RIP Reagent and Control Preparation

PURPOSE

- This procedure provides instructions for preparation of reagents and procedural controls

ABBREVIATIONS

- BSC: BioSafety Cabinet
- BSL: BioSafety level
- MM: master mix
- NEGC: negative control
- POSC: positive control
- PPE: personal protective equipment
- RIP: Simplexa RSV & Influenza A/B PCR
- RT: room temperature
- UTM: universal viral transport media
- Area/Room 1: Clean room
- Area/Room 2: Processing room
- Area/Room 3: Amplification room

SAFETY CONSIDERATIONS

A. Standard precautions. Refer to MB 2.02 Biohazard Containment
B. Use of engineering controls: Refer to MB 3.01 Engineering Controls to Prevent Nucleic Acid Contamination

MATERIALS REQUIRED

Equipment	Reagents	Supplies
Room 1: Clean room ▪ -10 to -30° C freezer ▪ Laminar flow Hood ▪ Pipette-aide Room 2: Processing ▪ Refrigerator 2 – 8° C ▪ BSC BSL-2 ▪ -70° C freezer Room 3: Amplification ▪ 3M Integrated Cycler	Simplexa Flu A/B & RSV Direct kit MOL2651 Reaction Mix (24) 50 µl	Orange barrier wipes
	Simplexa Flu A/B & RSV Control Pack MOL1455 10 tubes, 100 µl	Nitrile gloves (powder-free)
	Negative control – UTM	1.5 microcentrifuge tubes
	Sani-Cloth Bleach wipes	Cryovial storage box
	70% alcohol	Serological pipettes, 5 and 10 ml
	5% Extran	Micro tube racks
	Universal viral transport media (UTM)	

PROCEDURE A: Follow the activity below for preparing Negative Control (NEGC)

Preparing NEGC

Activity	Step	Action	Related Doc
PPE	1	Wear lab coat and gloves dedicated to the Clean room 1	
Aliquot	2	Label cryo-storage box with contents ▪ Lot number (L/N), expiration date and date of preparation	
	3	Aliquot 300 µl of UTM into 1.5 microcentrifuge tubes	
Storage	4	Refrigerate aliquots in room 2	

PROCEDURE B: Follow the activity below for Reagent Handling

Preparing Master Mix (MM) and Positive Control (POSC)

Activity	Step	Action	Related Doc
Warm MM to RT	1	***MM must be used within 30 min after removing from freezer***	
	2	Wear lab coat and gloves dedicated to the Clean room 1	Refer to MB 9.03 for storage conditions and expiry dates
	3	Remove one MM for each sample to be tested from freezer	
	4	Remove lab coat and return to room 2	
	5	Thaw MM at room temperature ▪ Use within 30 min ▪ Do not refreeze	
	6	Gently flick tube to mix ▪ *Do not vortex*	
	7	Quick spin MM reagents before use	
POSC	8	Remove POSC from – 70° C	
	9	Thaw POSC at room temperature ▪ Do not refreeze	
	10	Gently flick tube to mix ▪ *Do not vortex*	
	11	Quick spin POSC before use	

PROCEDURE C: Follow the activity below for preparing miscellaneous reagents

Preparing miscellaneous reagents

<table>
<tr><th>Reagent</th><th>Step</th><th>Action</th></tr>
<tr><td rowspan="2">5% Extran Working solution

Room 2</td><td>1</td><td>Prepare in amplification room.
Caution: Protective eyewear must be worn when working with concentrated Extran</td></tr>
<tr><td>2</td><td>Make working solution as follows:
<table>
<tr><th>Working Volume</th><th>Conc. Extran</th><th>Water</th></tr>
<tr><td>2000 ml</td><td>100 ml</td><td>1900 ml</td></tr>
<tr><td>3000 ml</td><td>150 ml</td><td>2850 ml</td></tr>
<tr><td>4000 ml</td><td>200 ml</td><td>3800 ml</td></tr>
</table></td></tr>
<tr><td rowspan="2">70% alcohol

Room 3 or Recycling room</td><td>1</td><td>Prepare from 100% Dehydrant alcohol located in the Flammable cabinet in the Recycling room.</td></tr>
<tr><td>2</td><td>Make working solution as follows:
<table>
<tr><th>Working Volume</th><th>100% Dehydrant</th><th>Water</th></tr>
<tr><td>1000 ml</td><td>700 ml</td><td>300 ml</td></tr>
</table></td></tr>
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REFERENCES

1. Simplexa™ *Flu A/B & RSV* Direct Circular PI.MOL2650.IVD, Rev. F, 18-September-2015, DiaSorin/Focus Diagnostics, Cypress, CA 90630

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1	P. Ackerman	11.30.2016	Initial Version