# Simplexa RSV & Flu A/B 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**

|  |  |
| --- | --- |
| * BSC: BioSafety Cabinet
* BSL: BioSafety level
* Ct : crossing threshold
* IC : internal control
* LOD: level of detection
* MM : master mix
* NEGC : negative control
* PCR: polymerase chain reaction
 | * POSC: positive control
* PPE: personal protective equipment
* RIP: Simplexa RSV & Influenza A/B PCR
* UTM: universal viral transport media

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

#### MATERIALS

|  |  |  |
| --- | --- | --- |
| **Equipment** | **Reagents** | **Supplies** |
| Room 1: Clean room* -10 to -30° C freezer
* Laminar flow Hood

Room 2: Processing* Refrigerator 2 – 8° C
* BSC BSL-2
* -70⁰ C freezer
* 100 or 200 µl pipette

Room 3: Amplification* Liaison MDX
 | Simplexa Flu A/B & RSV Direct kit MOL2651* Reaction Mix (24) 50 µl
 | 2.0 mL cryovials |
| Simplexa Flu A/B & RSV Control Pack MOL1455* 10 tubes, 100 µl
 | Nitrile gloves (powder-free) |
| Negative control – UTM  | Filtered pipette tips, 100 or 200 µl |
| Sani-Cloth Bleach wipes | Gripper rack |
| 70% alcohol | Sharps disposal container |
| 5% Extran | Replacement Foil wedge |
| Known positive patient |  |
|  | Known negative patient |  |

**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 | Test new reagent lots and/or shipments before or concurrently with being placed in service. | MB 5.02 MOLB Standards of Practice |
| **RIP New Lot/Shipment Reagent** **verification** | 2 | Retest one known positive and one known negative patient sample from previous lot against the new reagent lot* + ***Note:*** *Select a positive sample with a Ct value between* ***30 – 33*** *to challenge the LOD and verify the sensitivity of the assay*
 | * + MB 9.09.F2 RIP QC worksheet
 |
|  | 3 | Test a POSC and NEGC using the new lot/shipment reagents |  |
| **30 day QC** | 4 | Test a POSC and NEGC with the in use reagents. Rotate instruments.  |  |
| **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 Flu A, Flu B and RSV  |
| d | Negative Reagent Control | negative  |

 | New Lot/Shipment Inventory Forms* + MB 9.09.F1 RIP Direct
	+ MB 9.09.F3 POSC
 |
|  | 6 | Record results on QC worksheet; staple QC worksheet to RIP 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 Focus
 | MB 9.05 Procedure H: *Repeat Testing* |
|  | POSC fails | * Target not detected
1. System/reagent failure
2. Repeat run; vortex patient samples prior to testing
3. Thaw new MM
4. Gently flick MM and POSC to mix before repeat testing
5. If POSC fails on repeat, thaw new POSC
 |  MB 9.06Simplexa Troubleshooting guide |
|  | NEGC fails | * NEGC contaminated
1. Repeat run
2. Replace NEGC if contamination is indicated; review patient graphs for low level contamination
3. Review specimen handling/processing technique including pipetting , glove contamination and possible aerosols creation
* IC not detected
1. System/reagent failure
2. Repeat run
 | [Simplexa Operator's Manual IVD](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212355.pdf) Appendix B: Troubleshooting |
|  | Known pos/neg sample fails | * Review amplification curve for inhibition, lost target or carryover contamination
1. Select new positive sample if target appears to be lost
2. Repeat testing
 |  |
|  | Problem unresolved | 1. Call Focus technical service at **1-800-838-4548**, Option #3
2. Notify technical director or designee
 |  |

**REFERENCES**

1. Simplexa™ 3M™ Integrated Cycler Studio 5.0 , 3M™ Integrated Cycler Operator Manual Reference 34-8710-8239-1, PI.MOL1101.IVD\_REV. F for use with IVD assays, Focus Diagnostics 2009-2012, Focus Diagnostics, Inc. Cypress, CA
2. Simplexa™ *Flu A/B & RSV* Direct Circular PI.MOL2650.IVD, Rev. F, 18-September-2015, Focus Diagnostics, Cypress, CA 90630
3. Children’s Hospitals and Clinics of MN Simplexa RSV & Flu A/B Direct Verification/Validation Study, 2016
4. CLSI *Molecular Diagnostic Methods for Infectious Diseases;* Approved Guideline – Second Edition, CLSI document MM3-A2, Wayne, PA, Clinical and Laboratory Standards Institute; 2006
5. 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 | 12.08.2016 | Initial Version |
|  | 1 | J. Laramie | 12.02.2016 | Biennial review: 10.01.18 JL |
|  | 2 | J. Laramie | 07.19.2019 | Added 30 day QC notes, removed parallel testing of new lots/shipments of the POSC |