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 rotated weekly
- 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 until it has been determined safe 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
- RVP Wipe Testing worksheet MB 3.02.F2
- BORDP Wipe Testing worksheet MB 3.02.F3

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 ▪ -20° 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 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 ▪ *Test expanded environmental sites during a contamination event*	BD Environmental Monitoring
Collection and sample processing	2	Place one sample buffer tube for each swab to be tested in tube rack; refer to procedure MRSA 005	MB 10.05 BD MAX MRSA Assay
	3	Number buffer tubes and swabs according to worksheet ▪ Number sample tubes 1 – nn ▪ Number corresponding swab 1 – nn	
	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 cm^2	
	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 assay protocol MRSA 005	MB 10.05 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 B and C ▪ Perform result "Look-Back" ▪ Document problem and corrective action	
Archive	11	Attach run-specific report to Test Form MB 3.02.F1; place in Service manual	

Table1: Interpret results according to the following criteria

Result	Interpretation of result
MRSA NEG	No MRSA DNA detected.
MRSA POS	MRSA DNA detected. Perform broth enrichment to determine if bacterial contamination is involved.
Unresolved	Presence of inhibitory substance or internal control amplicon contamination; repeat testing

PROCEDURE B: Follow the steps in the table below for broth enrichment

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 C: Follow the steps in the table below for cleaning following contamination

Decontamination procedure

Activity	Step	Action	Related doc
General	1	Gloves and disposable lab coat required	MB 3.03 Cleaning and Decontamination of Equip and work areas
	2	Use unidirectional motion when cleaning	
	3	Change gloves often during decontamination	
	4	Use 1% Sani-Cloth Bleach wipes on all surfaces ▪ 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 wipes ▪ Allow bleach to dry for 4 – 5 min ▪ Rinse with water followed by 70% alcohol	
	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. 5, for additional information	Procedure H Alternate Cleaning Solutions
	11	Discard disposable materials in hoods, i.e., pipette tips, waste containers, etc.	
Repeat testing	12	Repeat wipe testing	Procedure H Alternate Cleaning Solutions
	13	If samples continue to be positive for amplicon or genomic DNA/RNA, prepare a 1% v/v bleach/Alconox cleaning solution (Proc. H, pg. 5)	
	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 D: 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 MB 3.02.F2	
Worksheet	2	Print RVP Wipe testing worksheet MB 3.02.F2 ▪ *Test expanded environmental sites during a contamination event*	RVP Wipe testing MB 3.02.F2
	3	Aliquot 200 µl NFW into a 2 ml cryovial for each sample to be tested	

Activity	Step	Action	Related Doc
Sample processing	4	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	
	5	Swab area by rotating the swab 2 – 3 times across the surface, approx 10 cm^2	
	6	Using an orange barrier protector, break the swab off into labeled sample tube	
	7	Vortex 1 - 2 min at high speed (9)	
PCR	8	Do not extract; perform PCR testing directly from eluted sample according to RVP assay protocol	MB 11.05 RVP assay
Notification	9	If results are positive ▪ Notify section technical director and/or designee ▪ Proceed to Procedure C, pg. 3 ▪ Perform result "Look-Back" ▪ Document problem and corrective action	Procedure C
Archive	10	Attach run-specific report to RVP Test Form MB 3.02.F2; place in Service manual	

PROCEDURE F: 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 ▪ *Test expanded environmental sites during a contamination event*	
Sample processing	2	Aliquot 200 µl NFW into a 2 ml cryovial for each sample to be tested	BORDP Wipe Testing MB 3.02.F3
	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 cm^2	
	5	Using an orange barrier protector, break the swab off into labeled sample tube	
	6	Vortex 5 min at speed 9	
PCR	7	Perform PCR testing according to BORDP assay protocol	MB 6.05 BORDP assay
Notification	8	If results are positive ▪ Notify section technical Director and/or designee ▪ Proceed to Procedure C ▪ Perform result "Look-Back" ▪ Document problem and corrective action	Procedure C
Archive	9	Attach run-specific report to BORDP Test Form MB 3.02.F3; place in Service manual	

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: 200 µl 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 Cdiff assay MB 8.05 Grp A Strep assay MB 9.05 RSV, Influenza A, B 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	
	4	Swab area by rotating the swab 2 – 3 times across the surface, approx 10 cm^2	
	5	Using an orange barrier protector, break the swab off into labeled sample tube	
	6	Vortex 1 min at high speed	
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 C ▪ 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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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