PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

PCR Contamination Prevention, Environmental Monitoring, and Decontamination Procedure

Department of Microbiology

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1.0 Purpose

Due to the nature and sensitivity of nucleic acid amplification testing, practices are necessary to safeguard against contamination of laboratory equipment and surfaces used to process and perform the PCR assays. Environmental contamination with cells, target nucleic acid, or amplicons may lead to contamination of patient samples that could result in false-positive test results. Improper decontamination can lead to false-negative test results.

2.0 Reagents & Equipment

- BD MAX™ assay kits and BD MAX™ instrument
- BioFire™ assay kits and FilmArray® instrument
- Swab collection device
- Sterile DI water
- Sterile saline
- Vortex
- 1:10 bleach dilution, 70% isopropyl alcohol, distilled water
- Alcohol preps

3.0 Procedure for Contamination Prevention & Monitoring

3.1 Workflow Measures & Routine Cleanup

1. Always pre-plan, organize and segregate workflow. Workflow should proceed in a unidirectional manner, beginning in the specimen processing area and moving to the pre-amplification/amplification/detection area.
2. Separate PPE should be used in each area.
   - Gloves must be worn and changed frequently throughout testing. New gloves must be donned prior to handling specimens. New gloves should be donned again after entering the pre-amplification/amplification/detection area where the instrument is located. Gloves should be worn when removing completed reaction strips.
   - A general use (blue) lab coat may be worn in the specimen processing area. This lab coat should never be worn into the pre-amplification/amplification/detection area. A separate (white) lab coat should be worn in the room where the BD MAX™ is located. This coat should not leave the room except to exchange for a new white coat. Coats should be changed weekly or following contamination. A lab coat is not required in the room with the BioFire™ FilmArray® instrument. However, gloves must be worn and changed as described above.
3. Decontaminate work surfaces in the specimen processing area prior to beginning work and then again after processing has been completed. Wipe down with 1:10 diluted bleach followed by another wipe down with 70% alcohol. The alcohol cleanup helps remove residual bleach that could inhibit PCR reactions.
4. All initial specimen manipulation should occur in a biological safety cabinet.
5. Only one specimen should be open at a time. Do not transfer specimens or control material with more than one sample buffer open at a time. In the case of the GBS assay where the sample buffer tubes are not recapped, transfer the inoculated tubes into another rack away from the work area to avoid cross contamination.
6. Use pipette tips with aerosol barriers.
7. Pipette should be routinely cleaned by wiping them down using 70% alcohol preps. Wipe pipettes from the top to the bottom. Do not saturate pipettes.
8. Take care to preserve the purity of reagents. Avoid contamination from positive controls and specimens by following good laboratory practices.
9. Decontaminate surfaces after work is completed in the room with the instrument using 1:10 diluted bleach followed by a wipe down with DI water and then 70% alcohol. The water and alcohol cleanup helps remove residual bleach that could inhibit PCR reactions.
10. Discard and do not break the PCR cards or pouches after use. Each BD MAX™ card has two rows and can be used twice. Breaking these devices can release contamination into the lab environment.
11. Never touch any objects in the amplification area, including the computer mouse, keyboard, or SmartCycler instruments, and then travel back to the specimen processing area without washing your hands and donning a new pair of gloves. Doing so could contaminate the specimen processing area. Pens, pencils, batch logs, etc., that are used in the amplification area should not be carried to other areas of the lab.

3.2 Environmental Monitoring

1. Surfaces to be Tested

   Laboratory equipment and surfaces used to process and perform the nucleic acid amplification testing should be sampled. The following is a list of surfaces that should be swabbed and tested at least once per month or following a potential issue with contamination. More surfaces may be tested following a potential contamination event.

   - Biosafety cabinet work surface – for all assays
   - BioFire™ FilmArray® pouch loading station – for all FilmArray® assays
   - Micropipettor (handle and button press) – for all BD MAX™ assays
   - Vortex – for all BD MAX™ assays

- Work surface on the bench in the room where the BD MAX™ is located – for all BD MAX™ assays
- BD MAX™ computer keyboard and mouse – for all BD MAX™ assays
- BD MAX™ instrument drawers that hold the PCR cards – for all BD MAX™ assays

2. Sample Collection and Preparation
   - BD MAX™ Assays
     - Use one sample buffer tube for each swab to be tested.
     - Label each tube to identify the surface area swabbed.
     - Use a separate sample buffer tube for each environmental sample. A separate sample should be collected for each assay. Dip the swab into sterile DI water so that it is moistened. Swab the surface and/or area by rotating the swab 2 to 3 times across the surface (~ 10 cm^2).
     - After sampling the surface area, place the swab into the buffer and break off the upper portion of the shaft.
     - Recap the Cdiff and MRSA tubes with a septum cap. Recap the GBS sample with the screw cap.
     - Vortex for 1 min at high speed.
     - Remove the screw caps from the GBS sample tubes.
   - BioFire™ FilmArray® Assays
     - Use 2 mL of sterile saline. Dip the swab into sterile saline so that it is moistened. Swab the pouch loading station by rotating the swab 2 to 3 times across the surface (~ 10 cm^2). Use the same swab to sample the work surface in the biosafety cabinet.
     - After sampling the surface area, place the swab into the sterile saline and break off the upper portion of the shaft. This sample can be used for testing all assays.
     - Briefly vortex the sample.
3. Sample Testing
   - Load the samples according to the assay protocol. Use the name of each surface tested to identify the samples.
   - Start the run.
4. Interpretation of Test Results
   - A negative assay result indicates that no target nucleic acid was detected.
   - A positive assay result indicates that target nucleic acid was detected, and environmental contamination has occurred.
   - An unresolved assay result indicates the presence of an inhibitory substance or internal control amplicon.
5. Decontamination
   - Surfaces that test positive for target nucleic acid should be decontaminated. Surface samples that yield unresolved results should also be decontaminated and cleaned. Refer to the assay procedures and/or instrument user's manuals for cleaning instructions.
   - If an environmental surface tests positive for target nucleic acid, additional surveillance testing should be performed after decontamination to verify that decontamination was successful.

3.3 Positivity Rate

3.3.1 Monthly Monitoring

The rate of positivity for each assay is monitored monthly. If there is an increase above the expected rate of positivity, environmental testing should be performed to eliminate the possibility of amplicon contamination. Positivity thresholds have been established based on testing data from previous years. These thresholds may need to be reassessed to accommodate changes in patient populations that may impact the rate of positivity for each assay.

Assay	monthly % positive threshold
Cdiff	25
MRSA	10
Strep B	35

3.3.2 Within Each Run

The proportion of positive results within a run should also be considered. However, the test batches for some assays are often small, making it difficult to judge the significance of % positive. The % positive results should be considered primarily for batches of at least 10 specimens. If the % positive samples exceed the run thresholds listed below, all positive samples should be repeated for verification prior to reporting patient results.

Assay	% positive threshold within a run
Cdiff	50
MRSA	30
Strep B	70

4.0 References

1. BD MAX™ System User's Manual (2012/05) (02)
2. FilmArray® Respiratory Panel (RP) Instruction Booklet, RFIT-PRT-0103 Feb 2013.

5.0 Document Control History

Adopted/Approved by director (AR) 06/29/2011

Reviewed by supervisor (JC) 06/29/2009, 06/2011, 06/18/2013, Jason Ammons 7/2015

12/26/2011 Added more details for contamination prevention by segregating work areas.

06/17/2013 Updated procedure for assays on the BD MAX™.

10/08/2013 Updated to include surveillance testing on the BioFire™ FilmArray® assays.