# 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
* Respiratory Viral Panel (RVP) wipe testing is performed monthly
* Herpes Simplex Virus (HSV) wipe testing is performed monthly
* 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**

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| --- | --- |
| * NA: nucleic acid * NEGC: negative control * NFW: nuclease free water * HSV: Herpes Simplex Virus * BORDP: Bordetella pertussis and Bordetella parapertussis | * POSC: positive control * PPE: personal protective equipment * SB: 5% sheep blood agar |

## DOCUMENTATION/RECORDS

* Assay run-specific Results Report
* RVP Wipe Testing worksheet MB 3.02.F2
* BORDP Wipe Testing worksheet MB 3.02.F3
* HSV Wipe Testing worksheet MB 3.02.F6

## SAFETY CONSIDERATIONS

* Standard precautions

#### MATERIALS REQUIRED

|  |  |  |
| --- | --- | --- |
| **Equipment** | **Reagents** | **Supplies** |
| Room 2   * Refrigerator 2 – 8° C * VWR Multi-tube vortex * BSC BSL-2 * Vortex Mixer * 100 µl Concept pipettor * 10 µl pipette * Cold block * Simplexa reaction disks and sealer   Room 3   * + eSensor XT   + -20⁰ C freezer   + Simplexa thermocycler   + Pipettes, 20 µl, 200 µl   + Vortex Mixer   + UVP Hood   + Mini-centrifuge (2)   + ABI Thermocycler | HSV kits | Orange barrier wipes |
| eSensor XT-8 RVP kit | Nitrile gloves (powder-free) |
| BORDP Kits | Disposable lab coats |
| Household Bleach | Absorbent clothes |
| Eliminase | Test tube rack |
| Alconox | 2 ml Cryovial |
| 1% Sani-Cloth Bleach wipes (equivalent to 1:10 bleach solution) | BBL CultureSwab |
| 70% alcohol | Disposable lab coats |
| NFW | 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. I***  Decontamination procedure following a contamination  event |
|  | 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](https://starnet.childrenshc.org/References/labsop/molbio/engctl/mb-3.02.f2-rvp-wipe-testing-worksheet.pdf)  BORDP Wipe Testing  [MB 3.02.F3](https://starnet.childrenshc.org/References/labsop/molbio/engctl/mb-3.02.f3-bordp-wipe-testing-worksheet.pdf)  HSV Wipe Testing [MB 3.02.F6](https://starnet.childrenshc.org/References/labsop/molbio/engctl/mb-3.02.f6-hsv-direct-1-and-2-wipe-testing-worksheet.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 | |  |
|  | 10 | If any samples are positive consult with the Technical Specialist and/or Technical Director | |  |

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

*Simplexa Bordetella pertussis* and *Bordetella parapertussis Direct* PCR Environmental Testing

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Worksheet** | 1 | Print BORDP Wipe testing worksheet MB 3.02.F3   * *Test expanded environmental sites during a contamination event such as drawer handles, light switches, phones, etc.* | BORDP Wipe Testing  [MB 3.02.F3](https://starnet.childrenshc.org/References/labsop/molbio/engctl/mb-3.02.f3-bordp-wipe-testing-worksheet.pdf) |
|  | 2 | Aliquot 200 µl VTM 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 (VTM) |  |
|  | 6 | Vortex 10 seconds, speed 8 |  |
| **PCR** | 7 | Perform PCR testing according to the Simplexa Bordetella Direct assay protocol | [MB 13.0](https://starnet.childrenshc.org/References/labsop/molbio/bor/mb-13.0-simplexa-bordetella-direct-assay.pdf) Simplexa Bordetella Direct 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 G Decontamination** |
| **Archive** | 9 | Attach run-specific report to Test Form MB 3.02.F6; place in binder |  |

**PROCEDURE C:** Follow the steps in the table below to collect and test Simplexa HSV 1 and 2 Direct PCR environmental samples

*Simplexa HSV 1 and 2 Direct PCR Environmental Testing*

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Worksheet** | 1 | Print HSV Wipe testing worksheet MB 3.02.F6   * *Test expanded environmental sites during a contamination event such as drawer handles, light switches, phones, etc.* | HSV Wipe Testing  [MB 3.02.F6](https://starnet.childrenshc.org/References/labsop/molbio/engctl/mb-3.02.f6-hsv-direct-1-and-2-wipe-testing-worksheet.pdf) |
|  | 2 | Aliquot 200 µl VTM 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 (VTM) |  |
|  | 6 | Vortex 10 seconds, speed 8 |  |
| **PCR** | 7 | Perform PCR testing according to the Simplexa HSV 1 & 2 Direct assay protocol | [MB 12.0](https://starnet.childrenshc.org/References/labsop/molbio/hsv/mb-12.0-simplexa-hsv-1-and-2-direct-assay.pdf) Simplexa HSV 1 and 2 Direct Assay |
| **Notification** | 8 | If results are positive   * *Notify* section technical Director and/or designee * Proceed to Procedure F * Perform result “Look-Back” * Document problem and corrective action |  |
| **Archive** | 9 | Attach run-specific report to Test Form MB 3.02.F6; place in binder |  |

**PROCEDURE D:** Follow the steps in the table below to collect and test Simplexa COVID-19 Direct PCR environmental samples

*Simplexa COVID-19 Direct PCR Environmental Testing*

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Worksheet** | 1 | Print a COVID-19 Wipe testing worksheet MB 3.02.F6   * *Test expanded environmental sites during a contamination event such as drawer handles, light switches, phones, etc.* | COVID-19 Wipe Testing  [MB 3.02.F7](MB%203.02.F7%20COVID-19%20Direct%20Wipe%20Testing%20Worksheet.docx) |
|  | 2 | Aliquot 200 µl VTM 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 (VTM) |  |
|  | 6 | Vortex 10 seconds, speed 8 |  |
| **PCR** | 7 | Perform PCR testing according to the Simplexa COVID-19 Direct assay protocol | [MB 14.0](file:///G:\Lab%20Procedures\Molecular%20Procedure%20Manual\MB%2014.0%20COVID-19%20Direct\MB%2014.0%20Simplexa%20COVID-19%20Direct%20Assay.docx) Simplexa COVID-19 Direct Assay |
| **Notification** | 8 | If results are positive   * *Notify* section technical Director and/or designee * Proceed to Procedure F * Perform result “Look-Back” * Document problem and corrective action |  |
| **Archive** | 9 | Attach run-specific report to Test Form MB 3.02.F6; place in binder |  |

**PROCEDURE E:** 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](https://starnet.childrenshc.org/References/labsop/molbio/engctl/mb-3.03-cleaning-and-decontamination-of-equipment-and-work-areas.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 |  |
| **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 wipesAllow 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 wipes Allow 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 |  |
| **Repeat testing** | 13 | If samples continue to be positive for amplicon or genomic DNA/RNA, prepare a 1% v/v bleach/Alconox cleaning solution |
|  | 14 | Repeat steps 5-12 |
|  | 15 | Repeat procedure until all environmental swabs test negative |  |

**PROCEDURE F: 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 solution

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| --- | --- | --- |
| 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**

1. Pershing, David H., et al, Molecular Microbiology, Diagnostic Principles and Practices, 2nd edition, 2011, ASM Press, Washington, DC, pg. 134 - 135
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 |
|  | 10 | J. Laramie | 12.01.17 | -Eliminated 4 week monitoring of negative follow-up swabs  -Wipe testing frequency for all tests: monthly  -Added extraction and IC addition to RVP wipe checks  -Added testing for GAS, RIP, CDT  -Removed testing of additional targets section (for GAS, RIP, and CDT) |
|  | 11 | J. Laramie | 01.01.17 | -Specified what tubes to break swabs off into for RIP (VTM) and RVP (nuclease free water) wipe testing. |
|  | 11 | J. Laramie | 01.01.17 | Biennial review: JL 03.07.18 |
|  | 12 | J. Laramie | 02.26.19 | Removed MRSA and CDT wipe testing procedures |
|  | 13 | Susan DeMeyere | 4.24.19 | Added HSV testing |
|  | 14 | J. Laramie | 10.28.19 | Removed GASD and RIP |
|  | 15 | J. Laramie | 03.22.20 | Changed BORDP wipe testing from NFW matrix to VTM with the direct assay conversion |
|  | 16 | J. Laramie | 6.15.20 | -Removed RVP wipe testing  -Added COVID-19 wipe testing |