BORD Storage and Stability of Samples, Controls and Reagents

PURPOSE

- This procedure provides instructions for storage and stability of sample buffer tubes, controls and reagents.

SAFETY CONSIDERATIONS

- Standard precautions. Refer to MB 2.02 Biohazard Containment
- Use of engineering controls: Refer to MB 3.01 Engineering Controls to Prevent Nucleic Acid Contamination

ABBREVIATIONS

- BORD: Bordetella
- BORDP: Bordetella PCR
- Bp: Bordetella pertussis
- Bpp: Bordetella parapertussis
- BSC: BioSafety Cabinet
- BSL: BioSafety level
- MM: master mix
- NEGC: negative control
- NFW: nuclease free water
- PCTL: process control
- POSC: positive control
- PP: primer – pair
- RT: room temperature
- SEAC: Simplexa extraction and amplification control
- TE buffer: Tris – EDTA buffer
- Area/Room 1: Clean room
- Area/Room 2: Processing room
- Area/Room 3: Amplification room

MATERIALS REQUIRED

Equipment	Reagents	Supplies
Room 1: Clean room ▪ Refrigerator 2 – 8° C ▪ -10 to -30° C freezer ▪ Mini-centrifuge ▪ Laminar flow hood ▪ Eppendorf Repeater pipette Room 2: Processing ▪ Refrigerator 2 – 8° C ▪ BSC BSL-2 ▪ -70° C freezer	Bp primer pair (50 µl)	Orange barrier wipes
	Bpp primer pair (50 µl)	200 µl TE tube (1.5 ml micro-centrifuge tube)
	TA master mix (2 X 200 µl)	Nitrile gloves (powder-free)
	Bordetella Molecular Control (POSC) 2 X 50 µl	Cryovial storage box
	NFW (NEGC)	Test tube rack
	TE buffer 1X pH 8.0 (100 ml)	Sterile scissors
	SEAC ▪ Amplification Control DNA ▪ Amplification Control primer pair	Eppendorf pipette tip, 5 ml
		2.0 ml cryovials

PROCEDURE A: Follow the activity below for the proper storage of neat samples and samples in TE buffer

Storage and Stability of Processed Samples and Reagents

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Documents</th></tr>
<tr><td>Processed sample in TE buffer tube Room 2</td><td>1</td><td>Prepare NP swabs for testing
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>Number patients on BORDP worksheet in consecutive order</td></tr>
<tr><td>b</td><td>Number primary container and associated label with assigned test number on worksheet</td></tr>
<tr><td>c</td><td>Number cap of a 200 µl TE tube according to assigned number on worksheet</td></tr>
<tr><td>d</td><td>Properly label TE tube with patient aliquot label matching the number on the cap to the number on the label</td></tr>
<tr><td>e</td><td>Verify number on primary container before transfer</td></tr>
<tr><td>f</td><td>Cut the wire mini-tip swab into the TE buffer tube with corresponding number on cap</td></tr>
<tr><td>g</td><td>Vortex 5 min, vortex setting 9</td></tr>
</table></td><td>MB 1.01 Specimen Management</td></tr>
<tr><td>Aliquot Bronchs, nasal washes/aspirates</td><td>2</td><td>Number and label a 2.0 mL cryovial for each nasal wash/aspirate and bronch specimen to be tested
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>Number patients on BORDP worksheet in consecutive order</td></tr>
<tr><td>b</td><td>Number primary container and associated label with assigned number on worksheet</td></tr>
<tr><td>c</td><td>Number cap of a 2.0 mL cryovial according to assigned number on worksheet</td></tr>
<tr><td>d</td><td>Properly label cryovial with patient aliquot label matching the number on the cap to the number on the label</td></tr>
<tr><td>e</td><td>Vortex sample in original container until well mixed</td></tr>
<tr><td>f</td><td>Verify number on primary and secondary containers before transfer</td></tr>
<tr><td>g</td><td>Transfer sample to tube with corresponding number on cap</td></tr>
</table></td><td>Refer to MB 6.05 Proc. K for archiving samples</td></tr>
<tr><td>Sample Storage</td><td>3</td><td>Store processed samples as follows:
<table>
<tr><th>Step</th><th>Temperature</th><th>Stability</th></tr>
<tr><td>a</td><td>Room temperature</td><td>4 hours</td></tr>
<tr><td>b</td><td>2 – 8° C</td><td>5 days</td></tr>
<tr><td>c</td><td>-70° C</td><td>1 year</td></tr>
</table></td><td></td></tr>
</table>

PROCEDURE B: Follow the activity below for proper storage of reagents. Refer to Tables 1 – 4.

Information for Reagent Storage

Activity	Step	Action	Related Doc
General Information	1	*Clean gloves are required prior to handling new reagents* BORDP reagents are shipped frozen on dry ice ▪ Do not use reagents if thawed upon arrival ▪ Do not use reagents if vials have been damaged ▪ Contact **DiaSorin Customer Service at 1.800.838.4548** for shipping issues	

Activity	Step	Action	Related Doc
General Information	2	Store BORDP reagents at -10 to -30° C until expiration date located on the vial unless otherwise noted. Refer to Table 1.	MB 5.02 Standards of Practice Waste Management 912.04
	3	Discard reagents that have not been stored properly or have expired according to the Organizational Waste Management policy	
	4	Remove only the required amount of reagents from storage needed for testing.	
	5	Protect from excess heat and light; store in dark	
	6	Reagents are stable through the end of the expiration month as indicated on the packaging	
	7	Thaw reagents at room temperature before use	
	8	Once thawed, store reagents at 2 – 8° C up to 30 days ▪ ***Do not refreeze***	
	9	Do not allow contact with reactive vapors from bleach or Extran or dust as these may affect the performance.	
	10	Do not interchange the reagent tube caps	

Table 1: Simplexa BORDP Reagents

Reagent	Unopened Reagent		Stability	Opened Reagent		Stability
	Temp (° C)	Location		Temp (° C)	Location	
BORDP POSC (red)	-70	Room 2	expiry date	2 – 8	Room 2	30 days
TA MM (green)	-10 to -30	Room 1	expiry date	2 – 8	Room 1	30 days
Bp PP, conc. 20 µM (brown)	-10 to -30	Room 1	expiry date	2 – 8	Room 1	30 days
Bpp PP, conc. 15 µM (brown)	-10 to -30	Room 1	expiry date	2 – 8	Room 1	30 days
SEAC (blue)	-10 to -30	Room 1	expiry date	2 – 8	Room 1	30 days

Table 2: Molecular Grade Water (RNase and DNase free)

Reagent	Unopened/Opened		Aliquot Storage		In Use Aliquots	
	Temp	Location	Temp (° C)	Location	Temp (° C)	Location
Nuclease free water (NFW)	RT	Room 1	2 – 8	Room 1	2 – 30	Room 2

Table 3: TE Buffer and Aliquot Storage

Reagent	Unopened/Opened temp		Aliquot Storage		In Use Aliquots, temp (° C)	
	Temp	Location	Temp (° C)	Location	Temp (° C)	Location
TE buffer 1X	RT	Room 1	2 – 8	Room 1	2 – 30	Room 2

Table 4: Process Control Storage

Reagent	Temp (° C)	Location	Stability	Temp (° C)	Location	Stability
BORDP Process Control in matrix	≤ 70	Room 2	1 year	2 – 8	Room 2	7 days

REFERENCES

1. *Bordetella* PCR Clinical Verification and Validation Study performed at Children's Hospitals and Clinics of MN, 2015
2. Simplexa™ *Bordetella* Universal Direct Circular PI.MOL2700.IVD, Rev. F, 18-July-2012, Focus Diagnostics, Cypress, CA 90630
3. *Bordetella pertussis* Primer Pair (50 µl) ASR, Circular PI.MOL9006 Rev. B, 20-January-2011, Focus Diagnostics, Cypress, CA 90630
4. *Bordetella parapertussis* Primer Pair (50 µl) ASR, Circular PI.MOL9007 Rev. B, 07-February-2011, Focus Diagnostics, Cypress, CA 90630
5. Simplexa™ *Bordetella* Molecular Control, Circular PI.MOL8006 Rev. A, 06-Feb-2013, Focus Diagnostics, Cypress, CA 90630
6. Simplexa™ Extracton & Amplification Control Set, Circular PI.MOL9000, Rev. D, CE, 7 Mar 2013, Focus Diagnostics, Cypress, CA 90630

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1	P. Ackerman	1.23.16	Initial Version
2	P. Ackerman	07.19.16	Reformatted for CMS upload