

BioFire® COVID-19 Test Instructions for Use

REF

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

IVD

R_x Only



The Symbols Glossary is provided on Page 38 of this booklet.

For use under an Emergency Use Authorization (EUA) only

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

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INTENDED USE

The BioFire® COVID-19 Test is a nested multiplexed 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 of non-pooled specimens is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high or moderate complexity tests, and similarly qualified U.S. Department of Defense (DoD) and non-U.S. laboratories.

The BioFire COVID-19 Test is also for the qualitative detection of nucleic acid from SARS-CoV-2 in pooled samples containing up to eight nasopharyngeal swabs collected individually in transport media from individuals suspected of COVID-19 by their healthcare provider. Testing of pooled specimens is limited to DoD laboratories that meet the requirements to perform high complexity tests.

Specimens should only be pooled in areas with low SARS-CoV-2 prevalence, and when testing demand exceeds laboratory capacity or reagent availability. For pooled specimen testing, authorized laboratories will adhere to a protocol for ongoing monitoring of the pooling strategy or limit testing to individuals who are subjected to a detailed infection prevention and control plan.

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. Pooled samples with positive or equivocal results must be tested individually prior to reporting results. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

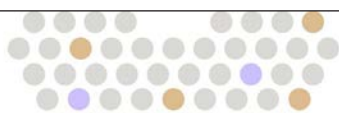
Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. Negative results from pooled samples should be reported as presumptive. Specimens with low viral genetic material may not be detected in pooled samples due to decreased sensitivity. If clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, the patient should be considered for individual testing.

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.

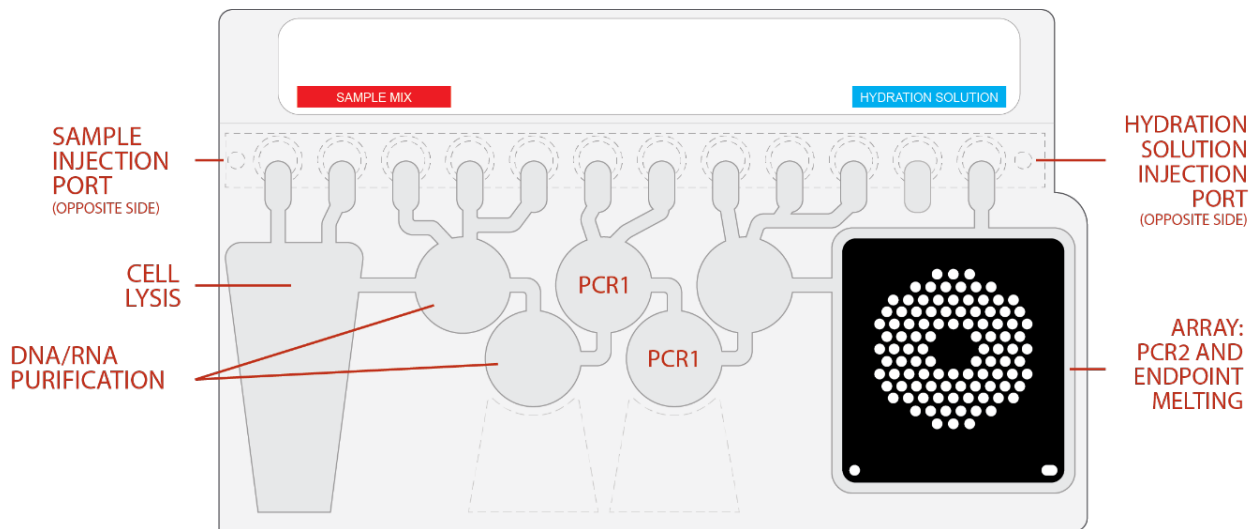


PRINCIPLE OF THE PROCEDURE

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.

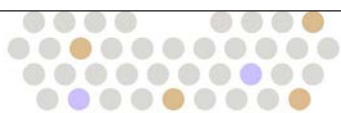
- Individually-packaged BioFire COVID-19 Test External Control (+) Vials
- Instructions and Documents
 - *BioFire COVID-19 Test External Control Kit – Quick Guide*

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. Testing of pooled specimens should only be performed in high complexity laboratories.
4. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
5. The BioFire COVID-19 Test has not been FDA cleared or approved but has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet the requirements to perform moderate and high complexity tests.
6. The BioFire COVID-19 Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
7. The BioFire COVID-19 Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner..
8. BioFire COVID-19 Test pouches are only for use with FilmArray 2.0 and FilmArray Torch systems.
9. BioFire COVID-19 External Control Kit (+) is only for use with FilmArray 2.0 and FilmArray Torch systems.
10. Always check the expiration date on the pouch. Do not use a pouch after its expiration date.
11. 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.
12. Bleach introduced in a sample may damage nucleic acids in the sample, which may lead to a false negative result.
13. 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 mucous 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.

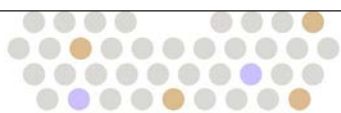
DO NOT PERFORM ADDITIONAL TESTING UNTIL THE AREA HAS BEEN DECONTAMINATED.

Precaution 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 specimen 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 Specimen Volume	0.3 mL (300 µL) per test
Transport and Storage	<p>Specimens 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 specimens 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

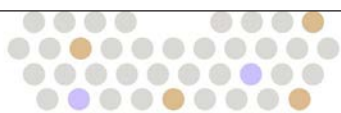
Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one BioFire COVID-19 Test pouch at a time and change gloves between samples and pouches. Once sample is added to the pouch, promptly transfer to the instrument to start the run. After the run 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.

Preparing Specimens for Pooling

Prior to considering specimen pooling, laboratories should evaluate pooling strategies based on population positivity rates (see section below on Specimen Pooling Implementation and Monitoring). Nasopharyngeal swabs which have been collected individually in transport media may be pooled. Pools of up to 8 specimens may be tested on the BioFire COVID-19 Test. Perform the following procedure when pooling specimens for testing.

1. Obtain an empty collection tube (collection tube is not provided).
2. Determine the appropriate volume of each specimen to add to the pool based on the number of specimens that will be pooled. The final volume of the pooled sample should be at least 750µL (to allow for one re-test as needed). Each specimen to be included in the pool should contribute an equal volume. For example, if pooling three specimens, 250 µL of each specimen should be pooled.
3. Transfer the determined volume of each individual specimen to the collection tube.
4. Mix the prepared sample pool.
5. Test the prepared sample pool according to the BioFire COVID-19 Test Procedure. Sample IDs should indicate that the sample was pooled.

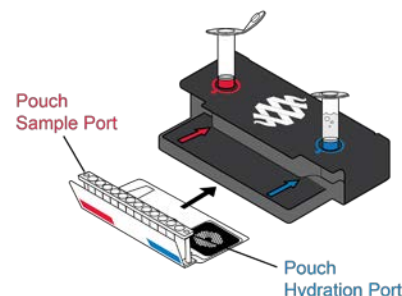


Step 1: Prepare Pouch

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.

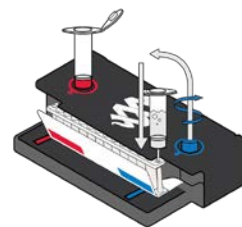
NOTE: The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

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.

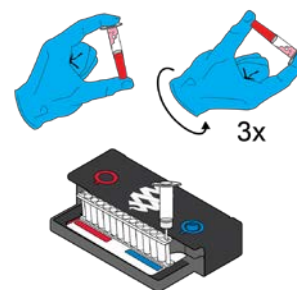
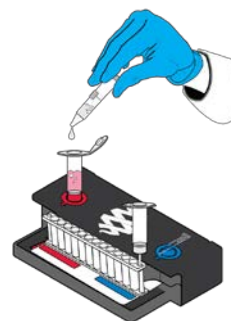
NOTE: Avoid touching the tube tip during handling, as this may introduce contamination.

- 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.

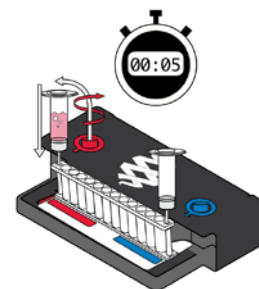


Step 4: Load Sample Mix

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.

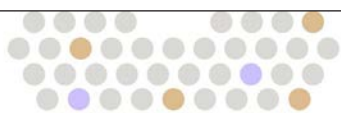
NOTE: The font color of the user name is red until the user name is recognized by the software.

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.

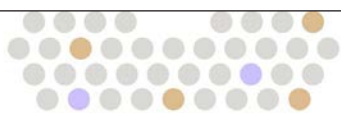
NOTE: The font color of the user name is red until the user name is recognized by the software.

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.

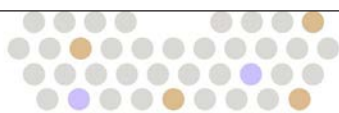
2. PCR2 Control

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 (T_m) 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 T_m 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 T_m 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

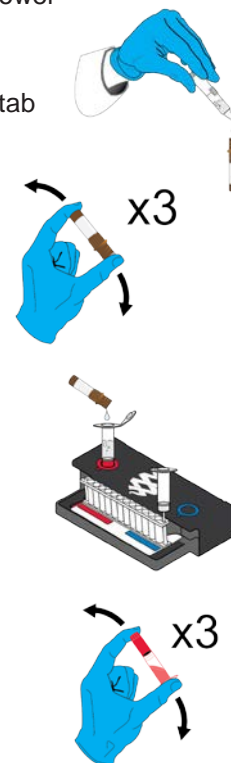


BioFire® COVID-19 Test External Control Procedure (+)

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.



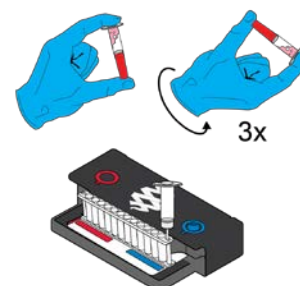
BioFire® COVID-19 Test External Control Procedure (-)

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.

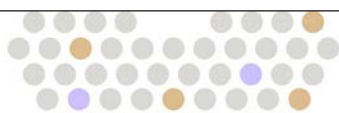
Assay Name	SARS-COV-2 Genomic Region
SARS-CoV-2a	ORF1ab
SARS-CoV-2d	ORF1ab
SARS-CoV-2e	ORF8

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 of the results, refer to Table 2 if testing individual specimens, or Table 3 if testing pooled specimens.

Interpretation When Testing Individual Specimens

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 the control assays have failed, all results are reported as 'Invalid' and retesting is required.

Table 2. Interpretation Rules for Individual Specimens

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.

Interpretation When Testing Pooled Specimens

If two or more assays are 'Detected', the test report result will be SARS-CoV-2 'Detected'. If only one of the assays is 'Detected', the test report result will be SARS-CoV-2 'Equivocal'. If either a 'Detected' or an 'Equivocal' result is obtained, individual reflex testing must be performed (each specimen included in the pooled sample must be tested following individual testing procedure). If all assays are 'Not Detected', the result on the test report will be SARS-CoV-2 'Not Detected'. A 'Not Detected' result should be considered presumptive. Specimens with low viral loads may not be detected when pooling samples due to decreased sensitivity. If clinical signs and symptoms are inconsistent with a negative result, the patient should be considered for individual testing. In cases where either or both control assays have failed, all results are reported as 'Invalid' and the pooled sample should be retested. If the result of the retest is 'Invalid', each specimen included in the pooled sample should be retested individually.

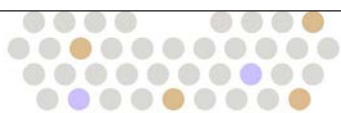




Table 3. Interpretation Rules for Pooled Samples

SARS-CoV-2 Interpretation	Assay Results	Action
Detected	3/3 Assays 'Detected' 2/3 Assays 'Detected'	Perform individual specimen reflex testing. Retest all specimens included in the sample pool individually.
Equivocal	1/3 Assays 'Detected'	Perform individual specimen reflex testing. Retest all specimens included in the sample pool individually.
Not Detected	0/3 Assays 'Not Detected'	Report the Results
Invalid	Invalid	Retest sample pool. If sample pool fails a second time, retest individual specimens. See Table 4, Interpretation of Internal Controls Field on the BioFire Test Report for instruction. If repeated errors occur, contact the BioFire Defense Customer Support Team.

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	Run Date:	31 Dec 2019 8:00 AM
Detected:	SARS-CoV-2	Internal Controls:	Passed
Equivocal:	None		
Result Summary			
Viruses			
✓ Detected	SARS-CoV-2		
✓ Detected	SARS-CoV-2a		
✓ Detected	SARS-CoV-2d		
✓ 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 4 provides additional information for each of the possible internal control field results.

Table 4. 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 5 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.

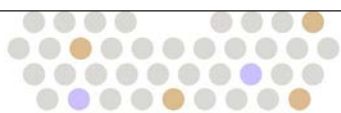
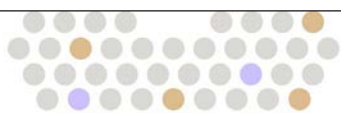


Table 5. Reporting of Results and Required Actions

SARS-CoV-2 Results	Explanation	Action
Detected	<p>The run was successfully completed</p> <p>AND</p> <p>The pouch controls were successful (Passed)</p> <p>AND</p> <p>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)</p>	Individual Specimen: Report results.
		Sample Pool: Perform individual specimen reflex testing. Retest all specimens included in the sample pool individually.
Not Detected	<p>The run was successfully completed</p> <p>AND</p> <p>The pouch controls were successful (Passed)</p> <p>AND</p> <p>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)</p>	Individual Specimen: Report results.
		Sample Pool: Report results.
Equivocal	<p>The run was successfully completed</p> <p>AND</p> <p>The pouch controls were successful (Passed)</p> <p>AND</p> <p>Only one of three assays was 'Detected' for the virus. The combination of 'Detected' and 'Not Detected' assay results were inconclusive</p>	Individual Specimen: Retest the original specimen 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.
		Sample Pool: Perform individual specimen reflex testing. Retest all specimens included in the sample pool individually.
Invalid	<p>The pouch controls were not successful (Failed)</p> <p>OR</p> <p>The run was not successful (Run Status displayed as: Aborted, Incomplete, Instrument Error or Software Error)</p>	Individual Specimen: See Table 4, Interpretation of Internal Controls Field on the BioFire Test Report for instruction.
		Sample Pool: Retest sample pool. If sample pool fails a second time, retest individual specimens. If repeated errors occur, contact the BioFire Defense Customer Support Team.



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
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 6 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.

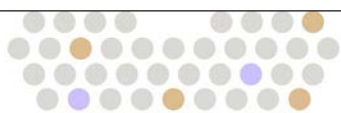
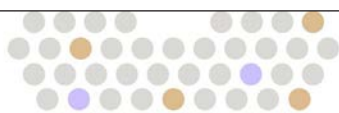


Table 6. BioFire COVID-19 Test External Control (+) Interpretation of Results

Result Interpretation	Result Analysis	Action																				
External Control (+) Passes	<table><tr><th colspan="2">Result Summary</th></tr><tr><td>✓ Detected</td><td>SARS-CoV-2</td></tr><tr><td>✓ Detected</td><td>SARS-CoV-2a</td></tr><tr><td>✓ Detected</td><td>SARS-CoV-2d</td></tr><tr><td>✓ Detected</td><td>SARS-CoV-2e</td></tr></table>	Result Summary		✓ Detected	SARS-CoV-2	✓ Detected	SARS-CoV-2a	✓ Detected	SARS-CoV-2d	✓ Detected	SARS-CoV-2e	Report the results.										
Result Summary																						
✓ Detected	SARS-CoV-2																					
✓ Detected	SARS-CoV-2a																					
✓ Detected	SARS-CoV-2d																					
✓ Detected	SARS-CoV-2e																					
BioFire COVID-19 External Test Control (+) Failed Result	<table><tr><th colspan="2">Result Summary</th></tr><tr><td>✖ Equivocal</td><td>SARS-CoV-2</td></tr><tr><td>✓ Detected</td><td>SARS-CoV-2a</td></tr><tr><td>Not Detected</td><td>SARS-CoV-2d</td></tr><tr><td>Not Detected</td><td>SARS-CoV-2e</td></tr></table> <table><tr><th colspan="2">Result Summary</th></tr><tr><td>Not Detected</td><td>SARS-CoV-2</td></tr><tr><td>Not Detected</td><td>SARS-CoV-2a</td></tr><tr><td>Not Detected</td><td>SARS-CoV-2d</td></tr><tr><td>Not Detected</td><td>SARS-CoV-2e</td></tr></table>	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.
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																					
BioFire COVID-19 External Control (+) Invalid Result	<table><tr><th colspan="2">Result Summary</th></tr><tr><td>⚠ Invalid</td><td>SARS-CoV-2</td></tr><tr><td>⚠ Invalid</td><td>SARS-CoV-2a</td></tr><tr><td>⚠ Invalid</td><td>SARS-CoV-2d</td></tr><tr><td>⚠ Invalid</td><td>SARS-CoV-2e</td></tr></table>	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.										
Result Summary																						
⚠ Invalid	SARS-CoV-2																					
⚠ Invalid	SARS-CoV-2a																					
⚠ Invalid	SARS-CoV-2d																					
⚠ Invalid	SARS-CoV-2e																					



LIMITATIONS

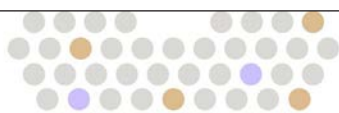
1. For *In Vitro* Diagnostic (IVD) Use under Emergency Use Authorization (EUA).
2. BioFire COVID-19 Test performance has only been established on the FilmArray 2.0 and FilmArray Torch systems.
3. The BioFire COVID-19 Test is a qualitative test and does not provide a quantitative value for the virus in 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>.

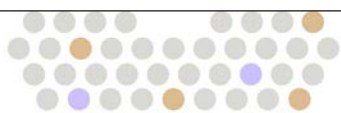
To assist clinical laboratories running the BioFire COVID-19 Test, the relevant Conditions of Authorization are listed below, and are required to be met by laboratories performing the EUA test.

- A. Authorized laboratories¹ using the BioFire COVID-19 Test will include with test result reports, 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 the BioFire COVID-19 Test 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 RT-PCR techniques and use appropriate personal protective equipment when handling this kit, and use the BioFire COVID-19 Test in accordance with the authorized labeling.
- G. For pooled specimen testing, authorized laboratories will adhere to a protocol for ongoing monitoring of the pooling strategy or limit testing to individuals who are subjected to a detailed infection prevention and control plan.
- H. Authorized laboratories using specimen pooling strategies when testing patient specimens with the BioFire COVID-19 Test will include with test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that *"Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing."*
- I. Authorized laboratories implementing pooling strategies for testing patient specimens must use the "Specimen Pooling Implementation and Monitoring" recommendations available in the authorized labeling to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.
- J. Authorized laboratories will keep records of specimen pooling strategies implemented including type of strategy, date implemented, and quantities tested, and test result data generated as part of the "Specimen Pooling Implementation and Monitoring". For the first 12 months from the date of their creation, such records will be made available to FDA within 48 business hours for inspection upon request, and will be made available within a reasonable time after 12 months from the date of their creation.
- K. 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.

¹ For ease of reference, the letter of authorization refers to "authorized laboratories" as follows: Testing of non-pooled specimens is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high or moderate complexity tests, and similarly qualified U.S. Department of Defense (DoD) and non-U.S. laboratories. Testing of pooled specimens is limited to DoD laboratories that meet the requirements to perform high complexity tests.



PERFORMANCE CHARACTERISTICS

Clinical Summary

The clinical performance was evaluated by testing individual archived nasopharyngeal swab specimens collected in transport media. The overall clinical performance including only individual clinical specimens is summarized in Table 7. The studies contributing to this summary are detailed below.

Table 7. Summary of Individual Clinical Specimens Evaluated with the BioFire COVID-19 Test

	PPA	%	NPA	%
Agreement	29/30	96.7%	71/71	100%
95% CI	[83.3-99.4%]		[94.9-100%]	

Testing of Archived Clinical Specimens

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 $100\% \times (TP / (TP + FN))$. Negative Percent Agreement (NPA) was calculated as $100\% \times (TN / (TN + FP))$. 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 8).

Table 8. BioFire COVID-19 Test Performance Summary

	PPA	%	NPA	%
Agreement	9/10 ^a	90.0%	5/5	100%
95% CI	[59.6-98.2%]		[56.6-100%]	

^a FN 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 Contrived Clinical 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 1x LoD (N=20), 10x LoD (N=5), and at 100x 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 9.

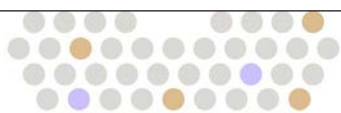


Table 9. Clinical Contrived and Negative Testing with the BioFire COVID-19 Test

	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.

Testing of Pooled Clinical Specimens

Archived specimens previously characterized as part of standard of care were used in testing. Twenty (20) specimens that returned 'SARS-CoV-2 Detected' results when tested on the CDC 2019-nCoV test were selected to represent a range of clinically relevant concentrations based on Ct values. An additional 160 specimens that returned 'SARS-CoV-2 Not Detected' results when tested on the CDC 2019-nCoV test were also selected.

Positive specimens were re-tested individually on the BioFire COVID-19 Test. Single individual positive specimens were combined with the negative specimens in pools of 5 and 8 specimens. Twenty pools of each size were tested. Pooled test results were compared to individual test results to evaluate the effect of pooling on SARS-CoV-2 detection. Results are shown in Table 10.

Table 10. Detection of SARS-CoV-2 in Pools of 5 or 8 Specimens (Binned by Ct Value)

Ct Value ^a Bins for Positive Samples	Individual Known Positive Samples		Pools of 5 Specimens		Pools of 8 Specimens	
	Detection Rate (PPA)	95% CI	Detection Rate (PPA)	95% CI	Detection Rate (PPA)	95% CI
Ct ≥ 35	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%
30 ≤ Ct < 35	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%	4/5 (80%)	37.6-96.4%
25 ≤ Ct < 30	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%
Ct < 25	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%
Overall	20/20 (100%)	83.9-100%	20/20 (100%)	83.9-100%	19/20 (95%)	76.4-99.1%

^a Ct values are from reconfirmation testing with the CDC 2019-nCoV test.

SARS-CoV-2 was detected by the BioFire COVID-19 Test in 20/20 (100% PPA) of the pools of 5 specimens and in 19/20 (95% PPA) of the pools of 8 specimens. For the single 8-pooled sample run in which SARS-CoV-2 was not detected, the positive specimen included in this pool had late amplification when tested individually and when included in a pool of 5 specimens, indicating analyte levels near the Limit of Detection (LoD).



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/coronaviruse/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 11. The LoD was determined to be $3.3\text{E}+02$ GC/mL ($2.2\text{E}-02$ TCID₅₀/mL), with a detection rate of 20/20 at $1 \times \text{LoD}$, and 14/20 at $0.1 \times \text{LoD}$ ($3.3\text{E}+01$ GC/mL; $2.2\text{E}-03$ TCID₅₀/mL).

Table 11. SARS-CoV-2 Virus Test Results at $1 \times$ and $0.1 \times$ LoD for the BioFire COVID-19 Test

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

FDA SARS-CoV-2 Reference Panel Testing

SARS-CoV-2 sensitivity and MERS-CoV cross-reactivity were evaluated using the FDA SARS-CoV-2 Reference Panel according to the standard protocol provided by the US FDA. The evaluation was performed using reference material (T1) and blinded samples. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The product LoD when using the FDA Reference Panel is presented in Table 12. No cross-reactivity with MERS-CoV was reported.

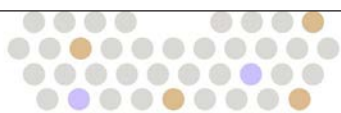
Table 12: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	NPS in transport medium	$5.4\text{E}+03$ NDU/mL ¹	N/A ²
MERS-CoV		N/A ²	ND ³

¹ NDU: Nucleic acid amplification test (NAAT) Detectable Units

² N/A: Not applicable

³ ND: Not detected



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 13.

Table 13. 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 diphtheria</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 Metapneumovirus (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 14 below.

Table 14. 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	Zeptomatrix 0810229CF	1.26E+06 TCID ₅₀ /mL	0/3	0/3	0/3	Not Detected
Human coronavirus OC43	Zeptomatrix 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	Zeptomatrix 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>	Zeptomatrix 0801530	2.63E+09 CFU/mL	0/3	0/3	0/3	Not Detected
<i>Mycobacterium tuberculosis</i> (attenuated strain)	Zeptomatrix 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>	Zeptomatrix 0801459	6.70E+09 CFU/mL	0/3	0/3	0/3	Not Detected
<i>Mycoplasma pneumoniae</i>	Zeptomatrix 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)	Zeptomatrix 0810050CF	3.39E+07 TCID ₅₀ /mL	0/3	0/3	0/3	Not Detected
Adenovirus 4 (species E)	Zeptomatrix 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)	Zeptomatrix 0810021CF	5.10E+07 TCID ₅₀ /mL	0/3	0/3	0/3	Not Detected
Human Metapneumovirus (hMPV)	Zeptomatrix 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	Zeptomatrix 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	Zeptomatrix 08010060BCF	1.70E+07 TCID ₅₀ /mL	0/3	0/3	0/3	Not Detected
Influenza A subtype H1	Zeptomatrix 0810036CFN	7.05E+04 TCID ₅₀ /mL	0/3	0/3	0/3	Not Detected
Influenza A subtype H3	Zeptomatrix 0810252CF	7.05E+04 TCID ₅₀ /mL (1.92E+08 copies/mL)	0/3	0/3	0/3	Not Detected
Influenza B	Zeptomatrix 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)	Zeptomatrix 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)	Zeptomatrix 0810237CF	1.58E+06 TCID ₅₀ /mL	0/3	0/3	0/3	Not Detected
Respiratory syncytial virus	Zeptomatrix 0810040ACF	1.05E+06 TCID ₅₀ /mL	0/3	0/3	0/3	Not Detected
Rhinovirus	Zeptomatrix 0810012CFN	1.26E+06 TCID ₅₀ /mL	0/3	0/3	0/3	Not Detected
<i>Pneumocystis jirovecii</i> (PJP)	ATCC PRA-159	1E+07 CFU/mL	0/3	0/3	0/3	Not Detected
<i>Candida albicans</i>	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 15a.

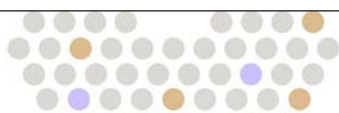
Substances listed below include endogenous substances that may be found in specimens at normal or elevated levels (e.g., blood, mucus/mucin, human genomic DNA), medications, washes or topical applications for the nasal passage, various swabs and transport media for specimen collection, and substances 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 15b for a list of competitive organisms tested.

Table 15a. 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 15b. Competitive Microorganisms Tested on FilmArray® RP2 Panel

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



SPECIMEN POOLING

Pooling Implementation

Pooling must only be performed by U.S. Department of Defense on individuals who are subjected to a detailed infection prevention and control plan prior to and during operations, or by laboratories that can adhere to a full protocol for ongoing monitoring of the pooling strategy per these Instructions for Use. Pooling of specimens allows for testing of more individuals with fewer reagents. When resource availability is sufficient to meet testing demand, laboratories should reconsider whether the risks of reduced test sensitivity with pooling continue to outweigh the benefits of resource conservation. Pooling of specimens should also be considered in context of the SARS-CoV-2 positivity rate within the test population. Higher positivity rates generally decrease the efficiency of pooling samples because specimens in positive pools must be retested individually. The BioFire COVID-19 Test has been validated for pooling up to eight samples.

Before implementing a pooling strategy, laboratories should determine the percent positivity rate of the testing population and choose an appropriate pooling sample size that is within the maximum validated pool size of eight samples.

Using historical data for individual specimens from the previous 7-10 days, the percent positivity rate ($P_{\text{individual}}$) can be determined by dividing the number of positive specimens by the total number of specimens tested during that date range.

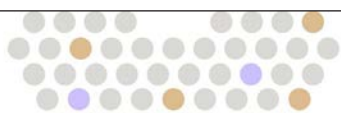
$$(P_{\text{individual}}) = (\text{Number of positive specimens} / \text{Number of specimens tested}) * 100$$

Refer to Table 16 to identify which pooling sample size provides the greatest testing efficiency for the determined $P_{\text{individual}}$ within the validated pool sizes for the assay. If $P_{\text{individual}}$ is 2% or less, then the largest validated pool size ($n=8$) should be used to maximize efficiency. If the $P_{\text{individual}}$ is greater than 25%, then pooling is not efficient and should not be implemented. An example of the efficiency calculation for 5-sample pooling uses the formula $F=1/(1+1/5-(1-P)^5)$, when $P_{\text{individual}}$ is 1%, the efficiency F is 3.46 for $n=5$. It means that 1,000 tests can cover testing of 3,460 patients on average.

Table 16. Testing Efficiency of Pooling

$P_{\text{individual}}$	n Corresponding to the Maximal Efficiency	Efficiency of n-Sample Pooling (maximum increase in number of tested patients)
1%–2%	8	5.11–3.65
3%–4%	6	3.00–2.60
5%–6%	5	2.35–2.15
7%–12%	4	1.99–1.54
13%–25%	3	1.48–1.10

If historical data for individual specimens from the previous 7-10 days are not available for a laboratory as described above, pooling may be implemented with the maximum pool size of ($n=8$). However, efficiency may not be maximized if $P_{\text{individual}}$ has not been determined.



Pooling Monitoring

Following the implementation of a pooling strategy, laboratories should evaluate performance of the strategy regularly to determine if the desired testing efficiency is still being achieved. Determination of the percent positivity rate in pools (P_{pools}) is required.

$$(P_{\text{pools}}) = (\text{Number of positive specimens in pools} / \text{Total number of specimens tested in pools}) * 100$$

For DoD Laboratories that Can Adhere to a Full Protocol for Ongoing Monitoring of the Pooling Strategy

Continue to monitor n-sample pooling strategy by calculating the positivity rate among patient samples during n-sample pooling ($P_{\text{pools-x}}$) for subsequent 7-10 day period based on n-sample pool testing. ($P_{\text{pools-x}}$) should be updated daily using a moving average.

Compare $P_{\text{pools-initial}}$ to $P_{\text{pools-x}}$. If $P_{\text{pools-x}}$ is less than 90% of $P_{\text{pools-initial}}$. ($P_{\text{pools-x}} < 0.90 \times P_{\text{pools-initial}}$), it is recommended that:

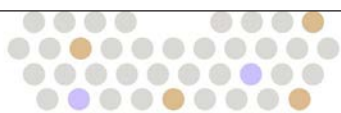
- The n-samples pooling should be re-assessed periodically by conducting a re-assessment study (described below).
- If P_{pools} is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate drops below.

Pooling Re-Assessment Study

Note: Individual testing as part of either re-assessment study option may be performed using a different and higher throughput EUA COVID-19 test.

Option 1 Stop n-sample pooling and return to individual testing. Patient samples should be prospectively individually tested until 10 consecutive positive samples have been collected. These individually tested samples should then be re-tested in a pool with one positive and n-1 negative samples.

Option 2 Continue n-sample pooling. Individual testing should be performed in parallel to the pooled testing until 10 consecutive positive samples are obtained. These positive samples should include both positive individual results generated from individual testing of samples from the non-negative sample pools following the n-sample pooling and deconvoluting workflow, and positive individual results obtained from individual testing of samples from the negative sample pools for the time period. Because non-negative pools require individual testing of samples included in the pool (samples in the positive pools will be tested as a part of normal n-sample pooling workflow), the study essentially consists of additionally testing individual samples from the pools with negative results.



For both options the following should be applied:

If the PPA between pooled-testing results and individual-testing results is $\geq 90\%$ (9 or 10 out of 10), then implementation of testing using n-sample pooling is acceptable.

If the PPA between pooled-testing results and individual-testing results is less than 90% then:

- If PPA $\leq 70\%$ (7 out of 10), reduce the pool size (consider a new n as n-1)
- If PPA is 80% (8 out of 10), to compensate for lost sensitivity, reduce the pool size (consider a new n as n-1) and continue with the reassessment testing until PPA of pooled compared to individual testing is not less than 90%. OR collect an additional 10 consecutive individually positive samples. Then, calculate the PPA from the combined data of 20 samples, between pooled-testing results and individual-testing results. If the PPA is $\geq 85\%$, then implementation of testing using n-sample pooling is acceptable.
- If PPA of at least 85% cannot be reached, cease pooling patient specimens.

If n-sample pooling is acceptable based on re-assessment, re-establish $P_{\text{individual}}$ in your laboratory by estimating the positivity rate from individual testing in the population from which the 10 (or 20) consecutive individual positive samples were collected. If the total number of samples (N^*) that needed to be tested to obtain the 10 (or 20) consecutive positive samples is stopped at the 10th (or 20th) positive sample, then the positivity rate of $10/N^*$ (or $20/N^*$) is overestimated. The positivity rate should be corrected by the following corresponding multiplier:

- Positivity rate for 10 samples is $(10/N^*) \times (10/11)$
- Positivity rate for 20 samples is $(20/N^*) \times (20/21)$.

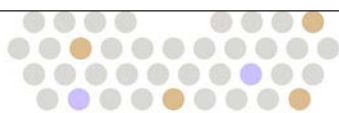
This updated new positivity rate should be used as $P_{\text{individual}}$ in the future laboratory monitoring.

For DoD Operations Unable to Adhere to a Full Protocol for Ongoing Monitoring of the Pooling Strategy

Individuals should be subjected to a detailed infection prevention and control plan prior to and during operations. This may include for example: restriction of movement, quarantine, isolation, continuous health monitoring programs and regular molecular SARS-CoV-2 surveillance testing by pooled or individual sample testing with the BioFire COVID-19 or other authorized molecular SARS-CoV-2 testing.

Continue to monitor n-sample pooling strategy by calculating the positivity rate among patient samples during n-sample pooling ($P_{\text{pools-x}}$) for subsequent 7-10 day period based on n-sample pool testing. ($P_{\text{pools-x}}$) should be updated daily using a moving average.


















Compare $P_{\text{pools-initial}}$ to $P_{\text{pools-x}}$. If $P_{\text{pools-x}}$ is less than 90% of $P_{\text{pools-initial}}$. ($P_{\text{pools-x}} < 0.90 \times P_{\text{pools-initial}}$), pooling may continue, but a new n-sample pooling size may need to be considered. If $P_{\text{pools-x}}$ is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate drops below.



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 	<i>In vitro</i> 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

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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.*