# *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
* 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
* -70⁰ 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 Controls ( Bp and Bpp PCTL, NEGC)  | Cryovial storage box |
| Negative control (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**

| **Activity** | **Step** | **Action** | **Related Documents** |
| --- | --- | --- | --- |
| Processed sample in TE buffer tubeRoom 2 | 1 | Prepare NP swabs for testing

|  |  |
| --- | --- |
| Step | Action |
| a | Number patients on BORDP worksheet in consecutive order |
| b | Number primary container and associated label with assigned test number on worksheet |
| c | Number cap of a 200 µl TE tube according to assigned number on worksheet |
| d | Properly label TE tube with patient aliquot label matching the number on the cap to the number on the label |
| e | Verify number on primary container before transfer |
| f | Cut the wire mini-tip swab into the TE buffer tube with corresponding number on cap |
| g | Vortex 5 min, vortex setting 9 |

 | MB 1.01 Specimen Management |
| Aliquot Bronchs, nasal washes/aspirates | 2 |

|  |  |
| --- | --- |
| Step | Action |
| a | Number patients on BORDP worksheet in consecutive order |
| b | Number primary container and associated label with assigned number on worksheet |
| c | Number cap of a 2.0 mL cryovial according to assigned number on worksheet |
| d | Properly label cryovial with patient aliquot label matching the number on the cap to the number on the label |
| e | Vortex sample in original container until well mixed  |
| f | Verify number on primary and secondary containers before transfer |
| g | Transfer sample to tube with corresponding number on cap |

Number and label a 2.0 mL cryovial for each nasal wash/aspirate and bronch specimen to be tested | Refer to MB 6.05 Proc. K for archiving samples |
| Sample Storage | 3 |

|  |  |  |
| --- | --- | --- |
| Step | Temperature | Stability |
| a | Room temperature | 4 hours |
| b | 2 – 8° C  | 5 days |
| c | -70⁰ C  | 1 year |

Store processed samples as follows:  |  |

**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** |
| --- | --- | --- | --- |
|  |  | *Clean gloves are required prior to handling new reagents*  |  |
| General Information | 1 | 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
 |  |
|  | 2 | Store BORDP reagents at -10 to -30° C until expiration date located on the vial unless otherwise noted. Refer to Table 1. | MB 5.02Standards of Practice |
| General Information | 3 | Discard reagents that have not been stored properly or have expired according to the Organizational Waste Management policy | Waste Management912.04 |
|  | 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 |
| 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: Positive (POSC) and Negative (NEGC) 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 |
|  | 3 | J. Laramie | 02.26.18 | -Added Bp and Bpp positive controls to reagent list, eliminated manufactured control-Changed negative controls to matrix (not NFW)-Added negative control to storage  |