# *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 tube  Room 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 5.02  Standards of Practice |
| General Information | 3 | Discard reagents that have not been stored properly or have expired according to the Organizational Waste Management policy | Waste Management  912.04 |
|  | 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

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| --- | --- | --- | --- | --- |
| 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 |