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

PURPOSE

* This procedure provides instructions for verifying reagent performance

**ABBREVIATIONS**

1. BOR: *Bordetella*
2. BORDP: *Bordetella* PCR
3. Bp: *Bordetella pertussis*
4. Bpp: *Bordetella parapertussis*
5. BSC: biosafety cabinet
6. F/T: freeze/thaw
7. IC: internal control
8. LOD: level of detection
9. MM: master mix
10. NEGC: negative control
11. NFW: nuclease free water
12. PCR: polymerase chain reaction
13. PCTL: process control
14. PP: primer – pair
15. POSC: positive control
16. PPE: personal protective equipment
17. SEAC: Simplexa extraction and amplification control
18. TE buffer: Tris – EDTA buffer

#### MATERIALS

| **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

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
* Gilson Concept pipette, 100 µl

Room 3: Amplification and detection* Focus Simplexa Integrated Cycler
 | 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 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 |

## 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:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMB003%20Engineering%20Controls%5CMB%20003.1%20Engineering%20Controls%20to%20Prevent%20Nucleic%20Acid%20Contamination.doc) Engineering Controls to Prevent Nucleic Acid Contamination

**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* POSC
* *Bordetella* PCTL
* TA MM
* SEAC
* TE buffer
 | [MB004.2](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMB004%20Quality%5CMB%20004.2%20Molecular%20Standards%20of%20Practice.doc) MOLB Standards of Practice |
| **PP, SEAC, TA MM, TE buffer** | 2 | Retest one known *B. pertussis* / *B. parapertussis* positive and one known negative patient sample buffer from current 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*
 | [MB005.8.F1](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CQC%20forms%5CSimplexa%5CSimplexa%20Bordetella%5CMB005.8.F1%20BOR%20New%20reagent%20QC%20worksheet.docx)BOR QC worksheet |
|  | 3 | Test a PCTL, POSC and NEGC using the new lot/shipment reagents |  |
| **POSC** | 4 | Test the new lot POSC in parallel with the old lot POSC before placing into service | [MB005.8.F2](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CQC%20forms%5CSimplexa%5CSimplexa%20Bordetella%5CMB005.8.F2%20Bp%20and%20Bpp%20POSC%20New%20Reagent%20QC%20Worksheet.docx)BOR POSC QC worksheet |
| **PCTL** | 5 | Test the new lot (prep date) PCTL in parallel with the old lot PCTL before placing into service | [MB005.8.F3](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CQC%20forms%5CSimplexa%5CSimplexa%20Bordetella%5CMB005.8.F3%20BOR%20PCTL%20New%20Reagent%20QC%20Worksheet.docx) BOR PCTL 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. | Positive Reagent Control | Positive for Bp and Bpp |
| d. | Negative Reagent Control | Negative |

 | New Lot/Shipment Inventory Forms* + [MB005.8.F4](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CQC%20forms%5CSimplexa%5CSimplexa%20Bordetella%5CMB005.8.F4%20Bp%20primer%20New%20lot%20inventory.docx) Bp PP
	+ [MB005.8.F5](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CQC%20forms%5CSimplexa%5CSimplexa%20Bordetella%5CMB005.8.F5%20Bpp%20primer%20New%20lot%20inventory.docx) Bpp PP
	+ [MB005.8.F6](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CQC%20forms%5CSimplexa%5CSimplexa%20Bordetella%5CMB005.8.F6%20Bhol%20primer%20New%20lot%20inventory.docx) Bhol PP
	+ [MB005.8.F7](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CQC%20forms%5CSimplexa%5CSimplexa%20Bordetella%5CMB005.8.F7%20BOR%20POSC%20Lot%20Inventory.docx) BOR POSC
	+ [MB005.2.F4](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CQC%20forms%5CSimplexa%5CMB005.2.F4%20%20SEAC%20Lot%20Inventory.docx) SEAC
	+ [MB005.2.F5](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CQC%20forms%5CSimplexa%5CMB005.2.F5%20TA%20MasterMix%20Lot%20Inventory.docx) TA MM
	+ [MB005.2.F7](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CQC%20forms%5CSimplexa%5CMB005.2.F7%20TE%20buffer%20Lot%20Inventory.docx) 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. |

***P*ROCEDURE 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 fails, contact Focus
 | [BOR 005](BOR%20005%20Simplexa%20Bordetella%20pertussis%2C%20parapertussis%20Assay.docx) Procedure I: *Repeat Testing* |
|  | POSC 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 fails, contact Focus
 | [BOR 006](BOR%20006%20Troubleshooting%20guide.docx)Simplexa Troubleshooting guide |
|  | NEGC fails | * Possible carryover or reagent contamination: Review pipetting technique, glove contamination, possible aerosol creation, and MM preparation
* Repeat testing; if fails, contact Focus
 |  |
|  | 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 Focus technical service at **1-800-838-4548**, Option #3
* Notify Technical Specialist 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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|  | Reviewed by | **Signature** | **Date** | Reviewed by | **Signature** | **Date** |
|  P. Ackerman | PA |  1.23.16 |  |  |  |
| Historical Record |  |
|   | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | P. Ackerman | 1.23.16 | Initial Version |
|  |  |  |  |
| **Distribution** |  |
|  | **Location** | **# Copies** | **Location** | **# Copies** |
|  | Molecular Diagnostics rm B422 | 1 | G:\Lab\Molecular Biology\Molecular Proc Manual\MB005.8 BORDP \BOR 009 | 1 |