RVP Eluate, Lysate and Reagent Storage and Stability

PURPOSE

- This procedure provides instructions for storage and stability of sample eluates, sample lysates and reagents.

SAFETY CONSIDERATIONS

- Standard precautions. Refer to MB 2.02 Biohazard Containment
- NucliSens Lysis buffer and EasyMag Wash buffer 1 contain guanidine thiocyanate. Guanidine thiocyanate is harmful by inhalation, in contact with skin and if swallowed. Contact with acid liberates cyanide gas.
- Avoid contact of Signal buffer on skin, eyes or mucous membranes. If contact occurs, flush with water for 15 min.

ABRREVIATIONS

- NA: Nucleic Acid
- NEGC: negative control
- NFW: nuclease free water
- RT-PCR: reverse transcription polymerase chain reaction
- 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: *Clean room* ▪ Adjustable pipettes ▪ Cold block ▪ Freezer, -20° C ▪ Laminar air-flow hood ▪ Refrigerator 2 – 8° C ▪ Vortex mixer Room 2: *Processing room* ▪ Adjustable pipettes ▪ BioHit 8 channel pipette ▪ Bio-Safety Cabinet (BSC) ▪ Cold Block ▪ Freezer, -70° C ▪ Mini-centrifuge ▪ NucliSens easyMag ▪ Refrigerator 2 – 8° C ▪ Tube racks, 1.5 – 2 ml ▪ Vortex mixer Room 3: *Amplification room* ▪ 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)
	Extraction Controls (H1, H3, RSV, FluB, hMPV)	PCR 8 tube strips with caps
	viral transport media (VTM)	easyMag vessel strips
	Sani-Cloth Bleach Wipes (10%)	BioHit pipette tips
	70% alcohol	BioHazard wipes
	5% Extran	
	MMQCI eSensor RVP Control Panel: Product No. M243	

PROCEDURE A: Follow the activity below for the proper storage of sample lysates and eluates

Storage and Stability of Lysates and Eluates

Activity	Step	Action	Related Documents
Lysates Room 2	1	**Lysates**: Perform On-Board Lysis buffer dispensing on the EasyMag Temperature: Room temp — Stability: 4 hr	MB 1.01 Specimen Management
Eluates (purified NA) Room 2	2	**Eluates:** After extraction, transfer to a 1.5 microcentrifuge tube within 30 min Room temp — 1 hr 2 – 8° C — 7 day – 70° C — 1 year	MB 2.01 Safe Work Practices
Frozen eluates (purified NA) Room 2	3	**Frozen eluate**: thaw sample in refrigerator; vortex briefly before use.	
	4	Sample testing should not be done after more than 2 freeze-thaw cycles.	

PROCEDURE B: Follow the activity below for proper storage of reagents. Refer to Tables 1 – 5.

Information for Reagent Storage

Activity	Step	Action	Related Doc
General Information		*Clean gloves are required prior to handling new reagents*	MB 5.02 Standards of Practice Waste Management 912.04
	1	RVP reagents are shipped frozen on dry ice ▪ Do not use reagents if thawed upon arrival ▪ Do not use reagents if vials have been damaged ▪ Contact *GenMark Customer Service at 1-800-373-6767*	
	2	Store reagents at recommended temperature until expiration date located on the original box unless otherwise noted. Refer to Tables 1 – 5.	
	3	Discard reagents that have not been stored properly or have expired according to the Organizational Waste Management policy	
	4	Do not mix kit reagents from different lots.	
	5	Remove only the required amount of reagents from storage needed for testing.	
	6	Protect from excess heat and light	
	7	Do not allow contact with reactive vapors from bleach or Extran or dust as these may affect the performance.	
Lysis Buffer	8	Lysis buffer may have a slight yellowish color that does not have an effect on test performance.	
	9	Check lysis buffer for crystals due to high salt concentration. ▪ Crystals will dissolve when the reagents are warmed at 37° C.	
MagSil	10	Do not freeze silica or store in magnetic rack. ▪ *Caution*: Binding performance will be decreased	

Activity	Step	Action	Related Doc
Freeze/Thaw (F/T) cycles	11	***Aliquot*** reagents during low volume times if the F/T cycle exceeds 5 X	

Season	Test Volume	No. of aliquots
Winter	High	Original tube (none)
Spring/Fall	Medium	Original tube (none)
Summer	Low	Original tube + 1

REAGENT STORAGE CONDITIONS

Table 1: eSensor RVP kit contents: Product No. MT005102

Reagent	Component	Packaging and Quanity	Storage		Stability	# F/T cycles
			Temp (° C)	Location		
eSensor RVP Cartridges	RVP cartridges	6 foil bags, 8 per bag	10 – 25	Room 3	4 weeks after bag opened	NA
eSensor RVP Amplification	RVP enzyme Mix	2 vials, 40 µl each	-15 to -30	Room 1	expiry date	5 X
	RVP PCR Mix	2 vials, 1000 µl each				
	MS2 Internal Control	2 vials, 300 µl each				
eSensor RVP Detection	RVP Signal Buffer	2 vials, 2200 µl each	-15 to -30	Room 3	expiry date	5 X
	Buffer 1	2 vials, 350 µl each				
	Buffer 2	2 vials, 700 µl each				
	Exonuclease	2 vials, 145 µl each				

Table 2: Molecular Grade Water (RNase and DNase free)

Reagent	Unopened/Opened		Aliquot Storage		In Use Aliquots	
	Temp	Location	Temp (° C)	Location	Temp (° C)	Location
Nuclease free water (NFW)	RT	Room 1	2 – 8	Room 1	2 – 30	Room 2

Table 3: easyMAG Extraction Reagents

Reagent	Unopened Reagent		Stability	Opened Reagent		Stability
	Temp (° C)	Location		Temp (° C)	Location	
Lysis buffer (store in dark)	2 – 30	Room 2	expiry date	2 – 30	Room 2	30 days
Buffer 1 (store in dark)	2 – 30	Room 2	expiry date	2 – 30	Room 2	30 days
Buffer 2 (store in dark)	2 – 30	Room 2	expiry date	2 – 30	Room 2	30 days
Buffer 3 (store in dark)	2 – 8	Room 2	expiry date	2 – 30	Room 2	30 days
MagSil	2 – 8	Room 2	expiry date	2 – 8	Room 2	expiry date

Table 4: Daily POSC/Extraction (EXC)/Negative (NEGC) Control

Reagent	Temp (° C)	Location	Stability	Temp (° C)	Location	Stability
POSC/ Process Control in matrix	≤ 70	Room 2	1 year Freeze/thaw: 1X	2 – 8	Room 2	7 days [hMPV: 2 days]
Negative Control, VTM (NEGC)	2 – 8	Room 1	NA	2 – 8	Room 2	expiry date

Table 5: MMQCI eSensor® XT-8™ RVP Control

Reagent	Unopened Reagent		Stability	Opened Reagent		Stability
	Temp (° C)	Location		Temp (° C)	Location	
MMQCI RVP Control Panel[3]	≤ -20	Room 2	expiry date	18 - 25	Room 2	Single use

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	5.6.2015	Initial Version
2	P. Ackerman	6.29.2015	Added Table 5: RVP Control
3	P. Ackerman	08.27.2016	Reformatted for CMS upload; changed logo