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 40 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

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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), or lower respiratory specimens (i.e., induced or expectorated sputum, endotracheal aspirates, bronchoalveolar lavage, or mini-bronchoalveolar lavage), 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. 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 saliva specimens or 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, high-complexity 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].

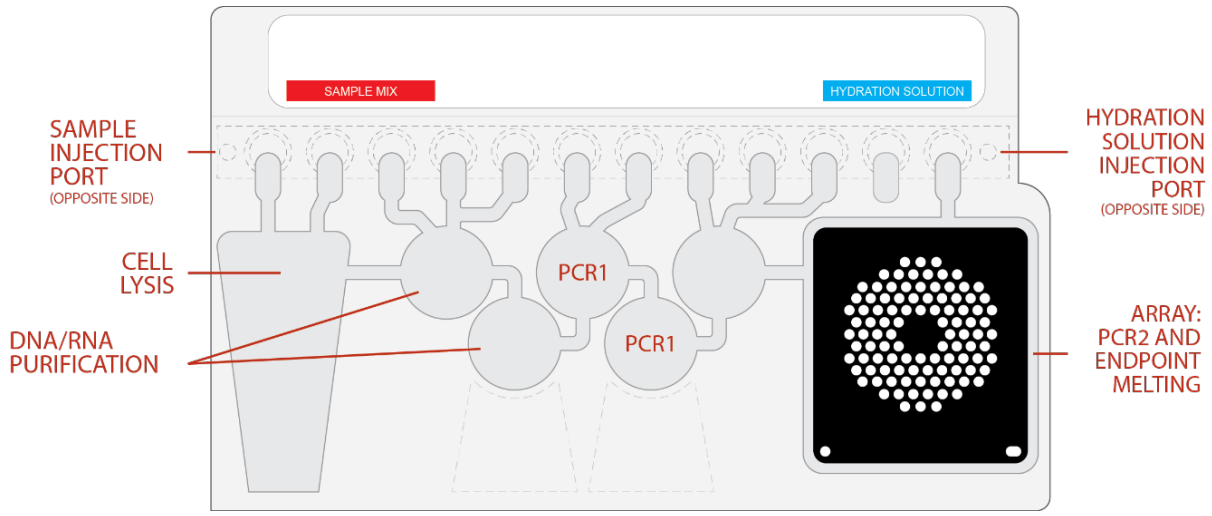
The BioFire COVID-19 Test is a qualitative test for use with the BioFire FilmArray 2.0 or Torch systems for the detection of SARS-CoV-2 RNA in upper respiratory (nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs), lower respiratory (sputum-like or BAL-like), and saliva specimens. 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
- *BioFire COVID-19 Test Lower Respiratory Quick Guide*
- *BioFire COVID-19 Test Upper Respiratory or Saliva Quick Guide*
- Documentation available at *www.biofiredefense.com/covid-19test*
    - *BioFire COVID-19 Test v1.1 Instructions for Use*

    NOTE: *Additional documentation is available online at www.biofiredefense.com/covid-19test*

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 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 Sample 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, 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 manipulating SARS-CoV-2 cultures.
- 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.
- Avoid collecting or handling specimens in areas that are exposed to SARS-CoV-2 vaccine material. Some vaccines may contain PCR-detectable genomic material. Contamination of specimens or testing materials with vaccine may cause false positive results.

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.
6. Always check the kit expiration date and do not use reagents beyond the expiration date printed on the pouch kit.

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 and Saliva 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). Pools of up to 8 specimens may be tested on the BioFire COVID-19 Test. Only upper respiratory specimens (i.e., nasopharyngeal, oropharyngeal, mid-turbinate nasal, or anterior nasal swabs), or saliva specimens which have been collected individually may be pooled. Upper respiratory and saliva specimens should never be pooled together. 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.

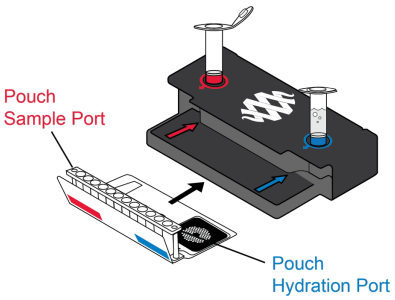
   NOTE: *Sample IDs should indicate that the sample was pooled.*

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.

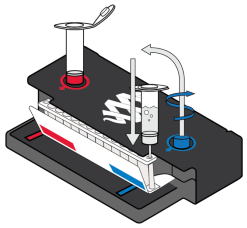
   NOTE: *The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.*

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.

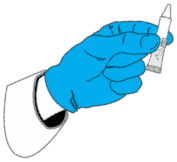
   - Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen.

   - 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 or Saliva 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.*

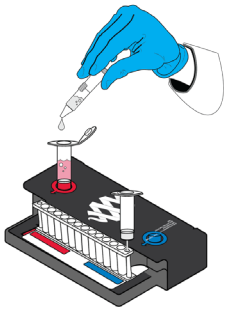


5. Add Sample Buffer to the Sample Injection Vial.
   - Hold the Sample Buffer Tube with the tip facing up.

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

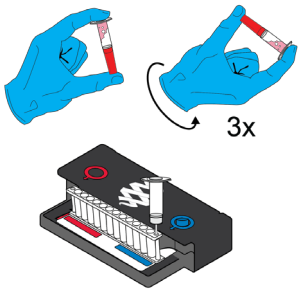
   - Firmly pinch at textured plastic tab on the side of the tube until the seal snaps.
   - Invert the tube over the Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

     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.*

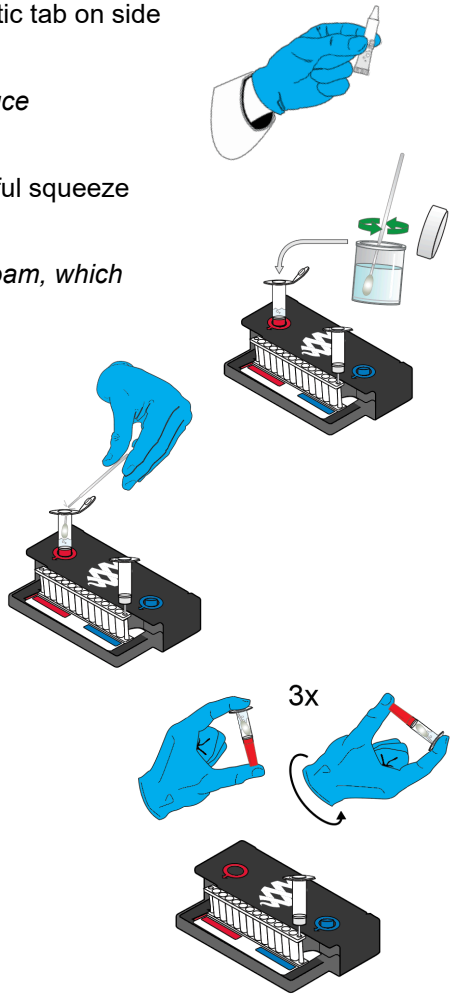
2. Dispense Sample Buffer into Sample Injection Vial using a slow, forceful squeeze followed by a second squeeze.

   ***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.

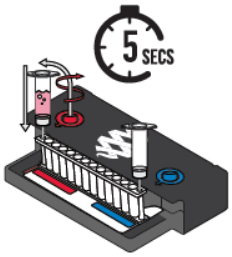
5. Invert the Sample Injection Vial 3 times and return to red well of Pouch Loading Station.



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 pouch hydration port to the pouch 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 pouch sample 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 confirm the Sample protocol. The BioFire COVID-19 Test uses a single Sample protocol for the testing of all clinical sample types.

   NOTE: *Two additional protocols are provided for use with the BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test v1.1. It is necessary to select the appropriate protocol* ***prior*** *to running the test. The Positive External Control and Negative External Control protocols are only for use with the BIOFIRE SHIELD Control Kit and should not be used to test clinical samples or other types of controls. Refer to the BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test v1.1 Instructions for Use for procedures to prepare and run BIOFIRE SHIELD Controls.*
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.
6. Select and confirm the Sample protocol. The BioFire COVID-19 Test uses a single Sample protocol for the testing of all clinical sample types.

   NOTE: *Two additional protocols are provided for use with the BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test v1.1. It is necessary to select the appropriate protocol* ***prior*** *to running the test. The Positive External Control and Negative External Control protocols are only for use with the BIOFIRE SHIELD Control Kit and should not be used to test clinical samples or other types of controls. Refer to the BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test v1.1 Instructions for Use for procedures to prepare and run BIOFIRE SHIELD Controls.*
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 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.

External Controls

Good laboratory practices recommend running positive and negative controls regularly. External controls should be used in accordance with laboratory protocols and the appropriate accrediting organization requirements, as applicable. Evaluation of external controls is also 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 FilmArray instrument. The BioFire COVID-19 Test should not be used for patient specimen testing if the external controls do not produce the expected results.

The BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test v1.1 (Part No. 424062) is an optional surrogate external assayed quality control for monitoring test performance. 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. The Positive External Control and Negative External Control protocols have been developed specifically for use with the BIOFIRE SHIELD Control Kit. Refer to the BIOFIRE SHIELD Control Kit for the BioFire COVID-19 Test v1.1 Instructions for Use for additional information.

Other commercially available control material may also be used according to the control manufacturer's instructions. When selecting a commercially available control material ensure that the positive control contains

the entire SARS-CoV-2 genome. Alternatively, previously characterized negative specimens, transport medium, saline, or phosphate-buffered saline 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. When evaluating these types of controls the Sample Protocol should be selected. The Positive External Control and Negative External Control protocols are only compatible with the BIOFIRE SHIELD Control Kit.

Refer to www.biofiredefense.com/covid-19test for guidance in selecting appropriate control materials and developing a laboratory verification protocol.

INTERPRETATION OF RESULTS

The BioFire COVID-19 Test consists of seven PCR assays designed to provide sensitive and specific detection of SARS-CoV-2 RNA. The gene target of each assay is shown in Table 1 below. 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-2c	ORF1ab
SARS-CoV-2d	ORF1ab
SARS-CoV-2e	ORF8
SARS-CoV-2f	ORF8
SARS-CoV-2g	S gene
SARS-CoV-2h	N gene

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'.

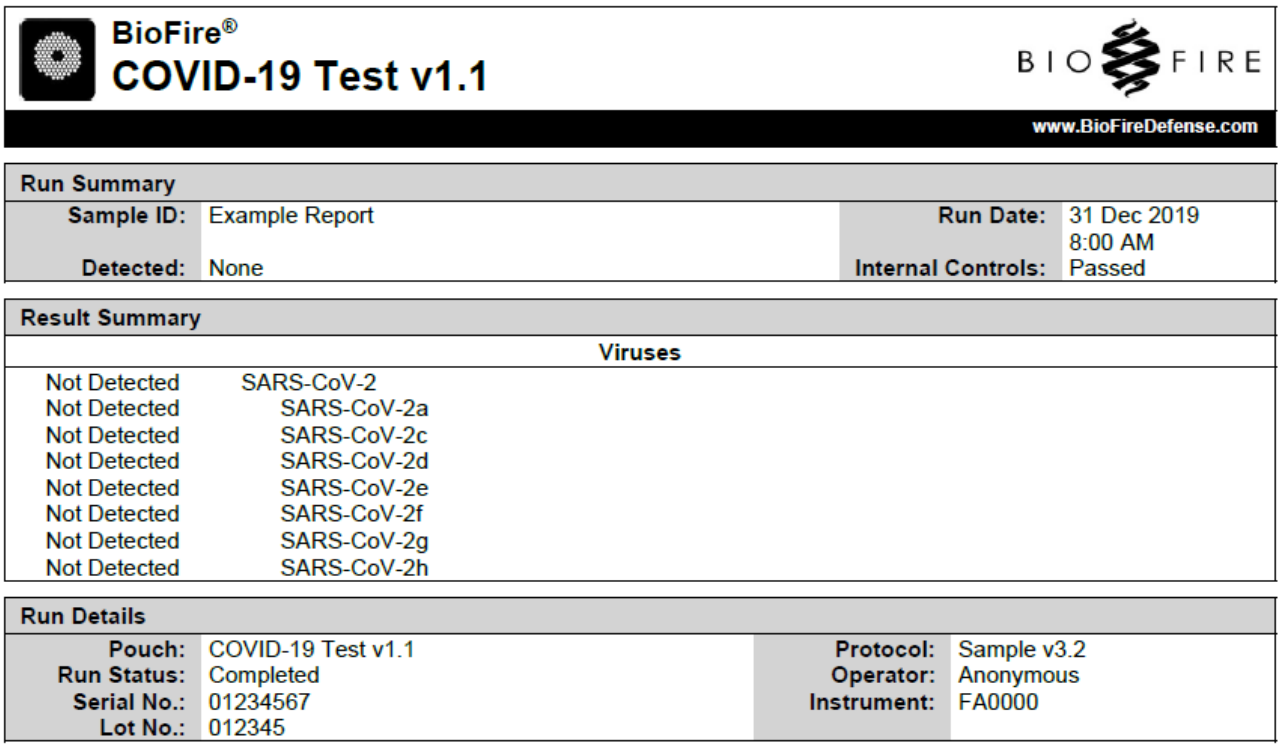
Overall Test Result

The results of each assay are combined to determine a final test result. If any of the seven SARS-CoV-2 assays are 'Detected', the overall test result (i.e., the SARS-CoV-2 result) will be 'Detected'. If none of the assays 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:	None	**Internal Controls:**	Passed

Result Summary	
	Viruses
Not Detected	SARS-CoV-2
Not Detected	SARS-CoV-2a
Not Detected	SARS-CoV-2c
Not Detected	SARS-CoV-2d
Not Detected	SARS-CoV-2e
Not Detected	SARS-CoV-2f
Not Detected	SARS-CoV-2g
Not Detected	SARS-CoV-2h

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 All 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. Note that 'Not Detected' results from pooled samples should be reported as presumptive negative. 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 been validated for testing of the following individual specimens: upper respiratory swabs (nasopharyngeal, oropharyngeal, anterior nasal), unprocessed sputum specimens, and saliva specimens; performance with other specimen types is unknown.
4. The BioFire COVID-19 Test has been validated for pooled sample testing only for the following specimen types: nasopharyngeal swabs and saliva.
5. 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.
6. Use the BioFire Sample Swab Kit when testing lower respiratory specimens with the BioFire COVID-19 Test. Performance of the BioFire COVID-19 Test has not been evaluated with other swabs.
7. 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.
8. 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.
9. 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.
10. Performance of the BioFire COVID-19 Test has not been established for monitoring treatment of infection.
11. 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.
12. 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.
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 laboratory and 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" protocol. 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)

Prospective Clinical Specimen Testing

The prospective clinical study evaluated the performance of the BioFire COVID-19 Test on nasopharyngeal swab (NPS) specimens in transport medium. Specimens were residual after standard of care (SoC) testing for SARS-CoV-2. A total of 523 specimens were tested at three study sites in the United States over four months (July–October 2020) during the COVID-19 global pandemic. Table 4 provides a summary of demographic information for the specimens included in the analyses.

Table 4. Overall and Per Site Demographic Analysis

		Overall	Site 1	Site 2	Site 3
Sex	Female	**253 (48.4%)**	141 (45.5%)	54 (50.0%)	58 (55.2%)
	Male	**267 (51.1%)**	169 (54.5%)	51 (47.2%)	47 (44.8%)
	Unknown	**3 (0.6%)**	0 (0%)	3 (2.8%)	0 (0%)
Age Range	0-18 years	**55 (10.5%)**	24 (7.7%)	18 (16.7%)	13 (12.4%)
	19-40 years	**170 (32.5%)**	102 (32.9%)	45 (41.7%)	23 (21.9%)
	41-60 years	**146 (27.9%)**	94 (30.3%)	32 (29.6%)	20 (19.0%)
	61+ years	**152 (29.1%)**	90 (29.0%)	13 (12.0%)	49 (46.7%)
Total		**523**	**310**	**108**	**105**

Specimens were de-identified and tested on the BioFire COVID-19 Test at clinical study sites. As a comparator for performance evaluation, specimens were also tested at clinical sites on FDA-authorized RT-PCR tests. Specimens could be refrigerated for up to three days before being tested but were never frozen before being tested on the BioFire COVID-19 Test or comparator. Specimens for which FP and/or FN results (i.e., discrepant results) were obtained when comparing the BioFire COVID-19 Test results to the comparator result were further investigated. A frozen aliquot of every specimen, regardless of comparator result, was transferred to a central reference laboratory (CRL) and tested on an independent FDA-authorized RT-PCR test. FP and FN results were compared to the initial SoC result and the CRL result to identify whether there was evidence for the presence of SARS-CoV-2. The prospective clinical study results are summarized in Table 5.

Table 5. BioFire COVID-19 Test Prospective Clinical Performance Summary

Positive Agreement (PPA)				Negative Agreement (NPA)			
TP	**FN**	**%**	**95% CI [a]**	**TN**	**FP**	**%**	**95% CI [a]**
68	1[b]	98.6%	92.2-99.7%	450	4[c]	99.1%	97.8-99.7%

[a] The binomial two-sided 95% confidence interval was calculated using the Wilson Score method.
[b] The FN specimen was negative for SARS-CoV-2 by SoC and CRL testing.
[c] Evidence of SARS-CoV-2 was found in one FP by SoC and CRL testing; evidence of SARS-CoV-2 was found in two additional FPs by CRL testing.

Testing of Archived Clinical Specimens

Archived upper respiratory specimens (25 nasopharyngeal swab, 10 anterior nasal swab, and 10 oropharyngeal swabs) collected in transport medium were tested using the BioFire COVID-19 Test. All specimens were collected from patients presenting with signs or symptoms of COVID-19 and were previously characterized for SARS-CoV-2 infection by another EUA RT-PCR test. Test results with the BioFire COVID-19 Test were compared to a highly sensitive EUA authorized test. For upper respiratory specimens, this study

achieved a PPA of 95.8% (23/24) and an NPA of 95.2% (20/21) with 95% Confidence intervals of 79.8-99.3%, and 77.3-99.2%, respectively.

Testing of Contrived Clinical NPS Specimens

In the absence of clinical positive samples at the beginning of the global SARS-CoV-2 pandemic, contrived testing was performed using negative clinical NPS specimens and contrived positive clinical NPS specimens. Contrived testing was performed using negative clinical NPS specimens and contrived clinical NPS specimens. A total of 70 spiked clinical specimens were tested at concentrations ranging from 1× LoD to 100× LoD. 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. 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'. This study achieved both a PPA (70/70) and NPA (66/66) of 100%, with 95% confidence intervals of 94.8-100% and 94.5-100%, respectively.

Testing of Pooled Clinical NPS 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 included. The samples represented 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 included.

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 6.

Table 6. Detection of SARS-CoV-2 in Pools of 5 or 8 NPS Specimens (Stratified by Ct Value)

	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%
	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%	4/5 (80%)	37.6-96.4%
	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%
	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%	5/5 (100%)	56.6-100%

[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 sputum specimens collected during the COVID-19 pandemic for standard of care were obtained from a commercial biorepository. Specimens were de-identified before testing on the BioFire COVID-19 Test and an FDA-authorized RT-PCR test as comparator. All (18/18) comparator-positive specimens were Detected by the BioFire COVID-19 Test, and 37/38 comparator-negative samples were Not Detected, resulting in 100% PPA and 97.4% NPA (Table 7).

Table 7. BioFire COVID-19 Test Performance Summary with Lower Respiratory Specimens

Sample Type	PPA			NPA		
	TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI
Sputum	18/18	100%	82.4-100%	37/38	97.4%	86.5-99.5%

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. Paired specimens were de-identified and saliva samples were tested on the same day on the BioFire COVID-19 Test. The NPS specimen result obtained from a highly sensitive EUA authorized RT-PCR test was considered the reference for performance calculations (Table 8). 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 8. BioFire COVID-19 Test Performance Summary with Paired Saliva and NPS Specimens

		NPS		BioFire COVID-19 Test Performance	95% CI
		Pos	Neg		
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.

Testing of Pooled Saliva Specimens

Archived saliva specimens collected from patients suspected of SARS-CoV-2 infection were purchased from commercial biorepositories. Since the BioFire COVID-19 Test does not generate Ct values, specimens were tested with an RT-PCR test EUA authorized for the testing of saliva samples (SalivaDirect) to ensure that the positive specimens represent a range of clinically relevant concentrations. These specimens were individually tested using the BioFire COVID-19 Test and categorized as positive or negative.

Each individual positive sample was then pooled with seven negative specimens into 8-sample pools. Negative pools consisted of eight negative specimens. Pooled test results were compared to the individual test results obtained from the BioFire COVID-19 Test to evaluate the effect of pooling on SARS-CoV-2 detection. SARS-CoV-2 was Detected in 19/21 (90.5% PPA) of positive pools. SARS-CoV-2 was Not Detected in 20/21 (95.2% NPA) of the pools that only contained negative specimens. Results are shown in Table 9 stratified by the Ct value derived from SalivaDirect testing of individual positive samples.

Table 9. Summary of Detection of SARS-CoV-2 in Positive and Negative Pools

Positive-Sample Pools Stratified by Ct Value[1]	Detected Rate (PPA)	95% CI
Ct ≥ 33.8[2]	5/5 (100%)	56.6-100%
28 ≤ Ct < 33.8	5/5 (100%)	56.6-100%
23 ≤ Ct < 28	5/5 (100%)	56.6-100%
Undetermined	4/6 (66.7%)	30.0-90.3%
All Positive-Sample Pools	**19/21 (90.5%)**	**71.1-97.3%**
Pools Containing Only Negative Specimens	**Not Detected Rate (NPA)**	**95% CI**
All Negative-Sample Pools	**20/21 (95.2%)**	**77.3-99.2%**

[1] Ct values are based on the result of testing individual positive specimens on the SalivaDirect Test, which has a mean Ct value of 36.8 at LoD.
[2] A Ct value of 41.1 was reported for one specimen by SalivaDirect, which is above the Ct cutoff value of 40.0. Because this sample was characterized as positive on the BioFire COVID-19 Test, it was binned with specimens of Ct ≥ 33.8.

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 from the USA-WA1/2020 isolate (BEI/NR-52286) 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
SARS-CoV-2 USA-WA1/2020 (infectious culture; WRCEVA)[a]	3.3E+02 GC/mL	2.2E-02 $TCID_{50}$/mL	20/20 (100%)
SARS-CoV-2 USA-WA1/2020 (heat-inactivated; BEI NR-52286)[b]	3.3E+02 GE/mL	4.3E-02 $TCID_{50}$/mL	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 isolate (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 95% (19/20).

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 isolate (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 heat-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 208,703 GISAID sequences from patient samples collected January through March 2022 and submitted to the database before April 12, 2022 were analyzed for mismatches co-occurring in all seven BioFire COVID-19 Test assays. Over the three-month collection time interval, there was negligible appreciation (0% prevalence) of any primer variants co-occurring on 5 or more assays. There was one primer variant identified with a single mismatch at a prevalence of >5% of sequences. Limited contrived testing showed no performance impacts for this mutation. One primer variant was found with two mismatches at 0.2% prevalence.

To address circulating strains with potential clinical importance, additional analyses were performed on all lineages designated as variants of concern (VOCs), variants of interest (VOIs) and variants under monitoring (VUM) by the WHO and the CDC. Based on monitoring up to April 2022, 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 current inclusivity analysis, customers should refer to the *BioFire COVID-19 Test SARS-CoV-2 Reactivity Technical Note* found on the website: www.biofiredefense.com.

Exclusivity (in silico)

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

Table 15. Organisms Evaluated in silico for Cross-Reactivity

Organisms Evaluated	
Human coronavirus 229E	*Adenoviridae*
Human coronavirus OC43	Legionellaceae
Human coronavirus HKU1	Mycoplasma
Human coronavirus NL63	Human metapneumovirus
SARS-coronavirus*	Human Influenza A virus
MERS-coronavirus	Influenza B virus
Adenovirus C1	Influenza C virus
Adenovirus 71	Parechovirus
Parainfluenza virus 1-4	*Corynebacterium diphtheriae*
Rhinovirus/Enterovirus	*Bacillus anthracis*
Enterovirus D68	*Moraxella catarrhalis*
Respiratory syncytial virus	*Neisseria elongata*
Chlamydia pneumoniae	*Leptospira spp.*
Haemophilus influenzae	*Chlamydia psittaci*
Legionella pneumophila	*Coxiella burnetii*
Mycobacterium tuberculosis	*Staphylococcus aureus*
Streptococcus pneumoniae	Homo sapiens
Streptococcus pyogenes	Herpes simplex virus type 1 (HSV-1)
Bordetella pertussis	Epstein-Barr virus (EBV)
Mycoplasma pneumoniae	Cytomegalovirus (CMV)
Pneumocystis jirovecii	*Porphyromonas gingivalis*
Candida albicans	*Bacteroides oralis*
Pseudomonas aeruginosa	*Nocardia* sp.
Staphylococcus epidermidis	*Streptomonas mutans*
Streptococcus salivarius	*Eikenella* sp.
Streptococcus mitis	*Neisseria* sp.
Streptococcus viridans	*Lactobacillus* sp.
Streptococcus anginosus	
Streptococcus sanguinis	
Streptococcus bovis	

Only near-neighbor non-human coronavirus genomes showed significant homology to assay-specific sets of primers and are predicted to be detected by the BioFire COVID-19 Test. It is unlikely that these isolates would be found in human respiratory samples; however, little is known about their potential to infect a human host. 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 GC/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
Parainfluenza virus 3	BEI NR-3233	5.10E+07 $TCID_{50}$/mL	None

Organism/Virus	Source/ID	Test Concentration	Cross-Reactivity Detected
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 collected specimens or introduced during specimen collection and testing were evaluated on the BioFire COVID-19 Test for their effect on pouch performance. Data from testing potentially interfering substances on the BioFire COVID-19 Test are presented in Table 17.

Substances tested included endogenous (e.g., blood, mucus/mucin, or human genomic DNA), and exogenous substances (e.g., medications, nasal washes, or substances used to clean work areas) that may be found in the clinical matrix. The concentration of substance tested was equal to or greater than the highest level expected to be in collected specimens.

None of the substances tested were shown to interfere with the BioFire COVID-19 Test.

Table 17. Substances Tested Demonstrating No Panel Interference on BioFire COVID-19 Test

Substance Tested		Concentration Tested
Toothpaste (Colgate Total) – Stannous fluoride 0.454%		2% v/v
Tobacco (Camel Snus)		10 mg/mL
Oral Rinse (Listerine) – Eucalyptol 0.092%, Menthol 0.042%, Methyl Salicylate 0.060%, Thymol 0.064%		1% v/v
Throat lozenges (Cepacol) – Benzocaine 7.5 mg, Dextromethorphan HBr 5 mg		2.2 mg/mL
Oral anesthetic and analgesic (Mouth Sore Relief) – Benzocaine 20%		1% v/v
Cough Drops – Menthol 5.4 mg		2.2 mg/mL
Cleanser (Dye-Free Antiseptic Cleanser) – Chlorhexidine Gluconate 4%		1% v/v
Nicotine		10 mg/mL
Mucin – Bovine submaxillary gland, type I-S Sigma M3895		5 mg/mL
Blood (human) – Human DNA		5% v/v
Leukocytes – Human DNA		1% v/v
Nasal spray (Wal-Four Nasal Spray) – Phenylephrine hydrochloride 1%		10% v/v
Afrin Nasal Spray – Oxymetazoline hydrochloride 0.05%		10% v/v
Saline Nasal Spray – NaCl 0.65% with preservatives (Phenylcarbinol, Benzalkonium Chloride)		10% v/v
Nasal corticosteroids	Beclomethasone	2 mg/mL
	Dexamethasone	1.5 mg/mL
	Flunisolide	2 mg/mL
	Triamcinolone	5 mg/mL
	Mometasone	1 mg/mL
Budesonide Nasal Spray – Budesonide 32 mcg/ spray		1% v/v
Allergy Nasal Spray – Fluticasone 50 mcg/ spray		1% v/v
Nasal gel (Zicam) – Luffa opperculata 4x, Galphimia glauca 4x, Sabadilla 4x		1% v/v
Sulfur		0.17 mg/mL
Allergy Relief (RhinAllergy) – Histaminum hydrochloricum 9C HPUS		10 mg/mL
Anti-viral drugs – Zanamivir		5.5 mg/mL
Antibiotic, Nasal ointment – Mupirocin		3.3 mg/mL
Antibacterial, systemic – Tobramycin		4 µg/mL
Transport Media	Remel M4 (R12503)	100%
	Remel M4RT (R12591)	100%
	Copan UTM-RT (UTM 330C)	100%

Substance Tested		Concentration Tested
	PrimeStore MTM (MTM-LH102)	100%
	Merit Medical Cultura Media (VDCV100)	100%
	Neuronics VTM (VTM-4:100)	100%
	Azer UTM (PFUTM-10)	100%
	Bartels FlexTrans (B102990D/DEL)	100%
	S2 VTM (5165)	100%
Diluents	Ethanol	10% v/v
	DMSO	10% v/v
	Methanol	10% v/v
	Chloroform	10% v/v
	DMF	10% v/v

SPECIMEN POOLING

Pooling Implementation

Pooling must only be performed by U.S. Department of Defense (DoD) on individuals who are subjected to a detailed infection prevention and control plan prior to and during operations, or by DoD laboratories that can adhere to a 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 or saliva 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}) = (Number of positive specimens in pools / Total number of specimens tested in pools) × 100%

For DoD Laboratories that Can Adhere to a 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 periods 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 $P_{pools\text{-}initial}$ (i.e., $P_{pools\text{-}x} / P_{pools\text{-}initial} < 0.90$), 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 positive percent agreement (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 $P_{pools\text{-}initial}$ (i.e., $P_{pools\text{-}x} / P_{pools\text{-}initial} < 0.90$), 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 Medical devices – Symbols to be used with information to be supplied by the manufacturer – Part 1: General requirements					
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
Use of Symbols in Labeling - 81 FR 38911, Docket No. (FDA-2013-N-0125)					
℞ 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
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-0134-05, May 2022

APPENDIX C

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7. Centers for Disease Control and Prevention. How to Report COVID-19 Laboratory Data. Centers for Disease Control and Prevention | COVID-19. Published April 4, 2022. Accessed May 19, 2022. https://www.cdc.gov/coronavirus/2019-ncov/lab/reporting-lab-data.html
8. CLSI. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline-Second Edition. CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
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	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.
05	May 2022	BioFire COVID-19 Test was expanded to include saliva pooling. BioFire COVID-19 Test v1.1 pouch module software was updated to use seven SARS-CoV-2 assays in result interpretation. IFU updated to remove reference of three assays and revised performance data. Intended use updated to include saliva pooling claims and remove mention of the BioFire COVID-19 Test v1.0.

[redacted]

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

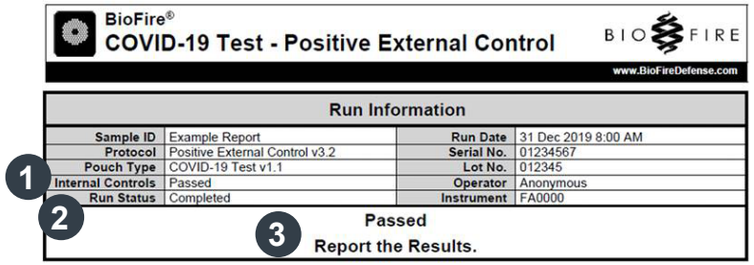
[redacted]

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-0113-04

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

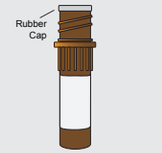
DO NOT DISCARD: Important product-specific information

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

REF 424062 (6 pk) **IVD**

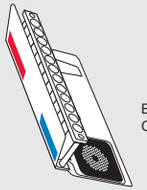
℞ Only

Package Contents

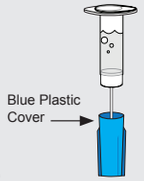


External Control Vial

Materials required but not provided

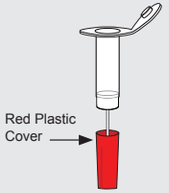


Pouch
BioFire® COVID-19 Test
Part No: 423744
423745

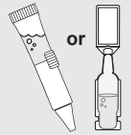


Hydration Injection Vial

Transfer Pipette



Sample Injection Vial



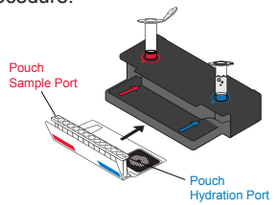
Sample Buffer Ampoule

Transport Medium, PBS or Saline

NOTE: 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

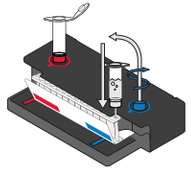
Refer to the *BioFire® COVID-19 Test Upper Respiratory Quick Guide STEP 1: Prepare Pouch*



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).

Add Sample Buffer to Positive External Control Vial

NOTE: *There are 2 possible designs of the Sample Buffer Ampoule*

c. Hold the Sample Buffer Ampoule with the tip facing up.

NOTE: *Do not touch the tip of the ampoule.*

d. Firmly pinch textured plastic tab on the side of the ampoule until the seal snaps or if there is no textured tab on the side, gently twist off the plastic tab on the tip.

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

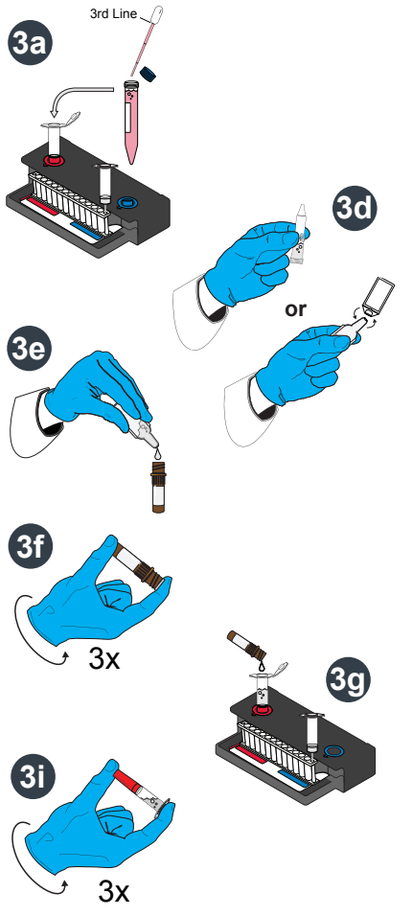
NOTE: Avoid generating excessive foam.

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

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

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

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



WARNING: Contact with sample buffer can cause serious eye damage and skin irritation and is harmful if swallowed.

j. 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

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

Add Sample Buffer to Sample Injection Vial.

NOTE: *There are 2 possible designs of the Sample Buffer Ampoule*

b. Hold the Sample Buffer Ampoule with the tip facing up.

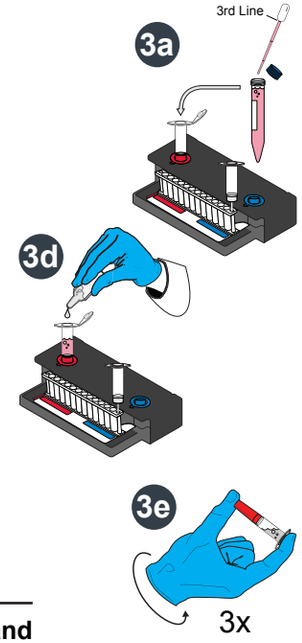
NOTE: *Do not touch the tip of the ampoule.*

c. Firmly pinch textured plastic tab on the side of the ampoule until the seal snaps or if there is no textured tab on the side, gently twist off the plastic tab on the tip.

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

NOTE: Avoid generating excessive foam.

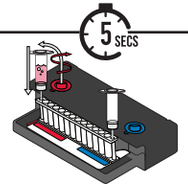
e. Tightly close lid of Sample Injection Vial and invert **3** times, return it to the red well of Pouch Loading Station.



WARNING: Contact with sample buffer can cause serious eye damage and skin irritation and is harmful if swallowed.

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.

BioFire® COVID-19 Test v1.1 Quick Guide

For use with BIOFIRE® FILMARRAY® 2.0 and BIOFIRE® FILMARRAY® TORCH Systems

DO NOT DISCARD: Important product-specific information

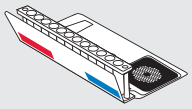
For in vitro diagnostic use under Emergency Use Authorization (EUA) only

LOWER RESPIRATORY

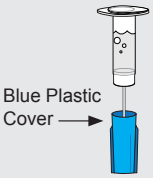
REF 423745 (6 pk)
423744 (30 pk)

IVD ℞ Only

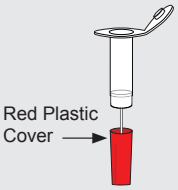
Package Contents



Pouch

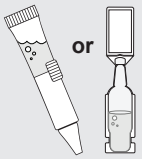


Hydration Injection Vial



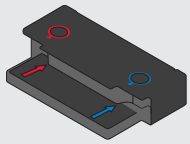
Sample Injection Vial

Transfer Pipette



Sample Buffer Ampoule

Materials required but not provided

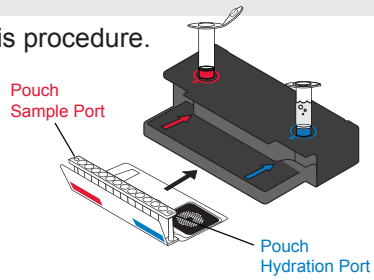


Pouch Loading Station
(included with Instrument)

Swab
Kit Part No. 424063

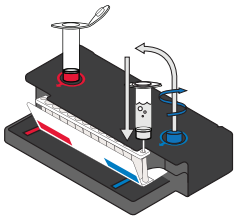
NOTE: Use clean gloves and other Personal Protective Equipment (PPE) when performing this procedure.

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

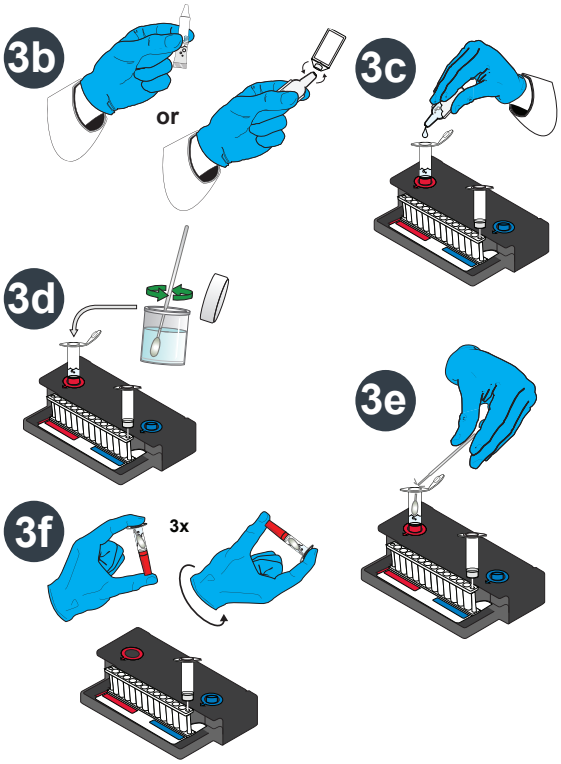


a. Unscrew Hydration Injection Vial from cover.
b. Remove Hydration Injection Vial, leaving blue plastic cover in Pouch Loading Station.
c. Insert Hydration Injection Vial into pouch hydration port.
d. Push down to puncture seal and wait as Hydration Solution is drawn into the pouch.

NOTE: Verify the pouch has been hydrated.

Step 3: Prepare Lower Respiratory Sample Mix

Add Sample Buffer



NOTE: There are 2 possible designs of the Sample Buffer Ampoule

a. Hold the Sample Buffer Ampoule with the tip facing up.

NOTE: Do not touch the tip of the ampoule.

b. Firmly pinch textured plastic tab on the side of the ampoule until the seal snaps or if there is no textured tab on the side, gently twist off the plastic tab on the 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. Use the Sample Swab to stir the entire specimen for **~10** seconds.

e. 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.

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

WARNING: Contact with sample buffer can cause serious eye damage and skin irritation and is harmful if swallowed.

BioFire® COVID-19 Test v1.1 Quick Guide

For use with BIOFIRE® FILMARRAY® 2.0 and BIOFIRE® FILMARRAY® TORCH Systems

LOWER RESPIRATORY

Step 4: Load Lower Respiratory Sample Mix

a. Unscrew Sample Injection Vial from red plastic cover.

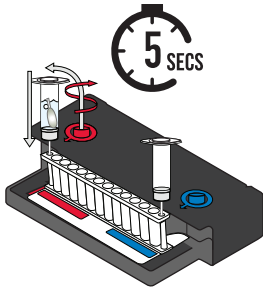
b. Wait for **5** seconds, then lift Sample Injection Vial, leaving red plastic cover in Pouch Loading Station.

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.

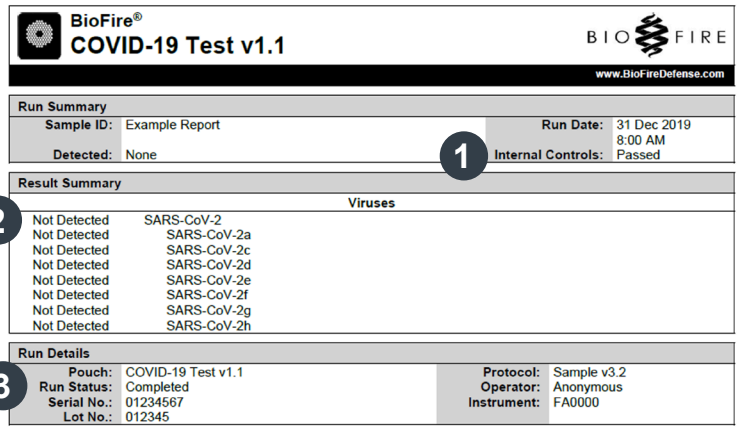
1. Internal Controls:
   - If 'Passed', results are valid.
   - If 'Failed' or 'Invalid', **RETEST SAMPLE** and refer to *Instructions for Use*.

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*.



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-0116-03

BioFire® COVID-19 Test v1.1 Quick Guide

For use with BIOFIRE® FILMARRAY® 2.0 and BIOFIRE® FILMARRAY® TORCH Systems

DO NOT DISCARD: Important product-specific information

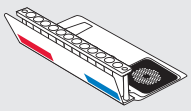
UPPER RESPIRATORY OR SALIVA

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

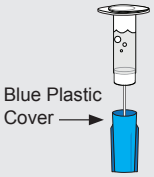
REF 423745 (6 pk)
423744 (30 pk)

IVD Rx Only

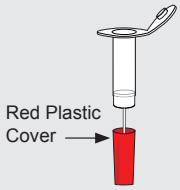
Package Contents



Pouch

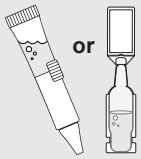


Hydration Injection Vial



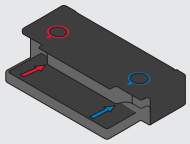
Sample Injection Vial

Transfer Pipette



Sample Buffer Ampoule

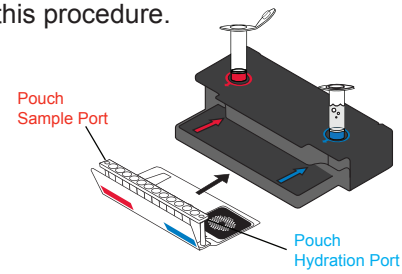
Materials required but not provided



Pouch Loading Station
(included with Instrument)

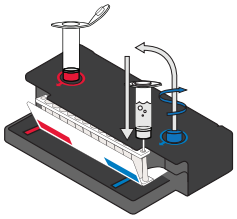
NOTE: Use clean gloves and other Personal Protective Equipment (PPE) when performing this procedure.

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.

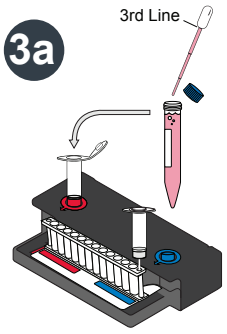
Step 2: Hydrate Pouch

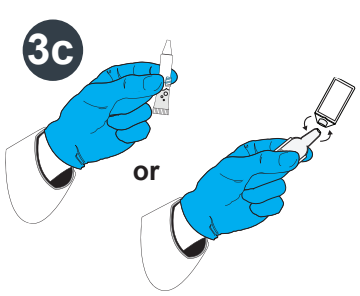


a. Unscrew Hydration Injection Vial from cover.
b. Remove Hydration Injection Vial, leaving blue plastic cover in Pouch Loading Station.
c. Insert Hydration Injection Vial into pouch hydration port.
d. Push down to puncture seal and wait as Hydration Solution is drawn into the pouch.

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.

Add Sample Buffer

NOTE: There are 2 possible designs of the Sample Buffer Ampoule

b. Hold the Sample Buffer Ampoule with the tip facing up.

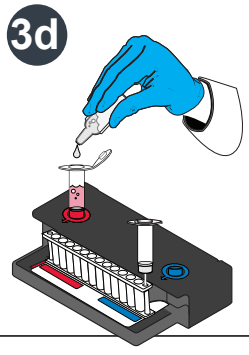
NOTE: Do not touch the tip of the ampoule.

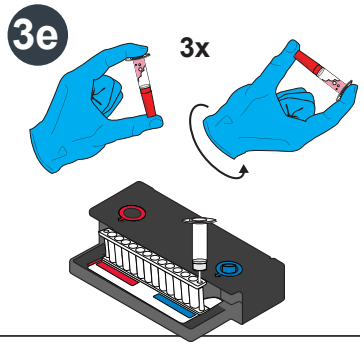
c. Firmly pinch textured plastic tab on the side of the ampoule until the seal snaps or if there is no textured tab on the side, gently twist off the plastic tab on the tip.

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

NOTE: Avoid generating excessive foam.

e. 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: Contact with sample buffer can cause serious eye damage and skin irritation and is harmful if swallowed.

BioFire® COVID-19 Test v1.1 Quick Guide

For use with BIOFIRE® FILMARRAY® 2.0 and BIOFIRE® FILMARRAY® TORCH Systems

UPPER RESPIRATORY OR SALIVA

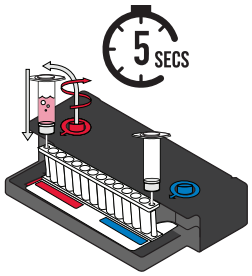
Step 4: Load Upper Respiratory Sample Mix

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.

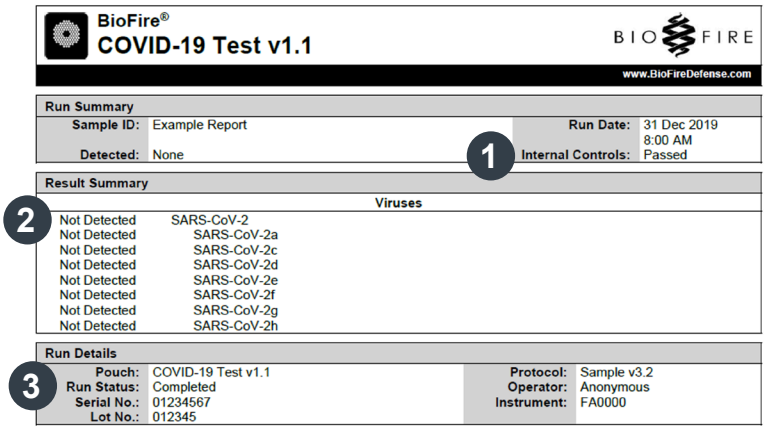
Step 6: Review Results

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*.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BIOFIRE
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Run Summary

Sample ID:	Example Report	Run Date:	31 Dec 2019 8:00 AM
Detected:	None	Internal Controls:	Passed

Result Summary

Viruses	
Not Detected	SARS-CoV-2
Not Detected	SARS-CoV-2a
Not Detected	SARS-CoV-2c
Not Detected	SARS-CoV-2d
Not Detected	SARS-CoV-2e
Not Detected	SARS-CoV-2f
Not Detected	SARS-CoV-2g
Not Detected	SARS-CoV-2h

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-0131-03

BIOFIRE DEFENSE

BioFire Defense, LLC | 79 West 4500 South, Suite 14, Salt Lake City, Utah 84107 USA | 801-262-3592 | www.BioFireDefense.com