eSensor Respiratory Viral Panel (RVP) Procedure

PURPOSE

- This procedure provides instructions for preparing samples, isolating nucleic acid, setting up the RT-PCR reaction, and running the *RVP* assay for the simultaneous detection of multiple respiratory viral nucleic acids in a sample

POLICY STATEMENT

- RVP testing is performed daily; samples must arrive by 0730 to provide same day results

ABBREVIATIONS

- EM: easyMAG
- EXC: extraction control
- F/T: freeze/thaw
- IC: internal control
- MM: master mix
- NA: Nucleic Acid
- NEGC: negative control
- NFW: nuclease free water
- RT-PCR: reverse transcription polymerase chain reaction
- POSC: positive control
- RSV: respiratory syncytial virus
- 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

DOCUMENTATION/RECORDS

- RVP Currents RUO Results Report
- RVP Detection Report, RUORV
- easyMAG Extraction Report
- LIS Incomplete and Completed worksheets
- Daily Maintenance Log

SAFETY CONSIDERATIONS

- Standard precautions for infectious agents. Refer to MB 2.02, Biohazard containment
- Use of engineering controls: Refer to MB 3.01 Engineering Controls to Prevent Nucleic Acid Contamination
- General Safety: MB 2.01 Safe Work Practices
- NucliSens EasyMAG Lysis Buffer and Wash Buffer 1 contain guanidine thiocyanate. Guanidine thiocyanate is harmful by inhalation, in contact with skin and if swallowed. Contact with acid liberates very toxic gas.
- *Caution:* Protective eyewear and PPE must be worn when working with concentrated Extran

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)	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

Equipment	Reagents	Supplies
Room 2 cont. ▪ 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	Extraction Controls (H1, H3, RSV, Flu B, hMPV) Sani-Cloth Bleach Wipes (10%) 70% alcohol 5% Extran MMQCI RVP Control Panel	easyMag disposable vessel strips and tips BioHit pipette tips BioHazard wipes Gripper rack Sharps disposal container

QUALITY CONTROL

A. Assay Controls

1. A POSC and NEGC must be included in each assay run.
2. The POSC serves as an extraction control and a reagent control.
3. Rotate POSC/EXC as follows:

Order	Viral Extraction Control
1	2009 H1N1
2	Seasonal Flu H3
3	Influenza B
4	RSV
5	hMPV

4. An IC is incorporated into each reaction mixture prior to extraction.
5. Include one POSC/EXC and one NEGC with each extraction run.
6. Bi – monthly (1st and 15th): Perform MMQCI eSensor RVP Control Panel[2]

B. QC Monitors:

Control	Control Monitor
Positive Control (POSC/EXC)	▪ POSC: Reagent failure and primer-probe integrity ▪ EXC: Lysis and/ or extraction failure; cross contamination
Negative Control (NEGC)	Reagent and/or environmental contamination, cumulative effect
Internal Control (IC)	PCR inhibition in specimen, reagent failure or process error

C. Before reporting patient results, all controls must yield valid results. Refer to MB 11.05, Refer to *Procedure I*, Evaluating and Interpreting Results.

PROCEDURE A: Follow the steps in the table below to organize and label samples

Numbering and Labeling

Activity	Step	Action	Related Doc
Sample Organization Room 2	1	Call worksheet **RVP**; use this worksheet for sample identification throughout testing.	MB 3.01 Engineering Controls
	2	Process up to 22 patient samples plus one POSC and NEGC per run. Position samples and controls as follows: Patient samples: 1 – nn POSC: 2nd to last position NEGC: Last tube	
Numbering	3	Using the RVP worksheet as a layout, organize patient samples and labels ▪ Number patients on worksheet (positions 1 – nn) ▪ Number each patient sample VTM tube according to worksheet. ▪ Number corresponding patient label according to worksheet ▪ Date one small label for each sample 1.5 ml micro-centrifuge tube ▪ Number one small label 1 – nn for eSensor cartridge	
Previously extracted	4	If sample(s), POSC and NEGC have been previously extracted, skip to *Procedure C*	
Tube sets	5	Each sample to be extracted requires one set of tubes: Patient: ▪ 2 ml cryo-vial; ▪ 1.5 ml micro-centrifuge tube (place in magnetic rack) POSC: ▪ 1.5 ml micro-centrifuge tube NEGC: ▪ 1.5 ml micro-centrifuge tube	
	6	Number caps of each set of patient sample tubes 1 – nn as needed; write POSC and NEGC on caps of last two 1.5 ml micro-centrifuge tubes	
Labeling	7	Label tube set, matching the number on the label to the number on the cap ▪ Place patient bar-coded label on 2 ml cryo-vial ▪ Placed dated small label on the 1.5 ml micro-centrifuge tube ▪ Label POSC and NEGC tubes with pre-printed labels	MB 1.01 Specimen Management
Transfer	8	Transfer patient samples into 2 ml cryo-vials with corresponding numbers.	
	9	Change gloves	

PROCEDURE B: Follow the steps in the table below for isolating nucleic acid

Extraction of Nucleic Acid, Room 2

Activity	Step	Action	Related Doc
Clean Room 2	1	Clean hood and equipment prior to processing, room 2 ▪ Sani-Cloth Bleach Wipes (10%) followed by water and 70% alcohol	RVP Workflow Guide
Reagents	2	Thaw IC at RT; vortex briefly and tap tube to settle contents ▪ one tube contains enough for 24 samples	
	3	Bring MagSil to room temp	

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Doc</th></tr>
<tr><td rowspan="4">Set-up easyMAG
Room 2</td><td>4</td><td>Set up the easyMAG instrument.<table><tr><th>Step</th><th>Prompt</th><th>Entry</th></tr><tr><td>a</td><td>Protocol</td><td>RVP</td></tr><tr><td>b</td><td>Sample type</td><td>Primary (on-board lysis)</td></tr><tr><td>c</td><td>Volume</td><td>0.200 mL</td></tr><tr><td>d</td><td>Eluate</td><td>60 µl</td></tr><tr><td>e</td><td>Matrix</td><td>Other</td></tr></table></td><td rowspan="4">MB 4.03
NucliSENS® EasyMag Procedure</td></tr>
<tr><td>5</td><td>Build worklist (Daily use icon): Scan bar-coded patient labels</td></tr>
<tr><td>6</td><td>Snap aspirator pipette strip(s) into easyMAG</td></tr>
<tr><td>7</td><td>Place easyMAG extraction strip(s) in carrier rack.
Consecutively number each well on the strip to correspond to patient samples, POSC and NEGC.</td></tr>
<tr><td rowspan="2">Prepare Samples</td><td>8</td><td>Add 200 µl of each sample, POSC & NEGC to related well avoiding air bubbles at the bottom of the well</td><td rowspan="2"></td></tr>
<tr><td>9</td><td>Change gloves after every 8 samples and when finished</td></tr>
<tr><td rowspan="3">Scan strip Barcodes</td><td>10</td><td>Snap extraction strip(s) into easyMAG</td><td rowspan="3"></td></tr>
<tr><td>11</td><td>Barcode strip location (A, B or C) and then barcode the extraction strip.</td></tr>
<tr><td>12</td><td>Touch the Silica icon, barcode the silica lot number and assign the lot number to the sample locations</td></tr>
<tr><td rowspan="2">Start lysis</td><td>13</td><td>Start lysis protocol (approx. 12 min)</td><td rowspan="2"></td></tr>
<tr><td>14</td><td>Change gloves</td></tr>
<tr><td rowspan="2">Add IC & silica</td><td>15</td><td>Vortex silica just prior to use and in-between strips</td><td rowspan="2"></td></tr>
<tr><td>16</td><td>After lysis, remove strips and pipette:
10 µl of internal control to each sample, changing tips between wells
50 µl of silica to each sample, changing tips between wells</td></tr>
<tr><td>Mix</td><td>17</td><td>Mix each strip after addition of silica with BioHit pipettor (P3) before advancing to next strip
Caution: avoid drips or aerosols that may cause cross-contamination</td><td></td></tr>
<tr><td rowspan="3">Start easyMAG

Tip: Set-up thermocycler during extraction</td><td>18</td><td>Snap extraction strips back into easyMAG</td><td rowspan="3"></td></tr>
<tr><td>19</td><td>Barcode strip location (A, B or C) and then barcode the strip.</td></tr>
<tr><td>20</td><td>Start extraction (approx. 34 – 40 minutes).</td></tr>
<tr><td>Freeze IC</td><td>21</td><td>Mark the cap of the IC to represent one F/T cycle; return to freezer
Maximum F/T cycles: 5 (split in two if necessary)</td><td></td></tr>
<tr><td rowspan="2">Clean
Room 2</td><td>22</td><td>Clean hood and equipment during extraction
Sani-Cloth Bleach Wipes (10%) followed by water and 70% alcohol</td><td rowspan="2"></td></tr>
<tr><td>23</td><td>Remove lab coat and change gloves; move to room 1</td></tr>
</table>

PROCEDURE C: Follow the steps in the table below for preparing the MM and setting up the RT-PCR reaction

MasterMix Preparation and RT-PCR Reaction Set-up, room 1

Activity	Step	Action	Related Doc
Room 1 **Thaw reagents**	1	Remove RVP enzyme and PCR mix from freezer ▪ Place enzyme in cold block; refrigerate until use ▪ Thaw PCR mix at RT up to 1 h	
	2	Clean hood and equipment prior to mm preparation, room 1 ▪ 5% Extran followed by 70% alcohol	
Prepare MM	3	Vortex PCR mix 2 – 5 s, making sure it is completely thawed	MB 11.04 Control and Reagent Preparation
	4	Centrifuge the enzyme and PCR mix; place both reagents in cold block	
	5	Prepare MM according to number of reactions needed; Refer to set-up table	
	6	Vortex MM and centrifuge ***Caution:*** Do not mix reagents by pipetting up and down	
Tip: Make MM while eluates sit for 10 min in magnetic rack	7	Mark the cap of the enzyme and PCR mix to represent one F/T cycle; return to freezer ▪ Maximum F/T cycles: **5**	
	8	Remove required number of PCR strip tubes for bag; reseal	
	9	Color code: Number PCR strip tubes 1 - nn; place in cold block	
	10	Pipette 30 µl of MM into each tube; close caps	
	11	***Note:*** Change gloves between strips of 8	
Clean Hood	12	Clean hood and pipettes with 5% Extran followed by alcohol	
	13	Remove lab coat and return to room 2 with prepared MM	
Note: ***Keep MM cold. Use MM within 30 min of preparation***			
Extraction completion	14	When the easyMAG displays **Finished,** remove the extraction strip(s); place in the carrier rack	MB 4.03 NucliSENS® EasyMag
Room 2	15	Set pipette at 70 µl	
Eluates	16	Transfer eluates to corresponding 1.5 micro-centrifuge tubes within 30 min; do not disturb silica button ▪ ***Caution:*** Silica inhibits amplification	
	17	Allow eluates to sit in magnetic rack for 10 min before setting up PCR reaction	

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Doc</th></tr>
<tr><td>Set up PCR Reaction
Room 2</td><td>18</td><td>Add 5 µl of patient eluates, POSC and NEGC in that order to PCR tubes, opening one tube at a time
<table><tr><th>Step</th><th>Action</th></tr><tr><td>a</td><td>Open tube and add eluate</td></tr><tr><td>b</td><td>Press cap firmly to close</td></tr><tr><td>c</td><td>Eject tip</td></tr><tr><td>d</td><td>Open next tube to prepare for loading
▪ Note: Tube serves as a location marker</td></tr><tr><td>e</td><td>Repeat a – d until all tubes complete</td></tr><tr><td>f</td><td>Change gloves between strips of 8</td></tr><tr><td>g</td><td>Vortex strips 5 s; return to cold block</td></tr><tr><td>h</td><td>Store unused portion of eluate at – 70° C when all testing is complete</td></tr></table></td><td></td></tr>
<tr><td rowspan="2">Clean
Room 2

Tip: Start PCR before cleaning in room 2
Procedure D</td><td>19</td><td>Clean hood and equipment
▪ Sani-Cloth Bleach Wipes (10%) followed by water and 70% alcohol</td><td></td></tr>
<tr><td>20</td><td>Change lab coat and gloves; move to room 3</td><td></td></tr>
</table>

PROCEDURE D: Follow the steps in the table below for *PCR* amplification

PCR Amplification

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Doc</th></tr>
<tr><td>Thermocycler Set-up
Room 3

Tip: Set-up before or during Extraction</td><td>1</td><td>Set up thermocycler; take run specific patient labels into room 3
<table><tr><th>Step</th><th>Key</th><th>Action/Entry</th></tr><tr><td>a</td><td>------</td><td>Turn on the thermocycler (power switch back right)</td></tr><tr><td>b</td><td>------</td><td>Set tube retainer tray on heat block (A1 upper left corner)</td></tr><tr><td>c</td><td>------</td><td>Spin PCR strip tubes 5 s</td></tr><tr><td>d</td><td>------</td><td>Place PCR strip in retainer tray</td></tr><tr><td>e</td><td>------</td><td>Close lid; pull handle down</td></tr><tr><td>f</td><td>F1</td><td>Select RUN</td></tr><tr><td>g</td><td>-----</td><td>↓ Highlight rvp rt-pcr program</td></tr><tr><td>h</td><td>F1</td><td>Select START</td></tr><tr><td>i</td><td>-----</td><td>Confirm reaction volume 35µl</td></tr><tr><td>j</td><td>F1</td><td>Select START</td></tr><tr><td>K</td><td>-----</td><td>RT-PCR program runs 3 hour</td></tr><tr><td>l</td><td>------</td><td>Change gloves</td></tr></table></td><td></td></tr>
<tr><td>Tip: Prepare “Hyb” soln before end of PCR
Procedure E

End of Run</td><td>2</td><td>When a run completes:
<table><tr><th>Step</th><th>Key</th><th>Action/Entry</th></tr><tr><td>a</td><td>------</td><td>Line beneath 4° C will be flashing ∞</td></tr><tr><td>b</td><td>Stop</td><td>Press the Stop key; the Confirm Stop screen appears</td></tr><tr><td>c</td><td>Stop</td><td>Press the Stop key again</td></tr><tr><td>d</td><td>------</td><td>The End of Run screen appears</td></tr><tr><td>e</td><td>F5</td><td>Select Exit to return to main menu</td></tr></table></td><td></td></tr>
</table>

Activity	Step	Action	Related Doc
Room 3	3	Slowly open lid; pull up handle to release and lift	
	4	***Caution:*** Tube caps may pop open when: ▪ The cover is opened quickly ▪ The block temperature is above 27° C	
Remove tubes	5	Remove PCR strips	
	6	Centrifuge strips for 10 s	
	7	Place PCR tubes in cold block for the exonuclease digestion	*Refer to Procedure E*
	8	Change gloves	
	9	Alternative: Amplified tubes can be refrigerated at 2 - 8° C for one week or frozen at -70° C for 1 month	

PROCEDURE E: Follow the activities below for preparing hybridization buffer

Hybridization Solution Preparation

Activity	Step	Action	Related Doc
Clean and thaw reagents 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	
	3	Vortex and centrifuge or tap lightly	
Prepare "Hyb" solution	4	Prepare hybridization buffer according to number of reactions needed; Refer to Hybridization buffer set-up table; stable up to 4 hours at RT	
	5	Label 2 ml tube "Hyb" (may need to prepare 2 tubes for sufficient volume)	

Step	Action
a	Add reagents to Hyb tube in order 1. Signal buffer 2. Buffer 1 3. Buffer2 (white precipitate will appear after addition)
b	Vortex at setting 10 for 3 – 5 s to clear precipitate
c	Centrifuge 3 – 5 s
d	***Note***: Warm with hands if precipitate does not disappear; vortex

Freeze reagents	6	Mark the cap of the buffer tubes to represent one F/T cycle	
	7	Change gloves; return detection reagents to freezer	

PROCEDURE F: Follow the steps in the table below for exonuclease digestion in room 3

Exonuclease Digestion

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related doc</th></tr>
<tr><td rowspan="2">Room 3</td><td>1</td><td>Remove the exonuclease from freezer; centrifuge and put in cold block
▪ Do not vortex</td><td rowspan="2"></td></tr>
<tr><td>2</td><td>Saturate orange BioHazardous wipe with 10% bleach; place in hood</td></tr>
<tr><td>Adding Exonuclease
Room 3</td><td>3</td><td>Slowly pipette 5µl of exonuclease, opening one tube at a time and touching bleach pad in-between tubes
▪ Caution: Change gloves immediately if contamination is suspected
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>Touch fingers to bleach pad between PCR tubes</td></tr>
<tr><td>b</td><td>Open tube slowly ; avoid touching the inside of cap</td></tr>
<tr><td>c</td><td>Pipette exonuclease slowly and evenly into tube, mid-way down</td></tr>
<tr><td>d</td><td>Press cap firmly to close</td></tr>
<tr><td>e</td><td>Eject tip</td></tr>
<tr><td>f</td><td>Open next tube to prepare for loading
▪ Note: Tube serves as a location marker</td></tr>
<tr><td>g</td><td>Repeat steps a – f until exonuclease is added to all tubes</td></tr>
<tr><td>h</td><td>Change gloves between strips of 8 and when leaving the hood</td></tr>
<tr><td>i</td><td>Vortex strips and centrifuge PCR tubes, 5 s each</td></tr>
<tr><td>j</td><td>Return PCR strip(s) to thermocycler</td></tr>
</table></td><td>eSensor User Manual (installed on the XT-8, HELP button)</td></tr>
<tr><td rowspan="3">Exonuclease program

Tip: set up XT-8, label cartridges during Exo-digest</td><td>4</td><td>Select exo-digest program, confirm 40 µl and start
▪ Refer to Procedure D, step 2</td><td rowspan="3">Refer to Procedures G, H</td></tr>
<tr><td>5</td><td>Run time approx. 25 min</td></tr>
<tr><td>6</td><td>Change gloves</td></tr>
<tr><td>Clean</td><td>7</td><td>Clean hood and equipment
Sani-Cloth Bleach Wipes (10%) followed by water and 70% alcohol</td><td></td></tr>
<tr><td rowspan="3">End of Run</td><td>8</td><td>End of run: remove PCR tubes from thermocycler; Refer to Procedure D, steps 3 - 5</td><td rowspan="3"></td></tr>
<tr><td>9</td><td>Centrifuge tubes 10 s; place in 0.2 ml rack</td></tr>
<tr><td>10</td><td>Change gloves</td></tr>
</table>

PROCEDURE G: Follow the activities below for setting up detection cartridges, room 3

Setting up Detection Cartridges, room 3

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Doc</th></tr>
<tr><td rowspan="3">Label cartridges

Room 3</td><td>1</td><td>Label cartridges with small patient label numbered 1 - nn; place in cartridge tray</td><td rowspan="3"></td></tr>
<tr><td>2</td><td>If “Hyb” solution was prepared in advance, vortex and spin prior to use</td></tr>
<tr><td>3</td><td>Saturate orange BioHazardous wipe with 10% bleach; place in hood</td></tr>
<tr><td>Add “Hyb” solution to PCR tube, 100 µl</td><td>4</td><td>Slowly add 100 µl of “Hyb” solution to each PCR tube
Caution: Change gloves immediately if contamination is suspected
<table><tr><th>Step</th><th>Action</th></tr><tr><td>a</td><td>Touch fingers to bleach pad</td></tr><tr><td>b</td><td>Open tube slowly ; avoid touching the inside of cap</td></tr><tr><td>c</td><td>Pipette “Hyb” soln slowly and evenly into PCR tube, avoiding aerosols</td></tr><tr><td>d</td><td>Close cap</td></tr><tr><td>e</td><td>Eject tip</td></tr><tr><td>f</td><td>Open next tube to prepare for loading
Note: Tube serves as a location marker</td></tr><tr><td>g</td><td>Repeat steps a – e for additional tubes</td></tr><tr><td>h</td><td>Note: Change gloves between strips of 8</td></tr></table></td><td>RVP Technical Support and Troubleshooting</td></tr>
<tr><td rowspan="2">Add “Hyb” / Sample to cartridge, 125 µl</td><td>5</td><td>Pipette 125 µl of “Hyb” sample mix to corresponding cartridge<table><tr><th>Step</th><th>Action</th></tr><tr><td>a</td><td>Open caps on all cartridges by inverting tray</td></tr><tr><td>b</td><td>Touch fingers to bleach pad between PCR tubes</td></tr><tr><td>c</td><td>Open PCR tube slowly ; avoid touching the inside of cap</td></tr><tr><td>d</td><td>Pipette “Hyb” sample mix into cartridge</td></tr><tr><td>e</td><td>Close cap</td></tr><tr><td>f</td><td>Eject tip</td></tr><tr><td>g</td><td>Continue until all cartridges are loaded</td></tr><tr><td>h</td><td>Secure all caps with a Sharpie pen, checking that all are level</td></tr><tr><td>i</td><td>Note: Change gloves between trays of 8</td></tr></table></td><td rowspan="2"></td></tr>
<tr><td>6</td><td>Change gloves</td></tr>
<tr><td>Insert cartridges</td><td>7</td><td>Insert cartridges into eSensor XT-8; Refer to Procedure H</td><td></td></tr>
<tr><td>Clean</td><td>8</td><td>Decontaminate hood and equipment
Sani-Cloth Bleach Wipes (10%) followed by water and 70% alcohol
UV for 15 min</td><td></td></tr>
</table>

PROCEDURE H: Follow the activities below for testing on the eSensor Xt-8 instrument

eSensor XT-8 instrument

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Doc</th></tr>
<tr><td>Power ON</td><td>1</td><td>To turn instrument on, press round button near the base</td><td></td></tr>
<tr><td>Instrument set-up

Room 3</td><td>2</td><td>Set-up instrument
<table>
<tr><th>Step</th><th>Prompt</th><th>Action/Entry</th></tr>
<tr><td>d</td><td>------</td><td>Touch keyboard icon</td></tr>
<tr><td>e</td><td>Username</td><td>Enter username using on screen keyboard</td></tr>
<tr><td>f</td><td>Password</td><td>Enter password ***** (case sensitive)</td></tr>
<tr><td></td><td>-----</td><td>Touch Login icon</td></tr>
<tr><td>g</td><td>-----</td><td>Touch cartridge location slot A1</td></tr>
<tr><td>h</td><td>-----</td><td>Scan patient Acc. No. using label barcodes in consecutive order 1 – nn, LED: blue → orange</td></tr>
<tr><td>i</td><td>-----</td><td>Touch Reagent Barcode field</td></tr>
<tr><td>j</td><td>-----</td><td>Scan reagent barcode
▪ Located on the RVP Detection Reagent box
▪ Remove from RVP Detection Reagent box
▪ Place on Cartridge box cover</td></tr>
</table></td><td></td></tr>
<tr><td rowspan="4">Insert cartridges</td><td>3</td><td>Insert cartridge(s) logo side up; gently push until it clicks in place</td><td rowspan="4">RVP Common Issues and Solutions</td></tr>
<tr><td>4</td><td>Firmly slide the module lever to the left
Caution: If you feel resistance, do not continue to push or pull lever; check that the cartridge is seated correctly</td></tr>
<tr><td>5</td><td>Led lights will change from orange → yellow (ready)</td></tr>
<tr><td>6</td><td>Verify information boxes: If the RVP protocol does not appear, remove cartridge and repeat insertion</td></tr>
<tr><td rowspan="3">Start</td><td>7</td><td>Touch the Start to begin hybridization and scanning protocol</td><td rowspan="3"></td></tr>
<tr><td>8</td><td>Blinking Green: XT is checking connections; wait until flashing stops</td></tr>
<tr><td>9</td><td>Solid Green: testing is in progress; run time 42 min</td></tr>
<tr><td>LED Status</td><td>10</td><td>LED Color Chart
<table>
<tr><th>Color</th><th>State</th><th>Status</th><th>Action</th></tr>
<tr><td>Blue</td><td>Solid</td><td>Empty</td><td>Available; insert cartridge</td></tr>
<tr><td>Orange</td><td>Solid</td><td>Info needed</td><td>Enter Acc. No.</td></tr>
<tr><td>Yellow</td><td>Solid</td><td>Ready</td><td>Press Start button</td></tr>
<tr><td>Green</td><td>Flashing</td><td>Running</td><td>Checking connections</td></tr>
<tr><td>Green</td><td>Solid</td><td>Running</td><td>Test in progress</td></tr>
<tr><td>Blue</td><td>Flashing</td><td>Complete</td><td>Test complete</td></tr>
<tr><td>Red</td><td>Flashing</td><td>Error</td><td>Troubleshoot</td></tr>
</table></td><td></td></tr>
</table>

PROCEDURE I: Follow the activities below for run completion and interpretation of results

Run Completion and Results

Activity	Step	Action	Related Doc
Run Completion	1	Flashing blue LED: remove cartridge and place in ziplock bag; discard in red trash	
	2	Touch Reporting Tab	

Activity	Step	Action	Related Doc
Reports/results	3	Select search criteria ▪ Default criteria will display all reports generated on the current date ▪ Touch individual samples to be viewed/printed or Select All button	MB 11.06 Troubleshooting RVP Retest Recommendations RVP Technical Support and Troubleshooting RVP Common Issues and Solutions
Room 3	4	Select Report type: ▪ Currents RUO ▪ RUORV	
	5	***Review RVP Detection Report (RUORV) Summary for positive targets, errors and troubleshooting*	
	6	Review nA values on Currents RUO report ; print ***Note:*** Dashes in the threshold column (---) indicate an error	
	7	Attach printed reports to RVP worksheet and extraction report	
Interpretation	8	Interpretation of results on the RVP Detection Report: Table 1	
Valid run	9	Before reporting patient results, all controls must yield valid results Control / Assay Result / IC Result POSC / Target detected / NA NEGC / Target not detected / Pass	
Invalid Run	10	Invalid Run ▪ Failure of controls (POSC or NEGC) invalidates run ▪ Do not report patient results until problem is investigated and resolved ▪ Record problem/action in the QC failure log	MB 3.02 Wipe Testing for Contamination

Table 1: Interpretation of Results; for additional information refer to RVP Retest *Recommendations by Report Type*

Report	Result message	Possible Explanations	Action
RUORV	Positive	▪ Test successful ▪ Positive for indicated analyte	▪ **Report results ≥ 10 nA** ▪ **Review results 3 – 10 nA** before reporting for questionable results that may require repeat testing
RUORV	Target not detected	▪ Test successfully completed ▪ Internal control was detected ▪ Result was negative	▪ Report results
RUORV	Error for any target	▪ Electrode or instrument failure	▪ Contact GenMark technical support for daily password to retest cartridge, 1-800-373-6767, option 2 ▪ Repeat RT-PCR and XT-8 analysis once; use extracted sample
RUORV	Fail (internal control failure)	▪ Failed internal control of primary sample	▪ If one or more targets are positive in the sample, retest is not necessary
RUORV	Fail (internal control failure)	▪ Failed internal control of primary sample ▪ Specimen inhibition ▪ Poor amplification ▪ Poor extraction	▪ If no viral targets are positive, repeat extraction from primary sample after F/T cycle
RUORV	Flu A only, but no subtype, possible variant **Send to MDH	▪ Possible test successful but no subtype ▪ Subtype is not H1, H3 or 2009 H1N1 ▪ Poor amplification ▪ Poor extraction	▪ Re-extract sample and repeat testing ▪ Still no subtype, send to MDH. Sample may contain a novel or newly emerging Flu A virus
RUORV	Positive for Influenza A 2009 H1N1, target not detected for Influenza A	▪ Influenza A below the level of detection ▪ Possible contamination	▪ Re-extract sample and repeat testing ▪ Report results if retest remains positive for 2009 H1N1

Report	Result message	Possible Explanations	Action
RUORV	Positive for Influenza A and multiple subtypes	▪ Possible co-infection ▪ Possible contamination	▪ Re-extract primary sample and repeat testing
Currents	"Fail" for 2 or more internal controls in run	▪ Poor amplification ▪ Poor recovery from extracted sample ▪ System error	▪ Re-extract run and repeat testing
Currents	Failed POSC or NEGC	▪ Failed run ▪ Possible contamination	▪ Repeat run extraction, RT-PCR and XT analysis; do not report patient results

PROCEDURE J: Follow the activities below for instrument shutdown

eSensor® XT-8 Shutdown

Activity	Step	Action	Related Doc
Log out	1	Touch the **Log Out** button on the lower left side	
Shutdown	2	Touch the **Shutdown** button on the Login screen	
	3	The instrument will automatically shut off	
	4	Once turned off, place the dust cover on the instrument for protection	

METHOD PERFORMANCE

1. Clinical Performance: Children's validation/verification study (6)
    - NW/NASP – 100% sensitivity with comparator methods
    - Bronchoscopy specimens – 100% sensitivity with comparator methods
2. Analytical Sensitivity: 10^{-2} – 10^{3} $TCID_{50}$/mL

PROFICIENCY TESTING

- CAP IDR – Infectious Disease Respiratory Panel

ALTERNATE METHOD

1. Send specimens to Fairview University Infectious Disease Diagnostic Laboratory – Virology (UMMC-East Bank)
2. Fairview University code: RVPCR
3. CHC Sunquest Order code: MBAT
4. Logistics:
    - 2 NP swabs: VTM
    - Nasopharyngeal aspirate: VTM
    - Nasopharyngeal washing: 0.5 – 2 mL shipped refrigerated in sterile container or VTM
    - Bronchoalveolar lavage (BAL): 0.5 – 2 mL shipped refrigerated in sterile container or VTM

LIMITATIONS

1. Adenovirus C has been observed to cross-react with Adenovirus D (serotype 9) and F (serotype 41). If definitive speciation is necessary, an alternative method should be performed (sequence analysis).
2. Enterovirus D68 (2) and Poliovirus have been observed to cross-react with human rhinovirus. Both are members of the family of Picornaviridae that also includes human rhinovirus. If enteroviral or polio infection are suspected, alternate testing should be performed (cell culture).
3. This is a qualitative test and does provide quantitative information regarding virus detected.

4. Results from this test must be correlated with clinical history when evaluating the patient.
5. False negative results may occur due to loss of nucleic acid. Viral detection is dependent upon adequate specimen collection, transport, and handling.
6. Analyte targets may persist *in vivo*, independent of virus viability.
7. Live intranasal influenza virus vaccine may cause false positive results for Influenza A, H1, H3, 2009 H1N1, and Influenza B.
8. Variant influenza A H3N2 virus (H3N2v) will be detected as seasonal influenza A H3
9. This test should not be used as a test for cure.
10. There is a risk of false negatives due to sequence variation in the viral target.
11. This assay detects both viable and nonviable virus. Test performance depends on viral load in the specimen and may not correlate with cell culture performed on the same specimen.

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9. Elena B. Popowitch, O'Neill, S.S.,Miller, M.B., Comparison of the Biofire FilmArray RP, GenMark eSensor RVP, Luminex xTAG RVPv1, and Luminex xTAG RVP Fast Multiplex Assays for Detection of Respiratory Viruses, J Clin Micro, 51: 1528-1533, 2013
10. GenMark User Manual, installed on the XT-8 instrument

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1	P. Ackerman	05.02.15	Initial Version
2	P. Ackerman	08.27.16	Reformatted for CMS upload; changed logo; added troubleshooting hyperlinks