

# Performance Verification of the Comprehensive Respiratory Tract Microbiota (RTM) Panel Utilizing QCMD Control Samples And Repository Specimen

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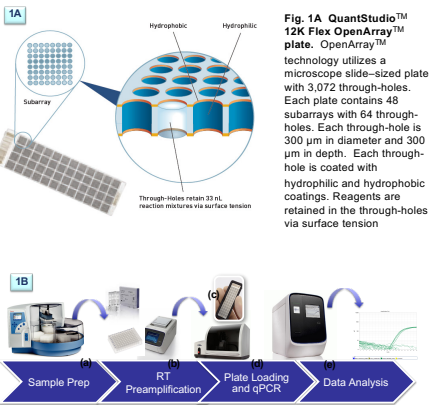
## INTRODUCTION

As one of the most common infections in humans, a respiratory infection is one high risk factor in pediatric, geriatric and immuno-compromised populations. Identification of the type of infection and pathogen(s) involved could help understanding the pathogenesis, severity and outcome of the infection. Currently, several respiratory panels are available in the market that utilize different technologies. To meet the needs of a wider respiratory pathogen coverage, higher sample throughput and content customization capability and lower cost, we developed a respiratory tract microbiota (RTM) panel that detects a total 42 pathogens, including viral, bacterial and fungal microorganisms on a nanofluidic device that can simultaneously interrogate all the targets on the panel via spatial multiplexing. After analytical performance, we also evaluated the panel with a large numbers of repository samples and Quality Control and Proficiency Test samples. These samples were previously characterized by other molecular testing methodologies. Concordance was analyzed and discordance was addressed by an orthogonal technology.

## MATERIALS AND METHODS

The repository specimen were acquired from various vendors. Quality Control and Proficiency Test samples were purchased from Qnostics (Glasgow, Scotland). Nucleic acids were extracted with MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit on King Fisher Flex System (Thermo Fisher Scientific). The extracted DNA/RNA samples were tested for the presence of pathogenic microorganisms on a Respiratory Tract Microbiota (RTM) panel that is spotted on OpenArray™ plates (Figure 1A) with one step reverse transcription (RT) and preamplification, and then followed by qPCR run on real-time instrument QuantStudio 12K (Figure 1B). The RTM panel covers 42 targets of viral, bacterial and fungal organisms (Table 1). Target detection was compared with the previous characterization. For discordant samples, capillary electrophoresis (CE) sequencing analysis was carried out following standard protocol.

Figure 1. OpenArray™ Technology and RTM Workflow



**Fig. 1A QuantStudio™ 12K Flex OpenArray™ plate.** OpenArray™ technology utilizes a microscope slide-sized plate with 3,072 through-holes. Each plate contains 48 subarrays with 64 through-holes. Each through-hole is 300 µm in diameter and 300 µm in depth. Each through-hole is coated with hydrophilic and hydrophobic coatings. Reagents are retained in the through-holes via surface tension.

**Fig. 1B RTM Workflow.** Nucleic acids were extracted King Fisher Flex System (a). RT-preamplification is performed on GeneAmp PCR system 9700™ (b). PCR mix is set up on OpenArray™ 384-Well Sample Plate and then is transferred onto OpenArray™ plate (c) by the QuantStudio™ OpenArray™ System (d). The sealed plate is placed on the QuantStudio™ 12K Flex system (e) for thermal cycling and data collection.

Table 1. List of Targeted Species in TaqMan® Respiratory Tract Microbiota (RTM) Panel

Microorganism Names				
DNA	Adenovirus	Influenza A pan	RNA Viral	
	Bocavirus	Influenza B		
	HHV8 (VZV)	Measles virus		
	HHV4 (EBV)	MERS		
	HHV5 (CMV)	Mumps virus		
	HHV6	RSVA specific		
	Human coronavirus 229E	RSVB specific		
	Human coronavirus HKU1	SARS		
	Human coronavirus NL63	<i>Pneumocystis jirovecii</i>		DNA Fungal
	Human coronavirus OC43	<i>Bordetella</i>		Viral
Human enterovirus D68 strain	<i>Bordetella holmesii</i>			
Human enterovirus pan	<i>Bordetella pertussis</i>			
Human metapneumovirus	<i>Chlamydia pneumoniae</i>			
Human parainfluenza virus 1	<i>Coxiella burnetii</i>			
Human parainfluenza virus 2	<i>Haemophilus influenzae</i>			
Human parainfluenza virus 3	<i>Klebsiella pneumoniae</i>			
Human parainfluenza virus 4	<i>Legionella pneumophila</i>			
Human parainfluenza virus 2	<i>Moraxella catarrhalis</i>			
Human rhinovirus	<i>Mycoplasma pneumoniae</i>			
Influenza A H1 seasonal	<i>Staphylococcus aureus</i>	DNA Bacterial		
Influenza A H3	<i>Streptococcus pneumoniae</i>			

The RTM panel covers 42 species, and most of them are from a wide range of viruses, both DNA and RNA viruses. It also includes some bacteria and one fungal targets. This is the most comprehensive respiratory panel currently available in the market.

## RESULTS

Table 2. RTM Correctly Identify Proficiency Test Control (QCMD) Samples.

Control Identity	Counts	Results
Adenovirus	1	Detected
Coronavirus - NL63	2	Both Detected
Coronavirus - OC43	1	Detected
Enterovirus 68	1	Detected
Human MPV	3	All Detected
Influenza A (H1N1)	1	Detected*
Influenza A	3	All Detected
Influenza B	2	Both Detected
Parainfluenza 1	1	Detected
RSV A	2	Both Detected
RSV B	2	Both Detected
Rhinovirus	2	Both Detected
<i>Bordetella pertussis</i>	1	Detected
<i>Haemophilus influenzae</i>	2	Both Detected
<i>Legionella pneumophila</i>	2	Both Detected
<i>Mycoplasma pneumoniae</i>	1	Detected
<i>Streptococcus pneumoniae</i>	2	Both Detected
Negative	3	Confirmed
Overall	31	31*

\* Influenza A H1N1 was detected by influenza A assay but not by influenza A H1 assay.

Three panels (Respl17, Respl17, and Respl117) of QCMD samples were used to evaluate accuracy of the RTM panel. The panels consists of both negative and positive controls covering common respiratory pathogens. The RTM panel was able to detect all identities of controls. One control influenza type A H1N1 was missed by influenza A H1 assay, but it was identify correctly by influenza A pan assay.

Table 3. High Positive Percent Agreement (PPA) and High Accuracy in RTM Panel

Targets	Claimed by Other Tests	Detected by RTM	PPA
Influenza A	4	4	100.0%
Influenza A (H1N1)	7	7	100.0%
Influenza A (H3)	41	41	100.0%
Influenza B	28	24	85.7%
RSV A	37	36	97.3%
RSV B	53	53	100.0%
RSV non-specified	2	2	100.0%
Rhinovirus - enterovirus	66	61	92.4%
HPiV-1	21	17	81.0%
HPiV-2	7	6	85.7%
HPiV-3	38	37	97.4%
HPiV	2	2	100.0%
hMPV	10	9	90.0%
Adenovirus	13	12	92.3%
Human coronavirus 229E	2	2	100.0%
Human coronavirus HKU1	1	1	100.0%
Human coronavirus OC43	1	1	100.0%
Sum	333	315	94.6%

A total of 316 repository samples (333 targets) retrospectively were tested by RTM panels. The RTM results were compared with characterization by other nucleic acid tests in market. Initial PPA is 94.6%. Sanger sequencing was conducted on all the 18 discordant targets - 13 of them support RTM results, 1 showed RTM missed the call, and 4 failed sequencing.

Table 4. Sequencing Summary Additional and Original Targets Detected by RTM

Sequenced Targets	Additional		Claimed	
	RTM Detected	Sequencing Confirmed	Sequencing Confirmed	RTM Detected
Adenovirus	1	1	1	1
CoV_229E	-	-	2	2
Flu_A_H3	3	3	6	6
Flu_B_pan	4	4	2	2
Flu_B_pan	1	1	2	1
hPIV1	-	-	4	4
hPIV3	4	4	7	7
RSV A	2	2	4	4
RSV B	3	3	6	6
Rhinovirus	19	19	31	31
CoV_HKU1	1	1	-	-
CoV_OC43	1	1	-	-
CoV_NL63	2	2	-	-
hMPV	1	1	-	-
<i>K. pneumoniae</i>	1	1	-	-
<i>H. influenzae</i>	14	14	-	-
HBoV	4	4	-	-
HHV4	4	4	-	-
HHV5	3	3	-	-
HHV6	11	11	-	-
<i>S. aureus</i>	11	11	-	-
<i>S. pneumoniae</i>	12	12	-	-
<i>M. catarrhalis</i>	1	1	-	-
Measles	1	1	-	-
<i>P. jirovecii</i>	3	3	-	-
Sum	107	107	65	64

RTM detects more targets than other platforms. A total of 222 Sanger sequencing runs were performed, including additional targets RTM detected and targets originally claimed by other platforms. Except for 50 sequencing attempts that did not generate sequencing results, the 172 sequencing results confirmed 100% of the additional targets and vast majority of the targets claimed by other tests.

Table 5. Representative Samples Showing RTM Detection and Sequencing Verification

Sample IDs	Benchmark Targets	Targets Detected by RTM	Sequencing Status	Sequencing Results	Ct Values with RTM
TM05395	Rhinovirus-ent	Rhinovirus	Performed	Confirmed	25.6
		<i>Haemophilus influenzae</i>	Performed	Confirmed	14.2
		<i>Staphylococcus aureus</i>	Performed	Confirmed	21.0
		<i>Streptococcus pneumoniae</i>	Performed	Confirmed	22.7
		Human herpesvirus 6 (HHV6)	Performed	Confirmed	26.3
TM05410	Rhinovirus-ent	<i>Klebsiella pneumoniae</i>	Performed	Confirmed	18.8
		Rhinovirus	Performed	Confirmed	20.7
		<i>Haemophilus influenzae</i>	Performed	Confirmed	20.5
		<i>Staphylococcus aureus</i>	Performed	No Result	27.3
		<i>Streptococcus pneumoniae</i>	Performed	Confirmed	13.1
TM05415	Rhinovirus-ent	Cytomegalovirus (VWV, HHV-5)	Performed	Confirmed	18.5
		Human bocavirus	Performed	Confirmed	25.2
		<i>Klebsiella pneumoniae</i>	Not performed	n/a	28.0
		Rhinovirus	Performed	Confirmed	16.8
		<i>Haemophilus influenzae</i>	Performed	Confirmed	17.8
TM05417	Rhinovirus-ent	<i>Staphylococcus aureus</i>	Performed	Confirmed	15.0
		Human bocavirus	Performed	Confirmed	13.7
		Adenovirus	Not performed	n/a	26.2
		Rhinovirus	Performed	Confirmed	17.6
		Human bocavirus	Performed	Confirmed	19.6
TM05407	Adenovirus	<i>K. pneumoniae</i>	Not performed	n/a	26.0
		<i>S. pneumoniae</i>	Not performed	n/a	26.7
		Adenovirus	Performed	Confirmed	7.0
		<i>Haemophilus influenzae</i>	Performed	Confirmed	11.0
		<i>Staphylococcus aureus</i>	Performed	Confirmed	14.6
TM05418	Influenza A	<i>Streptococcus pneumoniae</i>	Performed	Confirmed	20.8
		Human herpesvirus 6 (HHV6)	Performed	Confirmed	24.2
		Influenza Type A	Performed	Confirmed	23.1
		<i>Staphylococcus aureus</i>	Performed	Confirmed	21.4
		Flu A HB	Performed	Confirmed	24.4
TM05421	Flu A HB	<i>Haemophilus influenzae</i>	Performed	Confirmed	10.7
		<i>Staphylococcus aureus</i>	Performed	Confirmed	14.6
		<i>Streptococcus pneumoniae</i>	Performed	Confirmed	20.8
		Human coronavirus 229E	Performed	Confirmed	11.9
		<i>Haemophilus influenzae</i>	Performed	Confirmed	15.9
TM05402	Coronavirus 229 E	<i>Streptococcus pneumoniae</i>	Performed	Confirmed	26.2
		Human coronavirus 229E	Performed	Confirmed	19.1
		<i>Staphylococcus aureus</i>	Performed	Confirmed	22.2
		<i>Streptococcus pneumoniae</i>	Performed	Confirmed	16.1
		Cytomegalovirus (VWV, HHV-5)	Performed	No Result	26.7
TM05409	Coronavirus 229 E	Human herpesvirus 6 (HHV6)	Performed	No Result	26.8
		Human parainfluenza virus type 3	Performed	Confirmed	17.7
		<i>Haemophilus influenzae</i>	Performed	Confirmed	9.7
		Human herpesvirus 6 (HHV6)	Performed	Confirmed	29.9
		Human parainfluenza virus type 3	Performed	Confirmed	21.7
TM05403	Parainfluenza 3	Epstein-Barr virus (HHV4)	Performed	Confirmed	15.2
		Human coronavirus 229E	Performed	Confirmed	19.1
		Human coronavirus OC43	Not performed	n/a	24.7

Some of the representative samples with Sanger orthogonal verification are shown in Table 5, including the original characterized targets and additional targets detected by the RTM panel. All the original characterization are detected by RTM and confirmed by Sanger sequencing. Almost all additional targets by RTM are confirmed by Sanger sequencing except those with Ct values from RTM are higher than 25-26, which may reach the Sanger sequencing detection limit.

## CONCLUSIONS

- A large collection of TaqMan® assays have been developed to target respiratory potential pathogens. The Respiratory Tract Microbiota (RTM) panel is the most comprehensive PCR-based respiratory panel that is currently on market.
- We have evaluated the Respiratory Tract Microbiota (RTM) panel with clinical research repository specimens and QCMD reference samples.
- High Positive Percent Agreement (PPA) was observed between RTM results and other nucleic acid tests on market.
- Sanger sequencing was performed on both originally claimed targets and additional targets detected only by RTM and further confirmed the high accuracy of the RTM panel.
- In conclusion, the performance demonstrates high accuracy in identifying potential respiratory pathogens and provides an effective tool for researchers to understand pathogenically in respiratory tract infections.

## TRADEMARKS

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