# *Bordetella* Reagent and Process Control Preparation

**PURPOSE**

* This procedure provides instructions for preparation of reagents and procedural controls

**ABBREVIATIONS**

|  |  |
| --- | --- |
| * BORD: Bordetella * BORDP: *Bordetella* PCR * Bp: *Bordetella pertussis* * Bpp: *Bordetella parapertussis* * BSC: biosafety cabinet * Ct: crossing threshold * F/T: freeze/thaw * IC: internal control * MM: master mix * NEGC: negative control | * NFW: nuclease free water * PCR: polymerase chain reaction * PCTL: process control * PP: primer – pair * PPE: personal protective equipment * 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 |

## 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

#### MATERIALS REQUIRED

|  |  |  |
| --- | --- | --- |
| **Equipment** | **Reagents** | **Supplies** |
| Room 1: Clean room   * Laminar-flow hood, Clean rm 1 * Freezer, -10 to -30⁰ C * Refrigerator, 2 to 8⁰ C * Microcentrifuge * Nalgene cooling block * Vortex * Eppendorf Repeater pipette * Dedicated set of pipettes: 2 µl, 10 µl, 20 μl, 100 μl, 200 μl, and 1000 μl pipettes * Pipet-Aid   Room 2: Processing   * BSC, Process rm 2 * Refrigerator, 2 to 8⁰ C * Freezer, ≥ - 70⁰C * Nalgene cooling block * Vortex * Microcentrifuge * Dedicated set of pipettes: 2 µl, 10 µl, 20 μl, 100 μl, 200 μl, and 1000 μl pipettes   Room 3: Amplification and detection   * Liaison MDX   Location: Microbiology   * McFarland densitometer | TE buffer | Micro tube racks |
| Nuclease Free Water (NFW) | 2 ml cryovials |
| SEAC   * Internal control PP * Internal control DNA | Sterile filtered pipette tips for 10 µl, 20 µl, 100 μl, 200 µl, 1000 µl pipettes |
| Bp PP | Micro tubes 1.5 ml, RNase/DNase free |
| Bpp PP | Nitrile gloves (powder-free) |
| Bordetella Molecular Controls ( Bp and Bpp PCTL, NEGC) | Sharps disposal container |
| TA MasterMix | Gripper rack, rm 2 |
| Sani-Cloth Bleach wipes | Orange barrier wipes |
| 70% alcohol | 12X75 sterile plastic test tubes |
| 5% Extran | Sterile Q – Tipped applicator swabs |
| *Bordetella pertussis* ATCC 8467 | 50 ml sterile conical tube |
| *Bordetella parapertussis* ATCC 9305 | Eppendorf 5 ml tips |
|  | Serological pipettes, 5 and 10 ml |
|  |  |
|  |  |
|  |  |

**PROCEDURE A:** Follow the activities in the table below for Process Control preparation

Preparing Process Control Suspension

| **Activity** | **Step** | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Prepare** | 1 | Pour 10 ml of TE Buffer into a 50 ml sterile conical tube |  |
| **Matrix**  Room 2 | 2 | Add NFW to achieve a 27 ml suspension |  |
|  | 3 | If preparing a positive process control proceed to step 4.  If preparing a negative process control proceed to step 6. | MB 6.05 Bordetella PCR Assay |
| **0.5 McFarland**  **Micro** | 4 | Prepare a 0.5 McFarland suspension of *Bordetella pertussis* ATCC 8467 or *Bordetella parapertussis* ATCC 9305.   |  |  | | --- | --- | | Step | Action | | a | Pick isolated colonies (3 – 4 d growth) with sterile CultureSwab | | b | Suspend in saline; vortex | | c | Adjust suspension to 0.5 McFarland(~1.5 X 108 CFU/mL) using densitometer | | d | **Dilution 1**: make a 1:100 dilution of this suspension in NFW (~1.5 X 106 CFU/mL)   * Pipette 10 µl into 990 µl NFW * Vortex well | | e | **Dilution 2:** make a 1:100 dilution from dilution 1 in NFW (~1.5 X 104 CFU/mL)   * Pipette 40 µl into 4.0 mL NFW * Vortex well | | 0.5 McFarland Standard turbidity range = 0.5 – 0.63 |
| **Working suspension**  **Room 2** | 5 | **Dilution 3** (working suspension): Make a 1:10 dilution from dilution 2 (final concentration ~1.5 X 103 CFU/mL)   |  |  | | --- | --- | | Step | Action | | a | Pipette 3 mL from BORDP dilution 2 into 27 ml of TE Buffer and NFW suspension | | b | Mix well by inversion/vortexing | | c | Test suspension in duplicate prior to freezing (3 µl Bp suspension into 7 µl BORDP mm) | | d | Target control range: Ct values 27 -30 | | e | I f necessary, adjust suspension to obtain specified range with NFW; retest | | 1 log = ~ 3 Ct |
| **Aliquot and Freeze** | 6 | Label 1.5 ml micro-centrifuge tubes with contents, date of preparation (approx. 60 tubes), and expiration date (1 year). |  |
|  | 7 | Dispense 0.5 ml of working suspension into tubes |  |
|  | 8 | Freeze aliquots at –70° C. Stable for 1 year after preparation date. |  |
| **Decontaminate Hood** | 9 | Wipe down BSC with Bleach Sani – Cloth followed by water and 70% alcohol |  |
| **Room 2** | 10 | UV hood for 15 min |  |
| **Test aliquots**  **before use** | 11 | Before use:   * Thaw one BORDP PCTL or NEGC aliquot * Vortex * Perform direct testing 5 times |  |
|  | 12 | * Document Ct values on BORDP New Reagent Worksheet : MB 6.09.F3 (PCTL) or MB 6.09.F9 (NEGC) * Record relevant information on the appropriate New Lot Inventory Worksheet: MB 6.09.F6 (PCTL) or MB 6.09.F8 (NEGC) |  |
|  | 13 | Place worksheet and BORDP Segment report including graphs in *New Lot Inventory and QC* manual |  |
| **Stability** | 14 | Once thawed, process controls are stable for 7 days at refrigerated temperature |  |
|  | 15 | Do not refreeze (only 1 F/T cycle) |  |

**PROCEDURE B:** Follow the activities in the table below for aliquoting TE buffer (sample buffer tubes) and Nuclease Free Water (NFW) used for MM

Preparing TE buffer and NFW

| **Activity** | **Step** | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **PPE** | 1 | Wear lab coat and gloves dedicated to the Clean room 1 |  |
| **TE buffer** | 2 | Label cryo-storage box with contents   * BORDP TE buffer: reagent lot, expiration date and date of preparation |  |
| Room 1 | 3 | Aliquot the following amounts into 1.5 micro-centrifuge tubes   * BORDP Elution buffer: 200 µl of TE buffer into 1.5 ml micro-centrifuge tube |  |
| **Storage** | 5 | Refrigerate aliquots in room 1 |  |
|  | 6 | Keep working supply in room 2 |  |

**PROCEDURE C:** Follow the activity below for preparing master mix (MM)

Preparing RT-PCR Master Mix (MM)

| Activity | Step | **Action** |
| --- | --- | --- |
|  | 1 | ***MM must be used within 30 min of preparation****.* |
|  | 2 | Wear lab coat and gloves dedicated to the Clean room 1 |
| **Warm reagents to RmTemp** | 3 | Thaw Primer Probe mix, IC and the Master Mix at room temperature   * Protect from light * Use within 1 hour |
| Room 1 | 4 | Gently mix each component   |  |  | | --- | --- | | Component | Mixing action | | TA mm | Vortex 2 – 3 sec, setting 8 | | Bp PP | Gently flick | | Bpp PP | Gently flick | | IC DNA | Vortex 2 – 3 sec, setting 8 | | IC PP | Gently flick | |
|  | 5 | Quick spin reagents |
|  | 6 | Prepare MM in a 1.5 mL micro-centrifuge tube by combining the reagents according to **Table 1** |
|  | 7 | Gently vortex MM 2 – 3 sec to mix; vortex setting 8  ***Note:***Adjust mixing time according to volume. |
|  | 8 | Quick spin MM |
| **Refrigerate reagents** | 9 | Do not refreeze reagents; store in refrigerator up to 30 days  ***Note:*** Refer to procedure MB 6.03 for storage conditions and expiry dates |
| **Transport** | 10 | Transport to room 2 |
| Room 2 | 11 | Keep the MM in refrigerator or cooling block protected from light until PCR reaction set-up. |

**Table 1: BORDP Master Mix Table**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No. of samples** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **TA Master Mix (µl)** | 6 | 10 | 14 | 18 | 24 | 28 | 32 | 36 | 40 | 44 | 48 | 52 |
| **Bp Primer Mix (µl)** | 0.6 | 1 | 1.4 | 1.8 | 2.4 | 2.8 | 3.2 | 3.6 | 4 | 4.4 | 4.8 | 5.2 |
| **Bpp Primer Mix ( µl)** | 0.6 | 1 | 1.4 | 1.8 | 2.4 | 2.8 | 3.2 | 3.6 | 4 | 4.4 | 4.8 | 5.2 |
| **IC DNA (µl)** | 0.3 | 0.5 | 0.7 | 0.9 | 1.2 | 1.4 | 1.6 | 1.8 | 2 | 2.2 | 2.4 | 2.6 |
| **IC Primer Mix (µl)** | 0.3 | .05 | 0.7 | 0.9 | 1.2 | 1.4 | 1.6 | 1.8 | 2 | 2.2 | 2.4 | 2.6 |
| **NFW (µl)** | 2.7 | 4.5 | 6.3 | 8.1 | 10.8 | 12.6 | 14.4 | 16.2 | 18 | 19.8 | 21.6 | 23.4 |
| **Total volume (µl)** | 10.5 | 17.5 | 24.5 | 31.5 | 42 | 49 | 56 | 63 | 70 | 77 | 84 | 91 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No. of samples** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| **TA Master Mix (µl)** | 56 | 60 | 66 | 70 | 74 | 78 | 82 | 86 | 90 | 94 | 98 | 102 |
| **Bp Primer Mix (µl)** | 5.6 | 6 | 6.6 | 7 | 7.4 | 7.8 | 8.2 | 8.6 | 9 | 9.4 | 9.8 | 10.2 |
| **Bpp Primer Mix ( µl)** | 5.6 | 6 | 6.6 | 7 | 7.4 | 7.8 | 8.2 | 8.6 | 9 | 9.4 | 9.8 | 10.2 |
| **IC DNA (µl)** | 2.8 | 3 | 3.3 | 3.5 | 3.7 | 3.9 | 4.1 | 4.3 | 4.5 | 4.7 | 4.9 | 5.1 |
| **IC Primer Mix (µl)** | 2.8 | 3 | 3.3 | 3.5 | 3.7 | 3.9 | 4.1 | 4.3 | 4.5 | 4.7 | 4.9 | 5.1 |
| **NFW (µl)** | 25.2 | 27 | 29.7 | 31.5 | 33.3 | 35.1 | 36.9 | 38.7 | 40.5 | 42.3 | 44.1 | 45.9 |
| **Total volume (µl)** | 98 | 105 | 115.5 | 122.5 | 129.5 | 136.5 | 143.5 | 150.5 | 157.5 | 164.5 | 171.5 | 178.5 |

**PROCEDURE D:** Follow the activity below for preparing miscellaneous reagents

Preparing miscellaneous reagents

| Reagent | Step | **Action** |
| --- | --- | --- |
| 10% Bleach | 1 | Prepare in dish room. Made daily. |
| Dish room | 2 | Make working solution as follows:   |  |  |  | | --- | --- | --- | | Step | Reagent (10% bleach) | Volume | | 1 | House hold bleach (5 – 6 %) | 500 ml | | 2 | Water | 2000 ml | | 3 | Alconox (add for contamination clean-up) | 25 g |   Labeling:  Refer to: MB 2.01 Safe Work Practices in Molecular |
| **70% alcohol** | 1 | Prepare from 100% Dehydrant alcohol located in the Flammable cabinet in the Recycling room. |
| Room 3 or Recycling room | 2 | Make working solution as follows:   |  |  |  | | --- | --- | --- | | Working Volume | 100% Dehydrant | Water | | 1000 ml | 700 ml | 300 ml |   Labeling:  Refer to: MB 2.01 Safe Work Practices in Molecular  Expiration: 1 year |
| **5% Extran** |  | Make working solution as follows:   |  |  |  | | --- | --- | --- | | Step | Reagent (Extran 300) | Volume | | 1 | Extran 300 | 50 ml | | 2 | Water | 950 ml |   Labeling:  Refer to: MB 2.01 Safe Work Practices in Molecular  Expiration: 1 year |

**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 | 07.20.16 | Reformatted for CMS upload; prev BOR 004 |
|  | 3 | P. Ackerman | 03.29.17 | Instrument name change from Focus Integrated Cycler to DiaSorin Liaison MDX; fixed hyperlinks for SharePoint upload |
|  | 4 | J. Laramie | 02.26.18 | -Edited preparation of negative control matrix (changed from NFW to negative sample matrix)  -Added *B. parapertussis* ATCC 9305 to reagent list and to positive control preparation |
|  | 5 | J. Laramie | 05.16.18 | Eliminated the use of nasal matrix for control prep |
|  | 6 | J. Laramie/M. Meyer | 08.21.18 | -Updated to include expiration of frozen controls (1 year)  -Biennial review: 08.06.2018 JL/MM |
|  | 7 | J. Laramie | 08.21.18 | Added bleach prep and reagent labeling notes |
|  | 8 | J. Laramie | 8.29.18 | Updated target Ct range |
|  | 9 | J. Laramie | 8.21.19 | Added duplicate testing of control before freezing |