# Wipe Testing for Amplicon or Nucleic Acid Contamination

**PURPOSE**

* This procedure provides instruction for environmental monitoring of laboratory equipment and surfaces for DNA/RNA and amplicon contamination

#### POLICY STATEMENT

* *Bordetella* wipe testing is performed monthly
* MRSA wipe testing is performed monthly
* RVP wipe testing is performed on a weekly rotating schedule
* Other targets are performed as determined
* Notify section technical director and/or designee regarding positive or unresolved results
* Discontinue patient testing during a contamination event if the technical director has determined that it is unsafe to continue

**ABBREVIATIONS**

|  |  |
| --- | --- |
| * CMA: ChromAgar MRSA
* NA: nucleic acid
* NEGC: negative control
* NFW: nuclease free water
 | * POSC: positive control
* PPE: personal protective equipment
* SB: 5% sheep blood agar
 |

## DOCUMENTATION/RECORDS

* Assay run-specific Results Report
* MRSA Wipe Testing worksheet [MB 3.02.F1](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212215.pdf)
* RVP Wipe Testing worksheet [MB 3.02.F2](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212216.pdf)
* BORDP Wipe Testing worksheet [MB 3.02.F3](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212217.pdf)

## SAFETY CONSIDERATIONS

* Standard precautions

#### MATERIALS REQUIRED

|  |  |  |
| --- | --- | --- |
| **Equipment** | **Reagents** | **Supplies** |
| Room 2* Refrigerator 2 – 8° C
* VWR Multi-tube vortex
* BSC BSL-2
* Vortex Mixer
* !00 µl Concept pipettor
* 10 µl pipette

Room 3* + BD MAX instrument
	+ eSensor XT
	+ -20⁰ C freezer
	+ Simplexa thermocycler
	+ Pipettes, 20 µl, 200 µl
	+ Vortex Mixer
	+ UVP Hood
	+ Mini-centrifuge (2)
	+ ABI Thermocycler
 | BD MAX MRSA XT kit; refer to MB005.6 MRSA | Orange barrier wipes |
| eSensor XT-8 RVP kit; refer to MB005.7 RVP | Nitrile gloves (powder-free) |
| BORDP ASR reagents; refer to MB005.8 BORDP | Disposable lab coats |
| 1% Sani-Cloth Bleach wipes (equivalent to 1:10 bleach solution) | Absorbent clothes |
| NFW  | Test tube rack |
| 70% alcohol | 2 ml Cryovial |
| Household Bleach | BBL CultureSwab |
| Eliminase | Disposable lab coats |
| Alconox | Pipette tips |
|  | Pipette disposal containers |
|  | BioHazard ZipLock baggies |

**PROCEDURE A:** Follow the general guidelines below for decontamination and cleaning

General guidelines for decontamination

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **PPE** | 1 | Change gloves frequently during the decontamination process, especially during the first steps of decontamination and before touching any clean surface | ***Refer to******Proc. F***Decontamination procedure following a contaminationevent |
|  | 2 | All PPE should be disposed of after decontamination |
| **Room 2****Processing** | 3 | Room 2: Processing: Perform swab testing on work surfaces and equipment, including but not limited to: | RVP Wipe testing [MB 3.02.F2](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212216.pdf)BORDP Wipe Testing [MB 3.02.F3](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212217.pdf)MRSA Wipe Testing worksheet [MB 3.02.F1](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212215.pdf) |
| * door handles
* pipettes
* work surfaces
 | * centrifuges
* vortexers
* anything with a button
 |
| **Room 3****Amplification** | 4 | Room 3: Amplification: Perform swab testing on work surfaces and equipment, including but not limited to: |
| * thermocycler block and lid
* door handles
* pipettes
* work surfaces
 | * centrifuges
* vortexers
* anything with a button
 |
| **Results** | 5 | Based on results of the wipe testing, focus decontamination efforts on the room(s) showing contamination |  |
|  | 6 | Repeat swab testing after cleaning to confirm decontamination |
|  | 7 | Re-run any swab that comes up positive for contamination and individual swabs of each component, such as pipettes, listed sites on worksheet for that area and possible expanded sites to narrow down the source |
|  | 8 | If samples continue to be positive for amplicon or genomic DNA/RNA, prepare a 1% v/v bleach/Alconox cleaning solution  |  |
|  | 9 | Repeat testing until all environmental swabs are negative |  |
| **Monitor** | 10 | Monitor environment weekly for 4 weeks following a contamination event |  |

**PROCEDURE B:** Follow the steps below to collect and test Respiratory Viral Panel PCR (RVP) environmental samples

RVP environmental testing

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Frequency** | 1 | Rotate environmental samples weekly; see worksheet [MB 3.02.F2](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212216.pdf) |  |
| **Worksheet** | 2 | Print RVP Wipe testing worksheet * *Test expanded environmental sites during a contamination event such as drawer handles, light switches, phones, etc.*
 | RVP Wipe testing [MB 3.02.F2](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212216.pdf) |
|  | 3 | Aliquot 1 mL NFW into a 2 ml cryovial for each sample to be tested |  |
| **Sample processing** | 4 | Transfer 1 ml NFW into a sterile tube to pre-moisten swabs prior to collection; can be used for multiple swabs4* Soak each swab in NFW for 5 s
 |  |
|  | 5 | Swab area by rotating the swab 2 – 3 times across the surface, approx 10 cm2  |  |
|  | 6 | Using an orange barrier protector, break the swab off into labeled sample tube |  |
|  | 7 | Mix 1 – 2 min, vortex speed 8 |  |
| **PCR** | 8 | Do not extract; perform PCR testing directly from eluted sample according to RVP assay protocol | [MB 11.05](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/RVP/212298.pdf)RVP assay  |
| **Notification** | 9 | If results are positive * *Notify* section technical director and/or designee
* Proceed to Procedure F, page 4
* Perform result “Look-Back”
* Document problem and corrective action
* Contact GenMark for further assistance if needed, 1-800-373-6767
 | **Procedure F****Decontamination** |
| **Archive** | 10 | Attach run-specific report to RVP Test Form [MB 3.02.F2](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212216.pdf); place in Service manual |  |

**PROCEDURE C:** Follow the steps in the table below to collect and test *Bordetella* PCR (BORDP) environmental samples

*Bordetella* PCR Environmental Testing

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Worksheet** | 1 | Print BORDP Wipe testing worksheet [MB 3.02.F3](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212217.pdf)* *Test expanded environmental sites during a contamination event such as drawer handles, light switches, phones, etc.*
 | BORDP Wipe Testing [MB 3.02.F3](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212217.pdf) |
|  | 2 | Aliquot 200 µl NFW into a 2 ml cryovial for each sample to be tested |
| **Sample processing** | 3 | Transfer 1 ml NFW into a sterile tube to pre-moisten swabs prior to collection; can be used for multiple swabs* Soak each swab in NFW for 5 s
 |  |
|  | 4 | Swab area by rotating the swab 2 – 3 times across the surface, approx 10 cm2  |  |
|  | 5 | Using an orange barrier protector, break the swab off into labeled sample tube |  |
|  | 6 | Mix 5 min, vortex speed 8 |  |
| **PCR** | 7 | Perform PCR testing according to BORDP assay protocol  | [MB 6.05](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/BOR/212260.pdf)BORDP assay  |
| **Notification** | 8 | If results are positive * *Notify* section technical Director and/or designee
* Proceed to Procedure F, page 4
* Perform result “Look-Back”
* Document problem and corrective action
 | **Procedure F Decontamination** |
| **Archive** | 9 | Attach run-specific report to BORDP Test Form [MB 3.02.F3](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212217.pdf); place in Service manual |  |

**PROCEDURE D:** Follow the steps in the table below to collect and test MRSA environmental samples

**MRSA environmental testing**

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Worksheet** | 1 | Print MRSA Wipe Testing worksheet [MB 3.02.F1](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212215.pdf)* *Test expanded environmental sites during a contamination event such as drawer handles, light switches, phones, etc.*
 |  |
| **Collection and sample processing** | 2 | Place one sample buffer tube for each swab to be tested in tube rack; refer to MRSA XT assay protocol [MB 10.05](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/MRSA/212321.pdf) | [MB 10.05](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/MRSA/212321.pdf)BD MAX MRSA Assay |
| 3 | Number buffer tubes and swabs according to worksheet* Number sample tubes 1 – nn
* Number corresponding swab 1 – nn
 |
| **Processing** | 4 | Transfer 1.5 ml NFW into a sterile tube to pre-moisten swabs prior to collection; can be used for multiple swabs* Soak each swab in NFW for 5 s
 |  |
|  | 5 | Swab area by rotating the swab 2 – 3 times across the surface, approx 10 cm2  |  |
|  | 6 | Using an orange barrier protector, break the swab off into sample buffer tube; place septum cap on tube |  |
|  | 7 | Vortex 1 min at high speed (10) |  |
| **Testing** | 8 | Test according to the BD MAX MRSA XT assay protocol [MB 10.05](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/MRSA/212321.pdf) | [MB 10.05](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/MRSA/212321.pdf)BD MAX MRSA Assay |
| **Interpretation** | 9 | Interpret results according to Table 1 (see below) |  |
| **Notification** | 10 | If results are positive or unresolved* *Notify* section technical Director and/or designee
* Proceed to Procedure E (determined by technical director) and Procedure F
* Perform result “Look-Back”
* Document problem and corrective action
 |  |
| **Archive** | 11 | Attach run-specific report to Test Form [MB 3.02.F1](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212215.pdf); place in Service manual |  |

**Table1: Interpret results according to the following criteria**

|  |  |
| --- | --- |
| Result | Interpretation of result |
|  | No MRSA DNA detected.  |
|   | MRSA DNA detected. Perform broth enrichment to determine if bacterial contamination is involved. |
| UNR | Presence of inhibitory substance (possible bleach build-up) or internal control amplicon contamination; repeat testing |

**PROCEDURE E:** Follow the steps in the table below for broth enrichment under the direction of the technical director

**Broth enrichment**

|  |  |  |  |
| --- | --- | --- | --- |
| Activity | **Step** | **Action** | **Related doc** |
| **Culture** | 1 | Perform broth enrichment to determine the possible source of contamination, live cell, DNA or amplicon |  |
|  | 2 | Add 2.0 ml of 6.5% NaCl broth to buffer tube containing swab |  |
|  | 3 | Incubate for 24 – 48 h at 35° C |  |
|  | 4 | Subculture to ChromAgar MRSA (CMA) and SB |  |
| **Identify** | 5 | Identify *S. aureus* and confirm MRSA colonies according to standard protocols |  |

**PROCEDURE F:** Follow the steps in the table below for cleaning following contamination

Decontamination procedure following contamination

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
|  | 1 | Gloves and disposable lab coat required | [MB 3.03](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212214.pdf) Cleaning and Decontamination of Equip and work areas |
| **General** | 2 | Use unidirectional motion when cleaning |
|  | 3 | Change gloves often during decontamination |
|  | 4 | Use 1% Sani-Cloth Bleach wipes on all surfaces or * Use swabs to reach inaccessible areas
* Use disposable Bleach Sani-Cloth for more accessible areas
 | For deeper cleaning, refer to **Procedure H**1% v/v bleach/Alconox solution |
| **Bench-tops, Hoods, Pipettes, and small equipment**Room 2 and 3 | 5 | Wipe bench-tops, BioSafety Hoods, centrifuges, vortex mixers, and pipettes with Sani-Cloth Bleach wipes Allow bleach to dry for 4 – 5 min * Rinse with water followed by 70% alcohol
 | Perform cleaning process twice before repeat wipe testing |
| 6 | Discard in biohazardous waste |  |
| 7 | Change gloves |  |
|  | 8 | Repeat step 5 with fresh Sani-Cloth Bleach wipesAllow bleach to dry for 4 – 5 min * Rinse with water followed by 70% alcohol
* Hoods: Turn on UV light for 15 min
 |  |
|  | 9 | Change gloves and discard disposable lab coat in biohazardous waste |  |
| **Racks, cold blocks** | 10 | Soak racks in 10% bleach for 5 min* Rinse well with water followed by 70% alcohol
* Refer to alternative cleaning solutions, pg. 6, for additional information
 |  |
|  | 11 | Discard disposable materials in hoods and on countertops, i.e., pipette tips, waste containers, pens, etc. |  |
|  | 12 | Repeat wipe testing | **Procedure H** Alternate Cleaning Solutions |
| **Repeat testing** | 13 | If samples continue to be positive for amplicon or genomic DNA/RNA, prepare a 1% v/v bleach/Alconox cleaning solution (Proc. H, page 6) |
|  | 14 | Repeat steps 5-12 |
|  | 15 | Repeat procedure until all environmental swabs test negative |  |
| **Weekly Monitoring** | 16 | Monitor environment weekly for 4 weeks following a contamination event |  |

**PROCEDURE G:** Follow the steps in the table below for additional targets

Additional targets

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
| **Frequency** | 1 | Frequency: as determined, i.e., positive NEGC, review of positive rates |  |
| **Elution** | 2  | Prepare tubes used for elution:* RNA viruses: 1 mL NFW in 2 ml cryovial
* Group A strep (GASDN): 250 µl TE in 2 ml cryovial
* C. difficile (CDT): Cdiff sample buffer tube
 | [MB 7.05](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/CDT/212309.pdf)Cdiff assay[MB 8.05](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/GAS/212288.pdf)Grp A Strep assay |
|  | 3 | Transfer 1 ml NFW into a sterile tube to pre-moisten swabs prior to collection; can be used for multiple swabs* Soak each swab in NFW for 5 s
 | [MB 9.05](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/RIP/212360.pdf)RSV, Influenza A, B assay |
|  | 4 | Swab area by rotating the swab 2 – 3 times across the surface, approx 10 cm2 |  |
|  | 5 | Using an orange barrier protector, break the swab off into labeled sample tube |  |
|  | 6 | Mix 1 min, vortex speed 8 |  |
| **PCR** | 7 | Do not extract; test directly from eluted sample  |  |
|  | 8 | Test and interpret according to target testing protocol |  |
| **Notification** | 9 | If results are positive * *Notify* section technical director and/or designee
* Proceed to Procedure F
* Perform result “Look-Back”
* Document problem and corrective action
 |  |
|  | 10 | Discontinue patient testing until it has been determined safe to continue |  |
|  | 11 | Repeat decontamination until results test negative |  |

**PROCEDURE H: Alternative Cleaning Solutions**

Freshly prepared 10% bleach has been shown to be extremely effective in destroying DNA contamination. However, there are alternatives available that are less corrosive and are recommended for cleaning instrumentation.

* 1% v/v bleach/Alconox solution (reagents located in dishwashing room)

|  |  |  |
| --- | --- | --- |
| Step | Reagent | Volume |
| 1 | Household bleach (5 – 6 %) | 500 ml |
| 2 | Water | 2000 ml |
| 3 | Alconox | 25 ml |

* ELIMINase – Decon Laboratories
* DNA *AWAY* – Molecular BioProducts, Inc
* DNAZap - Invitrogen Life Science Technologies

**REFERENCES**

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2. CLSI *Molecular Diagnostic Methods for Infectious Diseases;* Approved Guideline – Second Edition, CLSI document MM3-A2, Wayne, PA, Clinical and Laboratory Standards Institute; 2006
3. CLSI. Establishing Molecular Testing in Clinical Laboratory Environments. Approved Guideline November 2011; CLSI document MM19-A
4. *Good Molecular Practices Guide*, eSensor® Respiratory viral Panel, Clinical Micro Sensors, Inc. dba GenMark Diagnostics, Inc., 5964 La Place Court, Carlsbad, CA 92008, 1-800-373-6767, ww.genmarkdx.com

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| Historical Record |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | P. Ackerman | 12/20/2006 | Initial Version |
| 1.1 | P. Ackerman | 5/24/08 | Added MRSA wipe testing, added appendix 1 and 2 forms |
| 1.2 | P. Ackerman | 2/4/09 | Modified MRSA procedure to exclude the lysis step; modified appendix 2 form by increasing number of samples to be tested. Added MRSA interpretation table. |
|  | 1.3 | P. Ackerman | 7/1/09 | Modified procedure format |
|  | 5 | P. Ackerman | 7/7/2013 | Reformatted procedure; added proc. C and D, updated proc. F, added alternative cleaning solutions |
|  | 6 | P. Ackerman | 1.8.2014 | Remove SmartCycler information; revised for BD MAX |
|  | 7 | P. Ackerman | 7.8.15 | Added RVP information |
|  | 8 | P. Ackerman | 5.31.16 | Added BORDP Proc. F; formatted for CMS upload; changed logo; added technical director; changed proc. # from MB003.2 to MB 3.02; name change – added Amplicon |
|  | 9 | P. Ackerman | 6.17.17 | Added Proc. A General guidelines, reorganized remaining procedures |