assays are ‘Detected’. Positivity is evaluated by opening the report and confirming that ‘Detected’ is indicated to the left of each of the three assay names listed in the Result Summary.

If any of the three SARS-CoV-2 assays have a ‘Not Detected’ result, the External Control (+) fails and should be repeated. If the failure persists, contact BioFire Defense Customer Support for further instruction. Refer to Table 5 for interpreting the report Result Summary.

Laboratories may decide to perform negative control testing on the system. In this case, after testing negative material (e.g., transport media or saline), the user should open the report and confirm that ‘Not Detected’ is indicated to the left of all three assay names listed in the Result Summary. If any of the three SARS-CoV-2 assays have a ‘Detected’ result, the External Control (-) fails and should be repeated after a thorough cleaning of the area. If the error persists, contact BioFire Defense Customer Support for further instruction.



Table 5. BioFire COVID-19 Test External Control (+) Interpretation of Results

| Result Interpretation | Result Analysis | Action |
|---|---|--|
| External Control (+) Passes | Result Summary ✓ Detected SARS-CoV-2 ✓ Detected SARS-CoV-2a ✓ Detected SARS-CoV-2d ✓ Detected SARS-CoV-2e | Report the results. |
| BioFire COVID-19 External Test Control (+) Failed Result | Result Summary ↔ Equivocal SARS-CoV-2 ✓ Detected SARS-CoV-2a Not Detected SARS-CoV-2d Not Detected SARS-CoV-2e Result Summary Not Detected SARS-CoV-2 Not Detected SARS-CoV-2a Not Detected SARS-CoV-2d Not Detected SARS-CoV-2e | Repeat External Control (+) Testing. If the error persists, contact BioFire Defense Customer Support for further instruction. |
| BioFire COVID-19 External Control (+) Invalid Result | Result Summary ▲ Invalid SARS-CoV-2 ▲ Invalid SARS-CoV-2a ▲ Invalid SARS-CoV-2d ▲ Invalid SARS-CoV-2e | Repeat External Control (+) Testing. If the error persists, contact BioFire Defense Customer Support for further instruction. |



LIMITATIONS

1. For *In Vitro* Diagnostic (IVD) Use under Emergency Use Authorization (EUA).
2. BioFire COVID-19 Test performance has only been established on the FilmArray 2.0 and FilmArray Torch systems.
3. The BioFire COVID-19 Test is a qualitative test and does not provide a quantitative value for the virus in the sample.
4. The BioFire COVID-19 Test has not been validated for testing of samples other than nasopharyngeal swab (NPS) specimens in transport media.
5. A false negative BioFire COVID-19 Test result may occur when the concentration of virus in the sample is below the device limit of detection.
6. The detection of viral nucleic acid is dependent upon proper sample collection, handling, transportation, storage and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
7. There is a risk of false positive and false negative results caused by improperly collected, transported, or handled samples. The RNA process control and the PCR2 control will not indicate whether or not nucleic acid has been lost due to inadequate collection, transport, or storage of samples.
8. As with any molecular test, mutations within the target regions of SARS-CoV-2 could affect primer binding, resulting in failure to detect the presence of virus.
9. All three assays show 80% or greater homology to Bat coronavirus RaTG13 (accession: MN996532). In addition, the SARS-CoV-2e assay shows greater than 80% homology to Pangolin coronavirus isolate MP789 (accession: MT084071). It is unlikely that these isolates would be found in our sample matrix of nasopharyngeal swabs; however, little is known about their potential to infect a human host, or their evolutionary relationship to SARS-CoV-2.



CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The BioFire COVID-19 Test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website:
<https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd>

To assist clinical laboratories running the BioFire COVID-19 Test, the relevant Conditions of Authorization are listed below, and are required to be met by laboratories performing the EUA test.

- A. Authorized laboratories¹ using the BioFire COVID-19 Test will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- B. Authorized laboratories using the BioFire COVID-19 Test will use as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the BioFire COVID-19 Test are not permitted.
- C. Authorized laboratories that receive the BioFire COVID-19 Test will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- D. Authorized laboratories using the BioFire COVID-19 Test will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- E. Authorized laboratories will collect information on the performance of the BioFire COVID-19 Test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and BioFire Defense Product Support website <https://www.biofiredefense.com/product-support/filmarray-support/adverse-reporting-biofire-covid19-test/> any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the BioFire COVID-19 Test of which they become aware.
- F. All laboratory personnel using the BioFire COVID-19 Test must be appropriately trained in performing and interpreting the results of the BioFire COVID-19 Test, use appropriate personal protective equipment when handling this kit, and use the BioFire COVID-19 Test in accordance with the authorized labeling.
- G. BioFire Defense, LLC, authorized distributors, and authorized laboratories using the BioFire COVID-19 Test will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

¹For ease of reference, the letter of authorization refers to, “United States (U.S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests, and in U.S. laboratories certified under CLIA to perform high complexity tests, or in similarly qualified non-U.S. laboratories” as “authorized laboratories.”



PERFORMANCE CHARACTERISTICS

Clinical Performance

Clinical testing was performed using ten positive and five negative NPS specimens stored in transport media. The positive samples were collected from patients presenting with signs or symptoms of COVID-19, and previously identified as positive for SARS-CoV-2 by another test (nine specimens were determined positive by a validated laboratory developed test (NECOV19) and one was determined positive by the Roche cobas SARS-CoV-2 EUA Test). The negative samples were collected in 2018, and therefore were presumed negative for SARS-CoV-2. All samples were de-identified before testing on the BioFire COVID-19 Test.

Positive Percent Agreement (PPA) was calculated as $100\% \times (\text{TP} / (\text{TP} + \text{FN}))$. Negative Percent Agreement (NPA) was calculated as $100\% \times (\text{TN} / (\text{TN} + \text{FP}))$. Nine of out ten positive samples were Detected by the BioFire COVID-19 Test and five out of five negative samples were Not Detected, resulting in 90% PPA and 100% NPA (Table 6).

Table 6. BioFire COVID-19 Test Performance Summary

| Virus | PPA | | | NPA | | |
|------------|-------------------|-----|------------|--------------|------|-------------|
| | TP/(TP + FN) | % | 95% CI | TN/(TN + FP) | % | 95% CI |
| SARS-CoV-2 | 9/10 ^a | 90% | 59.6-98.2% | 5/5 | 100% | 56.6-100.0% |

^a Specimen had a late Ct value when originally evaluated on the NECOV19 test. When the FN sample was retested on the NECOV19 test, the result was negative. These results indicate a near-LoD level of SARS-CoV-2 virus and/or sample degradation after the first NECOV19 test and prior to the BioFire COVID-19 Test.

Testing of Clinical Contrived Specimens

Contrived testing was performed using 4 unspiked negative clinical specimens and 30 negative contrived clinical specimens spiked with live SARS-CoV-2 virus (cultured from the USA_WA1/2020 strain obtained from World Reference Center for Emerging Viruses and Arboviruses (WRCEVA)). The thirty (30) individual unique clinical samples were contrived at 1× LoD (N=20), 10× LoD (N=5), and at 100× LoD (N=5), and tested with the four (4) negative (unspiked) specimens. These 34 samples were tested randomized and in a blinded fashion. In addition, sixty-two (62) additional negative individual unique clinical specimens were also evaluated. All test results were as expected. The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) was determined by comparing the observed test results to the expected result. PPA and NPA are shown in Table 7.

Table 7. Clinical Contrived and Negative Testing with the BioFire COVID-19 Test

| | Agreement with Known Analyte Composition | | | |
|------------------|--|------|--------------------|------|
| | PPA | % | NPA | % |
| Agreement | 30/30 | 100% | 66/66 ^a | 100% |
| 95% CI | [88.6 – 100] | | [94.5-100] | |

^a For comparison, 52 of the 66 negative samples were evaluated on the FilmArray 2.0 and FilmArray Torch systems. No unexpected results were observed on either system. The NPA was 100% for the negative samples on both systems.



Limit of Detection

Tentative Limit of Detection (LoD) for the BioFire COVID-19 Test was determined by testing three-fold dilutions of quantified live SARS-CoV-2 virus (cultured from the USA_WA1/2020 strain obtained from World Reference Center for Emerging Viruses and Arboviruses (WRCEVA)). Viral genomic copies per mL (GC/mL) for SARS-CoV-2 virus stock was determined by quantitative RT-PCR using the WHO protocol (<https://www.who.int/docs/default-source/coronavirus/protocol-v2-1.pdf>). Subsequent testing at the tentative LoD was conducted to confirm reliable detection ($\geq 95\%$) at LoD, and loss of detection ($< 95\%$) when tested 10-fold below LoD ($0.1 \times \text{LoD}$). LoD testing was performed using the FilmArray 2.0 system. Results for LoD testing are shown in Table 8. The LoD was determined to be $3.3E+02$ GC/mL ($2.2E-02$ TCID₅₀/mL), with a detection rate of 20/20 at $1 \times \text{LoD}$, and 14/20 at $0.1 \times \text{LoD}$ ($3.3E+01$ GC/mL; $2.2E-03$ TCID₅₀/mL).

Table 8. SARS-CoV-2 Virus Test Results at 1× and 0.1× LoD for the BioFire COVID-19 Test

| xLoD | Concentration Tested | | Test Result (% Detection) | | |
|------|----------------------|------------------------|---------------------------|------------|--------------|
| | Genomic Copies/mL | TCID ₅₀ /mL | Detected | Equivocal | Not Detected |
| 1× | 3.3E+02 | 2.2E-02 | 20/20 (100%) | 0/20 (0%) | 0/20 (0%) |
| 0.1× | 3.3E+01 | 2.2E-03 | 14/20 (70%) | 5/20 (25%) | 1/20 (5%) |



Analytical Reactivity (*in silico* Inclusivity)

Inclusivity of the BioFire COVID-19 Test was analyzed *in silico* using bioinformatics to align all unique genomes from both NCBI and GISAID EpiCoV databases.

As of March 16, 2020, the majority of assay primers showed 100% homology to all available SARS-CoV-2 genome sequences. Four primers, across 2 of the 3 BioFire COVID-19 Test assays, showed single mismatches to sequences of SARS-CoV-2. In the majority of these cases these were single isolate sequences. In both assays the mismatches were single base pair differences. Such mismatches are well tolerated on the BioFire platform and the assays are predicted to be reactive in the BioFire COVID-19 test despite such mismatches.

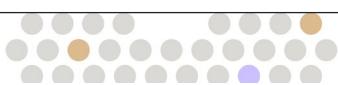
Analytical Specificity (*in silico* Exclusivity)

An *in silico* analysis was performed on the organisms listed in Table 9.

Table 9. Organisms Tested for Evaluation of BioFire COVID-19 Test *in silico* Cross-Reactivity

| Recommended Organisms | Additional Organisms |
|-----------------------------------|---|
| Human coronavirus 229E | Parechovirus |
| Human coronavirus OC43 | <i>Corynebacterium diphtheriae</i> |
| Human coronavirus HKU1 | <i>Bacillus anthracis</i> |
| Human coronavirus NL63 | <i>Moraxella catarrhalis</i> |
| SARS-coronavirus | <i>Neisseria elongata</i> |
| MERS-coronavirus | <i>Neisseria meningitidis</i> |
| Adenovirus | <i>Pseudomonas aeruginosa</i> |
| Human Metapneumonivirus (hMPV) | <i>Leptospira</i> |
| Parainfluenza virus 1-4 | <i>Chlamydia psittaci</i> |
| Influenza A & B | <i>Coxiella burnetii</i> |
| Enterovirus | <i>Staphylococcus aureus</i> |
| Respiratory syncytial virus | <i>Homo sapiens</i> |
| Rhinovirus | SARS-coronavirus |
| <i>Chlamydia pneumoniae</i> | Coronavirus |
| <i>Haemophilus influenza</i> | Recombinant SARSR-CoV |
| <i>Legionella pneumophila</i> | SARS2 |
| <i>Mycobacterium tuberculosis</i> | SARS coronavirus ExoN1 |
| <i>Streptococcus pneumonia</i> | SARS coronavirus wtic-MB |
| <i>Streptococcus pyogenes</i> | SARS coronavirus MA15 |
| <i>Bordetella pertussis</i> | SARS coronavirus MA15 ExoN1 |
| <i>Mycoplasma pneumoniae</i> | Bat <i>Betacoronavirus</i> SARS related virus |
| <i>Pneumocystis jirovecii</i> | Coronaviridae |
| <i>Candida albicans</i> | Coronavirinae |
| <i>Pseudomonas aeruginosa</i> | |
| <i>Staphylococcus epidermidis</i> | |
| <i>Staphylococcus salivarius</i> | |

All assays show 80% or greater homology to Bat coronavirus RaTG13 (accession: MN996532). In addition, the SARS-CoV-2e assay shows greater than 80% homology to Pangolin coronavirus isolate MP789 (accession: MT084071). It is unlikely that these isolates would be found in our sample matrix of nasopharyngeal swabs; however, little is known about their potential to infect a human host, or their evolutionary relationship to SARS-CoV-2. No other significant amplification of non-target sequences is predicted.

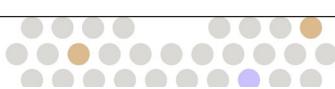


Analytical Specificity (Exclusivity)

Six viruses that are closely related to SARS-CoV-2 were tested, and none of the BioFire COVID-19 Test assays were cross-reactive to any of these viruses. More than 30 additional organisms were also tested, and none of the assays cross-reacted with any of these organisms. Results are shown in Table 10 below.

Table 10. Organisms Tested for Evaluation of BioFire COVID-19 Test Analytical Specificity

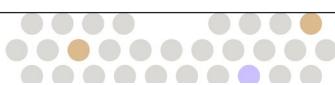
| Organism | ID | Test Concentration | Assay Detections | | | SARS-CoV-2 Test Result |
|---|------------------------|--------------------------------------|------------------|-------------|-------------|------------------------|
| | | | SARS-CoV-2a | SARS-CoV-2d | SARS-CoV-2e | |
| Human coronavirus 229E | Zeptometrix 0810229CF | 1.26E+06 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Human coronavirus OC43 | Zeptometrix 0810024CF | 9.55E+06 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Human coronavirus HKU1 (clinical specimen) | Clinical Isolate (NPS) | ~1.0E+08 copies/mL ^a | 0/3 | 0/3 | 0/3 | Not Detected |
| Human coronavirus NL63 | Zeptometrix 0810228CF | 2.51E+05 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| SARS-coronavirus (BSL3) | Culture (MRI Global) | 5.3E+08 GE/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| MERS-coronavirus (BSL3) | Culture (MRI Global) | 2.7E+08 GC/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| <i>Chlamydia pneumoniae</i> | ATCC 53592 | 2.90E+07 IFU/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| <i>Haemophilus influenzae</i> | ATCC 700223 | 4.20E+08 CFU/mL (7.42E+08 copies/mL) | 0/3 | 0/3 | 0/3 | Not Detected |
| <i>Legionella pneumophila</i> | Zeptometrix 0801530 | 2.63E+09 CFU/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| <i>Mycobacterium tuberculosis</i> (attenuated strain) | Zeptometrix 0801660 | 3.04E+07 CFU/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| <i>Streptococcus pneumoniae</i> | ATCC 6303 | 8.90E+07 CFU/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| <i>Streptococcus pyogenes</i> | ATCC 49399 | 4.65E+08 CFU/mL (8.25E+08 copies/mL) | 0/3 | 0/3 | 0/3 | Not Detected |
| <i>Bordetella pertussis</i> | Zeptometrix 0801459 | 6.70E+09 CFU/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| <i>Mycoplasma pneumoniae</i> | Zeptometrix 0801579 | 3.98E+07 CCU/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| <i>Pseudomonas aeruginosa</i> | ATCC 10145 | 5.68E+08 CFU/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| <i>Staphylococcus epidermidis</i> | ATCC 29887 | 7.43E+09 CFU/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| <i>Streptococcus salivarius</i> | ATCC 13419 | 7.38E+09 CFU/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Adenovirus 1 (species C) | Zeptometrix 0810050CF | 3.39E+07 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Adenovirus 4 (species E) | Zeptometrix 0810070CF | 7.05E+04 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |



| Organism | ID | Test Concentration | Assay Detections | | | SARS-CoV-2 Test Result |
|--|-------------------------|---|------------------|-------------|-------------|------------------------|
| | | | SARS-CoV-2a | SARS-CoV-2d | SARS-CoV-2e | |
| Adenovirus 7 (species B) | Zeptometrix 0810021CF | 5.10E+07 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Human Metapneumovirus (hMPV) | Zeptometrix 0810161CF | 1.78E+05 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Parainfluenza virus 1 | BEI NR-48681 | 8.0E+05 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Parainfluenza virus 2 | Zeptometrix 0810504CF | 1.10E+06 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Parainfluenza virus 3 | BEI NR-3233 | 5.10E+07 TCID ₅₀ /mL (7.0E+08 copies/mL) | 0/3 | 0/3 | 0/3 | Not Detected |
| Parainfluenza virus 4 | Zeptometrix 08010060BCF | 1.70E+07 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Influenza A subtype H1 | Zeptometrix 0810036CFN | 7.05E+04 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Influenza A subtype H3 | Zeptometrix 0810252CF | 7.05E+04 TCID ₅₀ /mL (1.92E+08 copies/mL) | 0/3 | 0/3 | 0/3 | Not Detected |
| Influenza B | Zeptometrix 0810239CF | 4.78E+06 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Enterovirus species A (EV71) | NCPV 0812215v | 5.0E+08 TCID ₅₀ /mL (3.8E+08 copies/mL) | 0/3 | 0/3 | 0/3 | Not Detected |
| Enterovirus species B (Echovirus 6) | Zeptometrix 0810076CF | 5.10E+07 TCID ₅₀ /mL (1.10E+08 copies/mL) | 0/3 | 0/3 | 0/3 | Not Detected |
| Enterovirus species C (Coxsackievirus A17) | ATCC VR-1023 | 7.90E+05 TCID ₅₀ /mL (3.17E+06 copies/mL) | 0/3 | 0/3 | 0/3 | Not Detected |
| Enterovirus species D (68) | Zeptometrix 0810237CF | 1.58E+06 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Respiratory syncytial virus | Zeptometrix 0810040ACF | 1.05E+06 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Rhinovirus | Zeptometrix 0810012CFN | 1.26E+06 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Pneumocystis jirovecii (PJP) | ATCC PRA-159 | 1E+07 CFU/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Candida albicans | ATCC MYA-2876 | 7.88E+08 CFU/mL | 0/3 | 0/3 | 0/3 | Not Detected |
| Pooled human nasal wash ^b | - | - | - | - | - | - |

^a The human coronavirus HKU1 used in this study was a previously collected clinical specimen. The concentration of virus in the sample was estimated based on the results of a previously performed real-time PCR test.

^b Pooled nasal wash was not evaluated in this study; however, approximately 50 negative residual NPS samples were evaluated during the clinical evaluation of the test, and no cross-reactivity of test assays to flora present in NPS samples was observed.



Interference

Potentially interfering substances that could be present in NPS specimens or introduced during specimen collection and testing were evaluated previously on the FilmArray® Respiratory 2 (RP2) Panel for their effect on pouch performance. The RP2 Panel and the BioFire COVID-19 Test use the same sample type; no interference testing has been performed for the BioFire COVID-19 Test. The data from the RP2 Panel interfering substances evaluation are summarized in Table 11a.

Substances listed below include endogenous substances that may be found in specimens at normal or elevated levels (e.g., blood, mucus/mucin, human genomic DNA), medications, washes or topical applications for the nasal passage, various swabs and transport media for specimen collection, and substances used to clean, decontaminate, or disinfect work areas. The concentration of substance added to the samples was equal to or greater than the highest level expected to be in NPS specimens.

None of the substances were shown to interfere with the RP2 Panel function and are not expected to interfere with the BioFire COVID-19 Test. However, it was observed that exposure of samples to bleach prior to testing could damage the organisms/nucleic acids in the sample, leading to inaccurate test results (lack of analyte detection). The effect of bleach was dependent on the concentration and/or length of time the bleach was allowed to interact with the sample.

Various commensal or infectious microorganisms typically found in NPS specimens were tested and did not interfere with the performance of the RP2 Panel Internal Controls. These organisms have not been tested on the BioFire COVID-19 Test but due to similarities in the internal control, they are not expected to interfere with the BioFire COVID-19 Test Internal controls. See Table 11b for a list of competitive organisms tested.

Table 11a. Substances Tested Demonstrating No Panel Interference in FilmArray® RP2 Panel^{ab}

| Substance Tested |
|--|
| Endogenous Substances |
| Human Whole Blood |
| Human Mucin (Sputum) |
| Human Genomic DNA |
| Exogenous Substances |
| Tobramycin (systemic antibiotic) |
| Mupirocin (active ingredient in anti-bacterial ointment) |
| Saline Nasal Spray with Preservatives (0.65% NaCl, Phenylcarbinol, Benzalkonium chloride) |
| Nasal Decongestant Spray (Oxymetazoline HCl 0.05%, Benzalkonium chloride, phosphate) |
| Analgesic ointment (Vicks® VapoRub®) |
| Petroleum Jelly (Vaseline®) |
| Snuff (Tobacco) |
| Disinfecting/Cleaning Substances |
| Bleach ^b |
| Disinfecting wipes (ammonium chloride) |
| Ethanol |
| DNAZap (Ambion™ AM9891G & AM9892G) |
| RNaseZap (Ambion™ AM9782) |



| Substance Tested |
|---|
| Specimen Collection Materials |
| Rayon Swabs (Copan 168C) |
| Nylon Flocked Swabs (Copan 553C) |
| Polyester Swabs (Copan 175KS01) |
| Calcium Alginate Swabs (Puritan 25-801 A 50) |
| M4® Transport Medium (Remel R12500, 3mL/tube) |
| M4-RT® Transport Medium (Remel R12506, 3 mL/tube) |
| M5® Transport Medium (Remel R12516, 3 mL/tube) |
| M6™ Transport Medium (Remel R12535, 1.5 mL/tube) |
| Universal Viral Transport vial (BD 220220, 3 mL/tube) |
| Sigma-Virocult™ Viral Collection and Transport System – Swabs and Transport Medium (Medical Wire MW951SENT) |
| ESwab™ Sample Collection and Delivery System – Swabs and Liquid Amies Medium (Copan 482C) |

^a Interfering substances were tested on the FilmArray RP2 Panel and have not been evaluated with the BioFire COVID-19 Test

^b ‘Not Detected’ results were reported for several FilmArray RP2 Panel analytes after incubation of the sample with 2% bleach for 10 minutes or overnight. It was concluded that interference resulted primarily from damage to the organism/nucleic acids in the sample, rather than inhibition or interference with pouch functions.

Table 11b. Competitive Microorganisms Tested on FilmArray® RP2 Panel

| Substance Tested | Concentration Tested |
|--|-----------------------------|
| Competitive Microorganisms typically found in NPS | |
| Coronavirus 229E | 1.7×10^4 TCID50/mL |
| Adenovirus A12 | 8.9×10^5 TCID50/mL |
| Parainfluenza Virus 3 | 6.6×10^5 TCID50/mL |
| <i>Bordetella pertussis</i> | 5.8×10^8 CFU/mL |
| Enterovirus D68 | 1.6×10^7 TCID50/mL |
| Echovirus 6 | 1.0×10^7 TCID50/mL |
| Respiratory Syncytial Virus | 4.2×10^4 TCID50/mL |
| <i>Staphylococcus aureus</i> | 2.5×10^7 CFU/mL |
| <i>Streptococcus pneumoniae</i> | 1.7×10^7 CFU/mL |
| <i>Haemophilus influenzae</i> | 6.2×10^7 CFU/mL |
| <i>Candida albicans</i> | 1.0×10^6 CFU/mL |
| Herpes Simplex Virus 1 | 1.6×10^6 TCID50/mL |
| Cytomegalovirus (CMV) | 1.2×10^6 TCID50/mL |

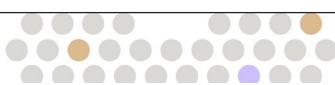


APPENDIX A

Symbols Glossary

The following symbols can be found on labeling for the FilmArray 2.0, FilmArray Torch, and BioFire COVID-19 Test Kits, kit components, BioFire COVID-19 Test External Control Kits (+), and throughout accompanying packaging.

| ISO 15223-1 Graphical symbols for use on equipment – Registered Symbols | | | | | |
|--|--|-----------|---|-----------|--|
| 5.1.1 | Manufacturer | 5.1.4 | Use-By date (YYYY-MM-DD) | 5.1.5 | Batch Code (Lot Number) |
| 5.1.6 | Catalog Number | 5.1.7 | Serial Number | 5.2.8 | Do Not Use if Package Is Damaged |
| 5.3.2 | Keep Away from Sunlight | 5.3.7 | Temperature Limit | 5.4.2 | Do not re-use |
| 5.4.3 | Consult Instructions for Use | 5.5.1 | In vitro Diagnostic Medical Device | 5.5.5 | Contains sufficient for <n> tests |
| United Nations Globally Harmonized System of Classification and Labeling of chemicals (GHS) (ST/SG/AC.10/30) | | | | | |
| | Corrosive (Skin Corrosion/Burns, Eye Damage, Corrosive to Metals) | | Exclamation Mark (Irritant, Acute Toxicity, Narcotic Effects, Respiratory Tract Irritant) | | Hazardous to the aquatic environment, long-term hazard |
| 81 FR 38911 | | | | | |
| Rx Only | Caution: Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner. | | | | |
| Manufacturer Symbols (BioFire Defense, LLC) | | | | | |
| | Positive Control | | COVID-19 symbol | | |



APPENDIX B

Contact and Legal Information

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



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DFA2-PRT-0080-02, April 2020

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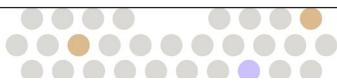
The purchase of this product includes a limited, non-transferable license under specific claims of one or more U.S. patents as listed on BioFire Defense's website (<http://www.biofiredefense.com/LegalNotices/>) and owned by the University of Utah Research Foundation and/or BioFire.



APPENDIX C

References

1. Biosafety in Microbiological and Biomedical Laboratories. December 2009. <http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf>. Accessed May 24, 2018.
2. CLSI. *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline-Fourth Edition*. CLSI document M29-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
3. Summary of Notifiable Diseases. MMWR available at <https://www.cdc.gov/>.
4. CIFOR Analysis of State Legal Authorities available at <http://www.cifor.us/>.
5. CLSI. *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline-Second Edition*. CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
6. CLSI. *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline-Fourth Edition*. CLSI document C24-Ed4. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.





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

Step 6: Review Results

Run Summary

① Internal Controls:

- If 'Passed', results are valid.
- If 'Failed' or 'Invalid', RETEST CONTROL and refer to *Instructions for Use*.

Result Summary

- #### ②
- All 3 assays must be 'Detected'
 - If any one assay is 'Not Detected', RETEST CONTROL and refer to *Instructions for Use*.

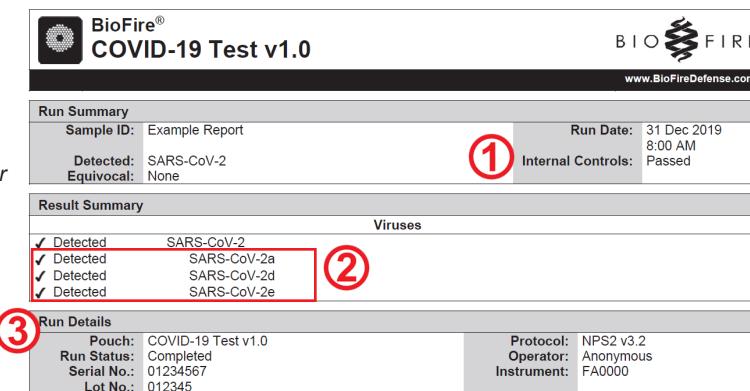
NOTE: External Control passes if all 3 assays are 'Detected' and the overall result is 'Detected'.

Run Details

③ Run Status:

- If 'Completed', run is complete.
- If 'Incomplete', 'Aborted', or any other error message, RETEST CONTROL, and refer to *Instructions for Use*.

NOTE: Refer to *Instructions for Use* for reporting information. If repeated error messages are obtained, contact Biofire Defense Technical Support.



DO NOT DISCARD: Important product-specific information

For use under an Emergency Use Authorization (EUA) only

REF 423748 (6 pk) **IVD**

Rx Only

Summary and Explanation of the Test

BioFire Defense provides an external positive assayed quality control kit to monitor the performance of the BioFire COVID-19 Test performed on FilmArray® 2.0 and FilmArray Torch® systems. Good laboratory practice recommends running positive and negative external controls regularly. Evaluation of external controls is recommended prior to using a new shipment or new lot of BioFire COVID-19 Test Kits. Evaluation of external controls is also recommended when there is a new operator, and following replacement/repair of a FilmArray 2.0 or FilmArray Torch system. External controls may also be used in initial laboratory validations of the FilmArray 2.0 or FilmArray Torch system used with the BioFire COVID-19 Test in accordance with appropriate federal, state, and local guidelines or accreditation requirements, as applicable.

Storage Instructions

Store at 15 - 30°C.

Procedure / Interpretation / Limitations



Users should refer to the *BioFire COVID-19 Test Instructions for Use* available on the BioFire Defense Website: www.biofiredefense.com/covid-19test
An optional verification protocol is also available on this website.

Questions / Support

If you have questions or need additional support, please contact BioFire Defense Technical Support at support@biofiredefense.com.

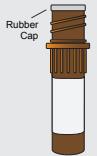
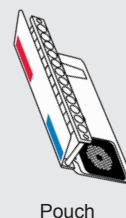
NOTE: Clean area thoroughly after testing External Positive Control to reduce risk of contamination.



C-19

Package Contents

Materials required but not provided


+ External Control Vial


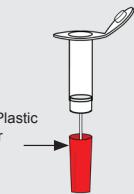
Pouch



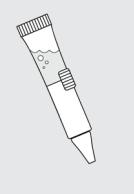
Hydration Injection Vial



Transfer Pipette



Sample Injection Vial



Sample Buffer Tube

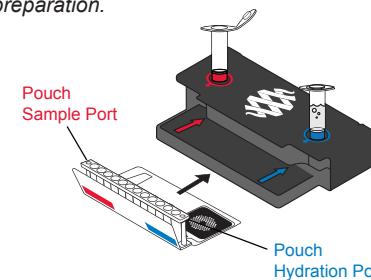


Transport Media or Saline

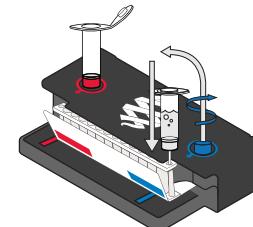
NOTE: FilmArray Instrument should be powered on and ready for use prior to pouch preparation.

Step 1: Prepare Pouch

Refer to the BioFire® COVID-19 Test Quick Guide for Step 1: Prepare Pouch

**Step 2: Hydrate Pouch**

Refer to the BioFire® COVID-19 Test Quick Guide for Step 2: Hydrate Pouch

**Step 3: Prepare External Control Mix**

- Use the Transfer Pipette to draw the Transport Media or Saline to the third line. Add to the **Sample Injection Vial**.
- Remove rubber cap from **External Control Vial (+)** and place on a clean surface (a paper towel may be used).
- Add Sample Buffer to **External Control Vial (+)**.
 - Hold Sample Buffer Tube tip facing up and firmly pinch at textured plastic tab on side of tube until seal snaps.

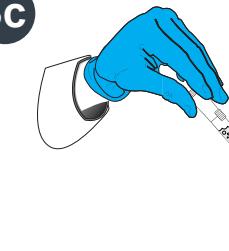
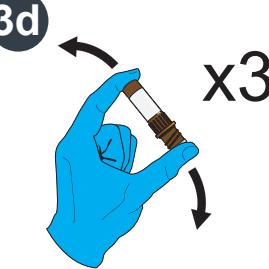
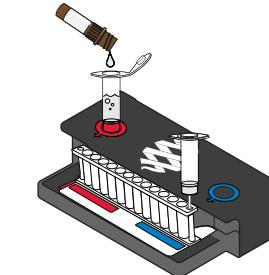
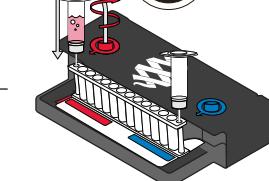
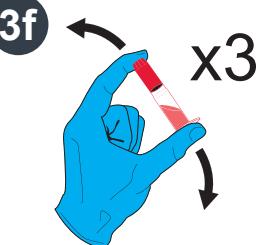
NOTE: Do not touch tube tip.

- Dispense Sample Buffer into **External Control Vial (+)** using a slow, forceful squeeze followed by a second squeeze.

NOTE: Avoid generating excessive foam.

- Recap **External Control Vial (+)** and invert 3 times to mix.
- Pour mixture of Sample Buffer and Control into the **Sample Injection Vial**.
 - Dispose of External Control Vial (+) and change gloves.**
- Tightly close lid of **Sample Injection Vial** and invert it 3 times; return it to the red well of Pouch Loading Station.

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

3c**3d****3e****3f****Step 4: Load Control Mix**

Refer to the BioFire® COVID-19 Test Quick Guide for Step 4: Load Sample Mix

Step 5: Run Pouch

Refer to the BioFire® COVID-19 Test Quick Guide for Step 5: Run Pouch





Step 6: Review Results

Run Summary - Displays information about the sample and a summary of the Internal Controls and test results.

1 Internal Controls:

- If 'Passed', results are valid.
- If 'Failed' or 'Invalid', RETEST SAMPLE and refer to *Instructions for Use*.

Test Interpretation:

- 2a** • If 'Detected', or 'Not Detected', report the results. (Figure 1)
- 2b** • If 'Equivocal', RETEST SAMPLE and refer to *Instructions for Use*. (Figure 2)

3 **Result Summary** - Displays test interpretation (Top Line) with detailed assay results.

4 **Run Details** - Displays information about the pouch, protocol, run status, operator, serial number, instrument, and lot number.

Run Status:

- If 'Completed', run is complete.
- If 'Incomplete', 'Aborted', or any other error message, RETEST SAMPLE and refer to *Instructions for Use*.

The screenshot shows the BioFire COVID-19 Test v1.0 software interface. At the top, it says "BioFire® COVID-19 Test v1.0" and "BIO FIRE www.BioFireDefense.com". The "Run Summary" section shows "Sample ID: Example Report", "Run Date: 31 Dec 2019 8:00 AM", and "Internal Controls: Passed". The "Result Summary" section under "Viruses" shows the following results:
 ✓ Detected SARS-CoV-2
 ✓ Detected SARS-CoV-2a
 ✓ Detected SARS-CoV-2d
 Not Detected SARS-CoV-2e
 The "Run Details" section shows:
 Pouch: COVID-19 Test v1.0
 Run Status: Completed
 Serial No.: 01234567
 Lot No.: 012345
 Protocol: NPS2 v3.2
 Operator: Anonymous
 Instrument: FA0000

Figure 1. 'DETECTED' Report

The screenshot shows the BioFire COVID-19 Test v1.0 software interface. At the top, it says "BioFire® COVID-19 Test v1.0" and "BIO FIRE www.BioFireDefense.com". The "Run Summary" section shows "Sample ID: Example Report", "Run Date: 31 Dec 2019 8:00 AM", and "Internal Controls: Passed". The "Result Summary" section under "Viruses" shows the following results:
 ↔ Equivocal SARS-CoV-2
 ✓ Detected SARS-CoV-2a
 Not Detected SARS-CoV-2d
 Not Detected SARS-CoV-2e
 The "Run Details" section shows:
 Pouch: COVID-19 Test v1.0
 Run Status: Completed
 Serial No.: 01234567
 Lot No.: 012345
 Protocol: NPS2 v3.2
 Operator: Anonymous
 Instrument: FA0000

Figure 2. 'EQUIVOCAL' Report

DO NOT DISCARD: Important product-specific information

For use under an Emergency Use Authorization (EUA) only

REF 423745 (6 pk)
423744 (30 pk)

IVD

Rx Only

Intended Use

The BioFire® COVID-19 Test is a nested multiplexed real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs in transport media from individuals suspected of COVID-19 by their healthcare provider. Please see www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd for the Instructions for Use.

Storage Instructions

Store at 15 - 30°C. Refer to the *BioFire COVID-19 Test Instructions for Use* before opening and preparing pouch for use.

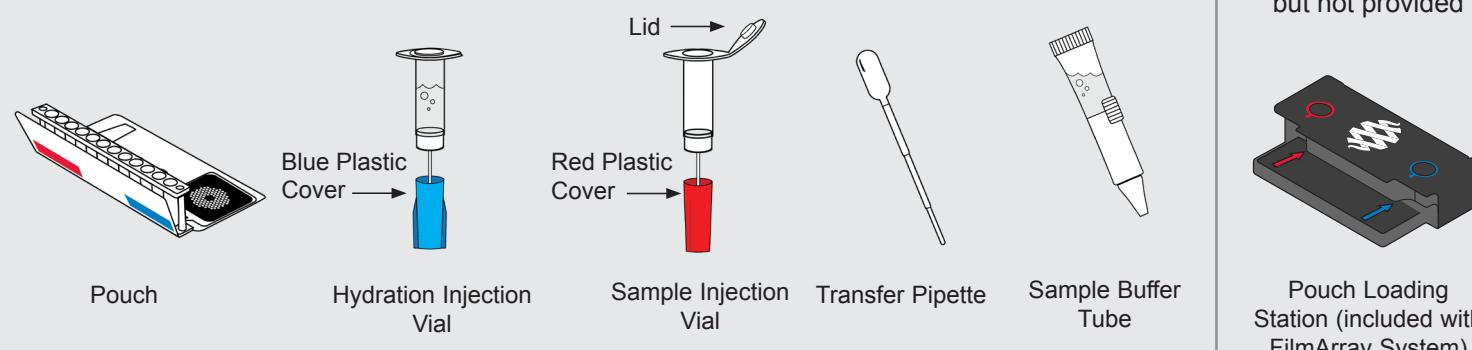
Procedure / Interpretation / Limitations

Users should refer to the *BioFire COVID-19 Test Instructions for Use* available on the BioFire Defense Website: www.biofiredefense.com/covid-19test

Questions / Support

If you have questions or need additional support, please contact BioFire Defense Technical Support at support@biofiredefense.com.

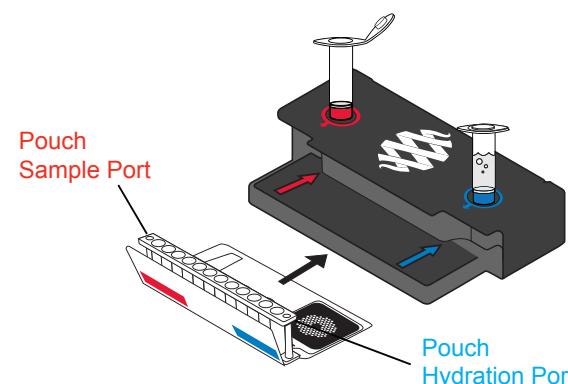


Package Contents

Use clean gloves and other Personal Protective Equipment (PPE) when performing this procedure.

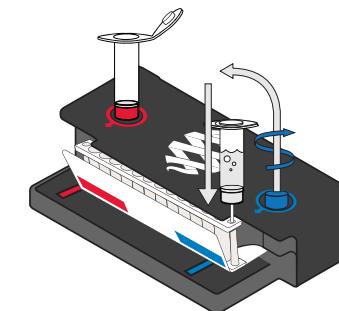
Step 1: Prepare Pouch

- Insert pouch into Pouch Loading Station.
- Place **Sample Injection Vial** into **red well**.
- Place **Hydration Injection Vial** into **blue well**.

**Step 2: Hydrate Pouch**

- Unscrew **Hydration Injection Vial** from cover.
- Remove **Hydration Injection Vial**, leaving **blue plastic cover** in Pouch Loading Station.
- Insert **Hydration Injection Vial** into **pouch hydration port**.
- Push down to puncture seal and wait as **Hydration Solution** is drawn into the pouch.

NOTE: Verify the pouch has been hydrated.

**Step 3: Prepare Sample Mix**

- Use the transfer pipette to draw specimen in transport media to the 3rd line. Add specimen to **Sample Injection Vial**.
- Hold **Sample Buffer Tube** tip facing up and firmly pinch at textured plastic tab on side of tube until seal snaps.

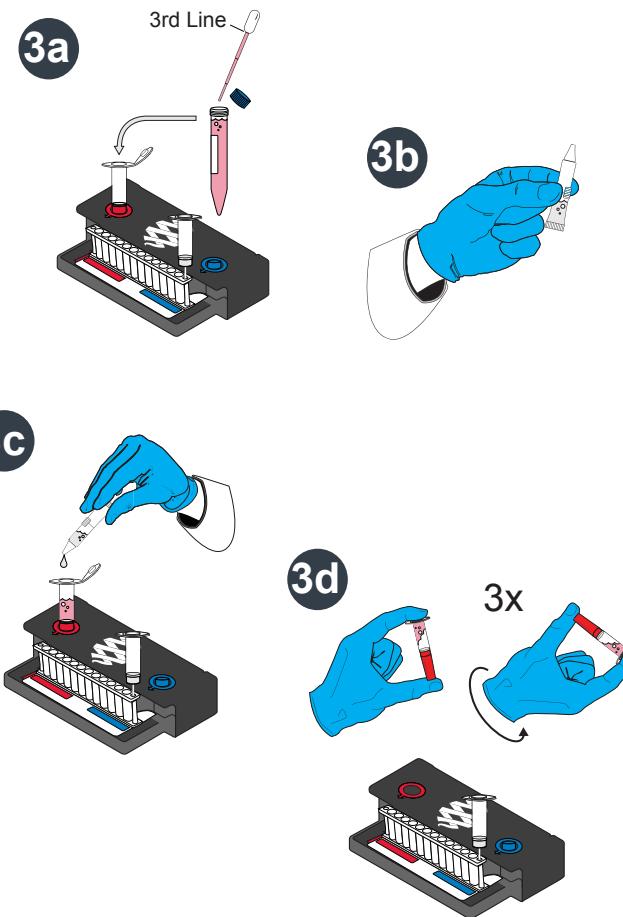
NOTE: Do not touch tube tip.

- Dispense **Sample Buffer** into **Sample Injection Vial** using a slow, forceful squeeze followed by a second squeeze.

NOTE: Avoid generating excessive foam.

- Tightly close the lid on the **Sample Injection Vial** and invert it 3 times, return it to the **red well** of Pouch Loading Station.

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

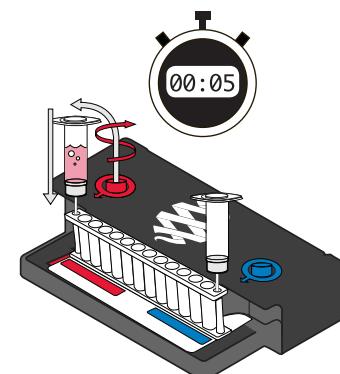
**Step 4: Load Sample Mix**

- Unscrew **Sample Injection Vial** from **red plastic cover**.
- Wait for 5 seconds, then lift **Sample Injection Vial**, leaving **red plastic cover** in Pouch Loading Station.

NOTE: Waiting five seconds decreases the contamination risk.

- Insert **Sample Injection Vial** into **pouch sample port**.
- Push down to puncture seal and wait as Sample Mix is drawn into the pouch.

NOTE: Verify the sample has been loaded.

**Step 5: Run Pouch**

- Discard the **Sample Injection Vial** and the **Hydration Injection Vial** in a biohazard sharps container.
- Follow instructions on computer for starting a test.



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

TECHNICAL
::: NOTE

Purpose

The Clinical Laboratory Improvement Amendments (CLIA) and the Clinical Laboratory Improvement Program (CLIP) establish 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 and CLIP regulations include a requirement for verifying the performance specifications of unmodified tests authorized for use under an Emergency Use Authorization (EUA) by the FDA. The BioFire® COVID-19 Test is for use only under EUA in U.S. laboratories certified under CLIA to perform moderate or high complexity tests.

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 which is detected by the BioFire® COVID-19 Test and may be easily modified or expanded to meet specific criteria. Day-to-day variation is evaluated by testing each sample on two separate days. To evaluate operator-to-operator variability, multiple laboratory technicians may test the same sample.

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

BioFire Intended Use

The BioFire® COVID-19 Test is a qualitative, multiplexed, nucleic acid-based in vitro diagnostic test intended for use with FilmArray® 2.0 and FilmArray® Torch Systems. The BioFire® COVID-19 Test is intended for detection of SARS-CoV-2 in nasopharyngeal swabs (NPS) in transport media from individuals with signs or symptoms of COVID-19.

The complete intended use statement and additional information about the use of the FilmArray system can be found in the *BioFire® COVID-19 Test Instructions for Use*.

TECHNICAL NOTE

Performance Verification: Overview

The procedures described below will generate Detected and Not Detected results for the BioFire® COVID-19 Test assays, and are example 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.

Two example performance verification procedures are described. The Simple Protocol was developed using the BioFire® COVID-19 Test External Control Kit. The Alternate Protocol describes the use of alternative materials as controls.

In addition to, or in place of, the verification scheme described here; a laboratory may choose to test clinical/patient samples to assess clinical sensitivity and sample matrix effects in its performance verification of the FilmArray® system. Pooled clinical/patient samples may be tested and used in place of control material.

Table 1. Overview of Verification Protocols

| Verification Protocol | Required Control Material | Sample Replicates | Expected Detected Results | Expected Not Detected Results | Days of Testing |
|-----------------------------|---|-------------------|---------------------------|-------------------------------|-----------------|
| Simple Protocol | BioFire® COVID-19 Test External Control Kit | 8 | 4 | 4 | 2 |
| Alternative Protocol | Alternative SARS-CoV-2 Source ^a | 8 | 4 | 4 | 2 |

^aAcceptable material may include live virus, inactivated virus, or genomic RNA at a concentration of 1.0×10^5 GE/mL.

Performance Verification: Materials

The following materials may be needed to perform 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 External Control Kit v1.0 (6 positive controls) ^a | 423748 |
| Genomic RNA, live virus, or inactivated virus from a SARS-CoV-2 isolate | BEI Resources NR-52285 (genomic RNA) or other source |

Technical Note

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www.BioFireDefense.com

Performance Verification Protocols

Both protocols can be followed to test a total of 8 samples, providing 4 positive results and 4 negative results on a single instrument. The estimated total time for completion of either verification example is 2 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 the individual laboratory. Results of the verification may be recorded in the verification records provided below.

Simple Protocol

The Simple Protocol utilizes the BioFire® COVID-19 Test External Control Kit which contains freeze-dried synthetic RNA transcripts designed to be amplified by the BioFire® COVID-19 Test.

Day 1

1. Obtain two BioFire® COVID-19 Test External Control vials and four BioFire® COVID-19 Tests.

NOTE: The tests should be run in a single day 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.

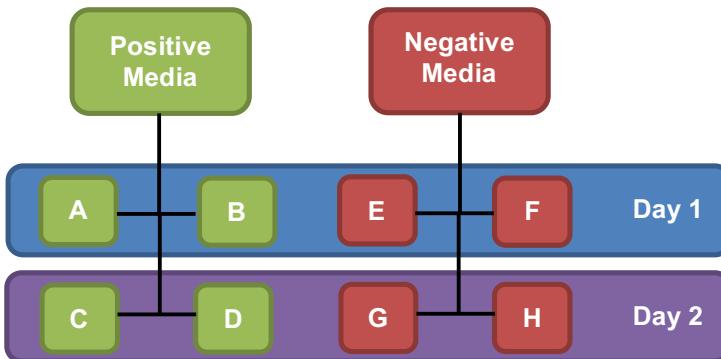
2. Follow the instructions in the *BioFire® COVID-19 Test External Control Quick Guide Use* to prepare and run 2 negative media samples on the BioFire® COVID-19 Test (e.g. samples E and F).
3. Follow the instructions in the *BioFire® COVID-19 Test External Control Quick Guide* to prepare and run 2 positive media samples on the BioFire® COVID-19 Test using the External Control material (e.g. samples A and B).

Day 2

4. To evaluate day-to-day variation, test the remaining positive media samples (e.g. samples C and D) and negative media samples (e.g. samples G and H) by repeating Steps 1-3 as in Day 1.

TECHNICAL NOTE

Figure 1. Simple Protocol Workflow



Alternative Protocol

The Alternative Protocol allows for the use of an alternative SARS-CoV-2 source material as a positive control. The material may be either live virus, inactivated virus, or genomic RNA. Known negative and positive clinical specimens may also be used, however the procedure may need to be modified. In order to obtain consistent results it is important that the material be accurately quantified.

Day 1

1. Using either transport media or saline solution, dilute the SARS-CoV-2 material in a new tube to obtain 0.75mL of sample material at a final concentration of 1.0×10^5 genomic equivalent copies/mL.

NOTE: Transport media is not recommended if using genomic RNA as a SARS-CoV-2 source material.

2. Obtain four BioFire® COVID-19 Tests.

NOTE: The tests should be run in a single day 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.

3. Follow the instructions in the *BioFire® COVID-19 Test Instructions for Use* or *Quick Guide* to prepare and run 2 negative media samples on the BioFire® COVID-19 Test (e.g. samples E and F) using either transport media or saline solution as the sample material.

NOTE: The media, (transport media or saline solution), in the negative samples should be the same sample media used to dilute the SARS-CoV-2 material.

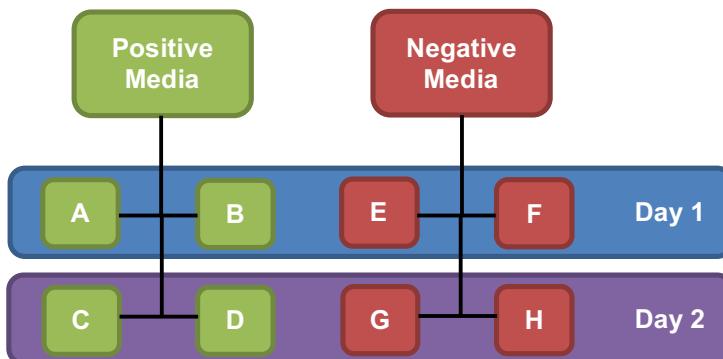
TECHNICAL NOTE

4. Follow the instructions in the *BioFire® COVID-19 Test Instructions for Use or Quick Guide* to prepare and run 2 positive media samples on the BioFire® COVID-19 Test using the SARS-CoV-2 positive material prepared in Step 1 (e.g. samples A and B).

Day 2

5. To evaluate day-to-day variation, test the remaining positive media samples (e.g. samples C and D) and negative media samples (e.g. samples G and H) by repeating Steps 1-4 as in Day 1.

Figure 2. Alternative Protocol Workflow



Expanding the Protocol

The protocol 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 and 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 samples to test. Four samples following the Day 1 testing plan may be sufficient (see Day 1 Testing above).

TECHNICAL NOTE

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
Phone: 1-801-262-3592
Fax: 1-801-447-6907



BioFire® COVID-19 Test Positive Verification Record

TECHNICAL
::: NOTE

Computer System Serial #: _____

BioFire® COVID-19 Test Kit Part #: _____

Lot #: _____

Sample/Control Material Source and Lot #: _____

Date: _____

| Organism | Instrument Serial # | Was the Organism Detected? | Date/Operator |
|------------|---------------------|---|---------------|
| SARS-CoV-2 | | <input type="checkbox"/> Yes <input type="checkbox"/> No | / |

Reviewed by:

Signature

Date

BioFire® COVID-19 Test Negative Verification Record

TECHNICAL
::: NOTE

Computer System Serial #: _____

BioFire® COVID-19 Test Kit Part #: _____

Lot #: _____

Date: _____

| Organism | Instrument Serial # | Was the Organism Detected? | Date/Operator |
|------------|---------------------|---|---------------|
| SARS-CoV-2 | | <input type="checkbox"/> Yes <input type="checkbox"/> No | / |

Reviewed by:

Signature

Date