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 room
- Area/Room 2: Processing room
- Area/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 ▪ -70° C freezer ▪ 100 or 200 µl pipette Room 3: Amplification ▪ 3M Integrated Cycler	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

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Doc</th></tr>
<tr><td rowspan="2">RIP New Lot/Shipment Reagent verification</td><td>2</td><td>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</td><td rowspan="2">MB 9.09.F2 RIP QC worksheet</td></tr>
<tr><td>3</td><td>Test a 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 9.09.F4 POSC QC worksheet</td></tr>
<tr><td rowspan="2">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>Positive Reagent Control</td><td>Positive for Flu A, Flu B and RSV</td></tr><tr><td>d</td><td>Negative Reagent Control</td><td>negative</td></tr></table></td><td rowspan="4">New Lot/Shipment Inventory Forms
MB 9.09.F1 RIP Direct
MB 9.09.F3 POSC</td></tr>
<tr><td>6</td><td>Record results on QC worksheet; staple QC worksheet to RIP segment report</td></tr>
<tr><td rowspan="3">Record</td><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>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

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related doc</th></tr>
<tr><td rowspan="3">Troubleshooting Failures</td><td rowspan="3">1</td><td colspan="2">Verify that the reagent performance is acceptable before implementation of a new lot and/or shipment</td><td rowspan="3">MB 9.05 Procedure H: Repeat Testing

MB 9.06 Simplexa Troubleshooting guide</td></tr>
<tr><td>If</td><td>Then</td></tr>
<tr><td>Any Control fails</td><td>Document observation/corrective action on QC log
Do not implement new lot/shipment
Repeat all testing; if repeat testing fails, contact Focus</td></tr>
<tr><td>POSC fails</td><td>Target not detected<ol type="a">
System/reagent failure
Repeat run; vortex patient samples prior to testing
Thaw new MM
Gently flick MM and POSC to mix before repeat testing
If POSC fails on repeat, thaw new POSC</td></tr>
</table>

Activity	Step	Action		Related doc
		NEGC fails	▪ NEGC contaminated a. Repeat run b. Replace NEGC if contamination is indicated; review patient graphs for low level contamination c. Review specimen handling/processing technique including pipetting , glove contamination and possible aerosols creation ▪ IC not detected a. System/reagent failure b. Repeat run	Simplexa Operator's Manual IVD Appendix B: Troubleshooting
		Known pos/neg sample fails	▪ Review amplification curve for inhibition, lost target or carryover contamination a. Select new positive sample if target appears to be lost b. Repeat testing	
		Problem unresolved	a. Call Focus technical service at **1-800-838-4548**, Option #3 b. 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

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1	P. Ackerman	12.08.2016	Initial Version