Online Supplement

Measurement Techniques and inclusion criteria for the study:
NT-proBNP was measured using the RAMP® diagnostic rapid kit (Response Biomedical Corp, Vancouver, BC, Canada) in whole blood specimens collected in EDTA tubes. RAMP assay uses quantitative immunochromatography and has a measurement range of 18 to 35,000 ng/L. Serum CRP was measured via a high-sensitivity assay on the Advia® 1800 Chemistry System analyzer (Siemens Healthcare GmbH, Erlangen, Germany), located in the Clinical Laboratory of St Paul's Hospital (Department of Pathology and Laboratory Medicine, Vancouver, BC). The analytical range of the assay is 0.2 to 200.0 mg/L including an auto-dilution capability on board the analyzer. In cases where the samples were over the analytical range, they were manually diluted. Complete blood count and differential were measured on whole blood specimens collected in EDTA tubes using the ADVIA ® 2120i Hematology System (Siemens Healthcare GmbH, Erlangen, Germany). Baseline lung function measurements were performed at the time of convalescence (i.e., at day 30 or day 90) for AECOPD patients. Spirometry was used to obtain lung function parameters after bronchodilator administration according to recommendations from ATS/ERS guidelines.[1] Sputum samples were collected on day-1 in OMNIgene®•ORAL (OM-505) tubes and stored in -80 ºC freezers until measurement. The tubes were thawed in a hot bath at 50 ºC for 1 hour and then placed in an air incubator at 24 ºC for 30 minutes. Next, sputolysin was added in 1:1 ratio to liquefy the samples; after which the samples were sub-aliquoted into 500 uL volumes and stored at -80 ºC.

For quantifying bacterial load, DNA was extracted from a 500 uL aliquot using the DNeasy Tissue and Blood extraction kit (Qiagen). The aliquot was centrifuged for 10 minutes at 7500 RPM to form a pellet. After the supernatant was discarded, the pellet was resuspended in 180 uL buffer ATL, according to manufacturer's instructions (DNeasy, Qiagen). DNA concentration was measured using Nanodrop. Droplet digital PCR (ddPCR) (Bio-Rad QX200) was used to quantify the bacterial load, which uses an EvaGreen qPCR assay with primers specifying the 293bp amplicon of the 16S rRNA gene.[2] Briefly, the following protocol was used: 1 cycle at 95°C for 5 minutes, 40 cycles at 95°C for 15 seconds and 60°C for 1 minute, 1 cycle at 4°C for 5 minutes, and 1 cycle at 90°C for 5 minutes all at a ramp rate of 2°C/second. Bio-Rad's T100 thermal cycler was used for the PCR step. A threshold cutoff of 10.000 and a 1/10 dilution of the samples were chosen based on preliminary experiments. Negative controls that comprised of DNase and RNase free water were used and ran alongside the samples. Finally, a correction factor using the formula ([average number of 16S copies per sample – average number of 16S copies in the negative control] / DNA concentration) was applied. In order to extract DNA and RNA simultaneously from the samples, QIAamp MinElute Virus Spin Kit (Qiagen) was used according to manufacturer's instructions. The nucleic acid concentration was then measured using Nanodrop. The extracted nucleic acid samples were sent in 50 uL volumes to Randox Laboratories (Crumlin, UK) for molecular pathogen detection.

The inclusion criteria were being 19 years of age or older, having a diagnosis of COPD, admission to a hospital for a COPD exacerbation or attending the clinic and not experiencing an exacerbation.

Principles and Procedure of the Randox Respiratory Multiplex Array
The Respiratory Multiplex Array II is designed to rapidly screen for the presence of 22 different respiratory pathogens simultaneously from one patient sample. The array is based on a combination of multiplex PCR, target hybridization and chemiluminescence to allow qualitative detection of respiratory pathogens within specimens. The array is approved for nasal swab, bronchoalveolar lavage or sputum specimens. The first step is nucleic acid extraction, and the recommended kit is QIAamp MinElute Virus Spin Kit (Qiagen). After that, a one-step process of reverse transcription combined with multiplex PCR amplification is performed to allow detection of both viral and bacterial nucleic acids within the specimen. If respiratory pathogens are present within the sample, target genes will be amplified to detectable levels. Amplified samples are then added to the Biochip permitting target gene sequences to hybridize to complementary probes spotted on specific regions of the Biochip surface. The Biochip is then imaged on the Evidence Investigator™ where onboard software will

identify the presence of respiratory pathogens within the sample. An extraction control (EC) is incorporated into the array which confirms successful sample nucleic acid extraction and PCR amplification.

Table S1: Pathogens Covered by the Randox Array

Bacteria	Viruses
Legionella pneumophila	Influenza A virus
Chlamydophila pneumoniae	Influenza B virus
Mycoplasma pneumoniae	Human respiratory syncytial virus A
Moraxella catarrhalis	Human respiratory syncytial virus B
Streptococcus pneumoniae	Human parainfluenza virus 1
Bordetella pertussis	Human parainfluenza virus 2
Haemophilus influenzae	Human parainfluenza virus 3
	Human parainfluenza virus 4
	Human coronavirus 229E/NL63
	Human coronavirus OC43/HKU1
	Human adenovirus A/B/C/D/E
	Human rhinovirus A/B/C
	Human enterovirus A/B/C
	Human bocavirus 1/2/3
	Human Metapneumovirus

Table S2: List of the Pathogens Detected for Each Patient in the Cohort

Patient	Pathogen 1	Pathogen 2	Pathogen 3	Pathogen 4
1	Negative			
2	HI	FLU B		
3	Negative			
4	HRV	HI	FLU B	
5	HRV			
6	HI			
7	Negative			
8	Negative			
9	HRV			
10	SP			
11	Negative			
12	HRV			
13	HI	SP	FLU B	
14	Negative			
15	SP			
16	Negative			
17	Negative			
18	Negative			
19	HI			
20	FLU A	HI	SP	
21	SP			
22	HRV			
23	Negative			
24	SP			
25	HI			
26	Negative			
27	RSV A	HI		
28	RSV A	HI	MCAT	
29	PIV 3			
30	HI			
31	HI			
32	HI			
33	FLU A	HI	SP	
34	FLU A	HRV	SP	CORO
35	Negative			
36	FLUA	HRV	SP	
37	PIV 4			
38	SP			
39	FLU A	HI	SP	
40	HI	MPV	SP	
41	Negative			
42	HI	SP		
43	HRV			
44	SP			
45	HI	SP		
46	HI	SP		

47	HI	
48	Negative	
49	HAV	RSV A
50	Negative	
51	HI	SP
52	Negative	
53	MCAT	SP
54	Negative	
55	HRV	SP
56	HRV	SP
57	PIV 4	
58	HI	
59	HI	
60	PIV 2	
61	SP	CORO
62	Negative	
63	HI	
64	HRV	
65	Negative	
66	FLU A	
67	HI	SP
68	HI	
69	HI	
70	HI	
71	HI	
72	Negative	

HI: haemophilus influenzae; SP: streptococcus pneumoniae; HRV: human rhinovirus; FLU: influenza virus; RSV: respiratory syncytial virus; MCAT: moraxella catarrhalis; PIV: parainfluenza virus; HAV: human adenovirus; MPV: metapneumovirus; CORO: coronavirus.

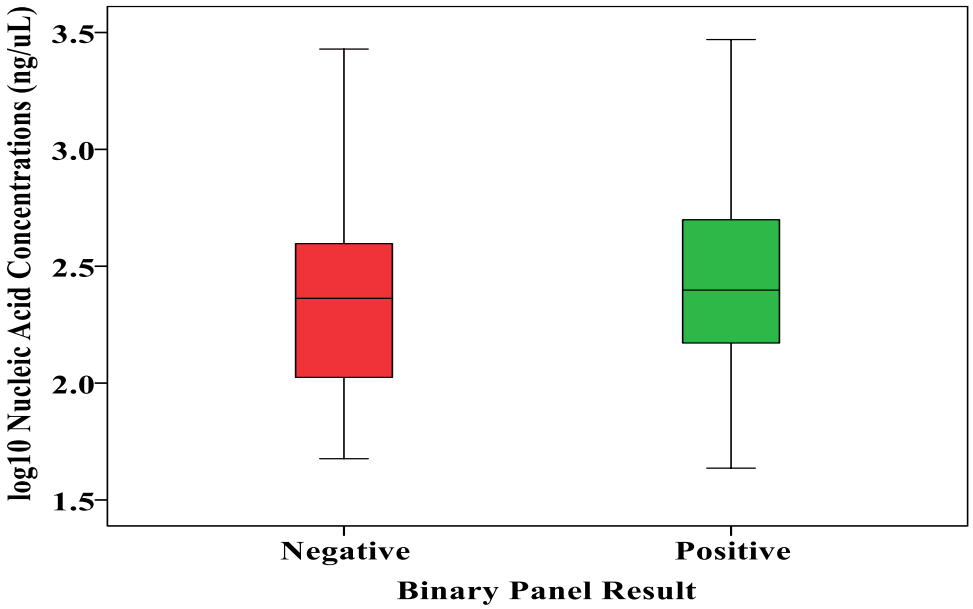

Figure S1: *Box plots representing the nucleic acid concentration in the patients in which no pathogens were detected and the ones with positive pathogen detection. There was no statistically significant difference between the groups (P=0.71).*

Table S3: Demographic and Clinical Data of Study Patients in the Pathogen-negative and Positive groups

	Negative Group	Positive Group	P-value
Age (years)	68.2 ± 11	64.8 ± 11.6	0.273
Male sex	55%	67.3%	0.414
BMI (kg/m^2)	22.7 ± 4.7	25.2 ± 7.5	0.404
Caucasian	85%	78.8%	0.744
Current smokers	45%	68.6%	0.102
Pack years	40 (15-60)	47 (37-70.5)	0.192
Cardiac comorbidities	35%	40.4%	0.790
Home O2 use	35%	14%	0.094
ICS use	75%	68.6%	0.774
16S copies (copies/ng/ul)	20.5 (5.5-108)	35.9 (8.7-84.9)	0.624
eGFR (mL/min/1.73 m^2)	78.6 ± 26	81.7 ± 25.4	0.652
FEV$_1$, percent predicted	44.6 ± 14.5	47.3 ± 17.5	0.613
FEV/FVC ratio (%)	74.4 ± 18.1	69.8 ± 16.2	0.447
GOLD 1	0%	4.9%	
GOLD 2	28.6%	39%	
GOLD 3	57.1%	36.6%	
GOLD 4	14.3%	19.5%	
NT-proBNP (ng/L)	1216.5 (311-1920.7)	369 (183-843)	**0.042**
NT-proBNP change at day-30*	-1102 [-1768 to 61]	-127.5 [-458 to -30]	**0.19**
CRP (mg/L)	25.7 (8.3-98.5)	56.5 (21.6-150.3)	0.382
Length of hospital stay (Days)	6.5 (5-17)	6 (3-8)	0.096
WBC (10^3 cells/uL)	11.1 (5.6-12.7)	8.7 (5.8-13.8)	0.851
Neutrophils (10^3 cells/uL)	8.8 (4.2-11.9)	6.3 (4.3-11.9)	1
Neutrophil%	84.6 (74.5-93.6)	85.1 (79-90.4)	0.939
Lymphocytes (10^3 cells/uL)	0.8 ± 0.5	0.8 ± 0.5	0.891
Lymphocyte%	11.4 ± 8.4	9.3 ± 5.5	0.377
Eosinophils (10^3 cells/uL)	0.007 (0-0.07)	0.025 (0.01-0.06)	0.139
Eosinophil %	0.1 (0-1.3)	0.2 (0.1-0.6)	0.246
Monocytes (10^3 cells/uL)	0.2 (0.1-0.7)	0.4 (0.2-0.6)	0.513

Monocyte%	4.8 ± 4.1	4.8 ± 3.1	0.984
Basophils (10^3 cells/uL)	0.01 (0-0.06)	0.02 (0.01-0.04)	0.695
Basophil%	0.2 (0.09-0.7)	0.2 (0.1-0.4)	0.988
RBC (10^6 cells/uL)	3.8 (3.1-4.4)	4.5 (4-4.8)	**0.029**
Hematocrit	32.5 (29.8-39)	39 (35.6-42.9)	**0.029**
Hemoglobin (g/L)	114 (95-133)	128.5 (111.5-138.5)	**0.031**
MCV	89.4 ± 8.7	88.4 ± 5.3	0.714
MCH	29.8 (27.9-31.5)	28.7 (26.9-31.4)	0.432
MCHC	335.5 (32.2-341.2)	330 (31.1-342.7)	0.590
Platelets (10^3 cells/uL)	229 (182-269)	213.5 (162.2-308.2)	0.761
RDW%	16.2 (14.4-20)	14.6 (13.9-16.1)	**0.025**

Data are represented as mean ±SD or median and interquartile ranges. ICS: inhaled corticosteroid; eGFR: estimated glomerular filtration rate; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; GOLD: global initiative for obstructive lung disease. *calculated as NT-proBNP taken at the 30th day from admission minus the NT-proBNP taken at the day of admission.

Table S4: Demographic and Clinical Data of Study Patients in the Pathogen-negative, Virus and Bacteria groups

	Negative Group	Virus Group	Bacteria Group	P-value
Age (years)	68.2 ± 11.1	64.5 ± 12.6	65.3 ± 10.8	0.533
Male sex	55%	61.5%	73.1%	0.459
BMI (kg/m^2)	22.7 ± 4.7	26.8 ± 9.8	23.9 ± 4.8	0.513
Caucasian	85%	80.8%	76.9%	0.930
Current smokers	45%	72%	65.4%	0.180
Pack years	40 (15-60)	40 (30-70)	57.5 (40-71.5)	0.178
Cardiac comorbidities	35%	42.3%	38.5%	0.953
Home O2 use	35%	16.7%	11.5%	0.161
ICS use	75%	64%	73.1%	0.683
16S copies (copies/ng/ul)	20.5 (5.5-108)	35.9 (9.7-97.2)	34.2 (6.5-86.9)	0.789
eGFR (mL/min/1.73 m^2)	78.6 ± 26	85.8 ± 27	77.8 ± 23.5	0.483
FEV$_1$, percent predicted	44.6 ± 14.5	51 ± 18.4	43.7 ± 16.2	0.340
FEV/FVC ratio (%)	74.4 ± 18.1	70.7 ± 16.8	69.1 ± 16.1	0.721
GOLD 1	0%	10%	0%	
GOLD 2	28.6%	45%	33.3%	
GOLD 3	57.1%	25%	47.6%	
GOLD 4	14.3%	20%	19%	
NT-proBNP (ng/L)	1216.5 (311-1920.7)	370 (199.7-1379.2)	310 (165-713)	0.081
NT-proBNP change at day-30*	-1102 [-1768 to 61]	-130.5 [-474 to -43.5]	-127.5 [-458 to-2.2]	0.391
CRP (mg/L)	25.7 (8.3-98.5)	40.1 (26-71.1)	76.8 (11.7-179.7)	0.618
Length of hospital stay (Days)	6.5 (5-17)	6 (3.75-13.5)	5 (3-7)	**0.046**
WBC (10^3 cells/uL)	11.1 (5.6-12.7)	6.5 (5.4-12.6)	10.4 (6.4-14.1)	0.745
Neutrophils (10^3 cells/uL)	8.8 (4.2-11.9)	5.5 (4.2-11.2)	6.5 (4.5-12.9)	0.812
Neutrophil%	84.6 (74.5-93.6)	85.2 (78.9-91.3)	85 (77.9-89.9)	0.966
Lymphocytes (10^3 cells/uL)	0.8 ± 0.5	0.7 ± 0.4	0.9 ± 0.6	0.666
Lymphocyte %	11.4 ± 8.4	9.6 ± 6.1	9.1 ± 5.2	0.669
Eosinophils (10^3 cells/uL)	0.007 (0-0.07)	0.03 (0-0.1)	0.02 (0.01-0.05)	0.326

Eosinophil %	0.1 (0-1.3)	0.3 (0.1-1.3)	0.2 (0.1-0.4)	0.460
Monocytes (10^3 cells/uL)	0.2 (0.1-0.7)	0.3 (0.2-0.5)	0.5 (0.2-0.6)	0.671
Monocyte%	3.4 (1.5-8.3)	4.6 (2.8-5.7)	3.5 (1.9-7.3)	0.889
Basophils (10^3 cells/uL)	0.01 (0-0.06)	0.01 (0-0.03)	0.02 (0.01-0.1)	0.152
Basophil%	0.2 (0.1-0.7)	0.2 (0.1-0.4)	0.3 (0.20-0.8)	0.155
RBC (10^6 cells/uL)	3.8 (3.1-4.4)	4.4 (4-4.6)	4.5 (3.8-5)	0.086
Hematocrit	32.5 (29.8-39)	38.9 (36.1-41.3)	39.4 (34.5-43.5)	0.089
Hemoglobin (g/L)	114 (95-133)	128 (111-135)	129 (111-144.5)	0.163
MCV	89.4 ± 8.7	88.4 ± 5.9	88.4 ± 4.9	0.898
MCH	29.8 (27.9-31.6)	29.2 (27.3-30.6)	28.2 (26.8-31.5)	0.719
MCHC	335.5 (32.2-341.2)	32.6 (31.1-340)	334 (30.8-343.5)	0.640
Platelets (10^3 cells/uL)	229 (182-269)	201 (180-301)	217 (138-347.5)	0.942
RDW	16.2 (14.4-20)	14.2 (13.8-14.9)	15 (14.1-16.4)	**0.046**

Data are represented as mean ±SD or median and interquartile ranges. ICS: inhaled corticosteroid; eGFR: estimated glomerular filtration rate; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; GOLD: global initiative for obstructive lung disease *calculated as NT-proBNP taken at the 30th day from admission minus the NT-proBNP taken at the day of admission.

Table S5: Length of Hospital Stay ANOVA Statistical Analysis Results.

Group	Number	Mean (SD) (log10)	F (*df*)	P-value	P-value Trend
Negative	20	0.9 (0.33)	3.21 (2,69)	0.046	0.017
Virus	26	0.8 (0.35)			
Bacteria	26	0.7 (0.28)			

ANOVA statistical analysis results for the length of hospital stay between the three groups (log10 transformed).

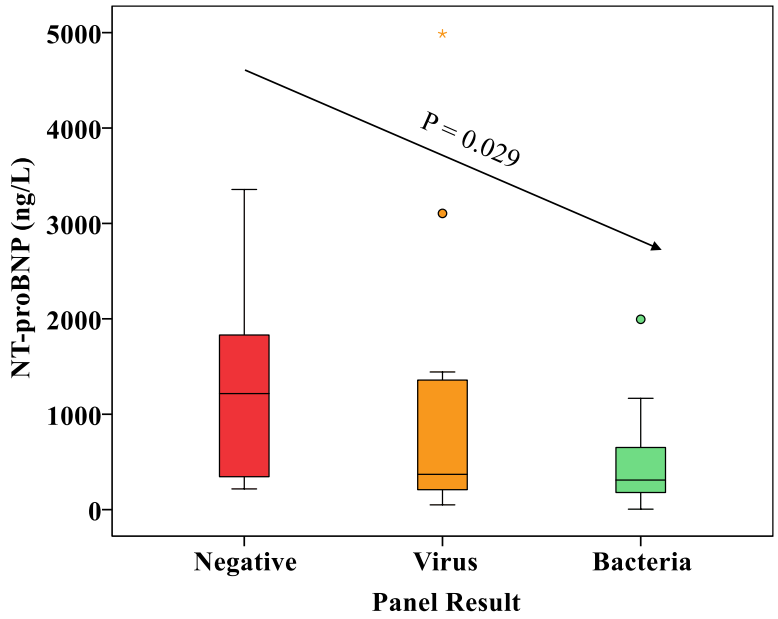

Figure S2: Box Plots Depicting NT-proBNP Concentrations in the Three Groups*. A statistically significant trend (P=0.029) in NT-proBNP concentrations was observed, with the pathogen-negative group having the highest concentrations, and the bacteria group having the lowest.*

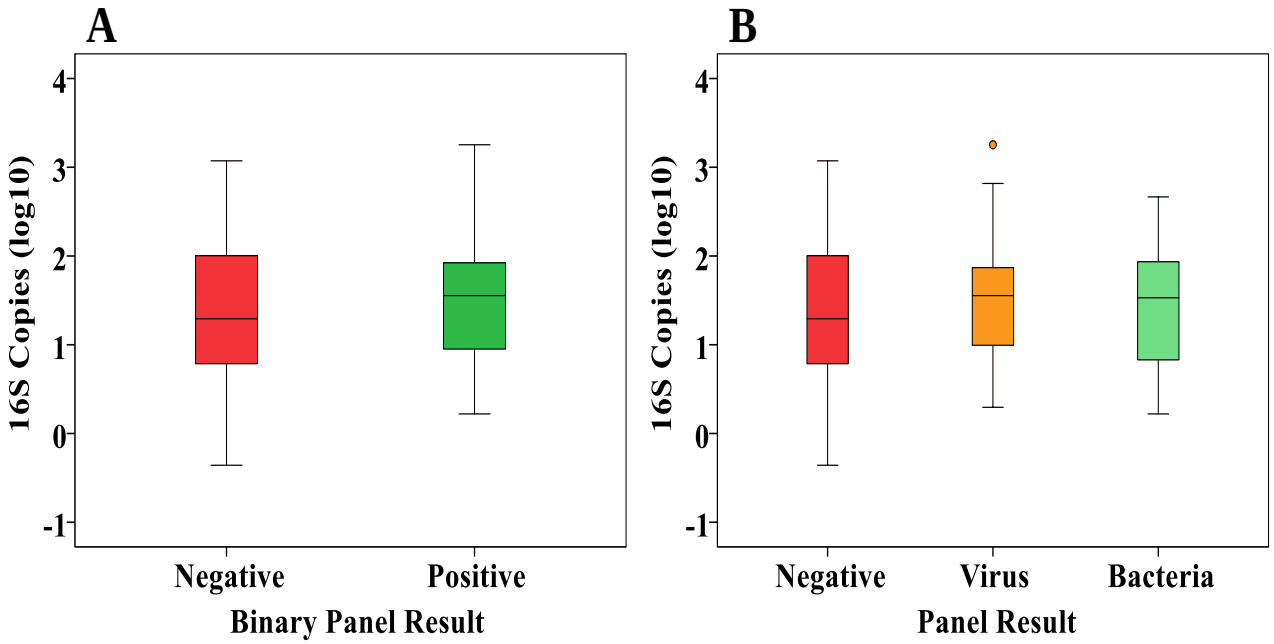

Figure S3: Bacterial Load Comparisons Between Groups. *Box plots depicting the bacterial load in different groups. A) Pathogen-negative (non-infectious) and positive (infectious) groups. B) The positive (infectious) group subdivided by viral detection. There were no statistically significant differences between groups in bacterial load.*

Table S6: Causes of Death in the Study.

Subject	Cause of Death
1	Cardiac Arrest
2	Fatal Arrythmia
3	End-stage COPD
4	End-stage COPD
5	Pneumonia
6	Subarachnoid Hemorrhage
7	Unknown
8	Unknown
9	Unknown
10	Unknown
11	Unknown
12	Unknown

Table S7: Randox Respiratory Multiplex Array II sensitivity and specificity

Target	RMA II Sensitivity	RMA II Specificity
Flu A	93.6	97.5
Flu B	93.8	99.4
RSV	95.1	93.6
PIV 1	100	98.5
PIV 2	100	99.3
PIV 3	53.8	98.9
PIV 4	100	100
HCV229E/ NL63	86.4	100
HCVOC43/ HKU1	96	97.1
HEV	50	100
HRV	74.6	93.9
HAV	100	93.7
HBOV	94.1	98.1
MPV	100	100
MP	98.7	98.3
CP	100	100
LP	100	100
SP	74.1	80
MC	70.8	81.4
HI	86.1	80.4
BP	100	98.7

Flu A, influenza type A. Flu B; influenza type B. RSV; respiratory syncytial virus. PIV; parainfluenza virus. HCV229E/ NL63; human corona virus type HCV229E/ NL63. HCVOC43/ HKU1; human corona virus type HCVOC43/ HKU1. HEV; human enterovirus. HRV; human rhinovirus. HAV; human adenovirus. HBOV; human bocavirus. MPV; metapneumovirus. MP; mycoplasma pneumoniae. CP; Chlamydophila pneumoniae. LP; legionella pneumophila. SP; streptococcus pneumoniae. MC; Moraxella catarrhalis. HI; Hemophilus influenzae. BP; Bordetella Pertussis.

The functional sensitivity and specificity of this assay was determined by comparison of results against TaqMan probe based quantitative Real Time PCR (qRT-PCR). A total of 436 nucleic acid extracts from clinical specimens were screened using TaqMan qRT-PCR prior to analysis with Respiratory Multiplex Array II. Results for this array were compared to those of the TaqMan qRT-PCR "gold standard" for each sample (the data was taken from the user manual for the panel [Respiratory Multiplex Array II EV3947 User Manual].

References

1. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J.* 2005;26(2):319-338.
2. Sze MA, Abbasi M, Hogg JC, Sin DD. A Comparison between Droplet Digital and Quantitative PCR in the Analysis of Bacterial 16S Load in Lung Tissue Samples from Control and COPD GOLD 2. *PLoS One.* 2014;9(10):e110351.