# *Simplexa™ Bordetella* New Lot and/or New Shipment Quality Control

## PURPOSE

- This procedure provides instructions for verifying reagent performance

## 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
- Ct: crossing threshold
- F/T: freeze/thaw
- IC: internal control
- LOD: level of detection
- MM: master mix
- NEGC: negative control
- NFW: nuclease free water
- PCR: polymerase chain reaction
- PCTL: process control
- PP: primer – pair
- POSC: positive control
- 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

## MATERIALS

| Equipment | Reagents | Supplies |
|---|---|---|
| Room 1: Clean room<br>▪ Laminar-flow hood, Clean rm 1<br>▪ Freezer, -10 to -30° C<br>▪ Refrigerator, 2 to 8° C<br>▪ Micro-centrifuge<br>▪ Nalgene cooling block<br>▪ Vortex<br>▪ Eppendorf Repeater pipette<br>▪ Dedicated set of pipettes: 2 µl, 10 µl, 20 µl, 100 µl, 200 µl, and 1000 µl pipettes<br>Room 2: Processing<br>▪ BSC, Process rm 2<br>▪ Refrigerator, 2 to 8° C<br>▪ Freezer, ≥ - 70°C<br>▪ Nalgene cooling block<br>▪ Vortex<br>▪ Micro-centrifuge<br>▪ Dedicated set of pipettes: 2 µl, 10 µl, 20 µl, 100 µl, 200 µl, and 1000 µl pipettes<br>▪ Gilson Concept pipette, 100 µl<br>Room 3: Amplification and detection<br>▪ Liaison MDX | TE buffer (Tris-EDTA) | Micro tube racks |
| | Nuclease Free Water (NFW) | 2 ml cryovials |
| | SEAC (*Simplexa* extraction and amplification control)<br>▪ Internal control primer (IC pp)<br>▪ Internal control DNA | Sterile filtered pipette tips for 10 µl, 20 µl, 100 µl, 200 µl, 1000 µl pipettes |
| | Bp Primer (Bp PP) | Micro tubes 1.5 ml, RNase/DNase free |
| | Bpp Primer (Bpp PP) | Universal disc |
| | *Bordetella* positive control (POSC) | Universal disc sealer |
| | BORD process control (PCTL) | Nitrile gloves (powder-free) |
| | TA MasterMix (TA MM) | Sharps disposal container |
| | Sani-Cloth Bleach wipes | Gripper rack, rm 2 |
| | 70% alcohol | Orange barrier wipes |
| | 5% Extran | Culturette swabs |

**PROCEDURE A:** Follow the activities for testing reagent reactivity in the table below

**New reagent lot and/or new shipment verification**

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Doc</th></tr>
<tr><td>Testing requirements</td><td>1</td><td>Reagent components from each new lot/shipment of the BORDP assay must be tested before placing them into service for equivalent performance with the reagents currently in use.<ul><li>B. pertussis Primer-Pair</li><li>B. parapertussis Primer- Pair</li><li>Bordetella POSC</li><li>Bordetella PCTL</li><li>TA MM</li><li>SEAC</li><li>TE buffer</li></ul></td><td>MB 5.02<br>MOLB Standards of Practice</td></tr>
<tr><td rowspan="2">PP, SEAC, TA MM, TE buffer verification</td><td>2</td><td>Retest one known B. pertussis / B. parapertussis positive and one known negative patient sample buffer from previous lot against the new reagent lot<ul><li>Note: Select a positive sample with a Ct value between 30 – 32 to challenge the LOD and verify the sensitivity of the assay</li></ul></td><td rowspan="2">MB 6.09.F1<br>BORDP QC worksheet</td></tr>
<tr><td>3</td><td>Test a PCTL, POSC and NEGC using the new lot/shipment reagents</td></tr>
<tr><td>POSC verification</td><td>4</td><td>Test the new lot POSC in parallel with the old lot POSC before placing into service</td><td>MB 6.09.F2<br>BORDP POSC QC Worksheet</td></tr>
<tr><td>PCTL verification</td><td>5</td><td>Test the new lot (prep date) PCTL in parallel with the old lot PCTL before placing into service</td><td>MB 6.09.F3<br>BORDP PCTL QC Worksheet</td></tr>
<tr><td>Results</td><td>5</td><td>Equivalent results must be obtained
<table>
<tr><th></th><th>Test Materials</th><th>Expected Results</th></tr>
<tr><td>a</td><td>Known positive sample/pt</td><td>positive</td></tr>
<tr><td>b</td><td>Known negative sample/pt</td><td>negative</td></tr>
<tr><td>c</td><td>Process Control</td><td></td></tr>
<tr><td>d</td><td>Positive Reagent Control</td><td>Positive for Bp and Bpp</td></tr>
<tr><td>e</td><td>Negative Reagent Control</td><td>Negative</td></tr>
</table></td><td rowspan="4">New Lot/Shipment Inventory Forms<ul><li>MB 6.09.F4 Bp PP</li><li>MB 6.09.F5 Bpp PP</li><li>MB 6.09.F6 BORDP POSC</li><li>MB 8.09.F4 SEAC</li><li>MB 8.09.F5 TA MM</li><li>MB 8.09.F7 TE buffer</li></ul></td></tr>
<tr><td rowspan="3">Record</td><td>6</td><td>Record results on QC worksheet; staple QC worksheet to BORD segment report</td></tr>
<tr><td>7</td><td>Verify that all reagents and materials meet expiration date and QC parameters as per CLSI document MM3-A2.</td></tr>
<tr><td>8</td><td>Check off inventory form</td></tr>
<tr><td></td><td>9</td><td>Archive result forms in New Lot Inventory and QC manual.</td></tr>
</table>

**PROCEDURE B:** Follow the activities for troubleshooting verification failures in the table below

**Performance Failures**

| Activity | Step | Action | | Related doc |
|---|---|---|---|---|
| Troubleshooting Failures | 1 | Verify that the reagent performance is acceptable before implementation of a new lot and/or shipment | | MB 6.05 Procedure I: *Repeat Testing*<br>MB 6.06 Simplexa Troubleshooting guide |
| | | If | Then | |
| | | Any Control fails | ▪ Document observation/corrective action on QC log<br>▪ Do not implement new lot/shipment<br>▪ Repeat all testing; if repeat testing fails, contact DiaSorin technical service | |
| | | POSC fails | ▪ Amplification failure: Review amplification curve for amplification of target<br>▪ Possible reagent or system failure: Review MM preparation and assay set-up<br>▪ Repeat testing; if repeat testing fails, contact DiaSorin technical service | |
| | | NEGC fails | ▪ Possible carryover or reagent contamination: Review pipetting technique, glove contamination, possible aerosol creation, and MM preparation<br>▪ Repeat testing; if repeat testing fails, contact DiaSorin technical service | |
| | | Known pos/neg sample fails | ▪ Review amplification curve for inhibition, lost target or carryover contamination<br>▪ Select new positive sample if target appears to be lost<br>▪ Repeat testing | |
| | | Problem unresolved | ▪ Call DiaSorin technical service at **1-800-838-4548**, Option #3<br>▪ Notify technical specialist/designee or technical director<br>▪ Document corrective action | |

## REFERENCES

1. Simplexa™ 3M™ Integrated Cycler Studio 5.0 , 3M™ Integrated Cycler Operator Manual Reference 34-8710-8382-9, PI.MOL1101.UD_REV. F for use with user defined assays, Focus Diagnostics 2009-2012, Focus Diagnostics, Inc. Cypress, CA
2. Clinical Verification and Validation Study performed at Children's Hospitals and Clinics of MN August 2014
3. CLSI *Molecular Diagnostic Methods for Infectious Diseases;* Approved Guideline – Second Edition, CLSI document MM3-A2, Wayne, PA, Clinical and Laboratory Standards Institute; 2006
4. CLSI *Establishing Molecular Testing in Clinical Laboratory Environments; Approved Guideline*, MM19-A, Vol. 31. No. 21, Wayne, PA, Clinical and Laboratory Standards Institute; 2011

### Historical Record

| Version | Written/Revised by: | Effective Date: | Summary of Revisions |
|---|---|---|---|
| 1 | P. Ackerman | 1.23.16 | Initial Version |
| 2 | P. Ackerman | 07.24.16 | Reformatted for CMS upload |
| 3 | P. Ackerman | 03.29.17 | Instrument name change from Focus Integrated Cycler to DiaSorin Liaison MDX; fixed hyperlinks for SharePoint upload |