BioFire® COVID-19 Test Master Table of Contents

BioFire Defense, LLC
Salt Lake City, Utah, USA

BioFire® COVID-19 Test v1.0 Instructions for Use (also contains BioFire® COVID-19 Test External Control Kit v1.0 Instructions for Use)

BioFire® COVID-19 Test v1.0 Quick Guide

BioFire® COVID-19 Test External Control Kit v1.0 Quick Guide

BioFire Defense, LLC
Salt Lake City, Utah, USA

REF

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

BioFire®

COVID-19 Test

Instructions for Use

v1.0

IVD

℞ Only

The Symbols Glossary is provided on Page 39 of this booklet.

For in vitro diagnostic use under an Emergency Use Authorization (EUA) only

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

Manufactured by
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INTENDED USE

The BioFire® COVID-19 Test is a nested, multiplexed RT-PCR test performed on the FilmArray® 2.0 and FilmArray® Torch Instrument Systems intended for the qualitative detection of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in non-pooled upper respiratory swab specimens (i.e., nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs) (versions v1.0 and v1.1) or lower respiratory specimens (induced or expectorated sputum, endotracheal aspirates, bronchoalveolar lavage, or mini-bronchoalveolar lavage) (version v1.1 only) collected from individuals suspected of COVID-19 by their healthcare provider. The test is also for use with saliva specimens collected without preservatives in a sterile container in a healthcare setting from individuals suspected of COVID-19 by their healthcare provider (version v1.1 only). 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 upper respiratory specimens (i.e., nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs) collected individually 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 upper respiratory, lower respiratory, and saliva specimens 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 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 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

SARS-CoV-2 is a positive-sense, single-stranded RNA virus. It caused a global pandemic as the etiological agent of Coronavirus Disease 2019 (COVID-19), which is primarily characterized by shortness of breath, fever, and pneumonia and may be fatal for individuals who are older or have underlying health conditions[1–3]. The virus is thought to be of zoonotic origin and is highly transmissible through the inhalation of respiratory droplets[2–4].

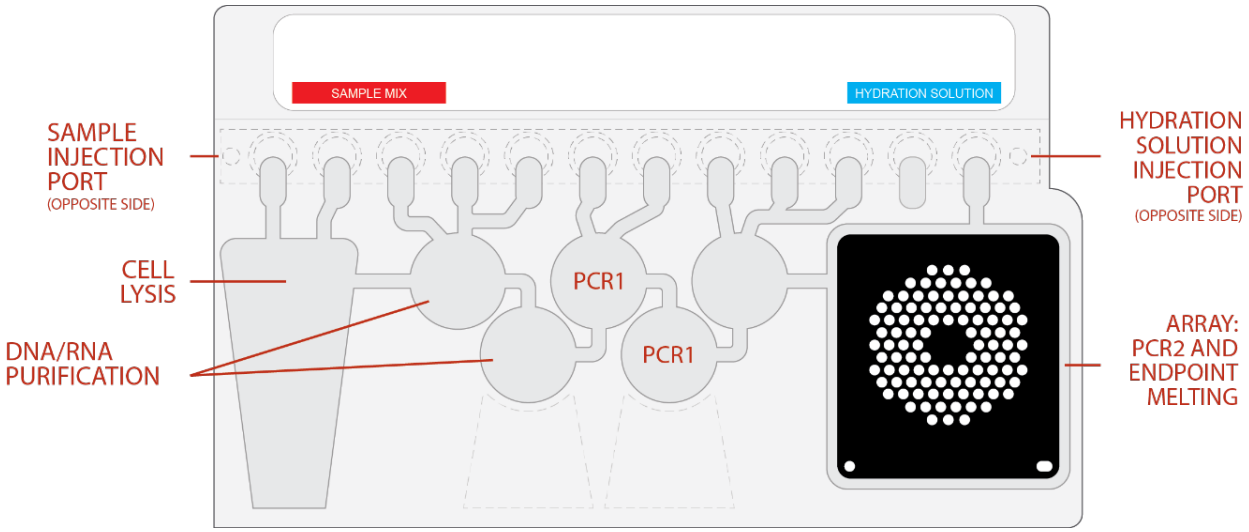
The BioFire® COVID-19 Test is a qualitative test on the FilmArray® 2.0 or FilmArray® Torch systems for the detection of the 2019 coronavirus (SARS-CoV-2) RNA in upper respiratory specimens (nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs) in transport media. The BioFire COVID-19 Test aids in the diagnosis of COVID-19 by testing samples in a time frame (~45 minutes) that allows the test results to be used in determining appropriate patient treatment and management. Internal controls are used to monitor all stages of the test process. The BioFire® COVID-19 Test External Control Kit v1.0 (+) 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 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 v1.0 Quick Guide*

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*

ADDITIONAL AVAILABLE MATERIALS

Each BioFire COVID-19 Test External Control Kit v1.0 (+) (sold separately) 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 v1.0 External Control Procedure.

- Individually-packaged BioFire COVID-19 Test v1.0 External Control (+) Vials
- Instructions and Documents
    - *BioFire COVID-19 Test External Control Kit v1.0 Quick Guide*

NOTE: *Instructions for the BioFire COVID-19 Test External Control Kit v1.0 are included in this document. Optional verification protocol (for laboratory verification which may not use External Control Kit) is available online at www.biofiredefense.com/covid-19test*

WARNINGS AND PRECAUTIONS

General Precautions

1. For *in vitro* diagnostic (IVD) use under Emergency Use Authorization only.
2. Positive results are indicative of the presence of SARS-CoV-2 RNA.
3. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
4. The BioFire COVID-19 Test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
5. 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.
6. The emergency use of the BioFire COVID-19 Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
7. 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.
8. BioFire COVID-19 Test pouches are only for use with FilmArray 2.0 and FilmArray Torch systems.
9. BioFire COVID-19 Test External Control Kit v1.0 (+) 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 mucus membranes. Change gloves often when handling reagents or samples.
2. Handle all samples and waste materials as if they were capable of transmitting infectious agents. Observe safety guidelines such as those outlined in:
   - CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*[5]
   - CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*.[6]
   - 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 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:

- Laboratory personnel may carry or shed SARS-CoV-2 asymptomatically and can inadvertently contaminate the specimen while it is being prepared. To avoid potential contamination, handle specimens in a biosafety cabinet. If a biosafety cabinet is not used, a dead air box (e.g., AirClean PCR workstation), a splash shield (e.g., Bel-Art Scienceware Splash Shield), or a face shield should be used when preparing specimens for testing.
- Do not handle samples or pouches in a biosafety cabinet which is used for SARS-CoV-2 culture or immunofluorescence testing.

- Laboratory personnel should wear a standard surgical mask (or equivalent) and should avoid touching the mask while handling specimens.
- 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.
- The BioFire COVID-19 Test assays may react with vaccines that contain specific segments of the pathogen genome or full genome or vaccines containing attenuated/inactivated pathogen, including vaccines for SARS-CoV-2. Avoid collecting or handling specimens in areas that are exposed to SARS-CoV-2 vaccine material. Particular care should be taken during these processes to avoid contamination.
- Clinical history of vaccine administration should be considered in the interpretation of results, particularly for vaccines administered by nasal spray.

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 in the appropriate BioFire FilmArray operator's manual.

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

Precaution Related to Public Health Reporting

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.[7] 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.

Laboratories within the U.S. and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

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	**Upper Respiratory Swabs** • Including nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs 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	Specimens should be processed and tested with the BioFire COVID-19 Test as soon as possible. If storage is required, samples can be held: • At room temperature for up to 4 hours (15-25ºC) • Refrigerated for up to 3 days (2-8ºC) • Frozen (≤-15ºC or ≤-70ºC) for up to 30 days

NOTE: *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 v1.0 Quick Guide* or the appropriate FilmArray operator's manual for more details.

Preparing Upper Respiratory 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). Only upper respiratory specimens (i.e., nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs) which have been collected individually 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.

   NOTE: *To avoid cross-contamination of specimens, use a new micropipette tip or disposable transfer pipette for each specimen.*
   NOTE: *Pipettes for the pooling procedure are not provided with the BioFire COVID-19 Test kit.*

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.

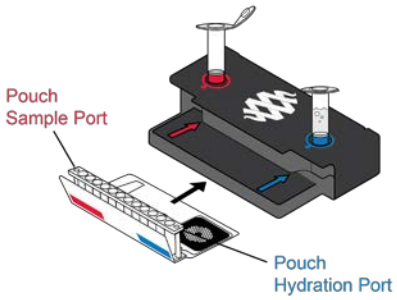
   NOTE: *Sample IDs should indicate that the sample was pooled.*

Step 1: Prepare Pouch

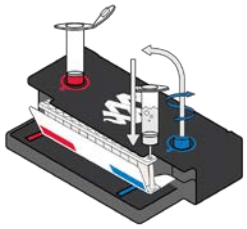
1. Thoroughly clean the work area and the FilmArray Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.
2. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.

   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, re-insert Hydration Injection Vial to ensure 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), re-insert Hydration Injection Vial to ensure 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*.

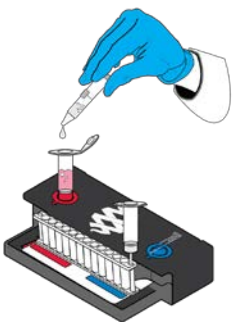
Step 3: Prepare Sample Mix

1. Thoroughly mix the upper respiratory 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.*

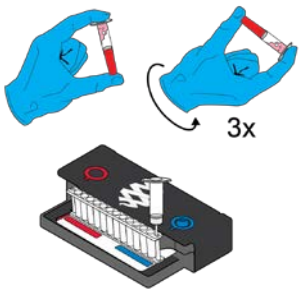


- Firmly pinch at textured plastic tab on the side of the tube until the seal snaps.
- Invert the tube over the red-capped Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

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.

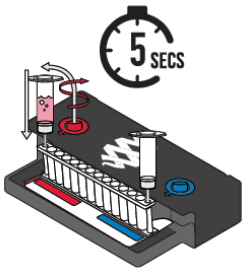
6. Tightly close the lid of the Sample Injection Vial.
7. Remove the Sample Injection Vial from the FilmArray Pouch Loading Station and invert the vial at least 3 times to mix.
8. Return the Sample Injection Vial to the red well of the FilmArray Pouch Loading Station.



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.

BioFire 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 v1.0 has a single NPS2 protocol available from the drop-down list. This is the correct protocol for all upper respiratory sample types (nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs).
6. Enter a username and password in the Name and Password fields.

   NOTE: *The font color of the username is red until the username 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.

BioFire 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 v1.0 has a single NPS2 protocol available from the drop-down list. This is the correct protocol for all upper respiratory sample types (nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs).
7. Enter operator username and password, then select Next.

   NOTE: *The font color of the username is red until the username is recognized by the software.*

8. Review the entered run information on the screen. If correct, select Start Run.

   Once the run has started, the screen displays a list of the steps being performed by the Module (instrument) and the number of minutes remaining in the run.

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

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

QUALITY CONTROL

Process Controls

Two process controls are included in each pouch:

1. **RNA Process Control**
   The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, PCR1, dilution, PCR2, and DNA melting. A positive control result indicates that all steps carried out in the BioFire COVID-19 Test were successful.

2. **PCR2 Control**
   The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that PCR2 was successful.

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

Monitoring Test System Performance

The FilmArray software will automatically fail the run if the melting temperature (Tm) for either the RNA Process Control or the PCR2 Control is outside of an acceptable range (80.3-84.4°C for the RNA Process Control and 73.8-78.2°C for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices.[8,9] Refer to the appropriate FilmArray operator's manual for instructions on obtaining control assay Tm values. The PCR2 Control is used in several FilmArray pouch types (e.g., RP2, BCID, GI, ME) and can therefore be used to monitor the system when multiple pouch types are used on the same FilmArray system or instrument.

BIOFIRE® COVID-19 TEST v1.0 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 v1.0 (+) 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 v1.0 (+) contains six (6) BioFire COVID-19 Test v1.0 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 v1.0 (+) 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 v1.0 External Control Procedure (+) below or *BioFire COVID-19 Test External Control Kit v1.0 Quick Guide*. To run a negative external control, use the BioFire COVID-19 Test and reference BioFire COVID-19 Test v1.0 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 the BioFire COVID-19 Test External Control Kit v1.0 (+) 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 (v1.0). 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.

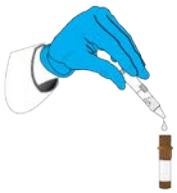
It is ultimately the responsibility of each laboratory to determine the frequency and type of material used for external control testing as part of the laboratory's Quality Control program.

Information on how to obtain optional external control material is posted on the BioFire Defense webpage: www.biofiredefense.com/covid-19test.

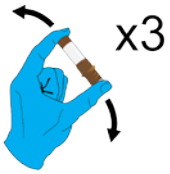
BioFire COVID-19 Test External Control Kit v1.0 (+)
Part Number: 423748

BioFire® COVID-19 Test v1.0 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).

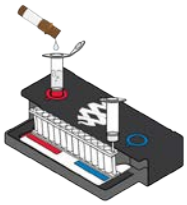


5. Add Sample Buffer to the External Control Vial (+).
    - Hold the Sample Buffer Tube tip facing up and firmly pinch at textured plastic tab on the side of the tube until the seal snaps.
    - Invert the Sample Buffer tube over the uncapped External Control Vial (+) and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

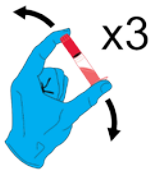


    ***NOTE**: Avoid generating excessive foam.*

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.



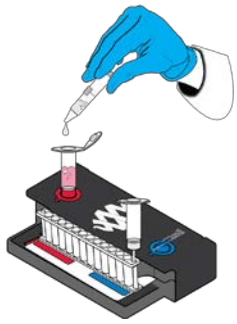
8. Tightly close lid of Sample Injection Vial and mix by gently inverting at least three (3) times.
9. Return Sample Injection Vial to red well of Pouch Loading Station.
10. Continue at Step 4 of the BioFire COVID-19 Test Procedure to load the pouch and run it on the FilmArray.



BioFire® COVID-19 Test v1.0 External Control Procedure (-)

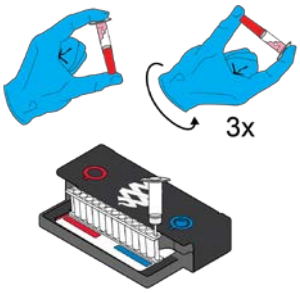
1. Follow Step 1 and Step 2 from the BioFire COVID-19 Test Procedure to prepare and hydrate the pouch.
2. Use the Transfer Pipette provided in the test kit to draw the transport media or saline to the third line (approximately 0.3mL) of the Transfer Pipette. Add to the Sample Injection Vial.

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

NOTE: *The BioFire COVID-19 Test results interpretation differs between versions v1.0 and v1.1. Consult the corresponding Instructions for Use. All BioFire COVID-19 Test v1.0 and v1.1 labeling is available on the BioFire Defense Product Support webpage: www.biofiredefense.com/covid-19test.*

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 (Tm) of the curve and compares it against the expected Tm range for the assay. If the software determines that the Tm of the curve is within the assay specification Tm range, the melt curve is called positive. If the software determines that the Tm of the curve is not in the appropriate Tm 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 Tm 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

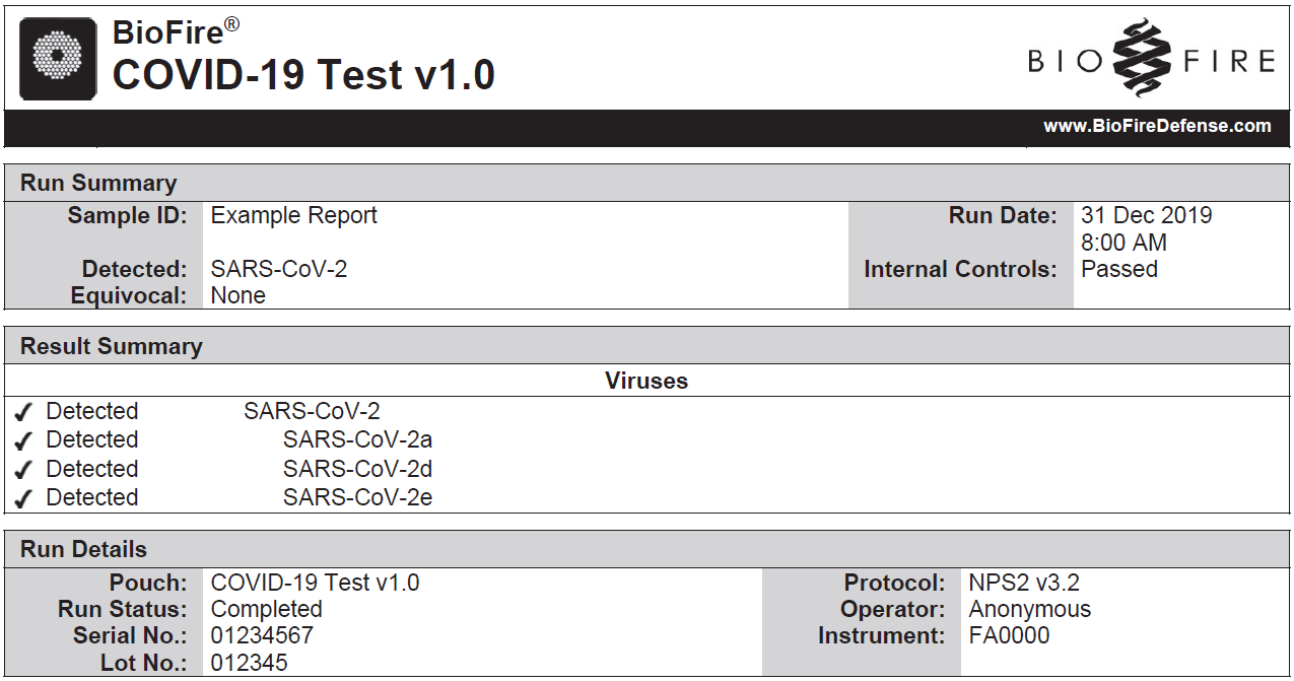
If two or more assays are 'Detected', the test report result will be SARS-CoV-2 'Detected'. If only one of the assays is 'Detected', the test report result will be SARS-CoV-2 'Equivocal'. If either a 'Detected' or an 'Equivocal' result is obtained, individual reflex testing must be performed (each specimen 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 retest result 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

BIOFIRE

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	The run was successfully completed AND The pouch controls were successful (Passed) AND Two or three assays for the virus were 'Detected' (i.e., met the requirements for a positive result described in the Assay Interpretation section above)	**Individual Specimen:** Report results.
		Sample Pool: Perform individual specimen reflex testing. Retest all specimens included in the sample pool individually.
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The three assays for the virus were 'Not Detected' (i.e., did not meet the requirements for a positive result described in the Assay Interpretation section above)	**Individual Specimen:** Report results.
		Sample Pool: Report results. Please note that 'Not Detected' results from pooled samples should be reported as presumptive. See Limitations for further information.
Equivocal	The run was successfully completed AND The pouch controls were successful (Passed) AND Only one of three assays was 'Detected' for the virus. The combination of 'Detected' and 'Not Detected' assay results were inconclusive	**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	The pouch controls were not successful (Failed) OR The run was not successful (Run Status displayed as: Aborted, Incomplete, Instrument Error or Software Error)	**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**
[1]Sample ID	New Example Id	Old Example Id	Anonymous	14 Dec 2019

Analysis of BioFire COVID-19 Test v1.0 External Control (+) Assays

The BioFire COVID-19 Test v1.0 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 v1.0 External Control Interpretation of Results

Result Interpretation	Result Analysis	Action
BioFire COVID-19 Test v1.0 External Control (+) Passes	**Result Summary** ✓ Detected SARS-CoV-2 ✓ Detected SARS-CoV-2a ✓ Detected SARS-CoV-2d ✓ Detected SARS-CoV-2e	Report the results.
BioFire COVID-19 Test v1.0 External Control (+) Failed Result	**Result Summary** ⇹ Equivocal SARS-CoV-2 ✓ Detected SARS-CoV-2a Not Detected SARS-CoV-2d Not Detected SARS-CoV-2e **Result Summary** Not Detected SARS-CoV-2 Not Detected SARS-CoV-2a Not Detected SARS-CoV-2d Not Detected SARS-CoV-2e	Repeat External Control (+) Testing. If the error persists, contact BioFire Defense Customer Support for further instruction.
BioFire COVID-19 Test v1.0 External Control (+) Invalid Result	**Result Summary** ⚠ Invalid SARS-CoV-2 ⚠ Invalid SARS-CoV-2a ⚠ Invalid SARS-CoV-2d ⚠ Invalid SARS-CoV-2e	Repeat External Control (+) Testing. If the error persists, contact BioFire Defense Customer Support for further instruction.

LIMITATIONS

1. For *In Vitro* Diagnostic (IVD) Use under Emergency Use Authorization (EUA) only.
2. The BioFire COVID-19 Test is a qualitative test and does not provide a quantitative value for the virus in the sample.
3. The BioFire COVID-19 Test v1.0 has not been validated for testing of individual specimens other than nasopharyngeal swabs collected in transport media or pooled specimens other than nasopharyngeal swabs.
4. BioFire COVID-19 Test performance has only been established on the FilmArray 2.0 and FilmArray Torch systems.
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 nucleic acid has been lost due to inadequate collection, transport, or storage of samples.
8. Performance of the BioFire COVID-19 Test has not been established for monitoring treatment of infection.
9. Viral nucleic acids may persist in vivo independent of virus viability. Detection of SARS-CoV-2 viral RNA targets does not imply that the virus is infectious or the causative agent for clinical symptoms.
10. As with any molecular test, mutations within the targeted regions of SARS-CoV-2 could affect primer binding, resulting in failure to detect the presence of virus.
11. 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, the patient should be considered for individual testing.
12. Based on in silico analysis all three SARS-CoV-2 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 the specified sample matrices; however, little is known about their potential to infect a human host, or their evolutionary relationship to SARS-CoV-2.
13. The performance of this device has not been assessed in a population immunized against COVID-19.
14. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
15. The BioFire COVID-19 Test External Control Kit v1.0 (+) is only for use with the BioFire COVID-19 Test (v1.0). It does not contain the entire genome of SARS-CoV-2. This product is not intended to replace the internal controls contained in the BioFire COVID-19 Test.
16. Quality control materials should be used in accordance with local, state, federal regulations and accreditation requirements.

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/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

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 laboratories[1] using the BioFire COVID-19 Test must 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 must use the BioFire COVID-19 Test as outlined in the authorized labeling. 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 must 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 must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories must 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 must 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 must 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 must 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 must 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 Nasopharyngeal Swab 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% x (TP / (TP +FN)). Negative Percent Agreement (NPA) was calculated as 100% x (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 with Nasopharyngeal Swabs

	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 1× LoD (N=20), 10× LoD (N=5), and at 100× LoD (N=5), and tested with the four (4) negative (unspiked) specimens. These 34 samples were tested randomized and in a blinded fashion. In addition, sixty-two (62) additional negative individual unique clinical specimens were also evaluated. All test results were as expected. The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) was determined by comparing the observed test results to the expected result. PPA and NPA are shown in Table 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 NPS 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 (≥95%) at LoD, and loss of detection (<95%) when tested 10-fold below LoD (0.1×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 3.3E+02 GC/mL (2.2E-02 $TCID_{50}$/mL), with a detection rate of 20/20 at 1×LoD, and 14/20 at 0.1×LoD (3.3E+01 GC/mL; 2.2E-03 $TCID_{50}$/mL).

Table 11. SARS-CoV-2 LoD Test Results for the BioFire COVID-19 Test - NPS

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

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	5.4E+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 (in silico)

Inclusivity of the BioFire COVID-19 Test was analyzed in silico using bioinformatics to survey all complete high-coverage genomes from the GISAID EpiCoV databases. A total of 541,884 GISAID sequences submitted before 01 June 2021 and collected after 01 March 2021, were analyzed for mismatches co-occurring in all three BioFire COVID-19 Test assays. Over the three-month collection time interval, there were no sequences with mutations occurring at a frequency of 0.1% or higher, within 10 bp of the 3' end of the primer, and on all three assays.

To address circulating strains with potential clinical importance, additional analyses were performed on all lineages designated as variants of interests (VOIs) or variants of concern (VOCs) by the CDC. Based on current monitoring, the emerging lineages are predicted to have minimal impact on the detection of SARS-CoV-2 by the BioFire COVID-19 Test. BioFire Defense is continuously monitoring emerging strains/sequence variants of SARS-CoV-2 and assessing predicated assay performance. For most up to date inclusivity analyses customers should refer to the website: www.biofiredefense.com.

Exclusivity (in silico)

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	*Corynebacterium diphtheria*
Human coronavirus HKU1	*Bacillus anthracis*
Human coronavirus NL63	*Moraxella catarrhalis*
SARS-coronavirus	*Neisseria elongata*
MERS-coronavirus	*Neisseria meningitidis*
Adenovirus	*Pseudomonas aeruginosa*
Human Metapneumonovirus (hMPV)	*Leptospira*
Parainfluenza virus 1-4	*Chlamydia psittaci*
Influenza A & B	*Coxiella burnetii*
Enterovirus	*Staphylococcus aureus*
Respiratory syncytial virus	*Homo sapiens*
Rhinovirus	SARS-coronavirus
Chlamydia pneumoniae	Coronavirus
Haemophilus influenza	Recombinant SARSr-CoV
Legionella pneumophila	SARS2
Mycobacterium tuberculosis	SARS coronavirus ExoN1
Streptococcus pneumonia	SARS coronavirus wtic-MB
Streptococcus pyogenes	SARS coronavirus MA15
Bordetella pertussis	SARS coronavirus MA15 ExoN1
Mycoplasma pneumoniae	Bat *Betacoronavirus* SARS related virus
Pneumocystis jirovecii	*Coronaviridae*
Candia albicans	*Coronavirinae*
Pseudomonas aeruginosa	
Staphylococcus epidermidis	
Staphylococcus salivarius	

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 respiratory sample matrix 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	Zeptometrix 0810229CF	1.26E+06 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Human coronavirus OC43	Zeptometrix 0810024CF	9.55E+06 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Human coronavirus HKU1 (clinical specimen)	Clinical Isolate (NPS)	~1.0E+08 copies/mL[a]	0/3	0/3	0/3	Not Detected
Human coronavirus NL63	Zeptometrix 0810228CF	2.51E+05 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
SARS-coronavirus (BSL3)	Culture (MRI Global)	5.3E+08 GE/mL	0/3	0/3	0/3	Not Detected
MERS-coronavirus (BSL3)	Culture (MRI Global)	2.7E+08 GC/mL	0/3	0/3	0/3	Not Detected
Chlamydia pneumoniae	ATCC 53592	2.90E+07 IFU/mL	0/3	0/3	0/3	Not Detected
Haemophilus influenzae	ATCC 700223	4.20E+08 CFU/mL (7.42E+08 copies/mL)	0/3	0/3	0/3	Not Detected
Legionella pneumophila	Zeptometrix 0801530	2.63E+09 CFU/mL	0/3	0/3	0/3	Not Detected
Mycobacterium tuberculosis (attenuated strain)	Zeptometrix 0801660	3.04E+07 CFU/mL	0/3	0/3	0/3	Not Detected
Streptococcus pneumoniae	ATCC 6303	8.90E+07 CFU/mL	0/3	0/3	0/3	Not Detected
Streptococcus pyogenes	ATCC 49399	4.65E+08 CFU/mL (8.25E+08 copies/mL)	0/3	0/3	0/3	Not Detected
Bordetella pertussis	Zeptometrix 0801459	6.70E+09 CFU/mL	0/3	0/3	0/3	Not Detected
Mycoplasma pneumoniae	Zeptometrix 0801579	3.98E+07 CCU/mL	0/3	0/3	0/3	Not Detected
Pseudomonas aeruginosa	ATCC 10145	5.68E+08 CFU/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	
Staphylococcus epidermidis	ATCC 29887	7.43E+09 CFU/mL	0/3	0/3	0/3	Not Detected
Streptococcus salivarius	ATCC 13419	7.38E+09 CFU/mL	0/3	0/3	0/3	Not Detected
Adenovirus 1 (species C)	Zeptometrix 0810050CF	3.39E+07 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Adenovirus 4 (species E)	Zeptometrix 0810070CF	7.05E+04 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Adenovirus 7 (species B)	Zeptometrix 0810021CF	5.10E+07 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Human Metapneumovirus (hMPV)	Zeptometrix 0810161CF	1.78E+05 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Parainfluenza virus 1	BEI NR-48681	8.0E+05 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Parainfluenza virus 2	Zeptometrix 0810504CF	1.10E+06 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Parainfluenza virus 3	BEI NR-3233	5.10E+07 $TCID_{50}$/mL (7.0E+08 copies/mL)	0/3	0/3	0/3	Not Detected
Parainfluenza virus 4	Zeptometrix 08010060BCF	1.70E+07 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Influenza A subtype H1	Zeptometrix 0810036CFN	7.05E+04 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Influenza A subtype H3	Zeptometrix 0810252CF	7.05E+04 $TCID_{50}$/mL (1.92E+08 copies/mL)	0/3	0/3	0/3	Not Detected
Influenza B	Zeptometrix 0810239CF	4.78E+06 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Enterovirus species A (EV71)	NCPV 0812215v	5.0E+08 $TCID_{50}$/mL (3.8E+08 copies/mL)	0/3	0/3	0/3	Not Detected
Enterovirus species B (Echovirus 6)	Zeptometrix 0810076CF	5.10E+07 $TCID_{50}$/mL (1.10E+08 copies/mL)	0/3	0/3	0/3	Not Detected
Enterovirus species C (Coxsackievirus A17)	ATCC VR-1023	7.90E+05 $TCID_{50}$/mL (3.17E+06 copies/mL)	0/3	0/3	0/3	Not Detected
Enterovirus species D (68)	Zeptometrix 0810237CF	1.58E+06 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Respiratory syncytial virus	Zeptometrix 0810040ACF	1.05E+06 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Rhinovirus	Zeptometrix 0810012CFN	1.26E+06 $TCID_{50}$/mL	0/3	0/3	0/3	Not Detected
Pneumocystis jirovecii (PJP)	ATCC PRA-159	1E+07 CFU/mL	0/3	0/3	0/3	Not Detected
Candida albicans	ATCC MYA-2876	7.88E+08 CFU/mL	0/3	0/3	0/3	Not Detected
Pooled human nasal wash [b]	-	-	-	-	-	-

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

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

Interference

Potentially interfering substances that could be present in NPS specimens or introduced during specimen collection and testing were evaluated previously on the FilmArray® Respiratory 2 (RP2) Panel for their effect on pouch performance. The RP2 Panel and the BioFire COVID-19 Test are used to evaluate NPS specimens; 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
DNA*Zap* (Ambion™ AM9891G & AM9892G)
RNase*Zap* (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	1.7×10^4 TCID50/mL
Adenovirus A12	8.9×10^5 TCID50/mL
Parainfluenza Virus 3	6.6×10^5 TCID50/mL
Bordetella pertussis	5.8×10^8 CFU/mL
Enterovirus D68	1.6×10^7 TCID50/mL
Echovirus 6	1.0×10^7 TCID50/mL
Respiratory Syncytial Virus	4.2×10^4 TCID50/mL
Staphylococcus aureus	2.5×10^7 CFU/mL
Streptococcus pneumoniae	1.7×10^7 CFU/mL
Haemophilus influenzae	6.2×10^7 CFU/mL
Candida albicans	1.0×10^6 CFU/mL
Herpes Simplex Virus 1	1.6×10^6 TCID50/mL
Cytomegalovirus (CMV)	1.2×10^6 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 authorized for pooling up to eight (8) upper respiratory 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 ($P_{individual}$) can be determined by dividing the number of positive specimens by the total number of specimens tested during that date range.

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

Refer to Table 16 to identify which pooling sample size provides the greatest testing efficiency for the determined $P_{individual}$ within the validated pool sizes for the assay. If $P_{individual}$ is 2% or less, then the largest validated pool size (n=8) should be used to maximize efficiency. If the $P_{individual}$ is greater than 25%, then pooling is not efficient and should not be implemented. The efficiency (F) of n-sample pooling for positivity rate (P) can be calculated with the following formula: $F= 1/(1+1/n-(1-P)^n)$. An example of the efficiency calculation for 5-sample pooling when P = 1% is: $F = 1/(1+1/5-(1-0.01)^5) = 4.02$. It means that 1,000 tests can cover testing of 4,020 patients on average.

Table 16. Testing Efficiency of Pooling

$P_{individual}$	n Corresponding to the Maximal Efficiency	Efficiency of n-Sample Pooling (maximum increase in number of tested patients)
1%–2%	8	4.94–3.65
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 $P_{individual}$ 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_{pools}) = (Number of positive specimens in pools / 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 the n-sample pooling strategy by calculating the positivity rate among patient samples during n-sample pooling ($P_{pools\text{-}x}$) for subsequent 7-10 day period based on n-sample pool testing. ($P_{pools\text{-}x}$) should be updated daily using a moving average.

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

- The n-sample pooling should be re-assessed by conducting a re-assessment study (described below).
- If $P_{pools\text{-}x}$ 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 ≥ 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 ≤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 ≥ 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 $P_{individual}$ 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*) × (10/11)
- Positivity rate for 20 samples is (20/N*) × (20/21).

This updated new positivity rate should be used as $P_{individual}$ 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 ($P_{pools\text{-}x}$) for subsequent 7-10 day period based on n-sample pool testing. ($P_{pools\text{-}x}$) should be updated daily using a moving average.

Compare $P_{pools\text{-}initial}$ to $P_{pools\text{-}x}$. If $P_{pools\text{-}x}$ is less than 90% of $P_{pools\text{-}initial}$. ($P_{pools\text{-}x} < 0.90 \times P_{pools\text{-}initial}$), pooling may continue, but a new n-sample pooling size may need to be considered. If $P_{pools\text{-}x}$ 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 Kit v1.0 (+), and throughout accompanying packaging.

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

APPENDIX B

Contact and Legal Information

Customer and Technical Support

Contact Us on the Web
http://www.BioFireDefense.com

Contact Us by Mail
79 West 4500 South, Suite 14
Salt Lake City, Utah USA
84107

Contact Us by E-mail
support@BioFireDefense.com

Contact Us by Phone
1-801-262-3592 – US and Canada
1-801-262-3592 – International

Contact Us by Fax
1-801-447-6907

BioFire Defense, LLC
79 West 4500 South, Suite 14
Salt Lake City, UT 84107 USA

 DFA2-PRT-0080-07, August 2021

APPENDIX C

References

1. Cucinotta D, Vanelli M. WHO Declares COVID-19 a Pandemic. Acta Bio Medica Atenei Parmensis. 2020;91(1):157-160. doi:10.23750/abm.v91i1.9397
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3. CDC | COVID-19. Accessed February 8, 2021. https://www.cdc.gov/coronavirus/2019-ncov/
4. Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-273. doi:10.1038/s41586-020-2012-7
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6. 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.
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9. 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.

REVISION HISTORY

Version	Revision Date	Description of Revision(s)
01	March 2020	Initial Release
02	April 2020	Instructions for Use (IFU) modified to include additional clinical performance data, as well as minor formatting edits.
03	September 2020	IFU updated to include FDA proficiency panel performance data.
04	October 2020	BioFire COVID-19 Test was updated to expand use with pooled NPS specimens per FDA authorization. IFU updated to include additional data supporting pooled specimens, as well as pooling monitoring and implementation plans.
05	February 2021	IFU updated to correct calculations in the pooled monitoring plan for NPS specimens and update language in Warnings and Precautions section to align with Emergency Use Authorization letter issued by FDA.
06	June 2021	IFU updated to support BioFire COVID-19 Test (v1.0) authorization with additional upper respiratory sample types.
07	August 2021	IFU updated to support pooling of upper respiratory specimens with the BioFire COVID-19 Test v1.0.

[redacted]

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

[redacted]

Step 6: Review Results

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

(1) Internal Controls:
- If 'Passed', results are valid.
- If 'Failed' or 'Invalid', RETEST SAMPLE and refer to *Instructions for Use*.

Test Interpretation:

(2a)
- If 'Detected', or 'Not Detected', report the results. (Figure 1)

(2b)
- If 'Equivocal', RETEST SAMPLE and refer to *Instructions for Use*. (Figure 2)

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

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

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

NOTE: *Refer to BioFire COVID-19 Test v1.0 Instructions for Use for reporting information. If repeated error messages are obtained, contact BioFire Defense Technical Support.*

BioFire® COVID-19 Test v1.0 — www.BioFireDefense.com

Run Summary			
Sample ID:	Example Report	Run Date:	31 Dec 2019 8:00 AM
(2a) Detected:	SARS-CoV-2	(1) Internal Controls:	Passed
Equivocal:	None		

(3) Result Summary	
Viruses	
✓ Detected	SARS-CoV-2
✓ Detected	SARS-CoV-2a
✓ Detected	SARS-CoV-2d
Not Detected	SARS-CoV-2e

(4) 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		

Figure 1. 'DETECTED' Report

BioFire® COVID-19 Test v1.0 — www.BioFireDefense.com

Run Summary			
Sample ID:	Example Report	Run Date:	31 Dec 2019 8:00 AM
(2b) Detected:	None	(1) Internal Controls:	Passed
Equivocal:	⬌SARS-CoV-2		

(3) Result Summary	
Viruses	
⬌ Equivocal	SARS-CoV-2
✓ Detected	SARS-CoV-2a
Not Detected	SARS-CoV-2d
Not Detected	SARS-CoV-2e

(4) 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		

Figure 2. 'EQUIVOCAL' Report

Conditions of Authorization

The BioFire COVID-19 Test v1.0 has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

The BioFire COVID-19 Test v1.0 has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of the BioFire COVID-19 Test v1.0 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.

BioFire® COVID-19 Test v1.0 Quick Guide

For use with FilmArray® 2.0 and FilmArray® Torch Systems

DO NOT DISCARD: Important product-specific information

For in vitro diagnostic use under an Emergency Use Authorization (EUA) only

REF 423745 (6 pk)
423744 (30 pk)

IVD

℞ Only

Intended Use

The BioFire® COVID-19 Test v1.0 is a nested multiplexed real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in non-pooled, upper respiratory swab specimens (nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs) in transport media from individuals suspected of COVID-19 by their healthcare provider. Please see www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd for the *BioFire COVID-19 Test v1.0 Instructions for Use*.

Storage Instructions

Store at 15 - 30ºC. Refer to the *BioFire COVID-19 Test v1.0 Instructions for Use* before opening and preparing pouch for use.

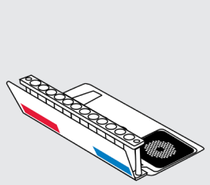
Procedure / Interpretation / Limitations

Users should refer to the *BioFire COVID-19 Test v1.0 Instructions for Use* available on the BioFire Defense Website: www.biofiredefense.com/covid-19test

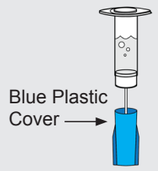
Questions / Support

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

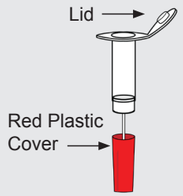
Package Contents



Pouch

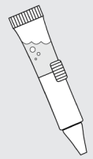


Hydration Injection Vial



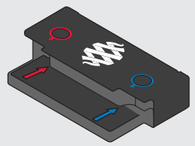
Sample Injection Vial

Transfer Pipette



Sample Buffer Tube

Materials required but not provided

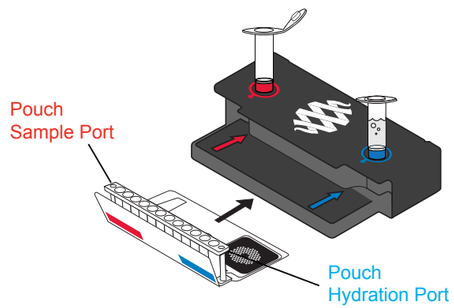


Pouch Loading Station (included with FilmArray System)

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

Step 1: Prepare Pouch

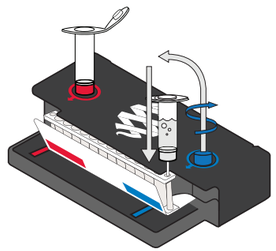
a. Insert pouch into Pouch Loading Station.
b. Place Sample Injection Vial into red well.
c. Place Hydration Injection Vial into blue well.



Step 2: Hydrate Pouch

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

NOTE: *Verify the pouch has been hydrated.*



BioFire® COVID-19 Test v1.0 Quick Guide

Step 3: Prepare Sample Mix

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

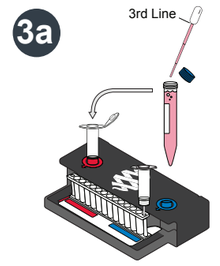
NOTE: *Do not touch tube tip.*

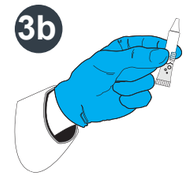
c. Dispense Sample Buffer into Sample Injection Vial using a slow, forceful squeeze followed by a second squeeze.

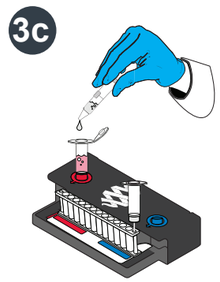
NOTE: *Avoid generating excessive foam.*

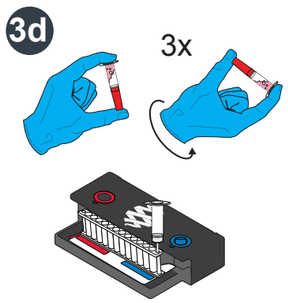
d. Tightly close the lid on the Sample Injection Vial and invert it 3 times, return it to the red well of Pouch Loading Station.

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









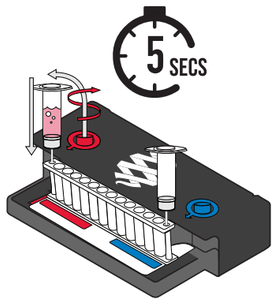
Step 4: Load Sample Mix

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

NOTE: *Waiting five seconds decreases the contamination risk.*

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

NOTE: *Verify the sample has been loaded.*



Step 5: Run Pouch

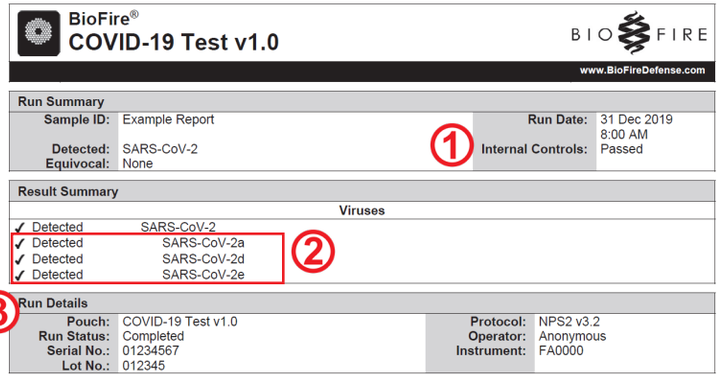
a. Discard the Sample Injection Vial and the Hydration Injection Vial in a biohazard sharps container.
b. Follow instructions on computer for starting a test.

BioFire® External Control Kit v1.0 Quick Guide
for BioFire COVID-19 Test v1.0

Step 6: Review Results

Run Summary

① Internal Controls:
- If 'Passed', results are valid.
- If 'Failed' or 'Invalid', RETEST CONTROL and refer to *BioFire COVID-19 Test v1.0 Instructions for Use*.


BioFire®
COVID-19 Test v1.0
BIOFIRE
www.BioFireDefense.com

Run Summary

Sample ID:	Example Report	Run Date:	31 Dec 2019 8:00 AM
Detected:	SARS-CoV-2	① Internal Controls:	Passed
Equivocal:	None		

Result Summary

	Viruses
✓ Detected	SARS-CoV-2
✓ Detected	SARS-CoV-2a
✓ Detected	SARS-CoV-2d
✓ Detected	SARS-CoV-2e

② ③

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		

Result Summary

②
- All 3 assays must be 'Detected'
- If any one assay is 'Not Detected', RETEST CONTROL and refer to *BioFire COVID-19 Test v1.0 Instructions for Use*.

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

Run Details

③ Run Status:
- If 'Completed', run is complete.
- If 'Incomplete', 'Aborted', or any other error message, RETEST CONTROL, and refer to *BioFire COVID-19 Test v1.0 Instructions for Use*.

NOTE: *Refer to BioFire COVID-19 Test v1.0 Instructions for Use for reporting information. If repeated error messages are obtained, contact Biofire Defense Technical Support.*

Conditions of Authorization

The BioFire COVID-19 Test External Control Kit v1.0 (+) has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

The emergency use of the BioFire COVID-19 Test External Control Kit v1.0 (+) 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.

DFA2-PRT-0084-03

DO NOT DISCARD: Important product-specific information

For in vitro diagnostic use under an Emergency Use Authorization (EUA) only

REF 423748 (6 pk) IVD

℞ Only

Summary and Explanation of the Test

BioFire Defense provides an external positive assayed quality control kit to monitor the performance of the BioFire COVID-19 Test v1.0 performed on FilmArray® 2.0 and FilmArray® Torch systems. Good laboratory practice recommends running positive and negative external controls regularly. Evaluation of external controls is recommended prior to using a new shipment or new lot of BioFire COVID-19 Test v1.0 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 v1.0 in accordance with appropriate federal, state, and local guidelines or accreditation requirements, as applicable.

Storage Instructions

Store at 15 - 30ºC.

Procedure / Interpretation / Limitations

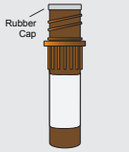
Users should refer to the *BioFire COVID-19 Test v1.0 Instructions for Use* available on the BioFire Defense Website: www.biofiredefense.com/covid-19test
An optional verification protocol is also available on this website.

Questions / Support

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

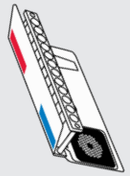
NOTE: ***Clean area thoroughly after testing External Positive Control to reduce risk of contamination.***

Package Contents

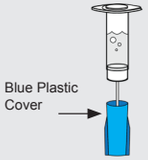


External Control Vial

Materials required but not provided

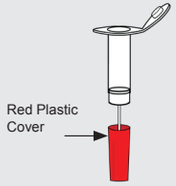


Pouch

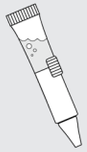


Hydration Injection Vial

Transfer Pipette



Sample Injection Vial



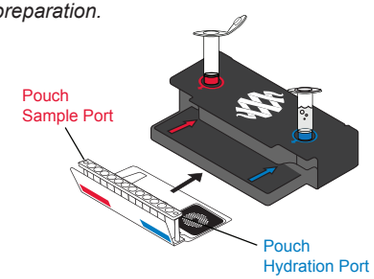
Sample Buffer Tube

Transport Media or Saline

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

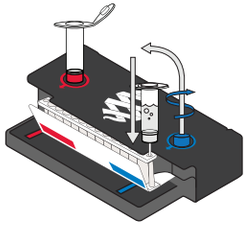
Step 1: Prepare Pouch

Refer to the *BioFire® COVID-19 Test v1.0 Quick Guide* for Step 1: Prepare Pouch



Step 2: Hydrate Pouch

Refer to the *BioFire® COVID-19 Test v1.0 Quick Guide* for Step 2: Hydrate Pouch



BioFire® COVID-19 Test v1.0 External Control Quick Guide

Step 3: Prepare External Control Mix

a. Use the Transfer Pipette to draw the Transport Media or Saline to the third line. Add to the Sample Injection Vial.

b. Remove rubber cap from External Control Vial (+) and place on a clean surface (a paper towel may be used).

c. Add Sample Buffer to External Control Vial (+).

- Hold Sample Buffer Tube tip facing up and firmly pinch at textured plastic tab on side of tube until seal snaps.

NOTE: *Do not touch tube tip.*

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

NOTE: *Avoid generating excessive foam.*

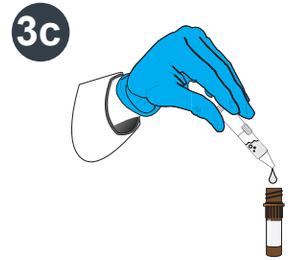
d. Recap External Control Vial (+) and invert 3 times to mix.

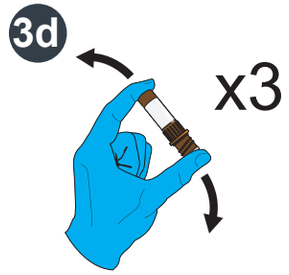
e. Pour mixture of Sample Buffer and Control into the Sample Injection Vial.

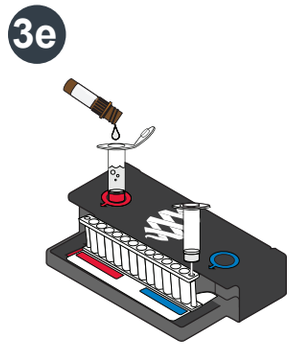
- **Dispose of External Control Vial (+) and change gloves.**

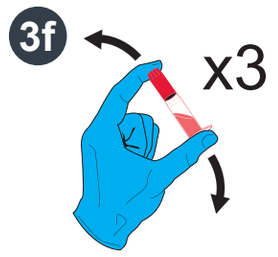
f. Tightly close lid of Sample Injection Vial and invert it 3 times; return it to the red well of Pouch Loading Station.

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



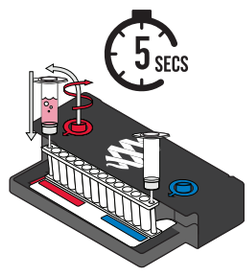






Step 4: Load Control Mix

Refer to the *BioFire® COVID-19 Test v1.0 Quick Guide* for Step 4: Load Sample Mix



Step 5: Run Pouch

Refer to the *BioFire® COVID-19 Test v1.0 Quick Guide* for Step 5: Run Pouch

BioFire® COVID-19 Test v1.1 Instructions for Use

BioFire® COVID-19 Test v1.1 Upper Respiratory Quick Guide

BioFire® COVID-19 Test v1.1 Lower Respiratory Quick Guide

BioFire Defense, LLC
Salt Lake City, Utah, USA

REF 423745 (6 pack test)
423744 (30 pack test)

BioFire®

COVID-19 Test

Instructions for Use

v1.1

IVD

℞ Only

The Symbols Glossary is provided on Page 39 of this booklet.

For in vitro diagnostic use under an Emergency Use Authorization (EUA) only

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

Manufactured by
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INTENDED USE

The BioFire® COVID-19 Test is a nested, multiplexed RT-PCR test performed on the FilmArray® 2.0 and FilmArray® Torch Instrument Systems intended for the qualitative detection of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in non-pooled upper respiratory swab specimens (i.e., nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs) (versions v1.0 and v1.1) or lower respiratory specimens (induced or expectorated sputum, endotracheal aspirates, bronchoalveolar lavage, or mini-bronchoalveolar lavage) (version v1.1 only) collected from individuals suspected of COVID-19 by their healthcare provider. The test is also for use with saliva specimens collected without preservatives in a sterile container in a healthcare setting from individuals suspected of COVID-19 by their healthcare provider (version v1.1 only). 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 upper respiratory specimens (i.e., nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs) collected individually 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 upper respiratory, lower respiratory, and saliva specimens 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 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 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

SARS-CoV-2 is a positive-sense, single-stranded RNA virus. It caused a global pandemic as the etiological agent of Coronavirus Disease 2019 (COVID-19), which is primarily characterized by shortness of breath, fever, and pneumonia and may be fatal for individuals who are older or have underlying health conditions[1–3]. The virus is thought to be of zoonotic origin and is highly transmissible through the inhalation of respiratory droplets[2–4].

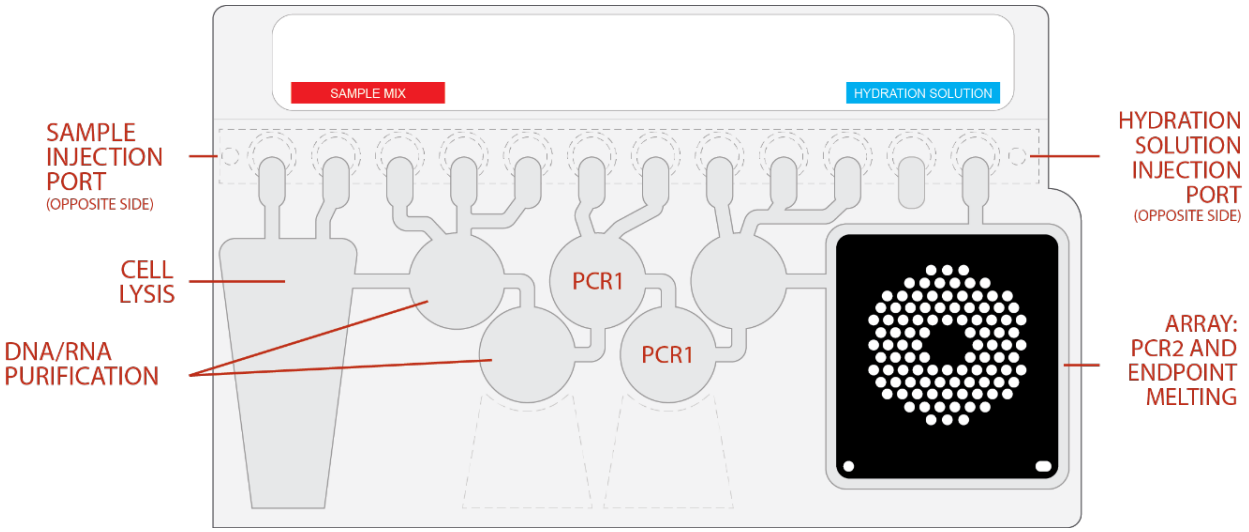
The BioFire COVID-19 Test is a qualitative test for use with the BioFire FilmArray 2.0 or Torch systems for the detection of SARS-CoV-2 RNA in upper respiratory (nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs), lower respiratory (sputum-like or BAL-like), and saliva specimens. The BioFire COVID-19 Test aids in the diagnosis of COVID-19 by testing samples in a time frame (~45 minutes) that allows the test results to be used in determining appropriate patient treatment and management. Internal controls are used to monitor all stages of the test process.

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 specimen. After sample collection, the user injects hydration solution, and sample combined with sample buffer into the pouch, places the pouch into a BioFire FilmArray instrument, and starts a run. The entire run process takes about 50 minutes. Additional details can be found in the appropriate BioFire 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; Part No. 423745) or 30 samples (30-test kit; Part No. 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 Upper Respiratory Quick Guide*
  - *BioFire COVID-19 Test Lower Respiratory Quick Guide*

NOTE: *When testing saliva specimens, refer to the BioFire COVID-19 Test Upper Respiratory Quick Guide.*

MATERIALS REQUIRED BUT NOT PROVIDED

- For saliva specimen collection: collection tubes without preservative
- For lower respiratory specimen testing: BioFire® Sample Swab Kit (Part No. 424063)

- BioFire® FilmArray® system including:
  - BioFire® FilmArray 2.0/Torch Instrument Systems and accompanying software
  - BioFire® FilmArray® Pouch Loading Station
- 10% bleach solution or a similar disinfectant
- 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*

ADDITIONAL AVAILABLE MATERIALS

- BIOFIRE® SHIELD™ Control Kit for the BioFire COVID-19 Test (Part No. 424062)
  - The BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test provides assayed positive controls for use in BioFire FilmArray System verifications.
  - See the BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test Instructions for Use for further information.

NOTE: *Known clinical samples, or simulated clinical samples containing inactivated virus or genomic RNA may also be used as external control testing material. Additional information may be found at www.biofiredefense.com/covid-19test.*

WARNINGS AND PRECAUTIONS

General Precautions

1. For in vitro diagnostic use under Emergency Use Authorization (EUA) only.
2. Positive results are indicative of the presence of SARS-CoV-2 RNA.
3. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
4. The BioFire COVID-19 Test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
5. 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.
6. The emergency use of the BioFire COVID-19 Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
7. 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.
8. BioFire COVID-19 Test pouches are only for use with BioFire FilmArray 2.0 and Torch systems.
9. Always check the expiration date on the pouch. Do not use a pouch after its expiration date.
10. 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 BioFire FilmArray instrument/module will be available and operational before unwrapping any pouches for loading.
11. Bleach introduced in a sample may damage nucleic acids in the sample, which may lead to a false negative result.
12. 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.
13. Patients should not eat, drink, use tobacco products, brush their teeth, use mouthwash, or chew gum for at least 30 minutes prior to providing a saliva specimen.
14. Saliva specimens must be collected in a healthcare setting under supervision of a healthcare provider.

Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable clean powder-free gloves. Protect skin, eyes, and mucus membranes. Change gloves often when handling reagents or samples.
2. Handle all samples and waste materials as if they were capable of transmitting infectious agents. Observe safety guidelines such as those outlined in:
   - CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*[5]
   - CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*[6]
   - 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 FilmArray Sample Buffer 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:

- Laboratory personnel may carry or shed SARS-CoV-2 asymptomatically and can inadvertently contaminate the specimen while it is being prepared. To avoid potential contamination, handle specimens in a biosafety cabinet. If a biosafety cabinet is not used, a dead air box (e.g., AirClean PCR workstation), a splash shield (e.g., Bel-Art Scienceware Splash Shield), or a face shield should be used when preparing specimens for testing.
- Do not handle samples or pouches in a biosafety cabinet which is used for SARS-CoV-2 culture or immunofluorescence testing.
- Laboratory personnel should wear a standard surgical mask (or equivalent) and should avoid touching the mask while handling specimens.
- Prior to processing specimens, thoroughly clean both the work area and the BioFire 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.
- Specimens 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.
- The BioFire COVID-19 Test assays may react with vaccines that contain specific segments of the pathogen genome or full genome or vaccines containing attenuated/inactivated pathogen, including vaccines for SARS-CoV-2. Avoid collecting or handling specimens in areas that are exposed to SARS-

CoV-2 vaccine material. Particular care should be taken during these processes to avoid contamination.
- Clinical history of vaccine administration should be considered in the interpretation of results, particularly for vaccines administered by nasal spray.

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.

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 in the appropriate BioFire FilmArray operator's manual.

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

Precaution Related to Public Health Reporting

Local, state and federal regulations for notification of reportable disease are continually updated and include organisms/viruses for surveillance and outbreak investigations[7]. 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.

Laboratories within the U.S. and its territories are required to report all SARS-CoV-2 results to the appropriate national public health authorities.

REAGENT STORAGE, HANDLING, AND STABILITY

1. Store the test, 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.

	Upper Respiratory and Saliva Specimens	Lower Respiratory Specimens
Specimen Types	**Upper Respiratory Swabs** • Including nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swab collected according to standard technique and immediately placed in 1-3 mL of transport medium, sterile normal saline (0.9%), or sterile phosphate-buffered saline. **Saliva** • Approximately 1-3 mL of saliva should be collected without preservatives in a sterile tube in a healthcare setting. • Patients should not eat, drink, use tobacco products, brush their teeth, use mouthwash, or chew gum for at least 30 minutes prior to providing a saliva specimen.	**Sputum-like specimens** • Includes induced or expectorated sputum, or endotracheal aspirate (ETA) collected according to standard technique. **Bronchoalveolar lavage (BAL)-like specimens** • Includes BAL, or mini-BAL collected according to standard technique.
Minimum Specimen Volume	Approximately 0.3 mL (300 µL) per test	Approximately 0.2 mL (200 µL) of specimen material will be captured by the Sample Swab per test (BioFire Sample Swab Kit; Part No. 424063).
Transport and Storage	Specimens should be processed and tested with the BioFire COVID-19 Test as soon as possible. If storage is required, samples may be held: • At room temperature for up to 4 hours (15-25ºC) • Refrigerated for up to 3 days (2-8ºC) • Frozen (≤-15ºC or ≤-70ºC) for up to 30 days	Specimens should be processed and tested with the BioFire COVID-19 Test as soon as possible. If storage is required, samples may be held: • Frozen (≤-70ºC) for up to 30 days

NOTE: *Specimens should NOT be centrifuged before testing. Lower respiratory specimens should NOT be pre-processed, treated with mucolytic or decontaminating agents (e.g., MycoPrep, Sputasol, Snap n' Digest, DTT, sodium hydroxide, oxalic acid, trypsin etc.), or placed into transport medium before testing.*

NOTE: *In accordance with good laboratory practice recommendations, institutions should follow their own established rules for acceptance/rejection of lower respiratory specimens (e.g., using Gram stain/Q-score) and therefore apply appropriate guidelines locally for acceptance/rejection of a sample for 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.

Upper respiratory and saliva specimens should be tested according to the Upper Respiratory testing procedures described below in Step 3a and the Upper Respiratory Quick Guide. Lower respiratory specimens should be tested according to the Lower Respiratory testing procedures described below in Step 3b and in the Lower Respiratory Quick Guide. Refer to the appropriate BioFire FilmArray operator's manual for additional information on the FilmArray instruments.

Preparing Upper Respiratory 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). Only upper respiratory specimens (i.e., nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs) which have been collected individually 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.

   NOTE: *To avoid cross-contamination of specimens, use a new micropipette tip or disposable transfer pipette for each specimen.*

   NOTE: *Pipettes for the pooling procedure are not provided with the BioFire COVID-19 Test kit.*
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.

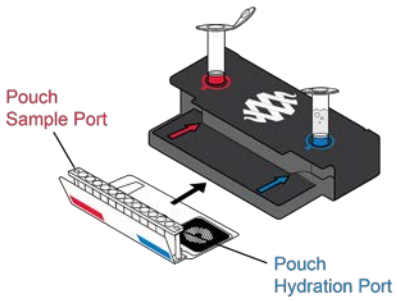
   NOTE: *Sample IDs should indicate that the sample was pooled.*

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.

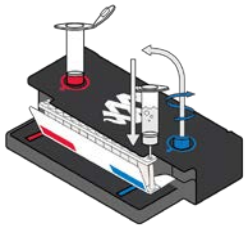
   NOTE: *The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.*

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, re-insert Hydration Injection Vial to ensure 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), re-insert Hydration Injection Vial to ensure 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 3a: Prepare Upper Respiratory Sample Mix

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

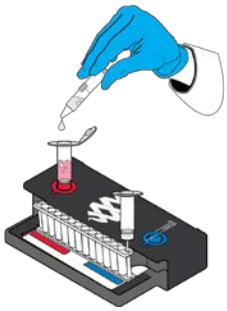
   NOTE: *DO NOT use the Transfer Pipette to mix the sample once it is loaded into the Sample Injection Vial.*

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.*

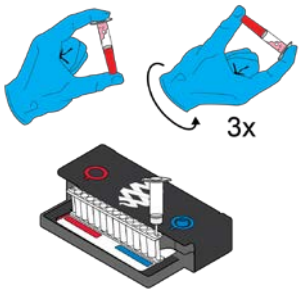
   - Firmly pinch at textured plastic tab on the side of the tube until the seal snaps.
   - Invert the tube over the Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

     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 3b: Prepare Lower Respiratory Sample Mix

1. Hold Sample Buffer tube tip facing up and firmly pinch at textured plastic tab on side of tube until seal snaps.

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

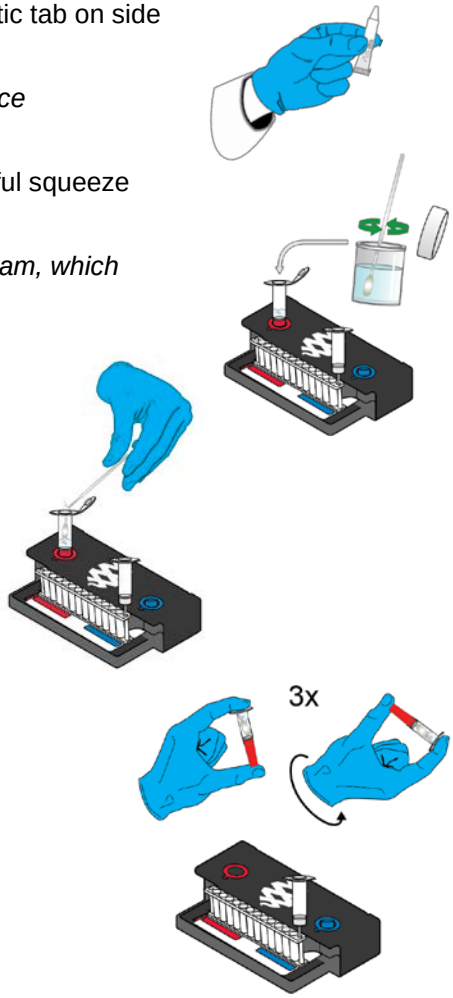
2. Dispense Sample Buffer into Sample Injection Vial using a slow, forceful squeeze followed by a second squeeze.

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

3. Use the Sample Swab (BioFire Sample Swab Kit, Part No. 424063) to stir the entire specimen for ~10 seconds.

4. Place the swab end into the Sample Injection Vial and break off the swab handle at the scored breakpoint. Discard the swab handle into an appropriate waste container and close Sample Injection Vial lid tightly.

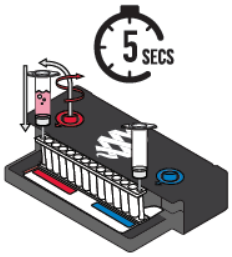
5. Invert the Sample Injection Vial 3 times and return to red well of Pouch Loading Station.



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

BioFire FilmArray 2.0

1. Ensure that the BioFire 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) and Pouch Type 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 from the Protocol drop down list. The BioFire COVID-19 Test v1.1 has a single Sample protocol available from the drop-down list for the testing of all sample types.

   NOTE: *There are three available protocols for use with the BioFire COVID-19 Test v1.1: Sample, Positive External Control, and Negative External Control. The Positive and Negative External Control Protocols are only for use with the BIOFIRE SHIELD Control Kit. It is necessary to ensure that the appropriate protocol is selected* ***prior*** *to running the test.*
6. Enter a username and password in the Name and Password fields.

   NOTE: *The font color of the username is red until the username 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 BioFire FilmArray database, and the test report can be printed, viewed, and/or saved as a PDF file.

BioFire FilmArray Torch

1. Ensure that the BioFire 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) and Pouch Type 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 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.

   Select and/or confirm the appropriate protocol from the Protocol drop down list. The BioFire COVID-19 Test v1.1 has a single Sample Protocol available from the drop-down list for the testing of all sample types.

   NOTE: *There are three available protocols for use with the BioFire COVID-19 Test v1.1: Sample, Positive External Control, and Negative External Control. The Positive and Negative External Control Protocols are only for use with the BIOFIRE SHIELD Control Kit. It is necessary to ensure that the appropriate protocol is selected* ***prior*** *to running the test.*

6. Enter operator username and password, then select Next.

   NOTE: *The font color of the username is red until the username 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 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.*

8. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.
9. The run file is automatically saved in the BioFire 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 BioFire FilmArray software will automatically fail the run if the melting temperature (Tm) for either the RNA Process Control or the PCR2 Control is outside of an acceptable range (80.3-84.4°C for the RNA Process Control and 73.8-78.2°C for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices.[8,9] Refer to the appropriate BioFire FilmArray operator's manual for instructions on obtaining control assay Tm values. The PCR2 Control is used in several FilmArray pouch types (e.g., RP2, BCID, GI, ME) and can therefore be used to monitor the system when multiple pouch types are used on the same BioFire FilmArray system or instrument.

External Controls

External Controls should be used in accordance with laboratory protocols and the appropriate accrediting organization requirements, as applicable. Transport medium, saline, or PBS may be used as an external negative control. Previously characterized positive specimens, or negative specimens spiked with inactivated virus may be used as external positive controls. Alternatively, the BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test (Part No. 424062), or other commercially available control material may be used; use according to the control manufacturer's instructions. Refer to www.biofiredefense.com/covid-19test for guidance in selecting appropriate control materials and developing a laboratory verification protocol.

INTERPRETATION OF RESULTS

NOTE: *The BioFire COVID-19 Test results interpretation differs between versions v1.0 and v1.1. Consult the corresponding Instructions for Use. All BioFire COVID-19 Test v1.0 and v1.1 labeling is available on the BioFire Defense Product Support webpage: www.biofiredefense.com/covid-19test.*

The BioFire COVID-19 Test consists of three independent and non-overlapping assays targeting two SARS-CoV-2 genes: ORF1ab and ORF8. The gene target of each assay is shown in Table 1 below. The assays are designed to detect SARS-CoV-2 specifically. Each assay exists on the PCR2 array of the pouch in multiple replicate wells.

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 Results

When PCR2 is complete, the BioFire FilmArray instrument performs a DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well of the PCR2 array (for more information see appropriate BioFire FilmArray operator's manual). The BioFire 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 BioFire FilmArray Software evaluates the DNA melt curve for each well of the PCR2 array. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve. If the software determines that the calculated Tm is within the range specified for the assay, the well is called positive; otherwise, the well is called negative.

Analysis of Replicates. The results of the replicate wells are then used to determine the result for each assay. If at least two of the wells associated with an assay are positive and if the melting temperatures for at least two of the positive wells are similar (within 1.0°C), the assay is called 'Detected'. If these criteria are not met, the assay is called 'Not Detected'.

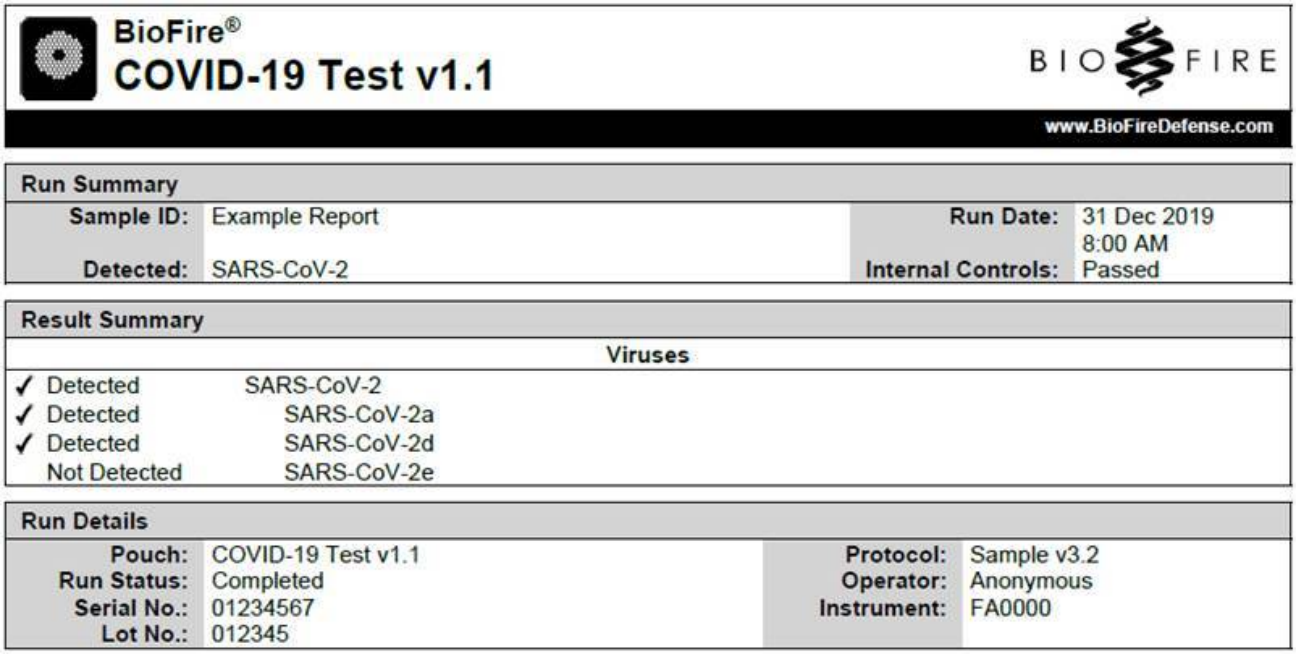
Overall Test Result

The results of each assay are combined to determine a final test result. If any of the assays are 'Detected', the overall test result (i.e., the SARS-CoV-2 result) will be 'Detected'. If none of the assays (SARS-CoV-2a, SARS-CoV-2d, or SARS-CoV-2e) are 'Detected', the overall test result will be 'Not Detected.

In cases where either one or both internal control assays have failed, all results are reported as '⚠ Invalid', and retesting is required.

BioFire COVID-19 Test Report

The BioFire COVID-19 Test report is automatically displayed upon completion of a test and can be printed or saved as a PDF file. Each report contains a Run Summary, a Result Summary, and a Run Details section. The test interpretation may be viewed in either the Run Summary or Result Summary sections, with only the Result Summary providing information for individual assays.



BioFire®
COVID-19 Test v1.1

BIOFIRE

www.BioFireDefense.com

Run Summary

Sample ID:	Example Report	**Run Date:**	31 Dec 2019 8:00 AM
Detected:	SARS-CoV-2	**Internal Controls:**	Passed

Result Summary

	Viruses
✓ Detected	SARS-CoV-2
✓ Detected	SARS-CoV-2a
✓ Detected	SARS-CoV-2d
Not Detected	SARS-CoV-2e

Run Details

Pouch:	COVID-19 Test v1.1	**Protocol:**	Sample 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 a detection summary.

A detection summary is displayed in the 'Detected' field. If the internal controls have passed and the overall test result is 'Detected' then the 'Detected' field will display 'SARS-CoV-2'. If the internal controls have passed and the overall test result is 'Not Detected' then the 'Detected' field will display 'None'. In the case of an incomplete run or failed internal controls, the 'Detected' field will display '⚠ Invalid'.

A summary of the internal control results is displayed in the 'Internal Controls' field. Internal controls are listed as 'Passed', 'Failed', or '⚠ Invalid'. Table 2 provides additional information for each of the possible internal control field results.

Table 2. 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 passed.	The test results are valid. Refer to the Result Summary section for instructions on how to interpret the results.
Failed	The run was successfully completed BUT At least one of the pouch controls failed.	Repeat the test using a new pouch. If the error persists, contact BioFire Defense Technical 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 BioFire FilmArray operator's manual or contact BioFire Defense Technical 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 overall test result for SARS-CoV-2 on the first line followed by each individual assay result. According to the result for each associated assay, 'Detected', 'Not Detected', or '⚠ Invalid' will be indicated to the left of each assay name. If any assay has a 'Detected' result, the overall SARS-CoV-2 result will also be 'Detected'. Table 3 provides an explanation for each interpretation and any follow-up necessary to obtain a final result. If an internal control assay has failed, or if the run was not successfully completed, all results will be '⚠ Invalid' in the Result Summary section.

Table 3. Reporting of Results and Required Actions

Overall SARS-CoV-2 Result	Explanation	Action
Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND One or more assays for the virus were 'Detected' (i.e., met the requirements for a 'Detected' result described in the Assay Interpretation section above)	**Individual Specimen:** Report results.
		Sample Pool: Perform individual specimen reflex testing. Retest all specimens included in the sample pool individually.
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The three assays for the virus were 'Not Detected' (i.e., did not meet the requirements for a 'Detected' result described in the Assay Interpretation section above)	**Individual Specimen:** Report results.
		Sample Pool: Report results. Please note that 'Not Detected' results from pooled samples should be reported as presumptive. See Limitations for further information.
Invalid	The pouch controls were not successful (Failed) OR The run was not successful (Run Status displayed as: Aborted, Incomplete, Instrument Error or Software Error)	**Individual Specimen:** See Table 2, 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 Technical Support Team.

Run Details

The **Run Details** section provides additional information about the run including pouch information (type, lot number, and serial number), run status (Completed, Incomplete, Aborted, Instrument 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. Any Sample IDs that have been changed will also be indicated by an asterisk in the BioFire FilmArray Database.

Change Summary				
Field	**Changed To**	**Changed From**	**Operator**	**Date**
[1]Sample ID	New Example Id	Old Example Id	Anonymous	14 Dec 2019

LIMITATIONS

1. For in vitro diagnostic use under Emergency Use Authorization (EUA) only.
2. The BioFire COVID-19 Test is a qualitative test and does not provide a quantitative value for the virus in the sample.
3. The BioFire COVID-19 Test has not been validated for testing of individual specimens other than upper respiratory swabs (nasopharyngeal, oropharyngeal, anterior nasal, mid-turbinate nasal), unprocessed sputum specimens, saliva specimens, or pooled specimens other than nasopharyngeal swabs.
4. When testing lower respiratory specimens with the BioFire COVID-19 Test, use the BioFire Sample Swab Kit. Performance of the BioFire COVID-19 Test has not been evaluated with other swabs.
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 nucleic acid has been lost due to inadequate collection, transport, or storage of samples.
8. Performance of the BioFire COVID-19 Test has not been established for monitoring treatment of infection.
9. Viral nucleic acids may persist *in vivo* independent of virus viability. Detection of SARS-CoV-2 viral RNA targets does not imply that the virus is infectious or the causative agent for clinical symptoms.
10. As with any molecular test, mutations within the targeted regions of SARS-CoV-2 could affect primer binding, resulting in failure to detect the presence of virus.
11. 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, the patient should be considered for individual testing.
12. Based on in silico analysis all three SARS-CoV-2 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 the specified sample matrices; however, little is known about their potential to infect a human host, or their evolutionary relationship to SARS-CoV-2.
13. The performance of this device has not been assessed in a population immunized against COVID-19.
14. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

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/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

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 laboratories[a] using the BioFire COVID-19 Test must include with test result reports of the BioFire COVID-19 Test, 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 must use the BioFire COVID-19 Test as outlined in the authorized labeling. 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 must 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 must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories must 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 must 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 must 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 must 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 must 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.

[a] For ease of reference, the letter of authorization refers to, "United States (U.S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, 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 (non-pooled specimens) and DoD laboratories that meet the requirements to perform high complexity tests (pooled specimens) as 'authorized laboratories'."

PERFORMANCE CHARACTERISTICS

Clinical Performance (Upper Respiratory Specimens)

Summary of Clinical Specimen Testing

The overall clinical performance on individually tested upper respiratory specimens is summarized in Table 4. The studies contributing to this summary are detailed below.

Table 4. Summary of Individual Upper Respiratory Clinical Specimens Evaluated with the BioFire COVID-19 Test

	PPA	%	95% CI	NPA	%	95% CI
Anterior Nasal Swab (ANS)	4/4	100%	51.0-100%	6/6	100%	61.0-100%
Nasopharyngeal Swab (NPS)	34/35	97.1%	85.5-99.5%	76/76	100%	95.2-100%
Oropharyngeal Swab (OPS)	5/5	100%	56.6-100%	5/5	100%	56.6-100%
Overall	43/44	97.7%	88.2-99.6%	87/87	100%	95.8-100%

Testing of Archived Clinical Specimens

Archived upper respiratory specimens were nasopharyngeal swab (NPS), anterior nasal swab (ANS), or oropharyngeal swab (OPS) in transport medium. All specimens were residual after standard of care that were either obtained from commercial biorepositories and tested at BioFire Defense, or were obtained and tested at the University of Nebraska Medical Center (UNMC).

Positive specimens and 71 negative specimens were collected from patients presenting with signs or symptoms of COVID-19, and previously characterized for SARS-CoV-2 infection by another EUA test. An additional five NPS specimens were collected in 2018, and were therefore presumed negative for SARS-CoV-2.

For the ten positive and five negative specimens tested at UNMC, the BioFire COVID-19 Test result was considered a True Positive (TP) or True Negative (TN) only when it agreed with the original EUA test result. All other specimens were tested at BioFire Defense on the BioFire COVID-19 Test and in parallel on the BioFire Respiratory 2.1 Panel (BioFire Diagnostics) as comparator. For all specimens, Positive Percent Agreement (PPA) was calculated as 100% x (TP / (TP+FN)) and Negative Percent Agreement (NPA) was calculated as 100% x (TN / (TN+FP)). Twenty-three (23) of out 24 positive specimens were Detected by the BioFire COVID-19 Test and all negative samples were Not Detected, resulting in 95.8% PPA and 100% NPA (Table 5).

Table 5. BioFire COVID-19 Test Performance Summary with Upper Respiratory Specimens

	PPA	%	95% CI	NPA	%	95% CI
Anterior Nasal Swab (ANS)	4/4	100%	51.0-100%	6/6[a]	100%	61.0-100%
Nasopharyngeal Swab (NPS)	14/15[b]	93.3%	70.2-98.8%	10/10	100%	72.3-100%
Oropharyngeal Swab (OPS)	5/5	100%	56.6-100%	5/5	100%	56.6-100%
Overall	23/24	95.8%	79.8-99.3%	21/21	100%	84.5-100%

[a] One specimen was initially positive by standard of care test, but negative by comparator and BioFire COVID-19 Test.

[b] FN specimen was positive with a late Ct value when originally evaluated for standard of care. When the FN sample was retested on the same standard of care test, the result was negative. These results indicate a near-LoD level of SARS-CoV-2 virus and/or sample degradation after the first standard of care test and prior to the BioFire COVID-19 Test

Testing of Contrived Clinical Specimens

Contrived testing was performed using negative clinical specimens and contrived clinical specimens. Positive Percent Agreement (PPA) was determined by comparing the expected 'Detected' results to the observed test results for samples contrived by spiking infectious virus into unique clinical specimens. A total of 70 spiked clinical specimens were tested: 40 of the spiked clinical specimens were tested as part of the LoD evaluation (24 at 1× and 4 each at 3×, 10×, 30×, and 100×LoD), and 30 additional spiked clinical specimens were contrived in a separate spiking event (20 at 1× and 5 each at 10× and 100×LoD). Negative Percent Agreement (NPA) was determined by comparing the observed test results for 66 SARS-CoV-2 negative clinical specimens (i.e. non-spiked) to the expected results of 'Not Detected'. PPA and NPA are shown in Table 6.

Table 6. Clinical Contrived and Negative Testing with the BioFire COVID-19 Test

	Agreement with known analyte composition			
	PPA	%	NPA	%
Overall Agreement	70/70	100%	66/66	100%
95% CI	**[94.8 – 100]**		**[94.5 – 100]**	

Testing of Pooled Clinical Specimens

Archived NPS 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-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (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 7.

Table 7. Detection of SARS-CoV-2 in Pools of 5 or 8 NPS 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).

Clinical Performance (Lower Respiratory Specimens)

Archived lower respiratory specimens were residual uncharacterized sputum collected during the COVID-19 pandemic for standard of care and obtained from a commercial biorepository. Specimens were screened with a SARS-CoV-2 RT-PCR assay at BioFire Defense, and positive and negative specimens were selected for the performance comparison.

Specimens were de-identified before testing on the BioFire COVID-19 Test and the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel as comparator. All (15/15) comparator-positive specimens were Detected by the BioFire COVID-19 Test, and 27/29 comparator-negative samples were Not Detected, resulting in 100% PPA and 93.1% NPA (Table 8).

Table 8. BioFire COVID-19 Test Performance Summary with Lower Respiratory Specimens

	PPA	%	95% CI	NPA	%	95% CI
Sputum	15/15	100%	79.6-100%	27/29[a]	93.1%	78.0-98.1%

[a] The two FP specimens were initially characterized as SARS-CoV-2-positive. Additional investigation by a separate PCR-based assay also found evidence of SARS-CoV-2 in both FP specimens.

Clinical Performance (Saliva Specimens)

Archived paired saliva and NPS specimens collected on the same day from the same individual were obtained from commercial biorepositories. The NPS specimen in each pair was previously characterized using a highly sensitive test with EUA for NPS; a limited number of saliva specimens were also characterized using a test with EUA for saliva. Paired specimens were de-identified and tested on the same day on the BioFire COVID-19 Test. The NPS specimen result was considered the reference for performance calculations (Table 9). SARS-CoV-2 was detected by the BioFire COVID-19 Test in 33/34 saliva/NPS specimen pairs (97.1% PPA). In three instances, SARS-CoV-2 was detected only in the saliva specimen of a matched pair (95.1% NPA).

Table 9. BioFire COVID-19 Test Performance Summary with Paired Saliva and NPS Specimens

		NPS		BioFire COVID-19	
		Pos	Neg	Test Performance	95% CI
Saliva	Pos	33	3[a]	33/34 97.1% PPA	85.1-99.5%
	Neg	1	58	58/61 95.1% NPA	86.5-98.3%
	Total	34	61		

[a] Evidence for SARS-CoV-2 was found in 1/3 negative NPS specimens upon retesting on an independent EUA assay.

Limit of Detection

The BioFire COVID-19 Test limit of detection (LoD) was determined using contrived samples containing known concentrations of inactivated or infectious SARS-CoV-2 material. The LoD concentration was first estimated based on results of serial dilutions spanning concentrations bracketing the anticipated LoD concentration. Additional dilutions were tested, if needed, to reach a concentration at which loss of detection could be observed. The LoD was then confirmed by testing 20 replicates at the estimated LoD concentration; LoD is defined as the lowest concentration of SARS-CoV-2 RNA that could be detected in at least 95% of replicates (at least 19/20 runs).

Upper Respiratory Specimens

Contrived samples were prepared with SARS-CoV-2 at known concentrations in NPS background. Results are shown in Table 10. The LoD for infectious SARS-CoV-2 material was determined to be 3.3E+02 GC/mL (2.2E-02 $TCID_{50}$/mL), with a detection rate of 100% (20/20); The LoD for heat-inactivated SARS-CoV-2 material was determined to be 3.3E+02 GE/mL (4.3E-02 $TCID_{50}$/mL), with a detection rate of 100% (20/20).

Table 10. SARS-CoV-2 LoD Test Results for the BioFire COVID-19 Test - NPS

Virus	LoD Concentration		Detection Rate
	GE/mL	$TCID_{50}$/mL	
SARS-CoV-2 USA-WA1/2020 (infectious culture; WRCEVA)[a]	3.3E+02	2.2E-02	20/20 (100%)
SARS-CoV-2 USA-WA1/2020 (heat-inactivated; BEI NR-52286)[b]	3.3E+02	4.3E-02	20/20 (100%)

[a] Obtained for culture in a biosafety level 3 laboratory from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA). Concentration determined by quantitative real-time PCR as described on the World Health Organization website: https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf

[b] Concentration determined by digital droplet PCR as indicated on the Certificate of Analysis provided by BEI Resources. $TCID_{50}$/mL was determined prior to inactivation.

Lower Respiratory Specimens

The LoD for lower respiratory specimens was determined by spiking heat-inactivated SARS-CoV-2 material from the USA-WA1/2020 variant (BEI/NR-52286) into residual sputum specimens verified for negativity. LoD testing results are shown in Table 11. The LoD was determined to be 1.0E+04 GE/mL (1.4E+00 $TCID_{50}$/mL), with a detection rate of 100% (20/20).

Table 11. SARS-CoV-2 LoD Test Results for the BioFire COVID-19 Test - Sputum

Variant (Source)	LoD Concentration[1]		# Detected/Total (% Detection)
	GE/mL	$TCID_{50}$/mL	
USA-WA1/2020 (BEI/NR-52286)	1.0E+04	1.4E+00	19/20 (95%)

[1] Concentration determined by digital droplet PCR as indicated on the Certificate of Analysis provided by BEI Resources. $TCID_{50}$/mL was determined prior to inactivation.

Saliva Specimens

The LoD for saliva specimens was determined by spiking heat-inactivated SARS-CoV-2 material from the USA-WA1/2020 variant (BEI/NR-52286) into confirmed negative saliva. Twenty replicates were individually contrived by spiking inactivated SARS-CoV-2 material into SARS-CoV-2-negative saliva. The LoD was determined to be 3.3E+02 GE/mL (2.9E-01 $TCID_{50}$/mL), with a detection rate of 100% (20/20).

Table 12. SARS-CoV-2 LoD Test Results for the BioFire COVID-19 Test - Saliva

Virus	LoD Concentration[1]		Detection Rate
	GE/mL	$TCID_{50}$/mL	
SARS-CoV-2 USA-WA1/2020 (heat-inactivated; BEI NR-52286)	3.3E+02	2.9E-01	20/20 (100%)

[1] Concentration determined by digital droplet PCR as indicated on the Certificate of Analysis provided by BEI Resources. $TCID_{50}$/mL was determined prior to inactivation.

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 U.S. 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 13. No cross-reactivity with MERS-CoV was reported.

Table 13. 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	5.4E+03 NDU/mL[a]	N/A[b]
MERS-CoV		N/A[b]	ND[c]

[a] NDU: Nucleic acid amplification test (NAAT) Detectable Units
[b] N/A: Not applicable
[c] ND: Not detected

Validation of Saline and PBS for use with Upper Respiratory Specimens

Sensitivity of the BioFire COVID-19 Test when testing NPS collected in either saline or PBS was evaluated by confirming the reliable detection (≥ 95%) of SARS-CoV-2 at 1× the LoD. For each medium, 20 samples were individually contrived by spiking inactivated SARS-CoV-2 from the USA-WA1/2020 isolate (BEI/NR-52286) into negative residual NPS specimens that had been collected in either saline or PBS. Each replicate was contrived at a concentration of 3.3E+02 GE/mL (1× LoD). Results are shown in Table 14. Reliable detection at 1× LoD was observed for both mediums.

Table 14. Summary of Results for Contrived NPS Specimens in PBS and Saline

Clinical Matrix	Testing Concentration	Detection Rate
NPS in PBS	3.3E+02 GE/mL (1× LoD)	20/20 (100%)
NPS in Saline	3.3E+02 GE/mL (1× LoD)	20/20 (100%)

Analytical Reactivity (in silico)

Inclusivity (in silico)

Inclusivity of the BioFire COVID-19 Test was analyzed in silico using bioinformatics to survey all complete high-coverage genomes from the GISAID EpiCoV database. A total of 541,884 GISAID sequences submitted before 01 June 2021 and collected after 01 March 2021, were analyzed for mismatches co-occurring in all three BioFire COVID-19 Test assays. Over the three-month collection time interval, there were no sequences with mutations occurring at a frequency of 0.1% or higher, within 10 bp of the 3' end of the primer, and on all three assays.

To address circulating strains with potential clinical importance, additional analyses were performed on all lineages designated as variants of interests (VOIs) or variants of concern (VOCs) by the CDC. Based on current monitoring, the emerging lineages are predicted to have minimal impact on the detection of SARS-CoV-2 by the BioFire COVID-19 Test. BioFire Defense is continuously monitoring emerging strains/sequence variants of SARS-CoV-2 and assessing predicated assay performance. For most up to date inclusivity analyses customers should refer to the website: www.biofiredefense.com.

Exclusivity (in silico)

An in silico analysis was performed on the organisms listed in Table 15.

Table 15 Organisms Tested for Evaluation of BioFire COVID-19 Test in silico Cross-Reactivity

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

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 respiratory sample matrix 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)

Organisms and viruses evaluated for exclusivity are shown in Table 16. None of the BioFire COVID-19 Test assays cross-reacted, including the six viruses that are closely related to SARS-CoV-2.

Table 16. Organisms and Viruses Tested for Evaluation of BioFire COVID-19 Test Analytical Specificity

Organism/Virus	Source/ID	Test Concentration	Cross-Reactivity Detected
Viruses (SARS-CoV-2 Related)			
Human coronavirus 229E	Zeptometrix 0810229CF	1.26E+06 $TCID_{50}$/mL	None
Human coronavirus HKU1	Clinical Specimen (NPS)	~1.0E+08 copies/mL[a]	None
Human coronavirus NL63	Zeptometrix 0810228CF	2.51E+05 $TCID_{50}$/mL	None
Human coronavirus OC43	Zeptometrix 0810024CF	9.55E+06 $TCID_{50}$/mL	None
Middle East Respiratory Syndrome coronavirus (MERS-CoV)	Culture (MRI Global)	2.7E+08 GE/mL	None
Severe Acute Respiratory Syndrome coronavirus (SARS-CoV)	Culture (MRI Global)	5.3E+08 GC/mL	None
Viruses			
Adenovirus 1 (species C)	Zeptometrix 0810050CF	3.39E+07 $TCID_{50}$/mL	None
Adenovirus 4 (species E)	Zeptometrix 0810070CF	7.05E+04 $TCID_{50}$/mL	None
Adenovirus 7 (species B)	Zeptometrix 0810021CF	5.10E+07 $TCID_{50}$/mL	None
Enterovirus species A (EV71)	NCPV 0812215v	5.0E+08 $TCID_{50}$/mL	None
Enterovirus species B (Echovirus 6)	Zeptometrix 0810076CF	5.10E+07 $TCID_{50}$/mL	None
Enterovirus species C (Coxsackievirus A17)	ATCC VR-1023	7.90E+05 $TCID_{50}$/mL	None
Enterovirus species D (68)	Zeptometrix 0810237CF	1.58E+06 $TCID_{50}$/mL	None
Human Metapneumovirus	Zeptometrix 0810161CF	1.78E+05 $TCID_{50}$/mL	None
Influenza A subtype H1	Zeptometrix 0810036CFN	7.05E+04 $TCID_{50}$/mL	None
Influenza A subtype H3	Zeptometrix 0810252CF	7.05E+04 $TCID_{50}$/mL	None
Influenza B	Zeptometrix 0810239CF	4.78E+06 $TCID_{50}$/mL	None
Parainfluenza virus 1	BEI NR-48681	8.0E+05 $TCID_{50}$/mL	None
Parainfluenza virus 2	Zeptometrix 0810504CF	1.10E+06 $TCID_{50}$/mL	None

Organism/Virus	Source/ID	Test Concentration	Cross-Reactivity Detected
Parainfluenza virus 3	BEI NR-3233	5.10E+07 $TCID_{50}$/mL	None
Parainfluenza virus 4	Zeptometrix 08010060BCF	1.70E+07 $TCID_{50}$/mL	None
Respiratory syncytial virus	Zeptometrix 0810040ACF	1.05E+06 $TCID_{50}$/mL	None
Rhinovirus	Zeptometrix 0810012CFN	1.26E+06 $TCID_{50}$/mL	None
Bacteria			
Bordetella pertussis	Zeptometrix 0801459	6.70E+09 CFU/mL	None
Chlamydia pneumoniae	ATCC 53592	2.90E+07 IFU/mL	None
Haemophilus influenzae	ATCC 700223	4.20E+08 CFU/mL	None
Legionella pneumophila	Zeptometrix 0801530	2.63E+09 CFU/mL	None
Mycobacterium tuberculosis (attenuated strain)	Zeptometrix 0801660	3.04E+07 CFU/mL	None
Mycoplasma pneumoniae	Zeptometrix 0801579	3.98E+07 CCU/mL	None
Pseudomonas aeruginosa	ATCC 10145	5.68E+08 CFU/mL	None
Staphylococcus epidermidis	ATCC 29887	7.43E+09 CFU/mL	None
Streptococcus pneumoniae	ATCC 6303	8.90E+07 CFU/mL	None
Streptococcus pyogenes	ATCC 49399	4.65E+08 CFU/mL	None
Streptococcus salivarius	ATCC 13419	7.38E+09 CFU/mL	None
Fungi			
Candida albicans	ATCC MYA-2876	7.88E+08 CFU/mL	None
Pneumocystis jirovecii	ATCC PRA-159	1E+07 CFU/mL	None
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 real-time PCR test.
[b] Pooled nasal wash was not evaluated in the exclusivity study; however, approximately 50 negative residual NPS samples were evaluated during the clinical evaluation, 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 are used to evaluate NPS specimens; 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 17a.

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 medium 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 17b for a list of competitive organisms tested.

Table 17a. Substances Tested Demonstrating No Panel Interference in FilmArray RP2 Panel [a]

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
DNA*Zap* (Ambion™ AM9891G & AM9892G)
RNase*Zap* (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] '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 17b. Competitive Microorganisms Tested on FilmArray RP2 Panel

Substance Tested	Concentration Tested
Competitive Microorganisms typically found in NPS	
Coronavirus 229E	1.7×10^4 TCID50/mL
Adenovirus A12	8.9×10^5 TCID50/mL
Parainfluenza Virus 3	6.6×10^5 TCID50/mL
Bordetella pertussis	5.8×10^8 CFU/mL
Enterovirus D68	1.6×10^7 TCID50/mL
Echovirus 6	1.0×10^7 TCID50/mL
Respiratory Syncytial Virus	4.2×10^4 TCID50/mL
Staphylococcus aureus	2.5×10^7 CFU/mL
Streptococcus pneumoniae	1.7×10^7 CFU/mL
Haemophilus influenzae	6.2×10^7 CFU/mL
Candida albicans	1.0×10^6 CFU/mL
Herpes Simplex Virus 1	1.6×10^6 TCID50/mL
Cytomegalovirus (CMV)	1.2×10^6 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 and authorized for pooling up to eight (8) Upper Respiratory 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 ($P_{individual}$) can be determined by dividing the number of positive specimens by the total number of specimens tested during that date range.

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

Refer to Table 18 to identify which pooling sample size provides the greatest testing efficiency for the determined $P_{individual}$ within the validated pool sizes for the assay. If $P_{individual}$ is 2% or less, then the largest validated pool size (n = 8) should be used to maximize efficiency. If the $P_{individual}$ is greater than 25%, then pooling is not efficient and should not be implemented. The efficiency (F) of n-sample pooling for positivity rate (P) can be calculated with the following formula: $F = 1/(1+1/n-(1-P)^n)$. An example of the efficiency calculation for 5-sample pooling when P = 1% is: $F = 1/(1+1/5-(1-0.01)^5) = 4.02$. It means that 1,000 tests can cover testing of 4,020 patients on average.

Table 18. Testing Efficiency of Pooling

$P_{individual}$	n Corresponding to the Maximal Efficiency	Efficiency of n-Sample Pooling (maximum increase in number of tested patients)
1%–2%	8	4.94–3.65
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 $P_{individual}$ 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.

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

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

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

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

- The n-sample pooling should be re-assessed by conducting a re-assessment study (described below).
- If $P_{pools\text{-}x}$ 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 ≥ 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 ≤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 ≥ 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 $P_{individual}$ 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*) × (10/11)
- Positivity rate for 20 samples is (20/N*) × (20/21)

This updated new positivity rate should be used as $P_{individual}$ 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 ($P_{pools\text{-}x}$) for subsequent 7-10 day period based on n-sample pool testing. ($P_{pools\text{-}x}$) should be updated daily using a moving average.

Compare $P_{pools\text{-}initial}$ to $P_{pools\text{-}x}$. If $P_{pools\text{-}x}$ is less than 90% of $P_{pools\text{-}initial}$ ($P_{pools\text{-}x} < 0.90 \times P_{pools\text{-}initial}$), pooling may continue, but a new n-sample pooling size may need to be considered. If $P_{pools\text{-}x}$ 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 BioFire FilmArray 2.0, BioFire FilmArray Torch, and BioFire COVID-19 Test Kits, kit components, and throughout accompanying packaging.

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

APPENDIX B

Contact and Legal Information

Customer and Technical Support

Contact Us on the Web
http://www.BioFireDefense.com

Contact Us by Mail
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Salt Lake City, Utah USA
84107

Contact Us by E-mail
support@BioFireDefense.com

Contact Us by Phone
1-801-262-3592 – US and Canada
1-801-262-3592 – International

Contact Us by Fax
1-801-447-6907

BioFire Defense, LLC
79 West 4500 South, Suite 14
Salt Lake City, UT 84107 USA

 DFA2-PRT-0134-04, August 2021

APPENDIX C

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REVISION HISTORY

Version	Revision Date	Description of Revision(s)
01	February 2021	Initial Release. BioFire COVID-19 Test was expanded to include additional specimen types, and updates to the software module (v1.1). IFU updated to include data supporting specimen types, changes to BioFire COVID-19 Test FilmArray report, reference to the updated BIOFIRE SHIELD Control Kit, and various edits to limitations and precautions related to these changes.
02	May 2021	Minor updates to revision table formatting.
03	June 2021	Minor clarifications to intended use, reporting information and document footer per FDA request.
04	August 2021	BioFire COVID-19 Test v1.1 was expanded to include use with saliva specimens. IFU updated to include data supporting use of saliva specimens as well as updated limitations and precautions sections related to the use of saliva specimens with the BioFire COVID-19 Test. Pooling sections were updated to indicate use with upper respiratory specimens. In silico inclusivity was also updated with an up- to-date-analysis.

[redacted]

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

[redacted]

BioFire® COVID-19 Test v1.1 Quick Guide

For use with FilmArray® 2.0 and FilmArray® Torch Systems

DO NOT DISCARD: Important product-specific information

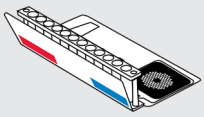
For in vitro diagnostic use under Emergency Use Authorization (EUA) only

UPPER RESPIRATORY

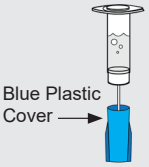
REF 423745 (6 pk)
423744 (30 pk)

IVD **℞ Only**

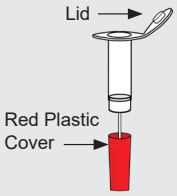
Package Contents



Pouch

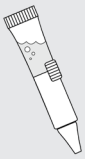


Hydration Injection Vial



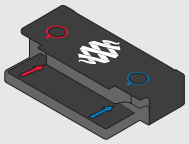
Sample Injection Vial

Transfer Pipette



Sample Buffer Tube

Materials required but not provided

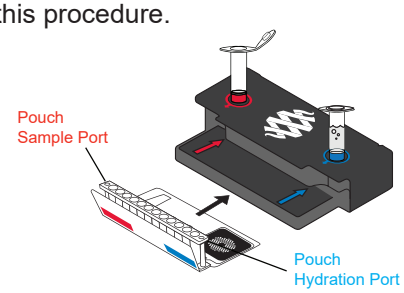


Pouch Loading Station
(included with FilmArray® System)

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

Step 1: Prepare Pouch

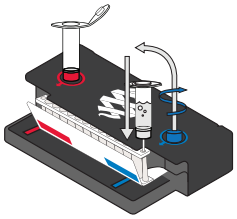
a. Insert pouch into Pouch Loading Station.
b. Place Sample Injection Vial into red well.
c. Place Hydration Injection Vial into blue well.



Step 2: Hydrate Pouch

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

NOTE: Verify the pouch has been hydrated.



Step 3: Prepare Upper Respiratory Sample Mix

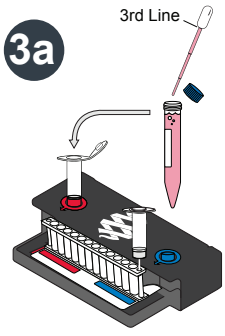
a. Use the transfer pipette to draw specimen to the 3rd line. Add specimen to Sample Injection Vial.
b. Hold Sample Buffer Tube tip facing up and firmly pinch at textured plastic tab on side of tube until seal snaps.

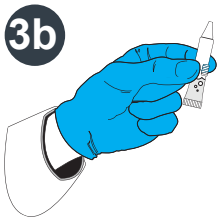
NOTE: Do not touch tube tip.

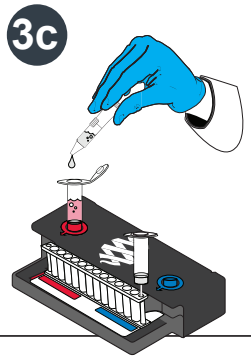
c. Dispense Sample Buffer into Sample Injection Vial using a slow, forceful squeeze followed by a second squeeze.

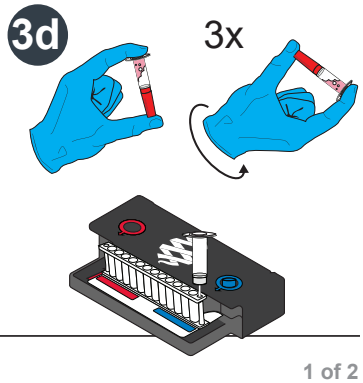
NOTE: Avoid generating excessive foam.

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









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

BioFire® COVID-19 Test v1.1 Quick Guide

For use with FilmArray® 2.0 and FilmArray® Torch Systems

UPPER RESPIRATORY

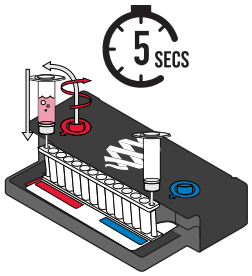
Step 4: Load Upper Respiratory Sample Mix

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

NOTE: Waiting **5** seconds decreases the contamination risk.

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

NOTE: Verify the sample has been loaded.



Step 5: Run Pouch

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

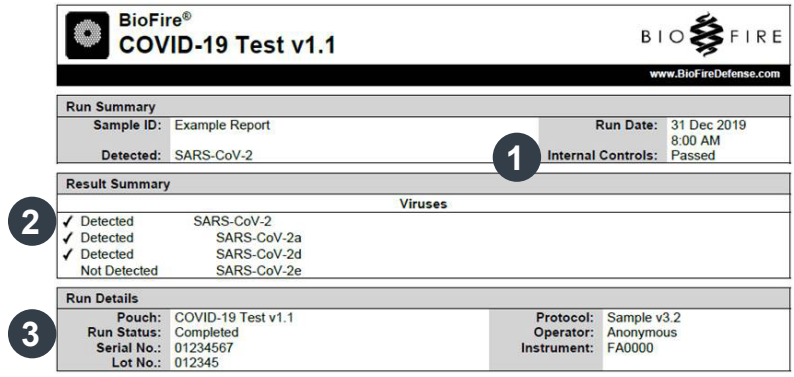
Step 6: Review Results

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

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

Result Summary - Displays overall SARS-CoV-2 test result on the first line, followed by each individual assay result.

2.
   - If overall 'SARS-CoV-2' test result is 'Detected' or 'Not Detected', report the results.
   - If 'Invalid', **RETEST SAMPLE** and refer to *Instructions for Use*.


BioFire® COVID-19 Test v1.1

BIOFIRE

www.BioFireDefense.com

Run Summary

Sample ID:	Example Report	Run Date:	31 Dec 2019 8:00 AM
Detected:	SARS-CoV-2	Internal Controls:	Passed

Result Summary

	Viruses
✓ Detected	SARS-CoV-2
✓ Detected	SARS-CoV-2a
✓ Detected	SARS-CoV-2d
Not Detected	SARS-CoV-2e

Run Details

Pouch:	COVID-19 Test v1.1	Protocol:	Sample v3.2
Run Status:	Completed	Operator:	Anonymous
Serial No.:	01234567	Instrument:	FA0000
Lot No.:	012345		

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

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

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

Conditions of Authorization

The BioFire COVID-19 Test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

The BioFire COVID-19 Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of the BioFire COVID-19 Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

DFA2-PRT-0131-01

BioFire® COVID-19 Test v1.1 Quick Guide

For use with FilmArray® 2.0 and FilmArray® Torch Systems

DO NOT DISCARD: Important product-specific information

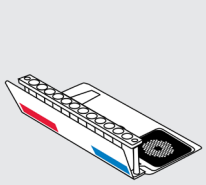
For in vitro diagnostic use under Emergency Use Authorization (EUA) only

LOWER RESPIRATORY

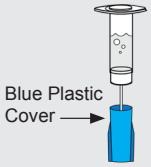
REF 423745 (6 pk)
423744 (30 pk)

IVD **Rx Only**

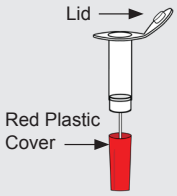
Package Contents



Pouch

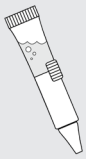


Hydration Injection Vial



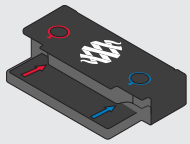
Sample Injection Vial

Transfer Pipette



Sample Buffer Tube

Materials required but not provided

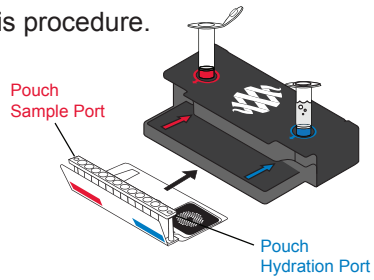


Pouch Loading Station
(included with FilmArray® System)

Swab
Kit Part No. 424063

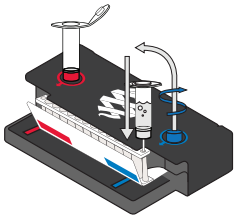
NOTE: Use clean gloves and other Personal Protective Equipment (PPE) when performing this procedure.

Step 1: Prepare Pouch



a. Insert pouch into Pouch Loading Station.
b. Place Sample Injection Vial into red well.
c. Place Hydration Injection Vial into blue well.

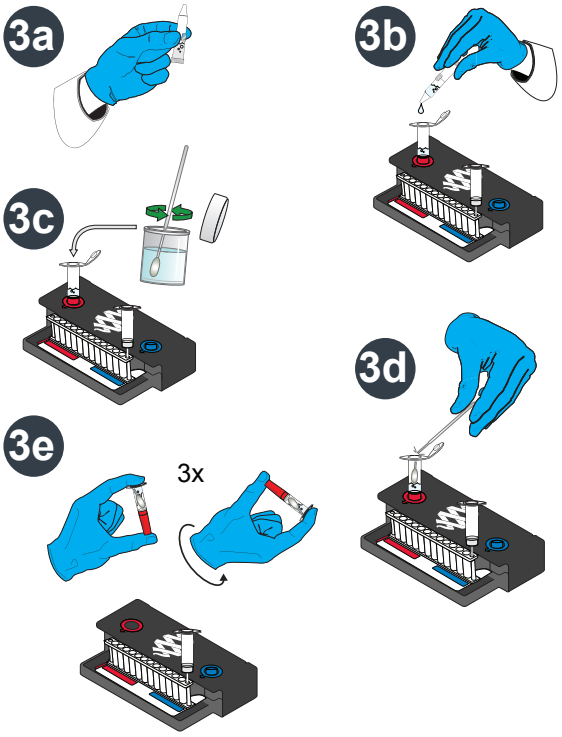
Step 2: Hydrate Pouch



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

NOTE: Verify the pouch has been hydrated.

Step 3: Prepare Lower Respiratory Sample Mix



a. Hold Sample Buffer tube tip facing up and firmly pinch at textured plastic tab on side of tube until seal snaps.

NOTE: Do not touch tube tip.

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

NOTE: Avoid generating excessive foam.

c. Use the Sample Swab to stir the entire specimen for **~10** seconds.

d. Place the swab end into the Sample Injection Vial then break off at the scored breakpoint. Discard the swab handle into an appropriate waste container and close Sample Injection Vial lid tightly.

e. Invert the Sample Injection Vial **3** times then return to red well of Pouch Loading Station.

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

BioFire® COVID-19 Test v1.1 Quick Guide

For use with FilmArray® 2.0 and FilmArray® Torch Systems

LOWER RESPIRATORY

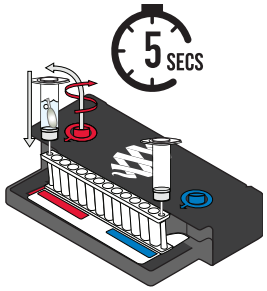
Step 4: Load Lower Respiratory Sample Mix

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

NOTE: Waiting **5** seconds decreases the contamination risk.

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

NOTE: Verify the sample has been loaded.



Step 5: Run Pouch

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

Step 6: Review Results

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

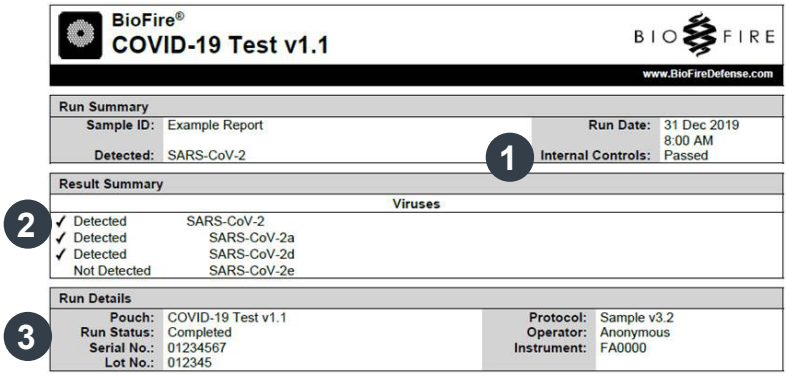
1. Internal Controls:
   - If 'Passed', results are valid.
   - If 'Failed' or 'Invalid', **RETEST SAMPLE** and refer to *Instructions for Use*.

Result Summary - Displays overall SARS-CoV-2 test result on the first line, followed by each individual assay result.

2.
   - If overall 'SARS-CoV-2' test result is 'Detected' or 'Not Detected', report the results.
   - If 'Invalid', **RETEST SAMPLE** and refer to *Instructions for Use*.

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

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


BioFire®
COVID-19 Test v1.1
BIOFIRE
www.BioFireDefense.com

Run Summary

Sample ID:	Example Report	Run Date:	31 Dec 2019 8:00 AM
Detected:	SARS-CoV-2	Internal Controls:	Passed

1

Result Summary

	Viruses
✓ Detected	SARS-CoV-2
✓ Detected	SARS-CoV-2a
✓ Detected	SARS-CoV-2d
Not Detected	SARS-CoV-2e

2

Run Details

Pouch:	COVID-19 Test v1.1	Protocol:	Sample v3.2
Run Status:	Completed	Operator:	Anonymous
Serial No.:	01234567	Instrument:	FA0000
Lot No.:	012345		

3

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

Conditions of Authorization

The BioFire COVID-19 Test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

The BioFire COVID-19 Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of the BioFire COVID-19 Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

DFA2-PRT-0116-01

BIOFIRE® SHIELD™ Control Kit for the BioFire COVID-19 Test v1.1 Instructions for Use

BIOFIRE® SHIELD™ Control Kit for the BioFire COVID-19 Test v1.1 Quick Guide

BioFire Defense, LLC
Salt Lake City, Utah, USA

REF 424062 (6 pack test)

BIOFIRE® SHIELD™ Control Kit
for the BioFire® COVID-19 Test
Instructions for Use v1.1

IVD

℞ Only

The Symbols Glossary is provided on Page 7 of this booklet.

For in vitro diagnostic use under an Emergency Use Authorization (EUA) only

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

Manufactured by

BioFire Defense, LLC

79 West 4500 South, Suite 14

Salt Lake City, UT 84107 USA

TABLE OF CONTENTS

INTENDED USE

The BIOFIRE® SHIELD™ Control Kit for the BioFire® COVID-19 Test is an optional surrogate external assayed quality control for monitoring performance of *in vitro* laboratory nucleic acid testing procedures using the qualitative BioFire COVID-19 Test on FilmArray® 2.0 and FilmArray® Torch systems.

The BIOFIRE SHIELD Positive External Control monitors for the presence of the PCR primers for SARS-CoV-2 assays in the BioFire COVID-19 Test. To reduce the risk of false positive results, the BIOFIRE SHIELD Positive External Control is composed of synthetic RNA sequences that produce signature melting temperature (Tm) values that are distinct from the Tm values produced by amplification of SARS-CoV-2 RNA.

External quality controls should be used in accordance with appropriate federal, state, and local guidelines or accreditation requirements, as applicable.

This product is not intended to replace the internal controls contained in the BioFire COVID-19 Test.

For use under an Emergency Use Authorization (EUA) only.

PRODUCT SUMMARY AND PRINCIPLE

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, when there is a new operator, and following replacement/repair of a BioFire FilmArray 2.0 or FilmArray Torch system. External controls may also be used in initial laboratory verification of BioFire FilmArray 2.0 or Torch systems used with the BioFire COVID-19 Test.

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 the selection of appropriate external control material and how to design a verification protocol is posted on the BioFire Defense webpage.

COMPOSITION

The BIOFIRE SHIELD Positive External Control is composed of a pool of synthetic RNA sequences that each produce a signature melting temperature (Tm) value that is distinct from the Tm value produced by SARS-CoV-2 RNA target to reduce the risk of false positive results. The BIOFIRE SHIELD Control Kit contains no biological hazards and is 100% non-infectious.

STORAGE AND STABILITY

- Store the BIOFIRE SHIELD Control Kit at room temperature (15-30°C). **DO NOT REFRIGERATE**.
- Avoid storage of any materials near heating or cooling vents, or in direct sunlight.
- Once the mylar bag containing the Positive External Control vial has been opened, the positive external control should be loaded as soon as possible.

MATERIALS PROVIDED

Each BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test contains sufficient reagents for six positive external control runs (6-pack; part no. 424062). Materials include:

- Individually packaged BIOFIRE SHIELD Positive External Control Vials for the BioFire COVID-19 Test
- Instructions and Documents
  - *BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test Quick Guide*

MATERIALS REQUIRED BUT NOT PROVIDED

- BioFire® FilmArray system including:
  - BioFire® FilmArray 2.0/Torch Instrument Systems and accompanying software
  - BioFire® FilmArray® Pouch Loading Station
- 10% bleach solution or a similar disinfectant
- Transport medium, PBS, or normal saline solution
- BioFire COVID-19 Test v1.1 (Part No. 423745 (6 pack test) or 423744 (30 pack test))

NOTE: *Negative controls may be run using negative matrix such as transport medium, PBS, or normal saline solution. Negative matrix is not included in this kit.*

NOTE: *Examples of verification protocols (for laboratory verifications with either the BIOFIRE SHIELD Control Kit or alternative external controls) are available online at www.biofiredefense.com/covid-19test.*

WARNINGS AND PRECAUTIONS

1. This BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test is designed for use only with the BioFire COVID-19 Test (v1.1) and should not be used with any other BioFire panel.
2. The BIOFIRE SHIELD Positive and Negative External Control FilmArray protocols should only be used to test external controls as described in the procedure section below. The Negative and Positive External Control protocols are only for use with the BIOFIRE SHIELD Control Kit.
3. The BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
4. 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;
5. The emergency use of the BIOFIRE SHIELD Control Kit for 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.
6. Always check the expiration date on the control kits. Do not use controls after the expiration date.
7. Although rare, contamination from small amounts of synthetic RNA in the BIOFIRE SHIELD Positive External Controls can contaminate the work area and may cause Negative External Controls to fail. For accurate tests results:
   a. Follow the instructions in the *BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test - Quick Guide* exactly.
   b. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable, powder-free gloves. Change gloves often when handling BIOFIRE SHIELD Positive External Control materials.

c. Decontaminate the work area after every use of an External Control.

8. Bleach introduced in a sample may damage nucleic acids in the sample and may result in a failed Positive External Control result.
9. Dispose of materials used in the assay, including reagents and used buffer vials, according the federal, state, and local regulations.

BIOFIRE SHIELD CONTROL PROCEDURE

Use clean gloves and other PPE when handling pouches and the BIOFIRE SHIELD Positive External Control. Only prepare one BioFire COVID-19 Test pouch at a time and change gloves between handling of External Controls and pouches. Refer to the *BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test Quick Guide* for detailed instructions on how to prepare and load the Positive and Negative External Controls. Once a Positive or Negative External Control is loaded into the pouch, promptly transfer the pouch to the instrument to start the run. Ensure the correct protocol is selected before starting the run. After the run is complete, discard the pouch in a biohazard container.

Refer to the appropriate BioFire *FilmArray operator's manual* for more details regarding the BioFire FilmArray System. Refer to the *BioFire COVID-19 Test Instructions for Use* for additional information about the BioFire COVID-19 Test.

***NOTE**: There are individual software protocols for the Positive and Negative External Controls. It is necessary to ensure that the appropriate protocol is selected prior to starting the test. DO NOT select the sample protocol.*

EXPECTED RESULTS

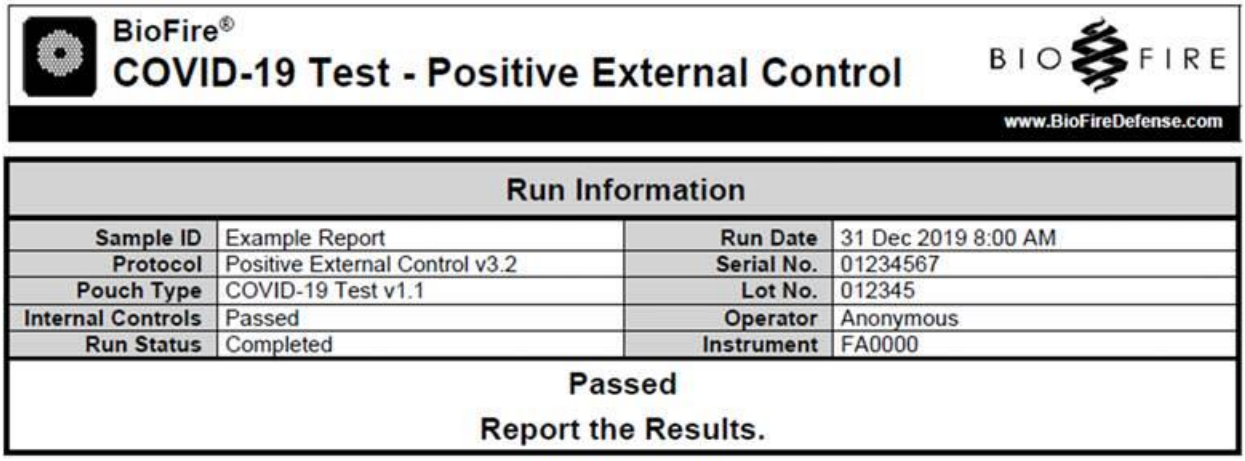
BioFire® COVID-19 Test External Control Reports

The BioFire COVID-19 Test External Control Report is automatically displayed upon completion of a run and can be printed or saved as a PDF file. Each report contains Run Information and results.

The **Run Information** section of the test report provides the Sample ID, time and date of the run, and the internal controls run status. Internal controls are listed as 'Passed', 'Failed', or 'Invalid'. For more information on internal controls, reference the *BioFire COVID-19 Test Instructions for Use*. The **Run Information** section also includes pouch information (type, lot number, and serial number), run status (Completed, Incomplete, Aborted, Instrument 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.

When performed properly, the BIOFIRE SHIELD External Controls should yield the result 'Passed' (See **Figures 1** and **2**). If the result is 'Failed', the operator will be instructed to retest the External Control once, either immediately (Positive External Control) or after decontaminating the workspace (Negative External Control) - See **Figures 3** and **4**. If the result is 'Invalid', the operator will be instructed to retest the External Control once and to contact Technical Support if the problem persists.

For further instructions on decontaminating the workspace, refer to the appropriate BioFire FilmArray operator's manual.



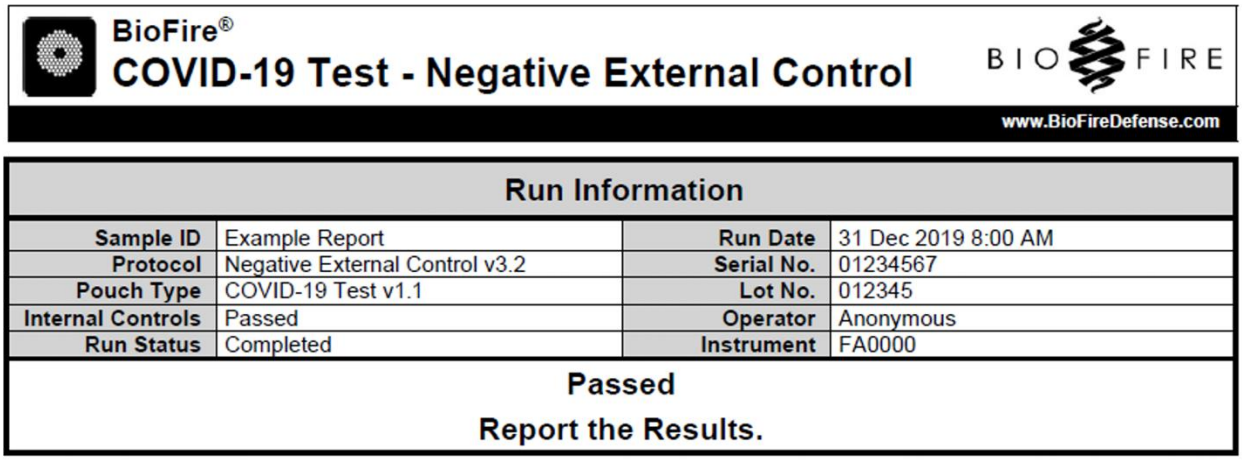
BioFire®
COVID-19 Test - Positive External Control
BIOFIRE
www.BioFireDefense.com

Run Information			
Sample ID	Example Report	Run Date	31 Dec 2019 8:00 AM
Protocol	Positive External Control v3.2	Serial No.	01234567
Pouch Type	COVID-19 Test v1.1	Lot No.	012345
Internal Controls	Passed	Operator	Anonymous
Run Status	Completed	Instrument	FA0000

Passed

Report the Results.

Figure 1. BioFire COVID-19 Test External Control Report for a passed Positive External Control



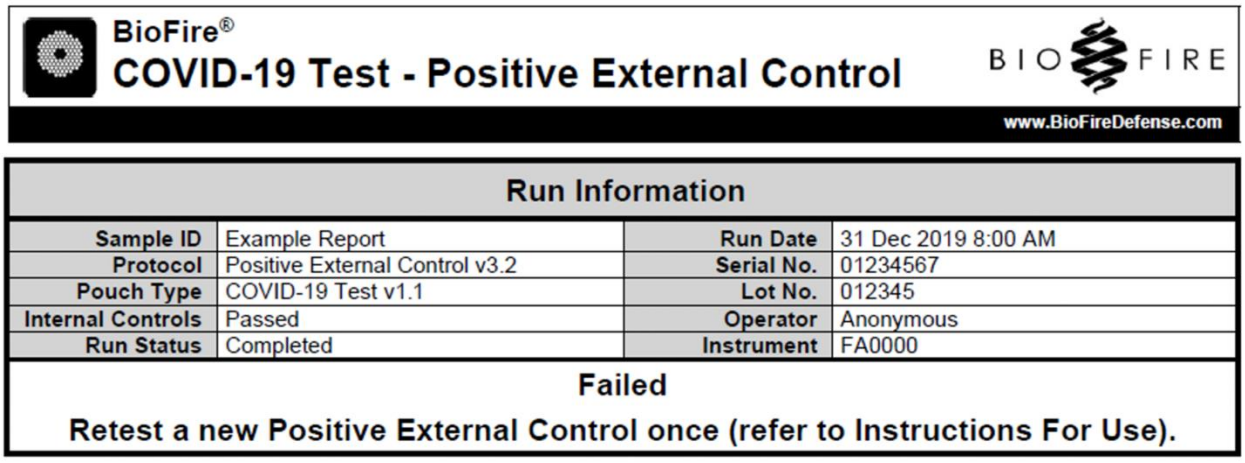
BioFire®
COVID-19 Test - Negative External Control
BIOFIRE
www.BioFireDefense.com

Run Information			
Sample ID	Example Report	Run Date	31 Dec 2019 8:00 AM
Protocol	Negative External Control v3.2	Serial No.	01234567
Pouch Type	COVID-19 Test v1.1	Lot No.	012345
Internal Controls	Passed	Operator	Anonymous
Run Status	Completed	Instrument	FA0000

Passed

Report the Results.

Figure 2. BioFire COVID-19 Test External Control Report for a passed Negative External Control



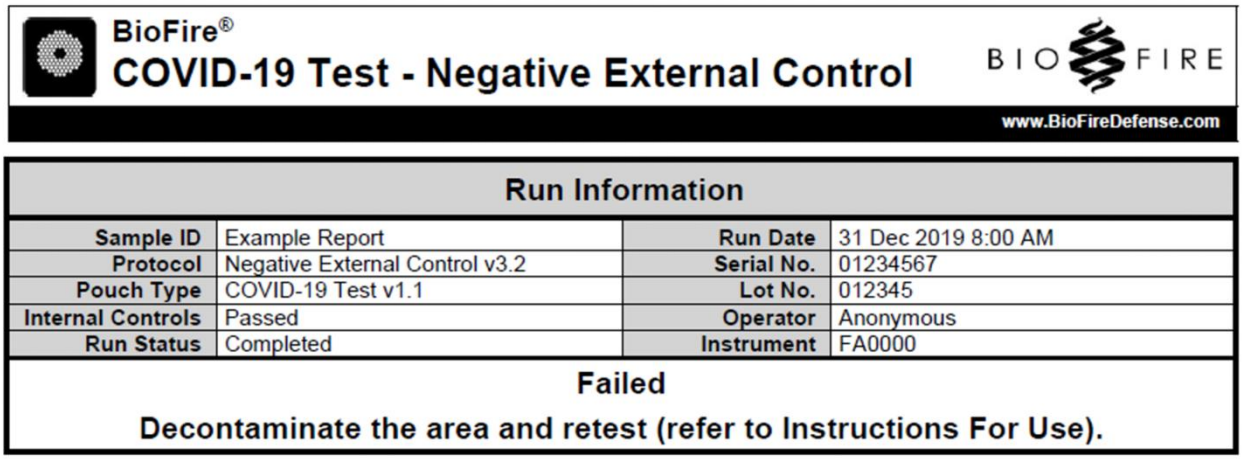
BioFire®
COVID-19 Test - Positive External Control
BIOFIRE
www.BioFireDefense.com

Run Information			
Sample ID	Example Report	Run Date	31 Dec 2019 8:00 AM
Protocol	Positive External Control v3.2	Serial No.	01234567
Pouch Type	COVID-19 Test v1.1	Lot No.	012345
Internal Controls	Passed	Operator	Anonymous
Run Status	Completed	Instrument	FA0000

Failed

Retest a new Positive External Control once (refer to Instructions For Use).

Figure 3. BioFire COVID-19 Test External Control Report for a Failed Positive External Control



BioFire®
COVID-19 Test - Negative External Control
BIOFIRE
www.BioFireDefense.com

Run Information			
Sample ID	Example Report	**Run Date**	31 Dec 2019 8:00 AM
Protocol	Negative External Control v3.2	**Serial No.**	01234567
Pouch Type	COVID-19 Test v1.1	**Lot No.**	012345
Internal Controls	Passed	**Operator**	Anonymous
Run Status	Completed	**Instrument**	FA0000

Failed

Decontaminate the area and retest (refer to Instructions For Use).

Figure 4. BioFire COVID-19 Test External Control Report for a Failed Negative External Control

Analysis of BIOFIRE SHIELD Positive and Negative External Control Assays

The BIOFIRE SHIELD Positive External Control passes when the software detects amplification within the specified melting temperature window for all three SARS-CoV-2 assays.

If amplification for one or more of the three SARS-CoV-2 assays is not detected, the Positive External Control fails and should be repeated. If the failure persists, contact BioFire Defense Technical Support for further instruction.

Laboratories may decide to perform negative control testing on the system. Negative control testing may be performed using transport medium, PBS, or saline in place of a patient sample. If amplification of Positive External Control material and/or SARS-CoV-2 RNA is detected, the Negative External Control fails and should be repeated after a thorough cleaning of the area. If the error persists, contact BioFire Defense Technical Support for further instruction.

LIMITATIONS

1. For in vitro diagnostic use under Emergency Use Authorization (EUA) only.
2. This product is only for use with the BioFire COVID-19 Test (v1.1). It does not contain the entire genome of SARS-CoV-2. This product is not intended to replace the internal controls contained in the BioFire COVID-19 Test.
3. Transport medium, PBS, or saline may be used as a Negative External Control. Negative External Controls are not provided.
4. Quality control materials should be used in accordance with local, state, federal regulations and accreditation requirements.

APPENDIX A

Symbols Glossary

The following symbols can be found on labeling for the BIOFIRE SHIELD Control Kit, and throughout accompanying packaging.

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

APPENDIX B

Contact and Legal Information

Customer and Technical Support

Contact Us on the Web
http://www.BioFireDefense.com

Contact Us by Mail
79 West 4500 South, Suite 14
Salt Lake City, Utah USA
84107

Contact Us by E-mail
support@BioFireDefense.com

Contact Us by Phone
1-801-262-3592 – US and Canada
1-801-262-3592 – International

Contact Us by Fax
1-801-447-6907

Ordering Information

BIOFIRE SHIELD Control Kit for use with the BioFire COVID-19 Test	
Kit Part No:	424062
Contents:	6 Positive External Control Vials

Revision History

Version	Revision Date	Description of Revision(s)
01	February 2021	Initial Release

BioFire Defense, LLC
79 West 4500 South, Suite 14
Salt Lake City, UT 84107 USA

 DFA2-PRT-0118-01, February 2021

[redacted]

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

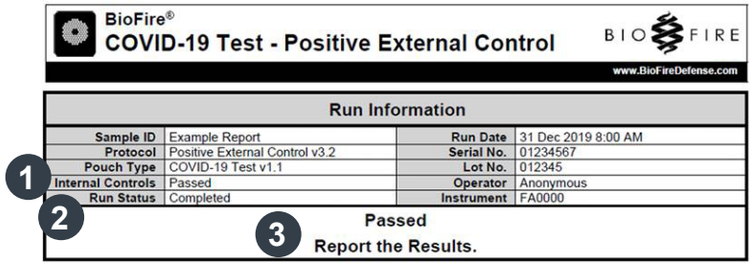
[redacted]

BIOFIRE® SHIELD™ Control Kit v1.1

Quick Guide for the BioFire® COVID-19 Test

STEP 6: Review Results

Run Information - Displays information about the pouch, protocol, run status, operator, serial number, instrument and lot number



1. **Internal Controls:**
   - If 'Passed', results are valid.
   - If 'Failed' or 'Invalid', **RETEST CONTROL** once.
2. **Run Status:**
   - If 'Completed', run is complete.
   - If 'Aborted', or any other error message, **RETEST CONTROL** once.
3. **External Control Results:**
   - If 'Passed', results are valid.
   - If 'Failed',
     - Positive External Control: **RETEST EXTERNAL CONTROL** once.
     - Negative External Control: Decontaminate the area; and **RETEST EXTERNAL CONTROL** once.
   - If 'Invalid', **RETEST EXTERNAL CONTROL** once.

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

Conditions of Authorization

The BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

The emergency use of the BIOFIRE SHIELD Control Kit for 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.

DFA2-PRT-0113-03

BIOFIRE DEFENSE
BioFire Defense, LLC | Salt Lake City, Utah, USA | 801-262-3592 | www.BioFireDefense.com

DO NOT DISCARD: Important product-specific information

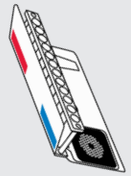
For in vitro diagnostic use under Emergency Use Authorization (EUA) only

REF 424062 (6 pk) **IVD**

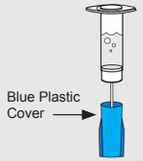
℞ Only

External Control Vial

Materials required but not provided

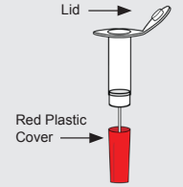


Pouch
BioFire® COVID-19 Test
Part No: 423744
423745

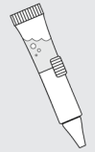


Hydration Injection Vial

Transfer Pipette



Sample Injection Vial



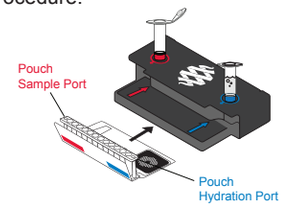
Sample Buffer Tube

Transport Medium, PBS or Saline

NOTE: FilmArray® Instrument should be powered on and ready for use prior to pouch preparation.
NOTE: Use clean gloves and other Personal Protective Equipment (PPE) when performing this procedure.

STEP 1: Prepare Pouch

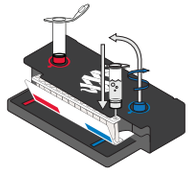
Refer to the *BioFire® COVID-19 Test Upper Respiratory Quick Guide STEP 1: Prepare Pouch*



STEP 2: Hydrate Pouch

Refer to the *BioFire® COVID-19 Test Upper Respiratory Quick Guide STEP 2: Hydrate Pouch*

- To prepare a Positive External Control, proceed to *STEP 3a*.
- To prepare a Negative External Control, proceed to *STEP 3b*.



STEP 3a: Prepare COVID-19 Positive External Control

a. Use the Transfer Pipette to draw the Transport Medium, PBS or Saline to the third line. Add to the Sample Injection Vial.

b. Remove rubber cap from Positive External Control Vial and place on a clean surface (a paper towel may be used).

c. Add Sample Buffer to Positive External Control Vial

1. Hold the Sample Buffer Tube with the tip facing up and firmly pinch at the textured plastic tab on the side of the tube until seal snaps.

NOTE: Do not touch tube tip.

2. Dispense Sample Buffer into Positive External Control Vial using a slow, forceful squeeze, followed by a second squeeze.

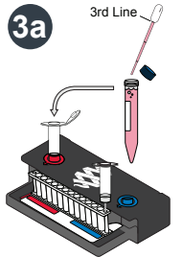
NOTE: Avoid generating excessive foam.

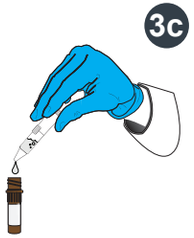
d. Recap Positive External Control Vial and invert it **3** times to mix.

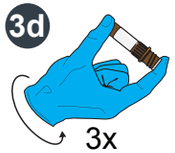
e. Pour mixture of Sample Buffer and Control into the Sample Injection Vial.

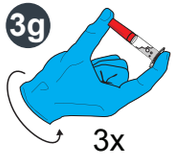
f. Dispose of Positive External Control Vial and change gloves.

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









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

h. Proceed to *STEP 4: Load Control Mix*.

BIOFIRE® SHIELD™ Control Kit v1.1 Quick Guide for the BioFire COVID-19 Test

STEP 3b: Prepare COVID-19 Negative External Control

i. Use the Transfer Pipette to draw the Transport Medium, PBS or Saline to the third line. Add to the Sample Injection Vial.

j. Add Sample Buffer to Sample Injection Vial.

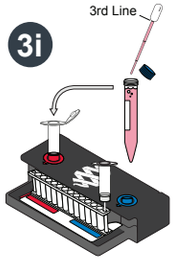
1. Hold the Sample Buffer Tube with the tip facing up and firmly pinch at the textured plastic tab on the side of the tube until seal snaps.

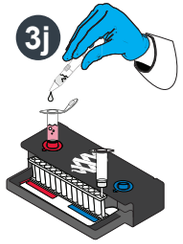
NOTE: Do not touch tube tip.

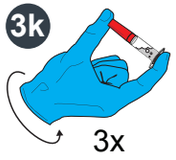
2. Dispense Sample Buffer into Sample Injection Vial using a slow, forceful squeeze, followed by a second squeeze.

NOTE: Avoid generating excessive foam.

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





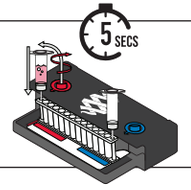


WARNING: Sample Buffer is harmful if swallowed and can cause serious eye damage and/or skin irritation.

l. Proceed to *STEP 4: Load Control Mix*.

STEP 4: Load Control Mix

Refer to the *BioFire® COVID-19 Test Upper Respiratory Quick Guide STEP 4: Load Sample Mix*



STEP 5: Run Pouch

Refer to the *BioFire® COVID-19 Test Upper Respiratory Quick Guide STEP 5: Run Pouch*

NOTE: Select either Positive External Control v3.2 or Negative External Control v3.2.