RVP Control and Reagent Preparation

PURPOSE

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

ABBREVIATIONS

- Ct: crossing threshold
- EXC: extraction control
- FABR: Flu A, B & RSV PCR
- Hyb: hybridization solution
- IC: internal control
- MM: master mix
- NA: Nucleic Acid
- NEGC: negative control
- NFW: nuclease free water
- RT-PCR: reverse transcription polymerase chain reaction
- PCTL: process control
- POSC: positive control
- RT: room temperature
- RVP: Respiratory Viral Panel
- VTM: viral transport media
- Area/Room 1: Clean room
- Area/Room 2: Processing room
- Area/Room 3: Amplification room

MATERIALS REQUIRED

Equipment	Reagents	Supplies
Room 1 ▪ Adjustable pipettes ▪ Cold block ▪ Freezer, -20° C ▪ Laminar air-flow hood ▪ Refrigerator 2 – 8° C ▪ Vortex mixer Room 2 ▪ Adjustable pipettes ▪ BioHit 8 channel pipette ▪ Bio-Safety Cabinet (BSC) ▪ Cold Block ▪ Freezer, -70° C ▪ Magnetic rack ▪ Mini-centrifuge ▪ NucliSens easyMag ▪ Refrigerator 2 – 8° C ▪ Tube racks, 1.5 – 2 ml ▪ Vortex mixer Room 3 ▪ Adjustable pipettes ▪ Cold Block ▪ Freezer, -20° C ▪ GenMark eSensor XT-8 instrument ▪ Mini-centrifuge ▪ PCR thermocycler ▪ PCR workstation ▪ Vortex mixer	eSensor *RVP* kit: Product No. MT005102	Sterile filtered 10 µl pipette tips
	easyMAG Lysis buffer, 2 ml	Sterile filtered 30 µl pipette tips
	easyMAG Buffer 1	Sterile filtered 100 µl pipette tips
	easyMAG Buffer 2	Sterile filtered 200 µl pipette tips
	easyMAG Buffer 3	Sterile filtered 1000 µl pipette tips
	MagSil	Micro tubes 1.5 ml, RNase/DNase free
	Molecular grade water, nuclease free	Nitrile gloves (powder-free)
	Viral transport media (VTM)	PCR 8 tube strips with caps
	Viral isolates: H1, H3, RSV, Flu B	easyMag disposable vessel strips and tips
	Patient hMPV sample	BioHit pipette tips
	Sani-Cloth Bleach Wipes (10%)	BioHazard wipes
	70% alcohol	Gripper rack
	Household bleach	Sharps disposal container
	MMQCI RVP Control Panel	

PROCEDURE A: Follow the activities in the table below for preparing RVP assay controls

Preparing RVP Control Panel, Positive/Extraction Controls, Internal Control, and Negative Control

Control	Step	Action
MMQCI RVP Control Panel[3] Room 2 1X use	1	The RVP control panel consists of 2 vials M244 and M245, single use only
	2	Allow the vials to warm to RT
	3	Vortex each vial for 5 s prior to use
	4	Spin for 5 s to pull down matrix
	5	Extract 200 µl supernatant including 10 µl IC using the EasyMag; final elution 60 µl
	6	Vortex the eluate for 5 sec; allow to sit in magnetic rack for 10 min
MMQCI Testing schedule	7	Analyze RVP control panel according to the RVP protocol ▪ Test with each new lot/shipment of RVP kits; record results MB 11.08.F2 ▪ Test weekly alternating M244 and M245; record results MB 11.08.F4
	8	Freeze eluates at -70° C
Internal Control Room 2 F/T 5X	1	No preparation necessary
	2	Remove Internal Control (IC) from -70° C freezer; warm to RT before use
	3	Add 10 µl to each sample in easyMag vessel to be extracted
	4	Mark cap after each use representing one F/T; return to -70° C freezer
	5	Freeze/thaw cycles up to 5X
Negative Control (NEGC) Room 1	1	Aliquot 300 µl VTM in 1.5 ml micro-centrifuge tubes
	2	Label tubes with NEGC and prep date using preprinted labels
	3	Label box with VTM lot number and expiry date
	4	Store at 2 – 8° C in room 1
	5	Store a 1 week working supply in room 2
RSV, 2009 H1, H3 and FluB PCTL/EXC Prepare virus Virology Lab	1	Cultivate from stock viral suspensions Virus / Cell line / CPE / Approx CX days 2009 H1N1 / RMK / 3 – 4+ / 3 Seasonal flu H3 / RMK / 3 – 4+ / 3 Influenza B / RMK / 3 – 4+ / 3 RSV / Hep-2 / 3 – 4+ / 3-4
	2	Scrape down cell culture tube to make a new stock suspension
Working suspension Room 2	3	Serially dilute each stock suspension using NFW to prepare a 10^{-4} working dilution (total volume approx 25 ml)
	4	Add 5 ml of VTM to the suspension; mix well
	5	Extract 200 µl of the working dilution (each control)
	6	Perform Simplexa FABR PCR testing to determine Ct value; target range 30 – 33

Control	Step	Action
Working suspension cont.	7	If necessary, adjust suspension to obtain projected range with NFW / VTM based on the previous Ct value ***Note:*** Each 10 fold dilution will increase the Ct value by approx 3 Ct.
	8	Repeat Simplexa FABR testing from new suspension.
Aliquot and freeze Room 2	9	Label a set of 1.5 mL micro-centrifuge tubes for each extraction control (H1, H3, RSV, FluB) using preprinted labels with prep date
	10	Pipette 1.1 mL of working suspension into tubes
	11	Store in –70° C freezer, EXC box
Test aliquots before use	12	Before use: ▪ Thaw one PCTL aliquot ▪ Test 5 X using Simplexa FABR ▪ Determine average Ct value
	13	Document Ct values on FABR/RVP PCTL New Reagent Worksheet MB 11.04.F1
	14	Test final dilution of each EXC on RVP assay; attach Currents Report (RUO) to worksheet
	15	Place worksheet, FABR Segment report including graphs and RVP Currents Reports in *New Lot Inventory and QC* manual
Stability	16	Once thawed, process control is stable for 5 days at refrigerated temperature
	17	Do not refreeze (only 1 F/T cycle)
hMPV PCTL/EXC RVP	1	Pool 2 - 3 known hMPV positive RVP (~nA 100) residual samples; mix well
	2	Serially dilute suspension using NFW to prepare a 10^{-4} working dilution (total volume approx 25 ml)
	3	Add 5 ml of VTM to the suspension; mix well
	4	Extract 200 µl of the working dilutions
	5	Perform hMPV RVP testing to determine nA value ▪ Final suspension: nA value between 70 – 100 ▪ Adjust if necessary by adding additional known positive or by diluting ▪ Mix well
	6	Repeat RVP testing
	7	If the nA value is within acceptable range, aliquot suspension
Aliquot/ freeze Room 2	8	Label a set of 1.5 mL micro-centrifuge tubes using preprinted labels with prep date
	9	Pipette 1 mL of working suspension into tubes
	10	Store in –70° C freezer, EXC box
Test aliquots before use	11	Before use: ▪ Thaw one PCTL aliquot ▪ Test 5X using RVP
	12	Document nA values on FABR/RVP PCTL New Reagent Worksheet MB 11.04.F1
	13	Place worksheet and Currents Reports in *New Lot Inventory and QC* manual
Stability	14	Once thawed, hMPV process control is stable for 2 days at 2 – 8° C, 1 year at ≤70° C
	15	Do not refreeze (only 1 F/T cycle)

Control	Step	Action
RVP PCTL/EXC Rotation	14	Rotate EXC as follows: Order: 1 — Viral Extraction Control: 2009 H1N1 2 — Seasonal Flu H3 3 — Influenza B 4 — RSV 5 — hMPV

PROCEDURE B: Follow the activity below for preparing master mix (MM)

Preparing RT-PCR Master Mix (MM)

Activity	Step	Action
MM Room 1	1	***MM must be used within 30 min of preparation***.
	2	Wear lab coat and gloves dedicated in the Clean room 1.
	3	Clean hood and equipment ▪ Sani-Cloth Bleach Wipes (10%) followed by water and 70% alcohol
	4	Thaw RVP PCR mix at RT up to 1 h. Place Enzyme mix in a cold block, refrigerated until use.
	5	Vortex PCR mix 3 – 5 s, making sure it is completely thawed
	6	Centrifuge the enzyme and PCR mix; place both reagents in cold block
	7	Prepare MM according to number of reactions needed including POSC and NEGC; Refer to Set-up Table MB 11.04.A1
	8	Vortex MM and centrifuge; place in cold block until use
Refreeze rgts	9	Refreeze reagents. Place a hatch mark on each cap to represent one F/T cycle (up to 5X)
MM calculations	10	Volume Calculations for MM: N = Total number of reactions in run including POSC and NEGC, MB 11.04.A1 Component: RVP PCR Mix — Volume/reaction: 28.6 µl — Calculation: 28.6 * N *1.1 = — Volume (µl): RVP Enzyme — 1.4 µl — 1.4 * N * 1.1 = Total volume — 30 µl — 30 * N * 1.1 =

PROCEDURE C: Follow the activity below for preparing Hybridization Solution

Preparing Hybridization Solution "Hyb"

Activity	Step	Action
Hyb solution Room 3	1	Clean hood and equipment ▪ Sani-Cloth Bleach Wipes (10%) followed by water and 70% alcohol
	2	Thaw Signal buffer, Buffer 1 and Buffer 2 at RT
	3	Vortex and centrifuge or tap lightly
	4	Prepare hybridization buffer according to number of reactions needed; Refer to Hybridization buffer set-up table MB 11.04.A1; stable up to 4 hours at RT

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th></tr>
<tr><td rowspan="3">Hyb solution Cont.</td><td>5</td><td>Label 2 ml tube “Hyb” (may need to prepare 2 tubes for sufficient volume)
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>Add reagents to Hyb tube in order
1. Signal buffer
2. Buffer 1
3. Buffer2 (white precipitate will appear after addition)</td></tr>
<tr><td>b</td><td>Vortex at setting 10 for 3 – 5 s to clear precipitate</td></tr>
<tr><td>c</td><td>Centrifuge 3 – 5 s</td></tr>
<tr><td>d</td><td>Note: Warm with hands if precipitate does not disappear; vortex</td></tr>
</table></td></tr>
<tr><td>6</td><td>Mark the cap of the buffer tubes to represent one F/T cycle</td></tr>
<tr><td>7</td><td>Change gloves; return detection reagents to -20^{o} C freezer</td></tr>
<tr><td>Hyb solution calculations</td><td>8</td><td>Volume Calculations for Hyb solution: N = Total number of reactions in run including POSC and NEGC, MB 11.04.A1
<table>
<tr><th>Component</th><th>Volume/reaction</th><th>Calculation</th><th>Volume (µl)</th></tr>
<tr><td>RVP Signal Buffer</td><td>70 µl</td><td>70 * N * 1.1 =</td><td></td></tr>
<tr><td>Buffer 1</td><td>10 µl</td><td>10 * N * 1.1 =</td><td></td></tr>
<tr><td>Buffer2</td><td>20 µl</td><td>20 * N * 1.1 =</td><td></td></tr>
<tr><td colspan="3">Total Hyb solution volume =</td><td></td></tr>
</table></td></tr>
</table>

PROCEDURE D: 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>10% Bleach
Dish room</td><td>1</td><td>Prepare in dish room.</td></tr>
<tr><td></td><td>2</td><td>Make working solution as follows:
<table>
<tr><th>Step</th><th>Reagent (10% bleach)</th><th>Volume</th></tr>
<tr><td>1</td><td>House hold bleach (5 – 6 %)</td><td>500 ml</td></tr>
<tr><td>2</td><td>Water</td><td>2000 ml</td></tr>
<tr><td>3</td><td>Alconox (add for contamination clean-up)</td><td>25 g</td></tr>
</table></td></tr>
<tr><td>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></td><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>
</table>

REFERENCES

1. eSensor® Respiratory viral Panel, PI1032 REV:D, December 2013, Clinical Micro Sensors, Inc. dba GenMark Diagnostics, Inc., 5964 La Place Court, Carlsbad, CA 92008, 1-800-373-6767, ww.genmarkdx.com
2. NucliSENS® Lysis Buffer, product circular 14900 E, 200292, September 2009.
3. eSensor XT-8 RVP Control Panel package insert; circular M243 102914.001, Maine Molecular Quality Controls, Inc. www.mmqci.com

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1	P. Ackerman	07.22.2015	Initial Version
2	P. Ackerman	08.27.2016	Reformatted for CMS upload; changed logo