A complete workflow solution for detecting respiratory tract microbiota using OpenArray technology

In this report, we show that:

- Applied Biosystems™ Qualified TaqMan® Assays for respiratory tract microbiota meet rigorous performance criteria
- Applied Biosystems™ OpenArray™ technology allows simultaneous interrogation of up to 42 respiratory tract microbes and 3 controls with the flexibility to customize content
- The Thermo Scientific™ KingFisher™ instrument, Applied Biosystems™ TaqMan® OpenArray™ plate, and Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System are part of a cost-effective, high-throughput workflow with minimal hands-on time

Introduction

Upper and lower respiratory tract infections are caused by a broad range of microbes, including RNA and DNA viruses, bacteria, and even fungi, and yet are often symptomatically similar. Detection of these pathogens can be challenging: immunoassays are limited to a small number of respiratory pathogens and lack sensitivity, whereas culture-based methods are labor intensive, have long turnaround times, and are prone to false-negative results due to fastidious growth in culture. While molecular testing is more sensitive, most commercially available tests are expensive, primarily focus on either viruses or bacteria, and lack the flexibility to customize target lists based on laboratory needs. In addition, concurrent prevalence of viral and bacterial pathogens is a growing concern and needs effective detection options.

To meet the need for more comprehensive coverage of respiratory pathogens, we introduce a panel-based molecular solution that detects a wide range of respiratory viruses, bacteria, and fungi in a single assay. The TaqMan OpenArray plate delivers a high-throughput, qPCR-based assay that can detect pathogenic organisms at very low concentrations. The flexible content can be customized to meet the needs of any laboratory. When combined with a KingFisher instrument and QuantStudio 12K Flex Real-Time PCR System, the plate offers a complete end-to-end solution for respiratory pathogen detection (Figure 1).

Figure 1. Workflow for detection of respiratory tract pathogens. The workflow shows extraction of total nucleic acid from respiratory tract samples using a KingFisher instrument and the Applied Biosystems™ MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit, followed by preamplification and then real-time PCR analysis using the TaqMan OpenArray plate on the QuantStudio 12K Flex system. Results provide a detected/not detected call for each of 42 targets that include bacteria, RNA and DNA viruses, and fungi, as well as for optional *B. atrophaeus* extraction and Xeno RNA spike-in controls.

Materials and methods

Total nucleic acid isolation from respiratory tract samples

The MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kit was used to isolate total nucleic acid (TNA) from respiratory samples. This kit was optimized for extraction of TNA from different microbe types that are found in respiratory samples (RNA and DNA viruses, bacteria, and fungi) and was shown to work well with respiratory sample types including nasopharyngeal swabs, nasopharyngeal aspirates, and bronchoalveolar lavage. TNA isolation from 96 samples using the KingFisher instrument took about 1.5 hours, with 30 minutes of hands-on time.

Detection of respiratory tract microbes on the OpenArray platform

Qualified TaqMan Assay designs and target sequences for respiratory tract microbiota underwent thorough bioinformatics selection and analysis to ensure high strain coverage and specificity. The assays have also undergone extensive performance testing with synthetic templates, nucleic acids extracted from whole-organism standards, and clinical research samples, to ensure that results are accurate and reproducible with high levels of sensitivity and specificity. For a complete list of assays included in the TaqMan Assay collection for respiratory tract microbiota, see Table 1.

OpenArray technology is a high-throughput, flexible-format system for real-time PCR that utilizes a microscope slide–sized stainless steel plate with 3,072 wells for individual 33 nL reactions, where TaqMan Assays are spotted according to customer specifications. The custom-format TaqMan OpenArray plates used in this study contain all 44 respiratory tract microbiota assays against 42 respiratory microbe targets plus 3 control assays; at least 3 technical replicates were run for each assay per plate. Control assays target the Applied Biosystems™ TaqMan® Universal Extraction Control Organism (*B. atrophaeus*), TaqMan® Universal RNA Spike-In/Reverse Transcription (Xeno) Control, and human RNase P gene (*RPPH1*). An inventoried Applied Biosystems™ TaqMan® OpenArray Respiratory Tract Microbiota Plate is available for the identification of 33 key respiratory tract microbial targets plus the 3 control assays. This 112-assay-format plate allows for 3 replicates to be run in parallel for all respiratory tract microbiota assays and for running up to 24 samples per plate.

All samples in this clinical research study were tested using our optimized protocol for respiratory tract microbiota profiling, which utilizes a reverse transcription plus preamplification step for highest sensitivity with the added benefit of sample conservation. Synthetic templates or purified genomic nucleic acid samples were first reverse-transcribed and preamplified as follows: 5 µL of each sample was combined with 2.5 µL of Applied Biosystems™ TaqPath™ 1-Step RT-qPCR Master Mix, CG, and 2.5 µL of Applied Biosystems™ TaqMan® PreAmp Pool, Respiratory Tract Microbiota, then reverse-transcribed and amplified for 14 cycles. Preamplified samples were diluted 1:10 with nuclease-free water, and then 2.5 µL of each diluted sample was combined with 2.5 µL of Applied Biosystems™ TaqMan® OpenArray™ Real-Time PCR Master Mix in an OpenArray™ 384-Well Sample Plate well. Each reaction was then transferred using the Applied Biosystems™ QuantStudio™ 12K Flex AccuFill™ System to subarrays on the TaqMan OpenArray plate. Plates were then run on the QuantStudio 12K Flex Real-Time PCR System, and data were analyzed by the instrument software.

For details on sample extraction, target and control assays, and running experiments, refer to the application guide “Respiratory tract microbiota profiling experiments: TaqMan Assays for respiratory tract microbiota profiling experiments using OpenArray plates” (Pub. No. MAN0017952).

Table 1. Categorization of respiratory tract microorganisms.

Organism type	Nucleic acid type	Assay ID	Assay name	Organism name
Virus	DNA	Vi99990001_po	AdV_1of2	Adenovirus 1/2*
		Vi99990002_po	AdV_2of2	Adenovirus 2/2*
		Vi99990003_po	HBoV	Human bocavirus
		Vi06439647_s1	HHV3	Human herpesvirus 3 (HHV3—varicella zoster virus)
		Vi06439675_s1	HHV4	Human herpesvirus 4 (HHV4—Epstein-Barr virus)
		Vi06439643_s1	HHV5	Human herpesvirus 5 (HHV5—cytomegalovirus)
		Vi06439627_s1	HHV6	Human herpesvirus 6 (HHV6)
	RNA	Vi06439671_s1	CoV_229E	Human coronavirus 229E
		Vi06439674_s1	CoV_HKU1	Human coronavirus HKU1
		Vi06439673_s1	CoV_NL63	Human coronavirus NL63
		Vi06439646_s1	CoV_OC43	Human coronavirus OC43
		Vi06439631_s1	EV_pan	Human enterovirus (pan assay)
		Vi06439669_s1	EV_D68	Human enterovirus D68
		Vi99990004_po	hMPV	Human metapneumovirus (hMPV)
		Vi06439642_s1	hPIV1	Human parainfluenza virus 1 (hPIV1)
		Vi06439672_s1	hPIV2	Human parainfluenza virus 2 (hPIV2)
		Vi06439670_s1	hPIV3	Human parainfluenza virus 3 (hPIV3)
		Vi99990005_po	hPIV4	Human parainfluenza virus 4 (hPIV4)
		Vi99990006_po	HPeV	Human parechovirus
		Vi99990014_po	RSVA	Human respiratory syncytial virus A (RSVA)
		Vi99990015_po	RSVB	Human respiratory syncytial virus B (RSVB)
		Vi99990007_po	RV_1of2	Human rhinovirus 1/2*
		Vi99990008_po	RV_2of2	Human rhinovirus 2/2*
		Vi99990011_po	Flu_A_pan	Influenza A
		Vi99990009_po	Flu_A_H1	Influenza A/H1-2009
		Vi99990010_po	Flu_A_H3	Influenza A/H3
		Vi99990012_po	Flu_B_pan	Influenza B
		Vi99990013_po	Measles	Measles virus
		Vi06439644_s1	MERS_CoV	Middle East respiratory syndrome coronavirus (MERS)
		Vi06439657_s1	Mumps	Mumps virus
		Vi06439634_s1	SARS_CoV	Severe acute respiratory syndrome coronavirus (SARS)
Bacterium	DNA	Ba06439624_s1	*Bordetella*	*Bordetella bronchiseptica, parapertussis,* or *pertussis*
		Ba06439621_s1	*B.holmesii*	*Bordetella holmesii*
		Ba06439623_s1	*B.pertussis*	*Bordetella pertussis*
		Ba06439616_s1	*C.pneumoniae*	*Chlamydophila pneumoniae*
		Ba06439618_s1	*C.burnetii*	*Coxiella burnetii*
		Ba06439625_s1	*H.influenzae*	*Haemophilus influenzae*
		Ba04932083_s1	*K.pneumoniae*	*Klebsiella pneumoniae*
		Ba06439617_s1	*L.pneumophila*	*Legionella pneumophila*
		Ba06439622_s1	*M.catarrhalis*	*Moraxella catarrhalis*
		Ba06439620_s1	*M.pneumoniae*	*Mycoplasma pneumoniae*
		Ba04646259_s1	*S.aureus*	*Staphylococcus aureus*
		Ba06439619_s1	*S.pneumoniae*	*Streptococcus pneumoniae*
Fungus	DNA	Fn06439626_s1	*P.jirovecii*	*Pneumocystis jirovecii*
Control	RNA	Ac00010014_a1	Xeno	Xeno RNA control
	DNA	Hs04930436_g1	*RPPH1*	Ribonuclease P RNA component H1
		Ba06596576_s1	*B.atrophaeus*	*Bacillus atrophaeus* or *subtilis,* subspecies *globigii*

* For adenoviruses and rhinoviruses, two assays are required for full strain coverage. For additional details on each assay, go to **thermofisher.com/taqman**.

Results

Sensitivity and linear dynamic range of TaqMan Assays

The sensitivity, efficiency, and linear dynamic range (LDR) of the TaqMan Assays for respiratory tract microbiota were evaluated using serial dilutions of the Applied Biosystems™ TaqMan® Respiratory Tract Microbiota Amplification Control, which is a linearized plasmid DNA control containing all target sequences. Preamplification and real-time PCR were performed on the amplification control without sample preparation, using the same optimized protocol used for respiratory tract samples and organism control samples. The amplification control dilution series, with input concentrations ranging from 10^5 to 0.1 copies/µL, was tested on custom-format TaqMan OpenArray plates covering all 44 respiratory tract microbiota assays plus control assays (Figure 2).

We achieved high sensitivity (limit of detection, LOD) down to 1–10 copies/µL of input per reaction for all respiratory tract microbiota assays (Figure 2), with minimal variation at lower concentrations. All assays demonstrated a LDR of 5 orders of magnitude (10^5 to 1 copies/µL) where R^2 was greater than 0.99 and PCR efficiency was very close to 100%. Representative LDR data plots are shown for four viral and two bacterial targets in Figure 3, with the standard error bars indicating low variation between replicates.

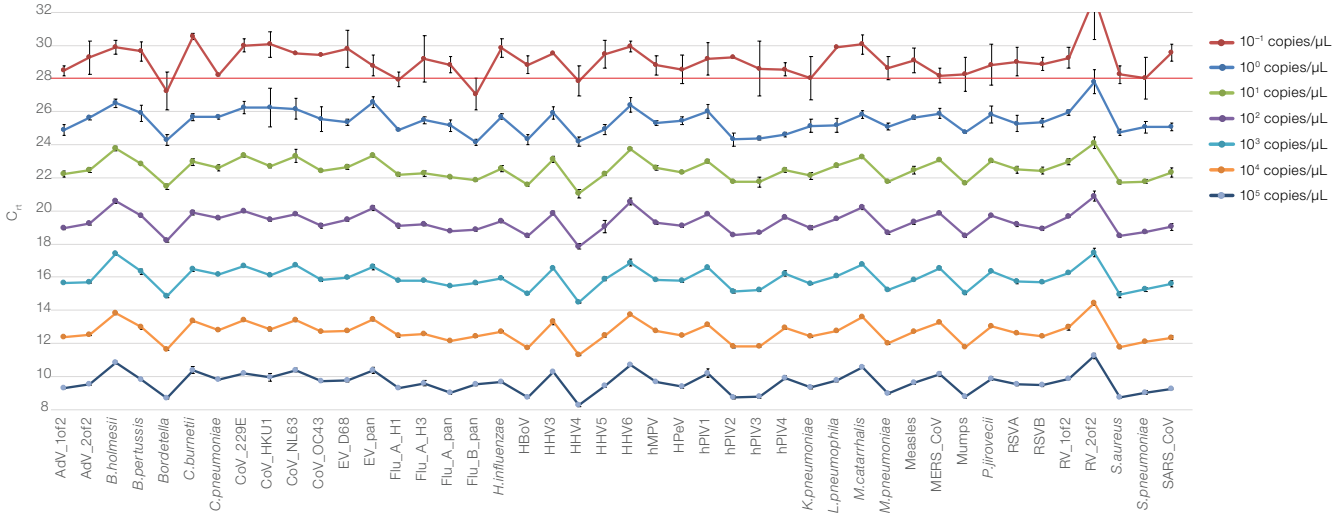

Figure 2. Limit of detection using the TaqMan Respiratory Tract Microbiota Amplification Control. Serial dilutions of 10^5 copies/µL down to 0.1 copies/µL of the amplification control were tested using the optimized preamplification plus real-time PCR protocol on TaqMan OpenArray plates (format 56) containing all 44 respiratory tract microbiota assays. Four technical replicates were run for each concentration. All assays were able to detect down to 1–10 copies/µL of target input using a C_{rt} threshold value of 28 (denoted by red line). Note: The RV_2of2 assay shows higher C_{rt} values and standard deviations than the RV_1of2 assay, as it is mismatched by 1 nucleotide with the amplification control rhinovirus sequence.

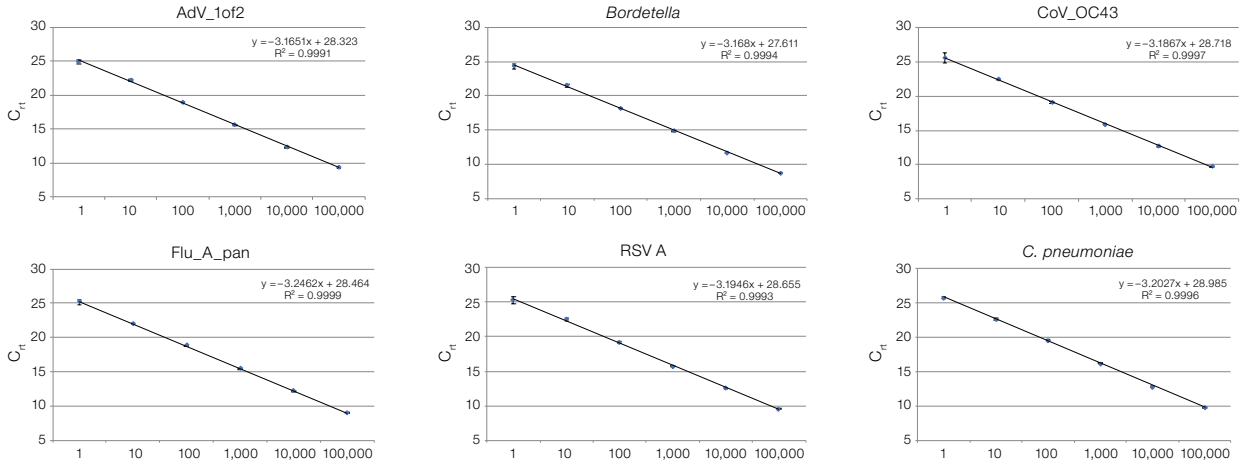

Figure 3. LDR results for representative TaqMan Assays targeting respiratory tract microbiota. Data from the TaqMan Respiratory Tract Microbiota Amplification Control shown in Figure 2 were used to calculate the LDR for the respiratory tract microbiota assays, using dilutions of 10^5 copies/µL down to 1 copy/µL. Plots for 6 representative assays are shown. All assays demonstrated an LDR of 5 orders of magnitude where R^2 was greater than 0.99 and PCR efficiency was approximately 100%.

The workflow LOD was also determined for a subset of the viral and bacterial respiratory target organisms, using low concentrations of 22 commercially available, enumerated whole organisms that were spiked into viral transport media. TNA was extracted using the MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kit, and sample aliquots underwent reverse transcription and preamplification followed by real-time PCR on the TaqMan OpenArray plate. Two-fold dilution series covering the estimated LOD per organism were tested for a total of 12 replicates, and LOD values were calculated by probit regression analysis. LODs were confirmed by testing an additional 12 extractions at 0.25X, 1X, and 4X LOD concentrations. The LOD results shown in Table 2 are similar to those provided in the literature for respiratory tract microbes detected by other qPCR platforms.

Table 2. LODs of TaqMan Assays for respiratory tract microbiota on TaqMan OpenArray plates.

Organism (strain)	Assay name	LOD (units/mL)*	95% confidence interval
Adenovirus (1)	AdV_1of2	7.81×10^{0}	(2.09×10^{0}, 2.92×10^{1})
Coronavirus 229E	CoV_229E	1.40×10^{-1}	(4.11×10^{-2}, 4.77×10^{-1})
Coronavirus NL63	CoV_NL63	2.94×10^{-3}	(1.02×10^{-3}, 8.44×10^{-3})
Coronavirus OC43	CoV_OC43	4.15×10^{-1}	(8.96×10^{-2}, 1.92×10^{0})
Enterovirus type 68 (2007 isolate)	EV_D68	4.01×10^{-2}	(1.26×10^{-2}, 1.28×10^{-1})
Enterovirus type 71 (2003 isolate)	EV_pan	6.95×10^{-2}	(1.70×10^{-2}, 2.85×10^{-1})
Human metapneumovirus (IA-2002)	hMPV	7.83×10^{-2}	(2.25×10^{-2}, 2.73×10^{-1})
Influenza A H1N1 (A/Brisbane/59/07)	Flu_A_pan	6.48×10^{-3}	(2.49×10^{-3}, 1.69×10^{-2})
Influenza A H1N1pdm (NY/03/09)	Flu_A_H1	4.87×10^{-3}	(1.27×10^{-3}, 1.87×10^{-2})
Influenza A H1N1pdm (NY/03/09)	Flu_A_pan	1.63×10^{-2}	(1.14×10^{-3}, 2.32×10^{-1})
Influenza A H3 (A/Wisconsin/67/05)	Flu_A_H3	1.91×10^{-2}	(4.45×10^{-3}, 8.21×10^{-2})
Influenza A H3 (A/Wisconsin/67/05)	Flu_A_pan	1.31×10^{-2}	(4.64×10^{-3}, 3.70×10^{-2})
Influenza B (B/Florida/04/06)	Flu_B_pan	6.11×10^{-2}	(1.96×10^{-2}, 1.91×10^{-1})
Parainfluenza 1	hPIV1	2.58×10^{-3}	(1.40×10^{-3}, 4.79×10^{-3})
Parainfluenza 2	hPIV2	2.70×10^{0}	(8.99×10^{-1}, 8.14×10^{0})
Parainfluenza 3	hPIV3	2.83×10^{0}	(9.13×10^{-1}, 8.75×10^{0})
Parainfluenza 4	hPIV4	1.85×10^{0}	(7.60×10^{-1}, 4.51×10^{0})
Respiratory syncytial virus A (2006 isolate)	RSVA	8.48×10^{-2}	(2.15×10^{-2}, 3.34×10^{-1})
Respiratory syncytial virus B (CH93(18)-18)	RSVB	2.62×10^{-1}	(6.86×10^{-2}, 1.00×10^{0})
Rhinovirus/enterovirus (1A)	RV_1of2	1.49×10^{-1}	(4.08×10^{-2}, 5.43×10^{-1})
Bordetella parapertussis (E595)	*Bordetella*	2.16×10^{4}	(3.74×10^{3}, 1.25×10^{5})
Bordetella pertussis (E431)	*B.pertussis*	9.87×10^{3}	(2.31×10^{3}, 4.21×10^{4})
Bordetella pertussis (E431)	*Bordetella*	9.63×10^{3}	(2.37×10^{3}, 3.91×10^{4})
Legionella pneumophila (Philadelphia)	*L.pneumophila*	2.12×10^{2}	(6.71×10^{1}, 6.70×10^{2})
Mycoplasma pneumoniae (M129)	*M.pneumoniae*	8.71×10^{0}	(1.38×10^{0}, 5.49×10^{1})

* LOD units are $TCID_{50}$/mL for viruses and CFU/mL for bacteria.

High specificity of TaqMan Assays for respiratory tract microbiota

TaqMan Assays for respiratory tract microbiota have undergone rigorous bioinformatic analysis to help ensure maximum strain coverage while minimizing the potential for off-target cross-reactivity. Each assay has been tested with on- and off-target genomic RNA or DNA isolated from target organisms (nucleic acid acquired from ATCC) in our inclusivity panel (Table 3). The inclusivity panel covers 29 of 42 (69%) respiratory tract microbiota targets; missing from this analysis were unculturable and biosafety level 3 and 4 organisms.

The respiratory tract microbiota assays provided highly specific results when screened simultaneously against the available subset of respiratory tract microbial genomes on TaqMan OpenArray plates (Table 4). Testing against nontarget organisms in an exclusivity panel also demonstrated no cross-reactivity of the respiratory tract microbiota assays with closely related species and other respiratory microbes (Table 5 and data not shown).

Table 3. Respiratory tract microbiota inclusivity controls.

Organism type	Nucleic acid type	Organism	ATCC ID*
Virus	DNA	Adenovirus C	VR-846D
		Adenovirus E	VR-1572D
		HHV3	VR-1367DQ
		HHV5	VR-538DQ
		Human coronavirus 229E	VR-740D
		Human coronavirus 0C43	VR-1558D
	RNA	Enterovirus D68	VR-1823D
		Enterovirus 71	VR-1432DQ
		Rhinovirus B	VR-1663DQ
		Influenza A virus (H1N1)	VR-1736D
		Influenza B virus (BY)	VR-1813D
		Measles virus	VR-24D
		Mumps virus	VR-106D
		hPIV1	VR-94D
		hPIV2	VR-92D
		hPIV3	VR-93D
		hPIV4b	VR-1377D
		RSVA	VR-1540D
		RSVB	VR-1803D
Bacterium	DNA	*Bordetella bronchiseptica*	BAA-588D-5
		Bordetella holmesii	51541_D2
		Bordetella parapertussis	BAA-587D-5
		Bordetella pertussis	9797D-5
		Chlamydophila pneumoniae	VR-1360D
		Haemophilus influenzae	51907DQ
		Klebsiella pneumoniae	700721DQ
		Legionella pneumophila	33152DQ
		Moraxella catarrhalis	25240D-5
		Mycoplasma pneumoniae	15531D
		Staphylococcus aureus	BAA-1718DQ
		Streptococcus pneumoniae	700669DQ

* Genomic nucleic acid controls were sourced from ATCC.

Table 4. Specificity testing of respiratory tract microbiota assays with the ATCC inclusivity panel.*

	Adenovirus C	Adenovirus E	*Bordetella holmesii*	*Bordetella pertussis*	*Bordetella parapertussis*	*Bordetella bronchiseptica*	*Chlamydophila pneumoniae*	Coronavirus 229E	Coronavirus OC43	Enterovirus D68	Enterovirus 71	Influenza A (H1N1)	Influenza B	*Haemophilus influenzae*	HHV3	HHV5	PIV1	PIV2	PIV3	PIV4b	*Klebsiella pneumoniae*	*Legionella pneumophila*	*Moraxella catarrhalis*	*Mycoplasma pneumoniae*	Measles	Mumps	RSVA	RSVB	Rhinovirus B	*Staphylococcus aureus*	*Streptococcus pneumoniae*
AdV_1of2	17.98																														
AdV_2of2		19.84																													
B.holmesii			17.44																												
B.pertussis				17.96																											
Bordetella				17.71	17.87	17.79																									
C.pneumoniae							15.09																								
CoV_229E								22.87																							
CoV_OC43									16.35																						
EV_D68										16.80																					
EV_pan										23.14	21.85																				
Flu_A_H1												16.70																			
Flu_A_pan												14.76																			
Flu_B_pan													15.87																		
H.influenzae														17.69																	
HHV3															14.48																
HHV5																15.68															
hPIV1																	16.76														
hPIV2																		15.96													
hPIV3																			15.72												
hPIV4																				16.56											
K.pneumoniae																					14.94										
L.pneumophila																						15.76									
M.catarrhalis																							15.75								
M.pneumoniae																								15.16							
Measles																									16.72						
Mumps																										16.76					
RSVA																											14.66				
RSVB																											27.45	16.63			
RV_1of2										18.04	19.70																		17.40		
RV_2of2																													18.29		
S.aureus																														13.68	
S.pneumoniae																															13.63

* Genomic RNA or DNA at 10^3 copies/µL from 31 ATCC cultivatable respiratory tract microbes were simultaneously screened against all 44 respiratory tract microbiota assays on TaqMan OpenArray plates. The microbial genomic samples are listed in columns and the target assays are listed in rows. The assays specifically amplified their intended targets, and no significant off-target amplifications were detected. The shaded boxes contain the average C_{rt} values (N = 3, C_q calculated by relative threshold method) for each assay–sample combination passing recommended filtration criteria for respiratory tract microbiota assays run with the preamplification plus qPCR protocol (where $C_{rt} \leq 28$, AmpScore ≥1.2, and C_q confidence ≥0.7). Note that the *Bordetella pertussis* sample is detected by both the *B.pertussis* and *Bordetella* assays and the influenza A H1N1 sample is detected by both the Flu_ A_H1 and Flu_A_pan assays. The enterovirus D68 sample is detected by both the EV_D68 and EV_pan assays, though at a much lower efficiency (difference of several C_{rt}) with the EV_pan assay, which does not detect all enterovirus D68 samples. The RV_1of2 assay detects the rhinovirus B sample as well as both enterovirus D68 and 71 samples. This is expected behavior, as the RV_1of2 assay detects both rhinovirus and enterovirus strains whereas the EV_D68 and EV_pan assays are specific for enterovirus strains. The RSVA sample is specifically detected by the RSVA assay and also detected at a much lower efficiency (C_{rt} difference of ~13) by the RSVB assay, due to the high sequence relatedness of RSVA and RSVB.

Table 5. Respiratory tract microbiota exclusivity controls.

Organism type	Nucleic acid type	Organism	ATCC ID*	Near neighbor or environment
Virus	DNA	Vaccinia virus	VR-1508D	Human respiratory pathogen
	RNA	Rubella virus	VR-315D	Human respiratory pathogen
		Rotavirus	VR-2018DQ	Human gastroenteric pathogen
Bacterium	DNA	*Psychrobacter cryohalolentis*	BAA-1226D-5	*Moraxella catarrhalis*
		Pasteurella multocida	700806	*Haemophilus influenzae*
		Raoultella planticola	33531	*Klebsiella pneumoniae*
		Achromobacter xylosoxidans	27061	*Bordetella bronchiseptica, pertussis, parapertussis, or holmesii*
		Blastomyces dermatitidis	26199D-2	Human respiratory pathogen
		Corynebacterium diphtheriae	700971D-5	Human respiratory pathogen
		Burkholderia cepacia	25416D-5	Human respiratory pathogen
		Neisseria meningitidis	700532D-5	Human respiratory pathogen
		Cryptococcus neoformans	MYA-565D-5	Human respiratory pathogen
		Staphylococcus saprophyticus	15305D-5	Human respiratory pathogen
		Streptococcus mitis	49456D-5	Human respiratory pathogen
Fungus		*Aspergillus fumigatus*	1022D	Human respiratory pathogen

* Genomic nucleic acid controls were sourced from ATCC.

Accurate identification of respiratory tract microbes in respiratory samples for clinical research

The sensitivity, specificity, and accuracy of the respiratory tract microbial assays on TaqMan OpenArray plates was further examined by testing with nearly 500 purchased clinical research respiratory samples that were previously characterized for respiratory tract microbes by various methods, including immunoassay, culture, and nucleic acid tests. The overall detection rate of the indicated respiratory tract organisms in these samples by our assays was very high. Shown in Table 6 are the results of concordance analysis conducted for the 400 samples in this set that had been characterized by commercial nucleic acid tests. Samples covering 17 key respiratory viruses were included in this study. A high positive percent agreement of over 97% with other detection platforms was observed.

Table 6. Clinical research sample testing: agreement with other nucleic acid test methods.

Pathogen	True positive*	False negative**	Positive percent agreement
Adenovirus	31	1	96.88%
Coronavirus 229E	2	0	100.00%
Coronavirus HKU1	1	0	100.00%
Coronavirus OC43	1	0	100.00%
Influenza A	6	0	100.00%
Influenza A H1	7	0	100.00%
Influenza A H3	54	0	100.00%
Influenza B	28	0	100.00%
hMPV	25	1	96.15%
hPIV	2	0	100.00%
hPIV1	20	1	95.24%
hPIV2	10	1	95.24%
hPIV3	45	1	97.83%
RSV	6	0	100.00%
RSVA	40	1	97.56%
RSVB	58	1	98.31%
Rhinovirus, enterovirus	75	5	93.75%
Total	411	12	97.16%

* Call from the vendor. Note: Four hPIV3 samples were mischaracterized by a commercial vendor as hPIV1 samples, as confirmed by qPCR, Sanger sequencing, and Ion Torrent™ next-generation sequencing (NGS). An additional 5 samples sold as influenza B samples by a commercial vendor were characterized as negative for influenza B and positive for either RSVA (2 samples), RSVB, influenza A H1, or no target, as confirmed by qPCR, Sanger sequencing, Ion Torrent NGS, and an orthogonal commercial molecular test platform. The orthogonally confirmed characterizations are tallied in the true-positive column for these samples.

** Call not detected by the respiratory tract microbiota assays.

The TaqMan Assay collection for respiratory tract microbiota simultaneously screens 42 respiratory organisms and includes other viral, bacterial, and fungal assays that are not included in the reference tests in our concordance study. In many of the clinical research samples, the respiratory tract microbiota assays detected additional targets that were either present or not present in the reference tests. To determine the veracity of these calls, Sanger sequencing was performed on over 200 additional targets as well as concordant target controls. However, 50 sequencing attempts did not generate sequencing results, either because the target was present in low amounts (e.g., high C_{rt} values) or because the sequencing primers did not detect the target. All 172 targets that generated sequencing results confirmed the identities of the targets that were detected by the respiratory tract microbiota assays (data not shown).

Of note is that 180 of the clinical research respiratory samples tested with respiratory tract microbiota assays on OpenArray plates were also tested on Applied Biosystems™ TaqMan® Array Cards. Results were highly concordant between the tests, demonstrating functional equivalence of the respiratory tract microbiota assays between platforms (for more information, see the application note "A complete workflow solution for detecting respiratory tract microbiota using TaqMan Array Cards", Pub. No. COL33409).

In addition to testing with clinical research samples, TaqMan OpenArray plates were tested with whole-organism proficiency test controls from Quality Control for Molecular Diagnostics (QCMD). Three panels of QCMD samples, which consisted of both negative and positive controls covering 17 common respiratory pathogens (Table 7), were used to evaluate the accuracy of the TaqMan Assays. All control organisms were detected for 100% concordance.

Table 7. TaqMan OpenArray plates tested with QCMD proficiency test control samples.

QCMD control identity	Sample count	TaqMan OpenArray plate results
Adv type 1	1	Detected
Coronavirus NL63	2	Both detected
Coronavirus OC43	1	Detected
Enterovirus 68	1	Detected
hMPV	3	All detected
Influenza type A (H1N1)	1	Detected*
Influenza type A	3	All detected
Influenza type B	2	Both detected
Parainfluenza type 1	1	Detected
RSV type A	2	Both detected
RSV type B	2	Both detected
Rhinovirus	2	Both detected
Bordetella pertussis	1	Detected
Haemophilus influenzae	2	Both detected
Legionella pneumophila	2	Both detected
Mycoplasma pneumoniae	1	Detected
Streptococcus pneumoniae	2	Both detected
Negative	3	Confirmed
Overall	32	All detected

* The influenza type A (H1N1) sample was detected only by the Flu_A_pan assay and not the Flu_A_H1 assay. Flu assays were developed for strains from 2013 onward to capture circulating strains; the Flu_A_H1 assay detects the 2009 pandemic H1N1 strain but may not detect other older strains. Sanger sequencing analysis of the QCMD influenza type A (H1N1) sample showed sequence mismatches with the Flu_A_H1 assay probe binding site, explaining the lack of detection. The sequence matched that of an influenza A H1 strain from 2008 that was not considered in assay design.

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Conclusions

- The Applied Biosystems™ real-time PCR solution for respiratory tract microbiota detection provides an accurate, reliable workflow for identification of a broad range of common and opportunistic respiratory pathogens.
- The TaqMan Assays for respiratory tract microbiota assays demonstrated accurate performance in numerous tests for sensitivity and specificity with different sample types.
- The MagMAX Viral/Pathogen Nucleic Acid Isolation Ultra Kit, optimized for microbial sample preparation, provides an automated solution for extracting total nucleic acid that can be analyzed using the panel of TaqMan Assays for respiratory tract microbiota.
- Qualified TaqMan Assays for respiratory tract microbiota, in combination with flexible-format, high-throughput TaqMan OpenArray plates, provide a low-cost solution for simultaneous detection of viral, bacterial, and fungal pathogens in respiratory tract infections.

Ordering information

Product	Quantity	Cat. No.
TaqMan OpenArray plates and assays		
TaqMan OpenArray Respiratory Tract Microbiota Plate (112-assay format, 24 samples)*	1 plate	A41237
TaqMan OpenArray Real-Time PCR Plate with Inventoried Assays (56-assay format, 48 samples)	1 (10 pack)	4471125
TaqMan OpenArray Real-Time PCR Plate with Inventoried Assays (112-assay format, 24 samples)	1 (10 pack)	4471126
TaqMan OpenArray Real-Time PCR Plate with Inventoried Assays (168-assay format, 16 samples)	1 (10 pack)	4471127
Controls and master mixes		
TaqPath 1-Step RT-qPCR Master Mix, CG	5 x 1 mL	A15299
TaqMan PreAmp Pool, Respiratory Tract Microbiota, 4X	1 x 1 mL	A41374
TaqMan OpenArray Real-Time PCR Master Mix	1 x 5 mL	4462164
TaqMan Universal RNA Spike In/Reverse Transcription (Xeno) Control	5 x 200 µL	A39179
TaqMan Universal Extraction Control Organism (*B. atrophaeus*)	3 vials/kit	A39180
TaqMan Respiratory Tract Microbiota Amplification Control	5 x 50 µL	A39178
Instrumentation and sample preparation		
KingFisher Flex Purification System with 96 Deep-Well Head	1 system	5400630
KingFisher Duo Prime Purification System	1 system	5400110
MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kit	100 preps	A42356
QuantStudio 12K Flex Real-Time PCR System with OpenArray Block	1 system	4472380
QuantStudio 12K Flex AccuFill System	1 system	4471021
Veriti 96-Well Thermal Cycler (or equivalent thermal cycler)	1 system	4375786

* This inventoried plate contains 33 assays to 31 key respiratory pathogens and assays to the TaqMan Universal Extraction Control Organism (*B. atrophaeus*), TaqMan Universal RNA Spike In/Reverse Transcription (Xeno) Control, and human RNase P gene (*RPPH1*). Plates contain 3 replicates of each respiratory tract microbiota assay and the Xeno assay, and 2 replicates of the *B. atrophaeus* and RNase P assays. Up to 23 samples and 1 control sample can be run per plate. Further details are available in the application guide (Pub. No. MAN0017952).

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