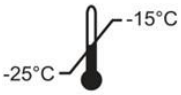
Instructions For Use

CE IVD *For In Vitro Diagnostic Use*



Store at -25°C to -15°C

REF RM-RT50

50

geneMAP™ Respiratory Master Panel

For Real-Time PCR

Multiplex Real-time PCR System for detection of respiratory pathogenes

(Inf A, Inf A (H1N1), Inf B, NL63, 229E, OC43, HKU1, AdV, MPV, HRV, HBoV, PIV1, PIV2, PIV3, PIV4, HEV, HPeV, RSV A/B, MP)

4-PLEX (FAM, VIC, ROX, CY5)

Validated on:

* Biorad® CFX96, CFX384 Real-time PCR System (Bio-Rad)
* ABI® 7500 Real-time PCR System (Thermo-Scientific)
* RotorGene Q5/Q6 Realtime PCR Systen (Qiagen)
* MIC qPCR System (Bio Molecular Systems)
* BaseTyper-Pentabse
* LightCycler480 II (Color Compansation)

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1. Intended Use

The geneMAP™ Respiratory Master Panel kit is qualitative in vitro assay (Multiplex qRT-PCR) for the detection of

1. Influenza A (Inf A),
2. Influenza A-H1pdm09 (H1N1),
3. Influenza B (Inf B),
4. Coronavirus NL63 (NL63),
5. Coronavirus 229E (229E),
6. Coronavirus OC43 (OC43),
7. Coronavirus HKU1 (HKU1),
8. Adenovirus (AdV),
9. Metapneumovirus A & B (MPV),
10. Human Rhinovirus A, B & C (HRV),
11. Human Bocavirus1,2,3,4 (HBoV),
12. Parainluenza Virus 1 (PIV1),
13. Parainfluenza Virus 2 (PIV2),
14. Parainfluenza Virus 3 (PIV3),
15. Parainfluenza Virus 4 (PIV4)
16. Human Enterovirus (HEV),
17. Human Parechovirus (HPeV),
18. Respiratory syncytial virus A & B (RSV A/B),
19. Mycoplasma pneumoniae (MP)

From Nasopharyngeal/Nasal aspirate, Nasopharyngeal swab, Oropharyngeal swab, Bronchoalveolar lavage, lower respiratory tract aspirates for people with or without clinical symptom associated with viral pneumonia.

The geneMAP™ Resipratory Master Panel Kit usage specifically for trained scientists and lab techinicans in Healtcare and Medical Laboratories.

2. Safety Instructions & General Warnings

- This kit must be used strictly in accordance with the instructions provided in this manual, and only in combination with validated reagents and instruments. Any off-label use of this product, as well as any modification of its components, will nullify Genmark's liability.
- Obey proper laboratory safety protocols when working with chemicals and specimens.
- The protocol can be performed by only professional and trained personal.
- Perform the protocol in a well-ventilated and well-lit environment.
- Store the kit and its components within recommended temperature range in de-frost refrigerators. Do not use no-frost refrigerators.
- Avoid skin contact with any of the reagents in the kit
- Wipe workspace surfaces with 10% bleach followed by 70% alcohol.
- Instruments may exhibit performance variations due to differences in electrical currents and power outlets, as well as the effects of maintenance and calibration. It is the responsibility of the user to ensure that all instruments are properly maintained and regularly calibrated according to the manufacturer's guidelines. Genmark disclaims responsibility for any performance issues arising from improper maintenance, calibration, or variations in electrical supply.
- All biological specimens should be handled as potentially infectious, following standard precautions. For guidelines on specimen handling, refer to the World Health Organization.
- Consult environmental waste personnel for proper disposal of used plates, consumables, and reagents, considering federal and local hazardous waste regulations. Check local and national disposal requirements.

3. Principles and Procedure Overview

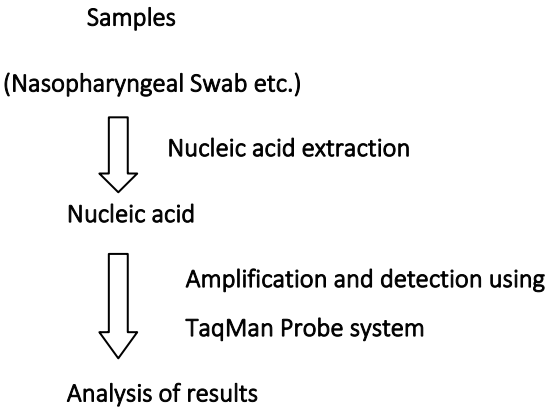
3.1 Principles

The polymerase chain reaction (PCR) is sensitive and specific TaqMan Probe technology with the use of DNA amplification technique, primer design and PCR optimization. The kit is, based on two main processes: nucleic acid extraction and PCR amplification of nucleic acid in the primer and probe mechanism of PCR machines by Real-time PCR. The kit is a real-time PCR test where respiratory pathogenes and Endogenous Control (EC) target is a multiplex realization that allows amplification of nucleic acids.

Pathogene	Targeted Gene
Influenza A (Inf A),	HA
Influenza A-H1pdm09 (H1N1),	HA
Influenza B (Inf B),	HA
Coronavirus NL63 (NL63),	N
Coronavirus 229E (229E),	N
Coronavirus OC43 (OC43),	N
Coronavirus HKU1 (HKU1),	N
Adenovirus (AdV),	Hexone gene
Metapneumovirus A & B (MPV),	N
Human Rhinovirus A, B & C (HRV),	UTR
Human Bocavirus1,2,3,4 (HBoV),	NS1
Parainluenza Virus 1 (PIV1),	Polymerase gene
Parainfluenza Virus 2 (PIV2),	Polymerase gene
Parainfluenza Virus 3 (PIV3),	Polymerase gene
Parainfluenza Virus 4 (PIV4)	Polymerase gene
Human Enterovirus (HEV),	Polyprotein gene
Human Parechovirus (HPeV),	UTR
Respiratory syncytial virus A & B (RSV A/B),	Matrix gene
Mycoplasma pneumoniae (MP)	MP3
Endogenous Control	Human RNaseP

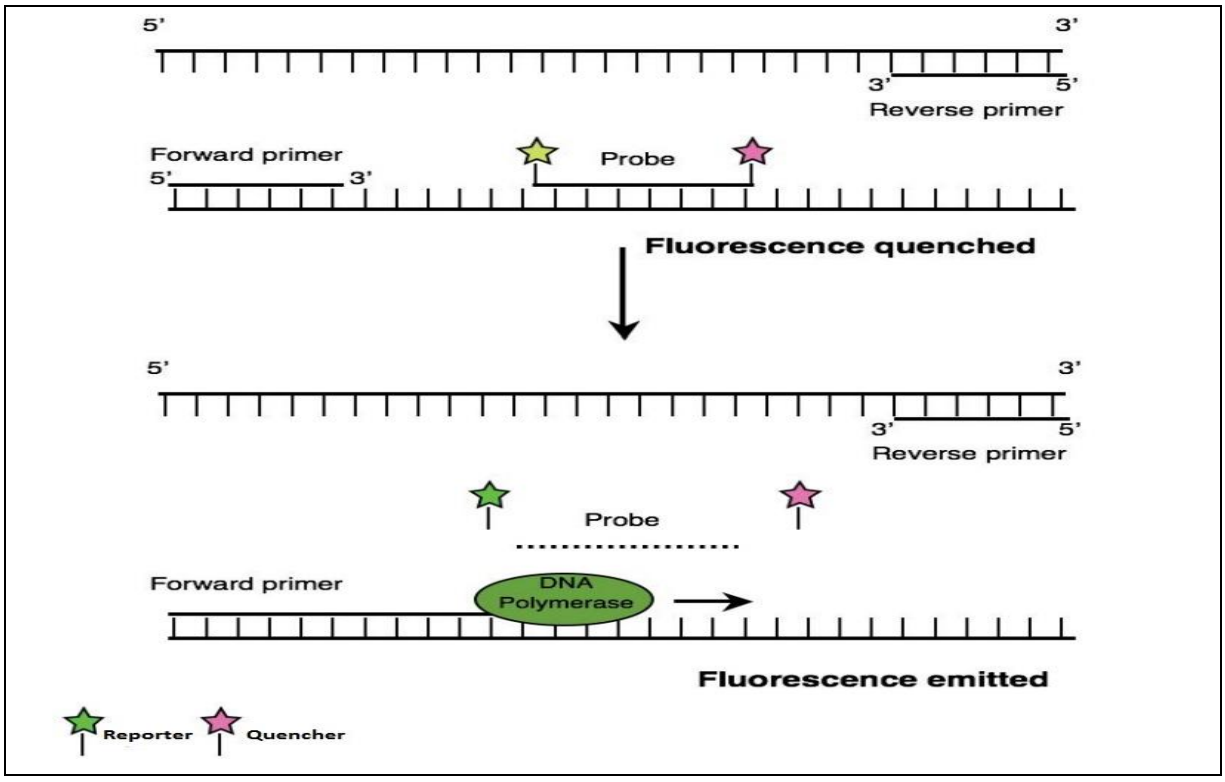
Table1: Primer Probe targets

Procedure Overview;

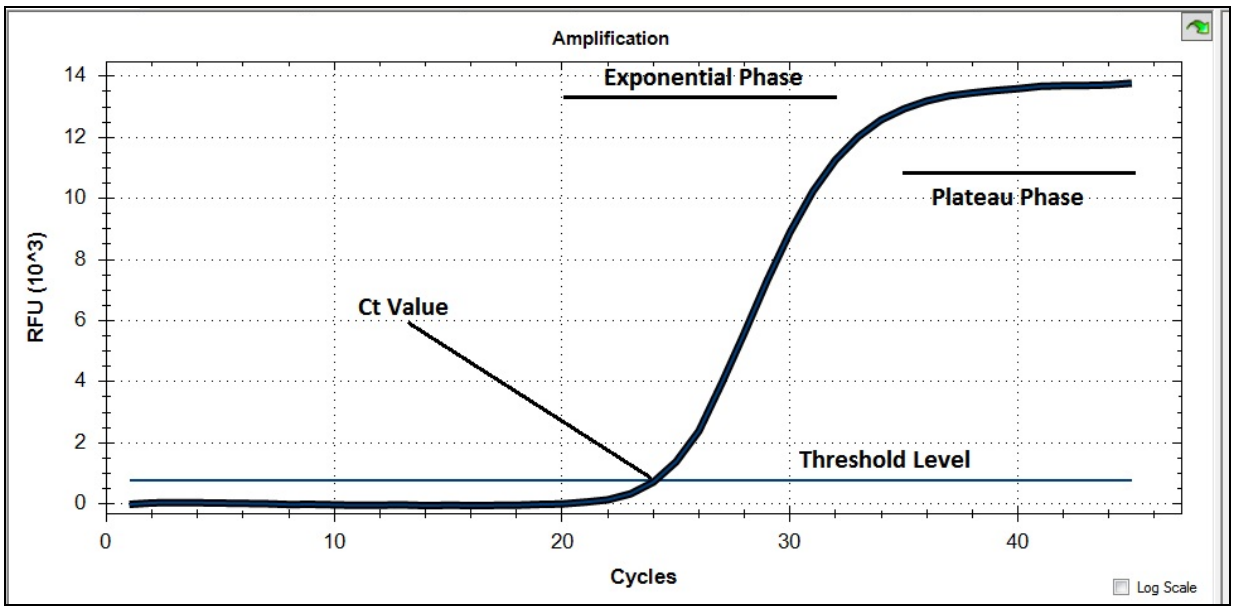


3.2 Technology

Hydrolysis (TaqMan) probes are the most common form of qPCR probes and are widely used in human, veterinary, and environmental diagnostics. These probes utilize a fluorescent dye at one end of the DNA oligonucleotide and a quencher at the other. During PCR, the probe specifically anneals to the target DNA sequence (from sample), which is flanked by the two primers. As DNA polymerase extends the new DNA strand, the probe is degraded by the 5' to 3' exonuclease activity of the polymerase, resulting in the fluorophore being separated from the quencher and emitting fluorescence. The more DNA present in the reaction, the earlier the fluorescence reaches a detectable level resulting in earlier Ct values.



Hydrolysis (TaqMan) Probe Technology.



Typical Amplification Plot of Real-time PCR in Linear Scale Graphic.

4. Background Information

Viral respiratory acute infections are common and contribute significantly to morbidity and mortality worldwide. Many different viruses can determine respiratory tract infections and most of them belong to the Orthomyxoviridae, Coronaviridae, Picornaviridae, Paramyxoviridae, Adenoviridae and Parvoviridae families. Viral respiratory infections may be either asymptomatic, or they may occur with mild symptoms or even cause severe diseases.

In patients with predisposing conditions, the outcome of these infections can be more severe and require hospitalization, sometimes even in intensive care units (ICUs), following the development of pneumonia and acute respiratory distress syndrome (ARDS). Respiratory viruses account for about 30% of pneumonia cases in adult patients, hospitalized in ICU, with mortality rates comparable to those of bacterial pneumonia. In children, especially those younger than 2 years, they frequently cause pneumonia.

A rapid and accurate etiological diagnosis is essential for prompt patient management, ruling out non-viral infection, limiting the spread of infections and, when available, initiating timely therapeutic treatments. For many years the diagnosis of viral respiratory tract infections has been made by non-molecular approaches such as antigen detection by direct immunofluorescence and viral culture. These methods, although effective and often complementary, are time-consuming, labour-intensive and, often, lack of sensitivity or specificity. Compared to classical methods, molecular methods have significantly improved the diagnosis of acute respiratory tract infections as they offer high sensitivity and provide specific results within a shorter period of time and for a larger number of pathogens. (1)

5. Reagents

Reagents contained in a kit are sufficient for 50 reactions

Description	No. of Reactions	No. of Tubes	Vol. Per Tube	Color of Caps	Description
2X qRT-Reaction Mix	300	2	1500 µl	Clear	• Buffer containing dNTPs, ddH2O, Taq Polymerase, Reverse Transcriptase and Uracil-DNA Glycosylase (UDG)
RM Panel Primer Probe Mix1	60	1	300 µl	Amber	• Primer Probe Sets, TE buffer
RM Panel Primer Probe Mix2	60	1	300 µl	Amber	• Primer Probe Sets, TE buffer
RM Panel Primer Probe Mix3	60	1	300 µl	Amber	• Primer Probe Sets, TE buffer
RM Panel Primer Probe Mix4	60	1	300 µl	Amber	• Primer Probe Sets, TE buffer
RM Panel Primer Probe Mix5	60	1	300 µl	Amber	• Primer Probe Sets, TE buffer
RNase Free Water	80	1	400 µl	Violet	• RNase Free Water for Negative Template Control
RM Panel Positive Control	20	1	100 µl	Red	Positive Control (PC): • Mixture of pathogen and IC clones

*Note: Do not subject the tubes to more than 5 freeze-thaw cycles.

6. Storage and Handling

All components of the kit must be stored at between -15°C /-25°C. All components are stable under the recommended storage conditions until the expiration date indicated on the label on the box. The performance of the kit components are not affected until 5 freeze and thaw. If reagents are to be used only intermittently, they should be stored in aliquots.

This product is shipped on frozen blue ice packs (+4 °C) and may thawed upon arrival.

Expiry date of the kit is one year from manufacter date.

7. Materials Required But Not Provided

- Disposable powder-free gloves (latex ornitrile)
- Pipettes (adjustable) and sterile pipette tips
- 1.5 mL microcentrifuge tubes
- Desktop centrifuge
- Vortex mixer
- Clean bench
- For Biorad CFX Instruments;
    - 96-Well Skirted PCR Plate, White well (Cat. No. HSP-9655, Biorad)
    - Permanent Clear Heat Seal (Cat. No. 1814035, Biorad)

For the other instruments please use 96 well plates and tubes recommended by device manufacturers.

8. Biosafety Information

- Handle all specimens as if infectious. Laboratory safety procedures must be taken when handling specimens.
- Thoroughly clean and disinfect all work surfaces with 0.5% sodium hypochlorite (in de-ionized or distilled water).
- Product components (product residuals, packaging) can be considered as laboratory waste.
- Dispose of unused reagents and waste in accordance with applicable federal, state, and local regulations.
- Manipulation of potentially infected specimens should be performed in a certified Class II BSC in a BSL-2 facility or higher. This includes aliquoting and/or diluting specimens and nucleic acid extraction procedures involving potentially infected specimens.
- Use appropriate personal protective equipment including but not limited to disposable gloves, laboratory coat/gown, and eye protection when handling specimens, reagents, pipettes, and other equipment.

9. Protocol

9.1 Specimen Collection, Storage, and Transport

All samples should be considered as potentially infectious material. Only sample materials collected, stored and transported in accordance with the following rules and instructions are permitted.

To ensure a high sample quality, samples should be transported as quickly as possible. The samples should be transported at the specified temperatures.

9.1.1 Specimen Collection

Nasopharyngeal swab and Oropharyngeal swab samples are examined for routine detection of common respiratory pathogens. The samples can be collected with flocked nylon swabs such as COPAN, Italy or Puritan (U.S).

Kit is validated on following mediums;

-Virus Transport Medium (VTM),

-Universal Transport Medium (UTM),

-Phosphate Buffer Saline (PBS),

-Saline Solution

-Steril Distilled Water samples.

9.1.2 Specimen Storage and Transport

Specimen	Storage*		Transport**	Note
	Temp.	Duration	Temp.	
Nasopharyngeal aspirate	2-8°C	3 days	2-8°C	Store any leftover specimens at ≤-20°C
Nasopharyngeal swab				
Oropharyngeal swab				
Bronchoalveolar lavage				

*: Performance may be affected by routine freezing or prolonged storage of specimens.
**: Specimens should also adhere to local and national instructions for transport of pathogenic material.

9.2 Nucleic Acid Extraction

Various manufacturers offer nucleic acid extraction kits. Use the correct protocol according to the manufacturers protocol. The following extraction kits have been validated for use with this kit.

9.2.1 Manual Nucleic Acid Extraction Kits

* Please use the recommended volumes of specimen and elution as indicated below. For all others, refer to the manufacturer's protocol.

Extraction Kit	Manufacturer	Cat. No	Recommended Vol.
QIAamp® MinElute® Virus Spin Kit*	QIAGEN	57704	Specimen:190µL Elution:100 µL
Ribo_spinvRD* (Viral RNA/DNA Extraction Kit)	GeneAll	302-150 SG1701	Specimen:290 µL Elution:100 µL

9.3 Preparation for Real-time PCR

-The correct tubes and caps must be used (see MATERIALS REQUIRED BUT NOT PROVIDED).
-Aerosol resistant filter tips and tight gloves must be used when preparing one-step RT-PCR reactions.
-Use extreme care to ensure no cross-contamination.
-Completely thaw all reagents at room temperature.
-Set up all reactions on ice to minimize the risk of RNA degradation.
-Briefly centrifuge the reagent tubes to remove drops from the inside of the cap.
-Prepare 1.5mL Microcenstrifuge tubes for Samples, PC and NTC
-Thaw the primer/probe mix tubes, positive control mixes, and 2X qRT- Reaction Mix.
-Vortex and spin down the tubes for about 5 seconds at room temperature.
-Five reactions are set up for each sample.
-The PCR reactions are setup in a total volume of 20 µl/sample.
-Reaction mixes for multiple samples (as well as control samples) should be pre-mixed as a master mix with 5% excess volume to compensate for pipetting losses.
-Alternatively, individual master mixes can be made with each primer/probe mix, with DNA samples added directly to the plate.
-The following reagents go into each 20 µl reaction:

Component	Volume
2X qRT-Reaction Mix	10 µL
Primer Probe Mix (1-5)	5 µL
Sample Nucleic Acid	5 µL
Total Volume	20µL

-Prepare a master mix for each sample as follows (calculated with 5% overage):

Reagent	Volume per Sample
2X qRT-Reaction Mix	52.5 µL
Sample Nucleic Acid or PC or NTC	27.5 µL

-Pulse vortex to mix and perform a quick spin down, at room temperature.
- Dispense 15 µl of the master mix per well into a single row (1-5) as shown in the Table 2.
- Add 5 µl of each primer/probe mix to its corresponding well.

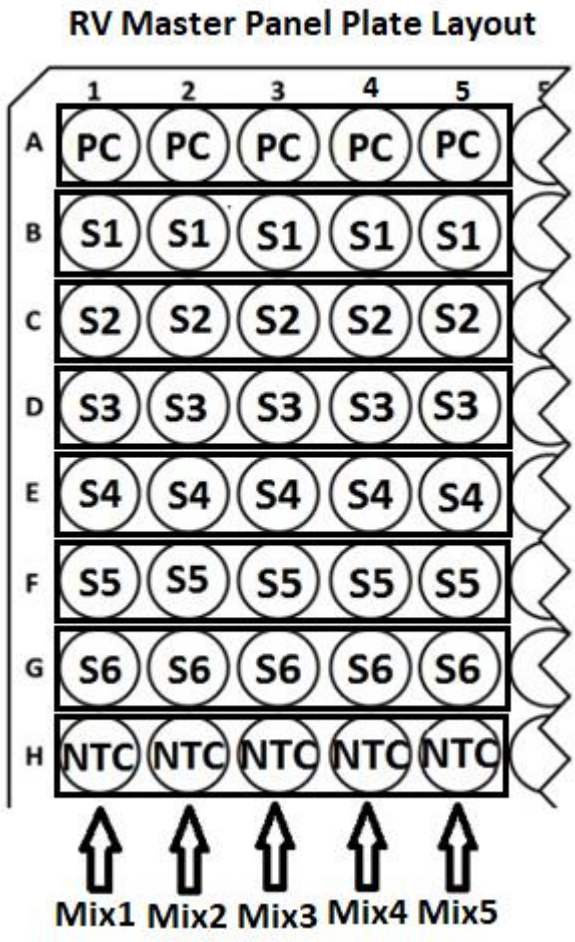


Table 2

Use a new sterile pipette tip for each sample.

* For Negative Control (NC), use 5 µL of RNase-free Water instead of sample's nucleic acid.
* For Positive Control (PC), use 5 µL of PC.
* Please be careful not to cross-contaminate the one-step RT-PCR Master Mix and samples with the Positive Control.
* The PCR tubes must be mixed by pipetting and centrifuged before running PCR reaction. It needs to be checked that liquid containing all PCR components is at the bottom of each PCR tube.

10. Real-time PCR Instrument Setup and Results Analysis

10.1 Real-time PCR System

10.1.1 Pre-settings for Data Analysis

A. Pre-settings for Fluorophore Selection and Thermal Cycling Conditions

Master Mix 1

Target	Reporter Fluorophore
Influenza A	FAM (Green)
Endogenous Control	VIC (Yellow)
H1N1	ROX (Orange)
Influenza B	CY5 (Red)

Master Mix 2

Target	Reporter Fluorophore
NL63	FAM (Green)
229E	VIC (Yellow)
OC43	ROX (Orange)
HKU1	CY5 (Red)

Master Mix 3

Target	Reporter Fluorophore
AdV	FAM (Green)
MPV	VIC (Yellow)
HRV	ROX (Orange)
HBoV	CY5 (Red)

Master Mix 4

Target	Reporter Fluorophore
PIV1	FAM (Green)
PIV2	VIC (Yellow)
PIV3	ROX (Orange)
PIV4	CY5 (Red)

Master Mix 5

Target	Reporter Fluorophore
HeV	FAM (Green)
HPeV	VIC (Yellow)
RSVA/B	ROX (Orange)
MP	CY5 (Red)

1) *For Biorad CFX96, QuantStudio Series and Other Four Channels Insturments*

Temperature	Time	Cycles	Data Collection
50˚C	10 min	1	
95˚C	4 min	1	
95˚C	10 sec	45	
59˚C	20 sec		FAM, VIC, ROX, CY5

2) *For ABI7500 (Standard Mode)*

Temperature	Time	Cycles	Data Collection
50˚C	10 min	1	
95˚C	4 min	1	
95˚C	10 sec	45	
59˚C	30 sec		FAM, VIC, ROX, CY5

Note1*: Passive reference dye should be set as "none"*

3) *For Rotorgene Q5/Q6 (Duration 75 min.)*

Temperature	Time	Cycles	Data Collection
50˚C	10 min	1	
95˚C	4 min	1	
95˚C	10 sec	45	
59˚C	20 sec		Green, Yellow, Orange, Red

Note1*: Please use only 72-well carousel, 36-well carousel does not recommended*

Note2: *Please perform Auto-Gain optimisation befor first acquisition. (Auto-Gain optimisation tube should be PC)*

4) *For Lightcycler480 (Roche)*

Temperature	Time	Cycles	Data Collection
50˚C	10 min	1	
95˚C	4 min	1	
95˚C	10 sec	45	
59˚C	20 sec		FAM (465-510), VIC/HEX (533-580), ROX (533-610), CY5 (618-660)

Note1: *4 channels Color Compansation must be performed before the studies.*

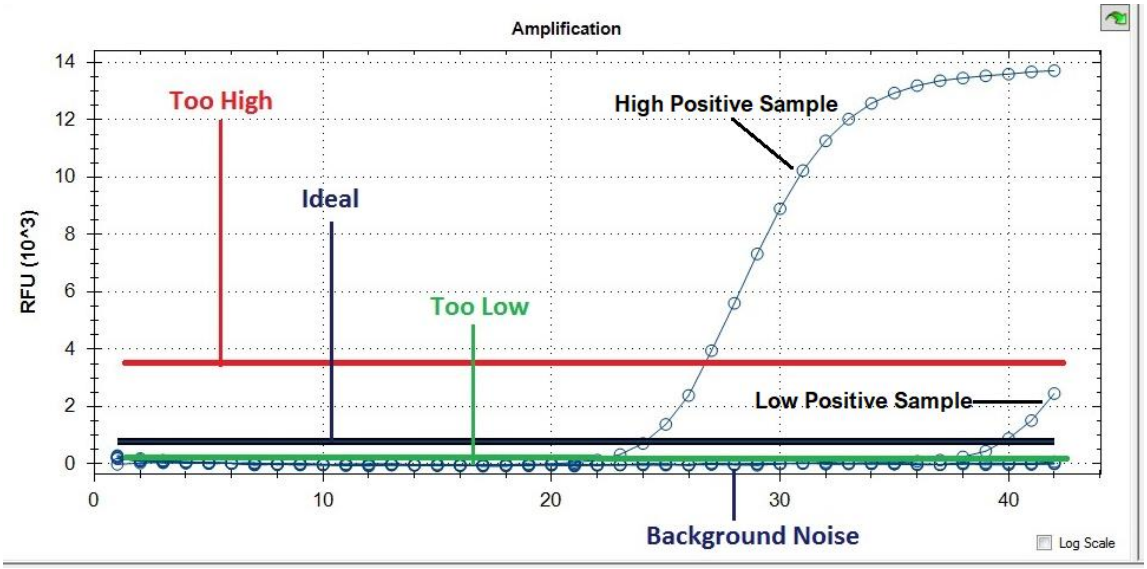
11. Results

11.1 General Rules of the Threshold Settings Manually

Normally the software-based methods will select a proper threshold, but in cases where the curves do not conform to the assumptions made by the algorithm, an incorrect threshold may be calculated but manually adjusting the threshold is highly recommended.

Ideally, the threshold should be set in the region where the just above the background noise or you can set it as 5% of positive control curve RFU height The threshold should not be so high that it crosses any of the plots where they are starting to plateau and are no longer linear.

To adjust the threshold for each dye collected must be set separately.



Example of ideal threshold level for Lineer Scale Graphic

If the threshold is too high, it gives false negative.

If the threshold is too low, it gives false positive.

11.2 Interpretation of Results

Mix 1

Target	Reporter Fluorophore	Sample	
		Ct Value	Result
InfA	FAM	<40	Positive (+)
		≥40 or NA	Negative (-)
RNaseP (EC)	VIC	<35	Positive (+)
		≥35 or NA	Negative (-)
H1N1	ROX	<40	Positive (+)
		≥40 or NA	Negative (-)
InfB	CY5	<40	Positive (+)
		≥40 or NA	Negative (-)

Mix 2

Target	Reporter Fluorophore	Sample	
		Ct Value	Result
NL63	FAM	<40	Positive (+)
		≥40 or NA	Negative (-)
229E	VIC	<40	Positive (+)
		≥40 or NA	Negative (-)
OC43	ROX	<40	Positive (+)
		≥40 or NA	Negative (-)
HKU1	CY5	<40	Positive (+)
		≥40 or NA	Negative (-)

Mix 3

Target	Reporter Fluorophore	Sample	
		Ct Value	Result
AdV	FAM	<40	Positive (+)
		≥40 or NA	Negative (-)
MPV	VIC	<40	Positive (+)
		≥40 or NA	Negative (-)
HRV	ROX	<40	Positive (+)
		≥40 or NA	Negative (-)
HBoV	CY5	<40	Positive (+)
		≥40 or NA	Negative (-)

Mix 4

Target	Reporter Fluorophore	Sample	
		Ct Value	Result
PIV1	FAM	<40	Positive (+)
		≥40 or NA	Negative (-)
PIV2	VIC	<40	Positive (+)
		≥40 or NA	Negative (-)
PIV3	ROX	<40	Positive (+)
		≥40 or NA	Negative (-)
PIV4	CY5	<40	Positive (+)
		≥40 or NA	Negative (-)

Mix 5

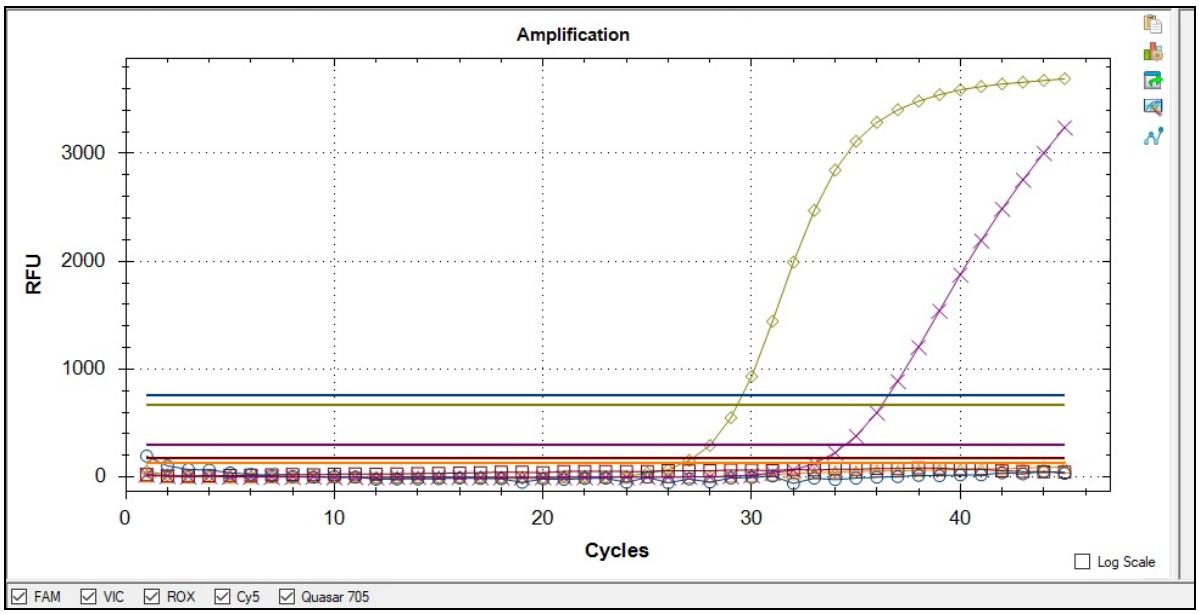
Target	Reporter Fluorophore	Sample	
		Ct Value	Result
HEV	FAM	<40	Positive (+)
		≥40 or NA	Negative (-)
HPeV	VIC	<40	Positive (+)
		≥40 or NA	Negative (-)
RSVA/B	ROX	<40	Positive (+)
		≥40 or NA	Negative (-)
MP	CY5	<40	Positive (+)
		≥40 or NA	Negative (-)

Interpretation	FAM	VIC/HEX	ROX	CY5	Reporting
Case 1 (Mix1)	+	+/-	-	-	InfA Positive
Case 1 (Mix2)	-	-	-	-	
Case 1 (Mix3)	-	-		-	
Case 1 (Mix4)	-	-	-	-	
Case 1 (Mix5)	-	-	-	-	

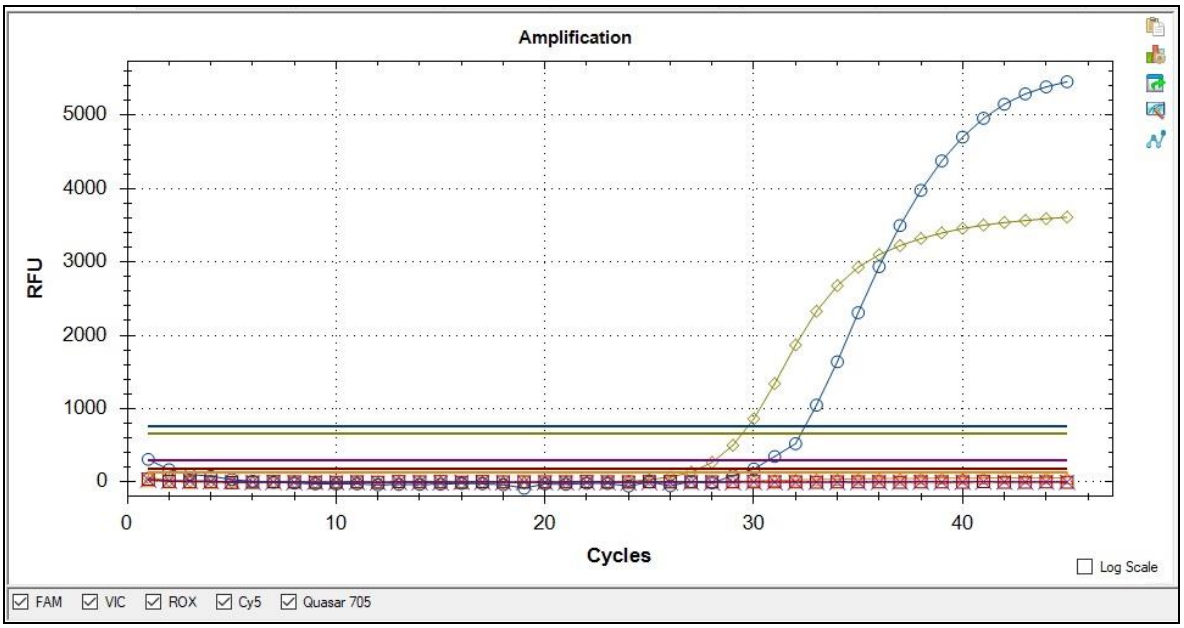
Interpretation	FAM	VIC/HEX	ROX	CY5	Reporting
Case 2 (Mix1)	+	+/-	+	-	InfA (H1N1) Positive
Case 2 (Mix2)	-	-	-	-	
Case 2 (Mix3)	-	-		-	
Case 2 (Mix4)	-	-	-	-	
Case 2 (Mix5)	-	-	-	-	

Interpretation	FAM	VIC/HEX	ROX	CY5	Reporting
Case 3 (Mix1)	-	+	-	-	OC43 & PIV4 Positive (Co-infection)
Case 3 (Mix2)	-	-	+	-	
Case 3 (Mix3)	-	-		-	
Case 3 (Mix4)	-	-	-	+	
Case 3 (Mix5)	-	-	-	-	

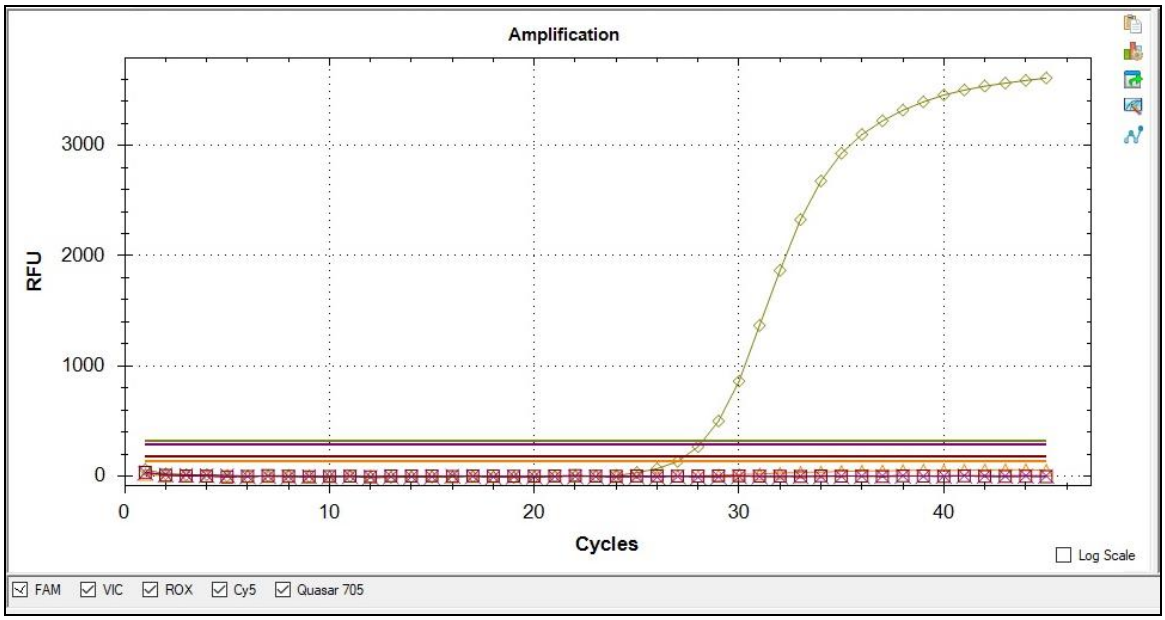
Interpretation	FAM	VIC/HEX	ROX	CY5	Reporting
Case 4 (Mix1)	-	-	-	-	Invalid (Failure of PCR or insufficent sampling step)
Case 4 (Mix2)	-	-	-	-	
Case 4 (Mix3)	-	-	-	-	
Case 4 (Mix4)	-	-	-	-	
Case 4 (Mix5)	-	-	-	-	



Mix1 Influenza B Positive: CY5 and VIC amplification curves are obsereved.



Mix1 Influenza A Positive: FAM and VIC amplification curves are observed.



Mix1, Mix2, Mix3, Mix4, Mix5 RV Master Panel Negative: only VIC (Internal Control) amplification curve are obsereved.

12. Performance Characteristics

12.1 Assay Specificity

12.1.1 *In Silico* Studies

In silico studies are summarized below;

No	Organism	In silico Analysis for % Identity target:s
1	Human respiratory syncytial virüs A/B	100% Match
2	Influenza A virus	100% Match
3	Influenza B virus	100% Match
4	Human coronavirus HKU1	100% Match
5	Human adenovirus (eg C1 Ad.71)	100% Match
6	Human rhinovirus	100% Match
7	Human metapneumovirus	100% Match
8	Human parainfluenza virüs (Type 1,2,3 &4)	100% Match
9	Enterovirus (e.g EV68)	100% Match
10	SARS-CoV-1	No alignment found
11	Middle East Respiratory Syndrome (MERS)	No alignment found
12	Epstein Barr Virus (EBV)	No alignment found
13	Human bocavirus	100% Match
14	*Streptococcus* (Taxid: 1301)	No alignment found
15	*Steptococcus pyogenes*	No alignment found
16	*Mycobacteria* (Taxid:85007)	No alignment found
17	*Mycoplasma* (Taxid:2085)	100% Match
18	*Legionella* (Taxid: 445)	No alignment found
19	*Bordetella pertussis*	No alignment found
20	*Pneumocycstis jirovecii (PJP)*	No alignment found

12.1.2 Clinical Studies

Cross-reactivity of geneMAP™ Respiratory Master Panel was tested using 21 viruses and bacteria as indicated below.

No	Organism	Source	Results
1	Respiratory syncytial virus A	TURKEY ISOLATE	Detected
2	Respiratory syncytial virus B	TURKEY ISOLATE	Detected
3	Influenza A virus (H3N2)	TURKEY ISOLATE	Detected
4	Influenza A virus (H1N1)	TURKEY ISOLATE	Detected
5	Influenza B virus	TURKEY ISOLATE	Detected
6	Human coronavirus NL63	TURKEY ISOLATE	Detected
7	Human coronavirus OC43	TURKEY ISOLATE	Detected
8	Human coronavirus 229E	TURKEY ISOLATE	Detected
9	Human coronavirus HKU1	TURKEY ISOLATE	Detected
10	Adenovirus	TURKEY ISOLATE	Detected
11	Human rhinoviruses	TURKEY ISOLATE	Detected
12	Human metapneumovirus	TURKEY ISOLATE	Detected
13	Parainfluenza 1	TURKEY ISOLATE	Detected
14	Parainfluenza 2	TURKEY ISOLATE	Detected
15	Parainfluenza 3	TURKEY ISOLATE	Detected
16	*Mycoplasma pneumoniae*	TURKEY ISOLATE	Detected
17	Human bocavirus	TURKEY ISOLATE	Detected
18	*Streptococcus pneumoniae*	TURKEY ISOLATE	Not detected
19	*Mycoplasma pneumoniae*	TURKEY ISOLATE	Detected
20	*Haemophilus influenzae*	TURKEY ISOLATE	Not detected
21	*Chlamydophila pneumoniae*	TURKEY ISOLATE	Not detected
22	*Human parechovirus*	TURKEY ISOLATE	Detected

12.2 Assay Sensitivity and Intra-Assay Reproducibility

Analytical Sensitivity/Limit of Detection summarized table below. Study has been performed with Recombinant Viral Partical or Synthetic Ultramer of target nucleic acids in Viral Transport Media (VTM) that consists of Tris-buffered saline, with added glycerol, anti-microbial agents and human proteins. Each assay at five sample template concentrations was repeated 25 times for each pathogenes

The Run performed with CFX96 PCR Insturment/BioRad Lab.

Pathogene	Limit Of Detection (Copies/mL)
Influenza A (Inf A),	500
Influenza A-H1pdm09 (H1N1),	750
Influenza B (Inf B),	500
Coronavirus NL63 (NL63),	250
Coronavirus 229E (229E),	350
Coronavirus OC43 (OC43),	200
Coronavirus HKU1 (HKU1),	500
Adenovirus (AdV),	500
Metapneumovirus A & B (MPV),	750
Human Rhinovirus A, B & C (HRV),	500
Human Bocavirus1,2,3,4 (HBoV),	200
Parainluenza Virus 1 (PIV1),	350
Parainfluenza Virus 2 (PIV2),	500
Parainfluenza Virus 3 (PIV3),	750
Parainfluenza Virus 4 (PIV4)	500
Human Enterovirus (HEV),	500
Human Parechovirus (HPeV),	500
Respiratory syncytial virus A & B (RSV A/B),	350
Mycoplasma pneumoniae (MP)	500

12.3 Clinical Evaulation

The clinical performance of the geneMAP™ Respiratory Master Panel assays was established in one site clinical evaluation. Fresh or freze-thaw clinical Nasopharyhngeal Swab (NPS) and Orapharyngeal Swab (OPS) specimens were tested with geneMAP™ Master Panel PCR Kit and one commercial CE-IVD marked kit has chosen as comparator. Results are summarized below.

1. Influenza-A (H1N1 & H3N2)

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	13	0	13
	Negative	0	65	65
Total		13	65	78

Statistic	Value	95% CI
Sensitivity	100.00%	75.29% to 100.00%
Specificity	100.00%	94.48% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Accuracy	100.00%	95.38% to 100.00%

2. Influenza-B

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	11	1	8
	Negative	0	65	25
Total		11	66	33

Statistic	Value	95% CI
Sensitivity	100.00%	71.51% to 100.00%
Specificity	98.48%	91.84% to 99.96%
Positive Predictive Value	91.67%	61.13% to 98.72%
Negative Predictive Value	100.00%	
Accuracy	98.70%	92.98% to 99.97%

3. PIV1,2,3 and 4

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	13	1	14
	Negative	0	65	65
Total		13	66	79

Statistic	Value	95% CI
Sensitivity	100.00%	75.29% to 100.00%
Specificity	98.48%	91.84% to 99.96%
Positive Predictive Value	92.86%	65.02% to 98.91%
Negative Predictive Value	100.00%	
Accuracy	98.73%	93.15% to 99.97

4. RSV-A

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	14	0	14
	Negative	0	65	65
Total		14	65	79

Statistic	Value	95% CI
Sensitivity	100.00%	76.84% to 100.00%
Specificity	100.00%	94.48% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Accuracy	100.00%	95.44% to 100.00%

5. RSV-B

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	5	0	5
	Negative	0	66	66
Total		5	66	71

Statistic	Value	95% CI
Sensitivity	100.00%	47.82% to 100.00%
Specificity	100.00%	94.56% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Accuracy	100.00%	94.94% to 100.00%

6. NL63

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	11	1	12
	Negative	0	65	65
Total		11	66	77

Statistic	Value	95% CI
Sensitivity	100.00%	71.51% to 100.00%
Specificity	98.48%	91.84% to 99.96%
Positive Predictive Value	91.67%	61.13% to 98.72%
Negative Predictive Value	100.00%	
Accuracy	98.70%	92.98% to 99.97%

7. OC43

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	5	0	5
	Negative	0	66	65
Total		5	66	71

Statistic	Value	95% CI
Sensitivity	100.00%	47.82% to 100.00%
Specificity	100.00%	94.56% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Accuracy	100.00%	94.94% to 100.00%

8. 229E

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	8	0	8
	Negative	0	66	66
Total		8	66	74

Statistic	Value	95% CI
Sensitivity	100.00%	63.06% to 100.00%
Specificity	100.00%	94.56% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Accuracy	100.00%	95.14% to 100.00%

9. HKU1

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	4	0	4
	Negative	0	65	65
Total		4	65	69

Statistic	Value	95% CI
Sensitivity	100.00%	39.76% to 100.00%
Specificity	100.00%	94.48% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Accuracy	100.00%	94.79% to 100.00%

10. AdV

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	7	0	7
	Negative	0	66	66
Total		7	66	73

Statistic	Value	95% CI
Sensitivity	100.00%	59.04% to 100.00%
Specificity	100.00%	94.56% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Accuracy	100.00%	95.07% to 100.00%

11. HBoV

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	9	0	9
	Negative	0	65	65
Total		9	65	74

Statistic	Value	95% CI
Sensitivity	100.00%	66.37% to 100.00%
Specificity	100.00%	94.48% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Accuracy	100.00%	95.14% to 100.00%

12. MPV

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	6	0	6
	Negative	0	65	65
Total		6	65	71

Statistic	Value	95% CI
Sensitivity	100.00%	54.07% to 100.00%
Specificity	100.00%	94.48% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Accuracy	100.00%	94.94% to 100.00%

13. HRV

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	19	1	20
	Negative	0	65	65
Total		19	66	85

Statistic	Value	95% CI
Sensitivity	100.00%	82.35% to 100.00%
Specificity	98.48%	91.84% to 99.96%
Positive Predictive Value	95.00%	73.09% to 99.25%
Negative Predictive Value	100.00%	
Accuracy	98.82%	93.62% to 99.97%

14. HeV

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	5	0	5
	Negative	0	65	65
Total		5	65	70

Statistic	Value	95% CI
Sensitivity	100.00%	47.82% to 100.00%
Specificity	100.00%	94.48% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Accuracy	100.00%	94.87% to 100.00%

15. HPeV

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Master Panel	Positive	8	0	8
	Negative	0	65	65
Total		8	65	73

Statistic	Value	95% CI
Sensitivity	100.00%	63.06% to 100.00%
Specificity	100.00%	94.48% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Accuracy	100.00%	95.07% to 100.00%

12.4 Reactivity/Inclusivity

An in silico inclusivity analysis of the geneMAP™ 's primers and probes was performed. All primer sets designed for detection of the targeted genes were tested against the complete available viruses genome sequence. The analysis demonstrated that the regions recognized by the designed primers and probes have 100% homology with all available target pathogen sequences from the National Center for Biotechnology Information (NCBI).

13. Limitations

Mutation in the target sequence of targeted pathogens or change in the sequence due to Virus Evolution can lead to false negative results.

False positive and false negative results can be caused by poor sample quality, incorrect sample collection, incorrect handling, incorrect laboratory processing, or restriction of testing technology.

The kit can not distinguish among RSVA and RSV B.

The kit performance has not been validated specimens from Urine, stool etc.

Interacting agents or PCR inhibitors can lead to false negative or Invalid results.

14. Interference Studies

Interference Studies study was performed to demonstrate that potentially interfering substances that may be found in the upper respiratory tract in symptomatic subjects (including over the counter medications) do not cross-react or interfere with geneMAP™ Respiratory Master Panel. Each substance was tested in triplicate in the absence or presence of each pathogens at 5 x LoD. The final concentration of the substances tested are documented in the Table below.

No	Interfering Substance	Source	Test Concentration	Interference (Yes/No)
1	Blood	Human	5%	No (negative 3/3, positive 3/3)
2	Mucin (bovine submaxillary gland, type I-S)	Sigma-Aldrich (Cat.No.M3895)	60 µg/ml	No (negative 3/3, positive 3/3)
3	Mupirocin (Antibiotic, nasal ointment)	Sigma-Aldrich (Cat.No.1448901)	6.5 mg/ml	No (negative 3/3, positive 3/3)
4	Oxymetazoline	Sigma-Aldrich (Cat.No.O2378)	15% (v/v)	No (negative 3/3, positive 3/3)
5	Tobramycin	Sigma-Aldrich (Cat.No.T4014)	5.0 µg/mL	No (negative 3/3, positive 3/3)
6	Zanamivir (Anti-viral drug-Relenza)	Sigma-Aldrich (Cat.No.SML0492)	3.5 mg/mL	No (negative 3/3, positive 3/3)
7	Oseltamivir (Anti-viral drug-Tamiflu)	Sigma-Aldrich (Cat.No.1479304)	25 mg/mL	No (negative 3/3, positive 3/3)

15. Revision History

Date of Last Edit: January 2025		
Change	**Affected Section**	**Page**
Added reaction numbers for the kit	4. Background Information	7
Added ∑ (total numbers of tests) symbol	Cover Page	1
Added Safety Instructions and General Warnings	2. Safety Instructions and General Warnings	3
Updated Information	5.Reagents	6
Thermal Profile Updated	10.1.1 Pre-settings for Data Analysis	12

16. References

1- Arvia et al. Detection of 12 respiratory viruses by duplex real time PCR assays in respiratory samples, Molecular and Cellular Probes, Volume 29, Issue 6, December 2015, Pages 408-413

17. Troubleshooting

geneMAP™ Respiratory Master Panel		
OBSERVATION	POSSIBLE CAUSES	SOLUTION
No signal in any flororphore	Fluorophores incompatible with protocol for data analysis	Select the correct fluorophores.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Presence of inhibitor	Repeat the test with the new extracted nucleic acid.
Amplification signals in Negative Control	Cross Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips and throughout the procedure and change tips between tubes. Repeat entire procedure from nucleic acid extraction with the new set of reagents.
No amplification signal in Positive Control	The fluorophores for data analysis does not comply with the protocol	Please select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect PCR mixture	Confirm that all components are added to the PCR mixture. Sensitivity is compromised with pre-composed premix. All reagents must be homogenized and spun down before use.
	Did not add sample's nucleic acid	Please carefully repeat the test.
	Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid into the correct PCR tubes and carefully repeat the test if necessary.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition (See page 6) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure and re-extract the nucleic acid. If the original specimen is not available, a new specimen must be collected.

18. Symbols Used

REF	Catalog Number
LOT	Lot/Batch Number
	Expiration Date
-15°C -25°C	Storage Conditions
	Manufactured by
CE IVD	Intended Use

19. Contact Information

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