# 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 |  |
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**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 Panel3 | 1 | The RVP control panel consists of 2 vials M244 and M245, single use only |
| Room 2 | 2 | Allow the vials to warm to RT |
| 1X use | 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 monthly alternating M244 and M245; record results [MB 11.08.F4](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/QC/212410.pdf) |
|  | 8 | Freeze eluates at -70⁰ C |
| Internal Control | 1 | No preparation necessary |
| Room 2 | 2 | Remove Internal Control (IC) from -70⁰ 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 -70⁰ C freezer |
| F/T 5X | 5 | Freeze/thaw cycles up to 5X |
| Negative Control | 1 | Aliquot 300 µl VTM in 1.5 ml micro-centrifuge tubes |
| **(NEGC)** | 2 | Label tubes with NEGC and prep date using preprinted labels |
| Room 1 | 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 |
|  | 3 | Serially dilute each stock suspension using NFW to prepare a 10-4 working dilution (total volume approx 25 ml) |
| **Working suspension** | 4 | Add 5 ml of VTM to the suspension; mix well |
| Room 2 | 5 | Extract 200 µl of the working dilution (each control) |
|  | 6 | Perform Simplexa FABR PCR testing to determine Ct value; target range 30 – 33 |
| **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** | 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 |
| Room 2 | 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](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/QC/212336.pdf) |
|  | 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) |
|  | 1 | Pool 2 - 3 known hMPV positive RVP (~nA 100) residual samples; mix well |
| **hMPV PCTL/EXC** | 2 | Serially dilute suspension using NFW to prepare a 10-4 working dilution (total volume approx 25 ml) |
| **RVP** | 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** | 8 | Label a set of 1.5 mL micro-centrifuge tubes using preprinted labels with prep date |
| Room 2 | 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](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/QC/212336.pdf) |
|  | 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) |
| **RVP PCTL/EXC Rotation** | 14 | Rotate EXC as follows:   |  |  | | --- | --- | | Order | Viral Extraction Control | | 1 | 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** |
| --- | --- | --- |
|  | 1 | ***MM must be used within 30 min of preparation****.* |
| **MM** | 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 |
| Room 1 | 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](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/RVP/212299.pdf) |
|  | 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](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/RVP/212299.pdf)   |  |  |  |  | | --- | --- | --- | --- | | Component | Volume/reaction | Calculation | Volume (µl) | | RVP PCR Mix | 28.6 µl | 28.6 \* N \*1.1 = |  | | 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** |
| --- | --- | --- |
|  | 1 | Clean hood and equipmentSani-Cloth Bleach Wipes (10%) followed by water and 70% alcohol |
| **Hyb solution** | 2 | Thaw Signal buffer, Buffer 1 and Buffer 2 at RT |
| Room 3 | 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](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/RVP/212299.pdf); stable up to 4 hours at RT |
| **Hyb solution**  **Cont.** | 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 | |
|  | 6 | Mark the cap of the buffer tubes to represent one F/T cycle |
|  | 7 | Change gloves; return detection reagents to -20⁰ C freezer |
| **Hyb solution calculations** | 8 | Volume Calculations for Hyb solution: N = Total number of reactions in run including POSC and NEGC, [MB 11.04.A1](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/RVP/212299.pdf)   |  |  |  |  | | --- | --- | --- | --- | | Component | Volume/reaction | Calculation | Volume (µl) | | RVP Signal Buffer | 70 µl | 70 \* N \* 1.1 = |  | | Buffer 1 | 10 µl | 10 \* N \* 1.1 = |  | | Buffer2 | 20 µl | 20 \* N \* 1.1 = |  | | Total Hyb solution volume = | | |  | |

**PROCEDURE D:** Follow the activity below for preparing miscellaneous reagents

Preparing miscellaneous reagents

| Reagent | Step | **Action** |
| --- | --- | --- |
| 10% Bleach | 1 | Prepare in dish room. |
| Dish room | 2 | Make working solution as follows:   |  |  |  | | --- | --- | --- | | Step | Reagent (10% bleach) | Volume | | 1 | House hold bleach (5 – 6 %) | 500 ml | | 2 | Water | 2000 ml | | 3 | Alconox (add for contamination clean-up) | 25 g | |
| **70% alcohol** | 1 | Prepare from 100% Dehydrant alcohol located in the Flammable cabinet in the Recycling room. |
| Room 3 or Recycling room | 2 | Make working solution as follows:   |  |  |  | | --- | --- | --- | | Working Volume | 100% Dehydrant | Water | | 1000 ml | 700 ml | 300 ml | |

**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](http://www.mmqci.com)

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| 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 |
|  | 3 | J. Laramie | 11.27.2017 | Changed MMQCI testing schedule: no testing with new lot/shipment. Rotation of panels monthly instead of weekly. |