Contamination Prevention and Decontamination

1. Introduction

The BIOFIRE® System is an automated in vitro diagnostic (IVD) system that utilizes nested multiplex polymerase chain reaction (PCR) and high-resolution melting analysis to detect and identify multiple nucleic acid targets from clinical specimens. PCR is a chemical and enzymatic amplification reaction that can create millions of copies of a specific DNA region from a small number of starting copies. As more copies are made, a DNA signal becomes detectable and the presence or absence of specific DNA in a sample can be determined.

The amplification process associated with PCR-based methods can lead to false positive results caused by contamination. Contamination refers to the unintended introduction of organisms or nucleic acids (including amplification products or "amplicons" from previous PCR reactions) into samples, reagents, or workspaces during clinical specimen collection, sample handling, or testing.

This document provides guidelines for reducing the risk of false positive results due to contamination when using the BIOFIRE System and panels to test clinical specimens and control materials. It also includes instructions for decontamination as part of routine cleaning or after a spill or other contamination event.

2. Contamination Prevention

Preventing Contamination During Specimen Collection:

The accurate and reliable results of diagnostic tests depend on the proper collection of specimens, free from contaminants. During specimen collection, healthcare workers and laboratory personnel should wear appropriate personal protective equipment (PPE), such as **disposable medical gloves and face masks**. Wearing gloves and masks helps prevent contamination from pathogens or normal flora carried by the health care worker or lab personnel, whether they show symptoms or not. Gloves and masks minimize the risk of contamination from normal skin bacteria (e.g. *Staphylococcus epidermidis*) and other organisms that may be transmitted by:

- Individuals with active respiratory infections or symptoms (e.g., runny nose, cough):
- Individuals with active or latent HSV-1 infection or lesions (e.g., cold sore).
- Healthy or asymptomatic individuals:
    - These individuals may actively shed normal flora or opportunistic pathogens (e.g., *S. pneumoniae*, *H. influenzae*) from the upper respiratory tract.[3]
    - Human rhinoviruses are genetically similar to enteroviruses and may be shed from the respiratory tract of healthy individuals.

Specimen collection and testing **should not** occur in areas where vaccines are administered or handled. Several vaccines contain PCR-detectable DNA, RNA, or (attenuated) pathogen (e.g. SARS-CoV-2 vaccines, Rotavirus vaccines, *Bordetella pertussis* acellular vaccines, etc.) and contamination of samples, work areas, or testing reagents with vaccine can cause false positive results. CDC offers guidance for minimizing *B. pertussis* vaccine contamination and these best practices would also be applicable to minimizing contamination from other vaccines:

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https://www.cdc.gov/pertussis/php/pcr-bestpractices/?CDC_AAref_Val=https://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-pcr-bestpractices.html

Handling of positive control (PC) or external quality control (EQC) materials should not occur in the same space as specimen collection. Most PC or EQC samples contain concentrated amounts of one or more organisms or nucleic acids that can contaminate samples, workspaces, or test reagents and may cause false positive results.

Consider the following precautions for these common specimen types (this is not an exhaustive list):

- **Cerebrospinal Fluid (CSF):**
  - Specimen collection should be performed in an enclosed room using sterile techniques.[1,2]
  - All individuals present in the room must wear masks.
  - Individuals with even minor illnesses, particularly those with active respiratory symptoms, should be excluded to prevent specimen contamination. [3]
  - While collecting the specimen:
    - Place sterile towels underneath the patient.[1]
    - Clean the skin with chlorhexidine or povidone-iodine.[1,2]
- **Blood Culture[4]:**
  - Blood cultures should be collected by staff members who have undergone comprehensive training and whose competency has been formally assessed.
  - Examine blood culture bottles for any signs of damage.
  - Do not use bottles with media that show signs of contamination, such as turbidity or excess gas pressure.
  - Verify the expiration date on the bottle and follow the proper collection protocol.
  - While collecting the sample:
    - Avoid collecting blood from venous or arterial catheters, as these devices may contain bacterial or fungi colonies.
    - Thoroughly disinfect the patient's skin with an appropriate disinfectant before collection.
- **Sputum[5]:**
  - Use sterile collection containers.
  - Patients should be in a private area to avoid exposing others to potential pathogens.
  - Ensure the collection area is clean, disinfected and well-ventilated.
- **Bronchoalveolar lavage (BAL)[6]:**
  - Ensure the bronchoscope and other equipment are properly sterilized.
  - Collect the lavage fluid in a sterile container, avoiding contact with the rim or inside the container.
  - Immediately seal the collection container following collection to prevent exposure.

Preventing Contamination During Sample Testing: Organisms, Vaccines, Positive Control (PC) and External Quality Control (EQC) Materials

The accurate and reliable results of diagnostic tests depend on the proper handling and testing of samples, free from contaminants.

During sample handling and testing, healthcare workers and laboratory personnel should wear appropriate personal protective equipment (PPE), such as **disposable medical gloves and face masks**. Wearing gloves and masks helps prevent contamination from pathogens or normal flora carried by the health care worker or lab personnel, whether they show symptoms or not. Gloves and masks minimize the risk of contamination from normal skin bacteria (e.g. *Staphylococcus epidermidis*) and other organisms that may be transmitted by:

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- Individuals with active respiratory infections or symptoms (e.g., runny nose, cough):
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- Healthy or asymptomatic individuals:
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    - Human rhinoviruses are genetically similar to enteroviruses and may be shed from the respiratory tract of healthy individuals.

Sample handling and testing **should not** occur in areas where vaccines are administered or handled. Several vaccines contain PCR-detectable DNA, RNA, or (attenuated) pathogen (e.g. SARS-CoV-2 vaccines, Rotavirus vaccines, *Bordetella pertussis* acellular vaccines, etc.) and contamination of samples, work areas, or testing reagents with vaccine can cause false positive results. CDC offers guidance for minimizing *B. pertussis* vaccine contamination and these best practices would also be applicable to minimizing contamination from other vaccines: https://www.cdc.gov/pertussis/php/pcr-bestpractices/?CDC_AAref_Val=https://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-pcr-bestpractices.html

Most PC or EQC samples contain concentrated amounts of one or more organisms or nucleic acids that can contaminate samples, workspaces, or test reagents and may cause false positive results. Handling of control materials should not occur in the same space as specimen collection. Lab personnel should disinfect the work area and change gloves before and after working with control materials. Thorough decontamination (see Section 3 Decontamination and Cleaning Procedures) should be performed if there are any spills, flicks, dropped tube caps, etc. that could disperse or aerosolize the control material and contaminate the work area.

All sample handling and testing (e.g. pouch loading) should be performed in a clean biosafety cabinet (BSC), a dead air box (e.g., AirClean PCR workstation), or behind a splash shield (e.g., Bel-Art Scienceware Splash Shields). If a BSC, dead air box, or splash shield is not available, a face shield should be used when handling and testing samples.

> **WARNING:** A BSC that is routinely used to perform viral, bacterial, or fungal culture is a **high contamination risk** and **should not** be used for sample handling or BIOFIRE test setup.

Prior to sample handling and testing, thoroughly clean the work area (including the BSC, dead air box, or splash shield) and the BIOFIRE® Pouch Loading Station using freshly prepared 10% bleach solution (1-part bleach to 9-parts water) or a similar disinfectant effective against nucleic acids. To avoid bleach residue build-up (potential PCR inhibition) wipe disinfected surfaces with distilled water.

> **WARNING:** Bleach should never be combined with Sample Buffer or sample waste as it can react with components of the buffer to generate a harmful gas.

After disinfecting the work area, change gloves and proceed with test set up. Handle samples and BIOFIRE pouches one-at-a-time. Completely set up a single BIOFIRE test and start the run before changing gloves, disinfecting the work area and Pouch Loading Station again, changing gloves, and then handling the next sample.

> **NOTE:** If you have touched your face or another potentially contaminated surface, change gloves before continuing with the sample loading and testing procedure.

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Preventing Contamination of Samples and Testing with Amplicon (copies of DNA from a previous test)

As with any PCR reaction, at the end of a test run, the BIOFIRE pouch can contain many millions of copies of nucleic acid segments (amplicon). Contamination of another sample or test reagents with amplicon from a used pouch does pose a risk for false positive results.

However, the self-contained, closed system design of the disposable BIOFIRE pouch significantly minimizes the risk of sample-to-sample or test-to-test amplicon carryover or contamination, as long as the pouch remains intact (no leaking, punctures, or breaks). If **pouch leaking or pouch breaks** are observed, **proceed immediately** to the actions and instructions provided in Section 3 **Decontamination and Cleaning Procedures**.

For routine testing with intact pouches, the guidelines described above for preventing contamination during specimen collection and sample testing will also minimize the risks of amplicon contamination. In addition, the following precautions will further minimize the risk of amplicon contamination:

- Handle samples and load pouches in an area separated from the BIOFIRE instruments and the area where used pouches are discarded.
- Avoid excessive handling of pouches after test runs.
- Discard used pouches in an appropriate biohazard container immediately after the run has completed.
- Avoid exposing pouches 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. Treat all broken pouches as capable of contaminating the work area. Change gloves and decontaminate the workspace around the instrument as described in the Decontamination and Cleaning Procedures section below. **Do not perform additional testing until the area has been decontaminated.** If you continue to experience pouch leaks, contact BIOFIRE Technical Support or an authorized distributor.

3. Decontamination and Cleaning Procedures

The following decontamination and cleaning procedures are general best practices for contamination prevention during routine use, if contamination is suspected (e.g. unexplained false positive results observed, especially if false result is consistent with a previously positive sample), and/or in the event of a sample spill or pouch leak/breakage. **Decontamination after a spill, leak, or break is critical to reduce the risk of false-positive results in subsequent runs.**

NOTE: Change gloves often during the decontamination process and before touching any clean surface or samples. All personal protective equipment (PPE) should be discarded if contamination of the PPE is suspected and after the decontamination process is completed.

Cleaning Materials

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This list provides the materials necessary for cleaning and decontamination:

- Fresh 10% bleach solution in a squeeze or spray bottle (1-part bleach to 9-parts water)
    - for full potency, mix a fresh 10% bleach solution frequently (daily is recommended)
    - alternately, bleach wipes or another disinfectant wipe effective against nucleic acids can be used
- Distilled water in a squeeze or spray bottle
- DNAZap™ or equivalent DNA degradation reagent
- Paper towels

CAUTION: Bleach should not come into contact with liquid or dried Sample Buffer from a BIOFIRE panel reagent kit. Bleach will chemically react with Sample Buffer and release a harmful gas (see Safety Data Sheets). Clean spilled or dried Sample Buffer with water prior to disinfecting with bleach.

CAUTION: Bleach and some other disinfectants may be corrosive or harmful to equipment, including the BIOFIRE Systems. Liquids and disinfectants should not be used inside the pouch slot of the BIOFIRE Systems.

If you are unsure whether a cleaning agent will be effective or whether it will react negatively with a substance or equipment/instruments, contact BIOFIRE Technical Support or an authorized distributor.

Procedure for Workspace Decontamination

Routine cleaning and decontamination of workspaces should focus on BSCs, benchtops, countertops, and touch surfaces of equipment in workspaces with the potential to be contaminated, including areas where patient specimens are collected and where control samples are stored and handled.

Workspace decontamination following a sample spill or pouch leak should primarily focus on the area of the spill or leak and any areas where contaminants could have been tracked or spread by normal foot traffic and touch transfer.

1. Put on a clean lab coat and gloves.
2. Wipe the surfaces and equipment (e.g. benchtop, BSC, vortex, pipettors, etc.) to be decontaminated with water.
3. Wipe surfaces and equipment with a paper towel soaked in a fresh 10% bleach solution (1-part bleach to 9-parts water; can be substituted with bleach or other disinfecting wipes, if necessary).
4. Let the disinfectant act on the surfaces for 5 minutes.
5. Change gloves.
6. Repeat the decontamination wipes (step 3 and 4) twice for a total of three bleach/decontamination wipes.
7. Wipe the decontaminated surfaces and equipment with paper towel soaked with distilled water (to remove bleach/disinfectant residue).
8. Wipe the area dry with a new paper towel.
9. Change gloves.
10. Treat the surfaces and equipment in the workspace with DNAZap or an equivalent nucleic acid degradation product. Follow the instructions for use of the product.
11. Change gloves.
12. Wipe the treated surfaces with paper towel soaked with distilled water and then wipe dry with a clean, dry paper towel.

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Procedure for Pouch Loading Station Decontamination

The Pouch Loading Station can be cleaned with water and bleach wipes, as described for workspace decontamination above, during daily use, and can also be submerged for decontamination on a regular basis (daily recommended):

1. Put on a clean lab coat and gloves.
2. Rinse the Pouch Loading Station with water.
3. Fill a sink or bin with water and add bleach to create a 10% bleach solution (1-part bleach to 9-parts water).
4. Submerge the Pouch Loading Station until completely covered with bleach solution. Soak for 15 minutes.
5. Remove Pouch Loading Station from sink or bin. Replace bleach solution with water.
6. Rinse the Pouch Loading Station by completely submerging in water two additional times.
7. Allow the Pouch Loading Station to dry before use.

Procedure for Disposal and Decontamination Related to Pouch Leak/Breakage

When a pouch leak or break is identified, act carefully and quickly to prevent the liquid from contaminating the instrument and work area.

1. If you are holding the leaking or broken pouch and are within reach of a biohazard container, immediately dispose of the pouch in the biohazard container (watch for drips or spills).
    a. Remove and dispose of your potentially contaminated gloves in the biohazard container. Remove potentially contaminated lab coat and other PPE.
2. If you are holding the leaking or broken pouch and are not within reach of a biohazard container, secure the pouch and any leaking liquid in a disposable container (e.g. ziplock-type bag) or, if no disposable container is available, on disposable absorbent material (e.g. lab bench liner or paper towel).
    a. Dispose of the contained leaking or broken pouch (including bag, container, or absorbent material) in the closest biohazard container.
    b. Remove and dispose of gloves. Remove potentially contaminated lab coat and other PPE.
    c. Put on clean gloves, lab coat and other PPE.
3. Block off the potentially contaminated workspace and/or instrument(s) so they are not used and traffic in the workspace is minimized until decontamination is complete (this will prevent further spread of the contamination).
4. Look for drips or spills on the floor or workspaces and follow workspace decontamination procedures below.

Procedure For BIOFIRE Instrument Decontamination

Routine cleaning and decontamination of the BIOFIRE instruments should focus on touch surfaces of the instrument and the surrounded benchtops, countertops, and workspaces, as described above. However, the decontamination procedure for returning an instrument for service (RMA) or instrument decontamination following a pouch leak or break may include more focus on exterior surfaces and well as limited interior (non-mechanical) instrument surfaces if liquid or dried liquid deposits are visible.

- When decontaminating an instrument, **use only damp paper towels** (soaked, but not dripping) to avoid getting liquid into the moving or functional parts of the instrument.
- **Never** spray or insert liquids, wet paper towels, or wet swabs into the pouch slot or other interior mechanical/optical compartments of the instrument.

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- Bleach and other disinfectants could be **corrosive or damaging to the instrument**.

Instrument decontamination for the purposes of returning for service (RMA) or following a pouch leak or break should proceed as follows:

1. Put on a clean disposable lab coat and gloves.
2. If a pouch is present in the instrument, remove the pouch and discard in a biohazard container. For broken or leaking pouches, contain the pouch and liquids in a bag or other container prior to biohazard disposal and avoid drips and spreading of the contaminating liquids.
3. Remove and dispose of gloves in the biohazard container. Put on clean gloves.
4. Remove and dispose of lab coat and other PPE. Put on a clean lab coat and other PPE.
5. Wipe instrument surfaces with a paper towel soaked with distilled water. Change gloves.
6. If there is liquid or dried liquid residue visible on the interior (non-mechanical) surfaces of the instrument (e.g. underside of the lid of the BIOFIRE® FILMARRAY® 2.0 System):
    a. Use a dry paper towel to gently dab and absorb visible liquid (do not wipe or spread the liquid with the towel). Immediately dispose of the paper towel in a biohazard container and change gloves. Follow with a wipe with a damp (not wet) paper towel (soaked with distilled water). Dispose of the paper towel in a biohazard container and change gloves.
    b. Use a damp (not wet) paper towel (soaked with distilled water) to wipe away dried liquids or residue from the interior (non-mechanical) surfaces of the instrument. Immediately dispose of the paper towel in a biohazard container and change gloves.

    CAUTION: If there is visible liquid or dried deposits in the pouch slot and other interior mechanical surfaces of the instrument, or if deposits on non-mechanical surfaces cannot be easily removed by wiping a damp paper towel, contact BIOFIRE Technical Support to request service for the instrument.
7. Decontaminate the instrument surfaces by wiping with a damp paper towel soaked with freshly prepared 10% bleach (1-part bleach to 9-parts water; can be substituted with bleach or other disinfecting wipes, if necessary).
    a. Flat, non-mechanical interior instrument surfaces (e.g. the underside of the BIOFIRE 2.0 System lid) can be disinfected by the same process as the exterior surfaces.
    b. Bleach and other disinfectants should not be used on other interior mechanical/optical surfaces of the instrument.
8. Let the disinfectant act on the surfaces for 5 minutes.
9. Change gloves.
10. Repeat the decontamination wipes (step 7 and 8) twice for a total of three bleach/decontamination wipes.
11. Wipe the decontaminated surfaces with paper towel soaked with distilled water (to remove bleach/disinfectant residue). Use only damp towels (not wet or dripping) to water wipe exterior or interior surfaces.
12. Change gloves.
13. Wipe the disinfected surfaces dry with a clean, dry paper towel.
14. Run a negative BIOFIRE panel pouch (see Section 5 Suspected False Positive Results caused by Contamination) to verify whether the decontamination procedure was effective, and the potential contamination has been resolved.

4. Contamination Monitoring/Swab Testing Procedure

Swabbing of workspaces or equipment and testing the swabs with one or more BIOFIRE panels to monitor for contamination may be conducted at regular intervals for routine monitoring and/or following the completion of decontamination procedures driven by suspected contamination events (including spills and pouch leaks). The

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frequency and details of swabbing and testing for contamination monitoring should be determined by the Laboratory Director.

- Using the procedures described above, decontaminate workspaces and touch surfaces used for specimen collection, as well as sample handling and testing. Workspaces may include: bio-safety cabinet (BSC) surfaces and sash, keyboards and mice, small equipment (vortexors, pipettors, etc.), drawers and drawer pulls, freezer/refrigerator handles, benchtops, door handles, Pouch Loading Station, and BIOFIRE instrument surfaces.

  WARNING: Bleach can be corrosive and is a known PCR inhibitor. Bleach residue from the decontamination procedure must be removed with distilled water wipes prior to swabbing or swab tests may fail.

- Determine the number and type of surfaces to be swabbed.
  - One swab should be used per specific area wished to be monitored and tested for contamination.
- Put on clean lab gloves, lab coat, and other PPE.
- Obtain the necessary BIOFIRE panel pouches, reagents, and Pouch Loading Station(s) for the number of swabs that will be tested.
- Obtain sterile 1.5 mL microcentrifuge tube(s) (or equivalent).
- Fill each microcentrifuge tube(s) with approximately 500 µL of molecular grade water.
- Unwrap individually packaged sterile flocked swab(s) (or equivalent) and wet each swab by submerging one or more swabs in the water in the tube(s).
  - Do not touch the swab with hands or allow the swab head to touch any surfaces.
  - Allow the swabs to sit, submerged in water, for approximately 1 minute.
- Run or swipe a wetted swab over the designated (and previously decontaminated) workspaces, surfaces, equipment, etc.

  CAUTION: Wet swabs should not be placed in the interior of the instrument.

- Each swab should be tested immediately with a BIOFIRE panel pouch:
  - Set up a pouch and other reagents in the Pouch Loading Station and place the wet swab (after swiping over the designated surfaces) in the (red) Sample Injection Vial.
  - Remove or break-off the swab shaft at the breakpoint (if applicable; the shaft must be short enough to be able to place the cap on the Sample Injection Vial).
  - Follow standard pouch loading instructions to dispense Sample Buffer from the Sample Buffer Ampoule in the Sample Injection Vial (to submerge the swab head). Tightly close lid of Sample Injection Vial.
  - Follow standard pouch loading instructions to hydrate the pouch using the (blue) Hydration Injection Vial.
  - Invert the Sample Injection Vial (containing the swab and Sample Buffer) 3 times to mix well.
  - Follow standard pouch loading instructions to load the swab sample into the pouch using the Sample Injection Vial.
  - Label and remove the hydrated and loaded pouch from the Pouch Loading Station and transport to a BIOFIRE System.
  - Follow instructions on the BIOFIRE System to insert a pouch and initiate the test run.
- Results of the swab(s) tested should be valid (pouch controls pass) and negative/Not Detected for all organisms/analytes.

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- If positive/Detected results are reported for one or more swab tests, repeat the Decontamination and Cleaning Procedures (above) and run a negative pouch (see Section 5 Suspected False Positive Results caused by Contamination), and/or repeat swab testing.
- Contact BIOFIRE Technical Support if positive/Detected results for swab testing (or a negative pouch) are not resolved after the second decontamination.

5. Suspected False Positive Results Caused by Contamination

If false positive results are identified and contamination is suspected, the specimen collection area(s), specimen handling, and pouch loading areas, the area around the BIOFIRE Instrument, and the touch surfaces of the instrument should be decontaminated as described in the Decontamination and Cleaning Procedures above.

After decontamination, a negative (no sample) pouch should be run to verify the decontamination process was successful:

1. Follow standard pouch loading instructions to prepare and hydrate a BIOFIRE panel pouch.
2. Add Sample Buffer to the Sample Injection Vial, but do not add a sample (Sample Buffer only, no water, swab, control material, or other sample should be added).
3. Continue with the standard instructions for loading the sample into the pouch from the Sample Injection Vial, label the pouch, and test with a BIOFIRE System.
4. Results of the negative pouch test should be valid (pouch controls pass) and negative/Not Detected for all organisms/analytes.
5. If positive/Detected results are reported for the negative pouch, repeat the Decontamination and Cleaning Procedures (above), with a focus on the pouch loading and testing/instrument areas, and run another negative pouch.
6. Contact BIOFIRE Technical Support if positive/Detected results for the negative pouch are not resolved after the second decontamination.

Technical Support Contact Information

bioMérieux is dedicated to providing the best customer support available. If you have questions or concerns about this process, please contact your local bioMérieux representative or your authorized distributor.

References

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