# *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
* PPE: personal protective equipment
* SEAC: Simplexa extraction and amplification control
* TE buffer: Tris – EDTA buffer

Area/Room 1: Clean roomArea/Room 2: Processing roomArea/Room 3: Amplification room |

#### MATERIALS

| **Equipment** | **Reagents** | **Supplies** |
| --- | --- | --- |
| Room 1: Clean room* Laminar-flow hood, Clean rm 1
* Freezer, -10 to -30⁰ C
* Refrigerator, 2 to 8⁰ C
* Micro-centrifuge
* 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

Room 2: Processing* BSC, Process rm 2
* Refrigerator, 2 to 8⁰ C
* Freezer, ≥ - 70⁰C
* Nalgene cooling block
* Vortex
* Micro-centrifuge
* Dedicated set of pipettes: 2 µl, 10 µl, 20 μl, 100 μl, 200 μl, and 1000 μl pipettes
* Gilson Concept pipette, 100 µl

Room 3: Amplification and detection* Liaison MDX
 | TE buffer (Tris-EDTA) | Micro tube racks |
| Nuclease Free Water (NFW) | 2 ml cryovials |
| SEAC (*Simplexa* extraction and amplification control)* Internal control primer (IC pp)
* 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 Molecular Controls ( Bp and Bpp PCTL, NEGC) | Universal disc sealer |
| TA MasterMix (TA MM) | Nitrile gloves (powder-free) |
| Sani-Cloth Bleach wipes | Sharps disposal container  |
| 70% alcohol | Gripper rack, rm 2 |
| 5% Extran | Orange barrier wipes |
| *Bordetella pertussis* ATCC 8467 | Culturette swabs |
| *Bordetella parapertussis* ATCC 9305 |
|  |

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

New reagent lot and/or new shipment verification

| **Activity** | **Step** | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Testing requirements** | 1 | 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. * *B. pertussis* Primer-Pair
* *B. parapertussis* Primer- Pair
* *Bordetella* PCTL
* *Bordetella* NEGC
* TA MM
* SEAC
* TE buffer
 | MB 5.02 MOLB Standards of Practice |
| **PP, SEAC, TA MM, TE buffer verification** | 2 | Retest one known *B. pertussis* / *B. parapertussis* positive and one known negative patient sample buffer from previous lot against the new reagent lot* + ***Note:*** *Select a positive sample with a Ct value between* ***30 – 32*** *to challenge the LOD and verify the sensitivity of the assay*
 | MB 6.09.F1BORDP QC worksheet |
|  | 3 | Test a PCTLs and NEGC using the new lot/shipment reagents |  |
| **PCTL and NEGC verification** | 4 | Test the new lot (prep date) in parallel with the old lot before placing into service | MB 6.09.F3BORDP PCTL QC WorksheetMB 6.09.F9 BORDP NEGC QC Worksheet |
| **Results** | 5 | Equivalent results must be obtained

|  |  |  |
| --- | --- | --- |
|  | Test Materials | Expected Results |
| a | Known positive sample/pt | positive |
| b | Known negative sample/pt | negative |
| c | Process Control | positive |
| d | Negative Reagent Control | negative |

 | New Lot/Shipment Inventory Forms* + MB 6.09.F4 Bp PP
	+ MB 6.09.F5 Bpp PP
	+ MB 6.09.F6 BORDP PCTL
	+ MB 6.09.F8 BORDP NEGC
	+ MB 8.09.F4 SEAC
	+ MB 8.09.F5 TA MM
	+ MB 8.09.F7 TE buffer
 |
|  | 6 | Record results on QC worksheet; staple QC worksheet to BORD segment report  |
| **Record** | 7 | Verify that all reagents and materials meet expiration date and QC parameters as per CLSI document MM3-A2. |
|  | 8 | Check off inventory form |
|  | 9 | Archive result forms in *New Lot Inventory and QC* manual. |

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

Performance Failures

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

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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 | 07.24.16 | 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 |
|  | 4 | J. Laramie | 02.26.18 | -Eliminated POSC notes (manufactured control) |