# Storage and Stability of Processed Samples, Controls and Reagents

**PURPOSE**

* This procedure provides instructions for storage and stability of sample buffer tubes and reagents.

## SAFETY CONSIDERATIONS

1. Standard precautions. Refer to [MB002.2](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMB002%20Safety%5CMB%20002.2%20v4%20Biohazard%20Containment.docx) Biohazard Containment
2. Use of engineering controls: Refer to [MB003.1](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMB003%20Engineering%20Controls%5CMB%20003.1%20Engineering%20Controls%20to%20Prevent%20Contamination.doc) Engineering Controls to Prevent Nucleic Acid Contamination

**ABBREVIATIONS**

1. BORDP: Bordetella PCR
2. Bp: Bordetella pertussis
3. Bpp: Bordetella parapertussis
4. BSC: BioSafety Cabinet
5. BSL: BioSafety level
6. MM: master mix
7. NEGC: negative control
8. NFW: nuclease free water
9. PCTL: process control
10. POSC: positive control
11. PP: primer – pair
12. RT: room temperature
13. SEAC: Simplexa extraction and amplification control
14. TE buffer: Tris – EDTA buffer

#### 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 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 Specimens 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 001.1](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMB001%20Specimen%20Management%5CMB001.1%20Specimen%20Management%20in%20Molecular.doc) Specimen Management |
| Aliquot Bronchs, nasal washes/aspirates |  |

|  |  |
| --- | --- |
| 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 specimen in original container until well mixed  |
| f | Verify number on primary and secondary containers before transfer |
| g | Transfer specimen 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 BOR 005 Proc. K for archiving samples |
| Sample Storage | 3 |

|  |  |
| --- | --- |
| Temperature | Stability |
| Room temp | 5 days\*\* |
| 2 – 8° C  | 5 days |
| -70⁰ C | 1 year |

Store samples as follows: \*\***Bronch specimens are stable at RT up to 4 h** |  |

**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 **Focus** **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 004.2](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMB004%20Quality%5CMB%20004.2%20Molecular%20Standards%20of%20Practice.doc) 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](http://khan.childrensmn.org/Manuals/Policy/900/005314.asp) |
|  | 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

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