Clinical Evaluation of the BioFire® FilmArray® Pneumonia Panel *plus*

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Background

The BioFire FilmArray Pneumonia Panel *plus* identifies select organisms and antimicrobial resistance (AMR) genes in sputum (including endotracheal aspirate) and BAL (including mini-BAL) specimens using nested, multiplexed PCR followed by melting curve analysis.

Viruses and atypical bacteria are reported qualitatively as Detected or Not Detected. The AMR genes are also reported qualitatively (Detected/Not Detected), but only if one or more applicable bacteria (i.e. potential carriers of the AMR gene) are also detected in the sample.

For bacteria assays, the BioFire Pneumonia Panel *plus* targets single copy genes and reports an approximate quantity of organism (i.e. bacterial DNA in copies/mL).

AMR Gene Reporting

AMR Gene Result	Applicable Bacteria
***mecA/C* and MREJ**	*Staphylococcus aureus*
CTX-M	*Acinetobacter calcoaceticus-baumannii* complex
IMP	*Enterobacter aerogenes*
KPC	*Enterobacter cloacae* complex
NDM	*Escherichia coli*
OXA-48-like	*Klebsiella oxytoca*
VIM	*Klebsiella pneumoniae*
	Pseudomonas aeruginosa
	Proteus spp.
	Serratia marcescens

Bacteria Reporting

Assay Result		Reported Result and Bin Result	
Negative OR	<10^3.5 copies/mL	Not Detected	
Positive AND	≥10^3.5 – <10^4.5 copies/mL	Detected	10^4 copies/mL
Positive AND	≥10^4.5 – <10^5.5 copies/mL	Detected	10^5 copies/mL
Positive AND	≥10^5.5 – <10^6.5 copies/mL	Detected	10^6 copies/mL
Positive AND	≥10^6.5 copies/mL	Detected	≥10^7 copies/mL

Specimen Enrollment

A prospective clinical evaluation was conducted between October 2016 – July 2017 at 8 sites in the United States and enrolled a total of 1682 specimens (846 BAL and 836 sputum). Patient population included adults and children as well as hospitalized, out-patient, and ED.

Reference Methods

Pneumonia Panel	Reference / Comparator Method(s)
Bacteria	Method 1 – Sensitivity and Specificity performance: Quantitative reference culture (qRefCx)[a] Method 2 – Bin performance: quantitative PCR/sequencing method (qMol)
AMR Genes	qMol (from specimen nucleic acid extract)
Atypical Bacteria and Viruses[b]	Conventional PCR & Sequencing

[a] Specimens were plated at dilutions over four logs; bacteria considered "Detected" if enumerated at 10^3.5 CFU/mL or greater

[b] MERS-CoV assumed negative; not circulating in US during testing period

Performance: Viruses compared to Conventional PCR+seq

Analyte	Specimen Type	Panel Det.	Sensitivity/PPA TP/(TP+FN)	Sensitivity/PPA %	Specificity/NPA TN/(TN+FP)	Specificity/NPA %
Adenovirus	BAL	8	8/8	100	837/837	100
	Sputum	15	13/17	76.5	815/817	99.8
Coronavirus	BAL	31	18/21	85.7	810/823	98.4
	Sputum	34	28/32	87.5	796/802	99.3
Human Metapneumovirus	BAL	9	8/8	100	836/837	99.9
	Sputum	21	20/21	95.2	812/813	99.9
Human Rhinovirus/ Enterovirus	BAL	63	52/54	96.3	771/782	98.6
	Sputum	109	96/96	100	717/730	98.2
Influenza A	BAL	13	10/10	100	830/833	99.6
	Sputum	16	13/13	100	819/822	99.6
Influenza B	BAL	6	5/6	83.3	837/838	99.9
	Sputum	14	12/12	100	821/823	99.8
Middle East respiratory syndrome coronavirus (MERS-CoV)	BAL	0	0/0	-	846/846	100
	Sputum	0	0/0	-	836/836	100
Parainfluenza Virus	BAL	18	16/18	88.9	824/826	99.8
	Sputum	30	28/29	96.6	804/806	99.8
Respiratory Syncytial Virus	BAL	3	3/3	100	841/841	100
	Sputum	47	43/43	100	787/791	99.5

Discrepancy investigation summary: Evidence of analyte presence was observed in 5/5 FN BAL, 10/10 FN sputum, 21/31 FP BAL, and 24/33 FP sputum specimens by various methods.

Performance: Bacteria compared to qRefCx

Analyte	Specimen Type	Panel Det.	Sensitivity/PPA TP/(TP+FN)	Sensitivity/PPA %	Specificity/NPA TN/(TN+FP)	Specificity/NPA %
***Acinetobacter calcoaceticus-baumannii* complex**	BAL	7	0/0	-	839/846	99.2
	Sputum	28	10/11	90.9	807/825	97.8
Enterobacter aerogenes	BAL	13	6/7	85.7	832/839	99.2
	Sputum	12	3/4	75.0	823/832	98.9
***Enterobacter cloacae* complex**	BAL	23	11/12	91.7	822/834	98.6
	Sputum	32	11/12	91.7	803/824	97.5
Escherichia coli	BAL	20	12/12	100	826/834	99.0
	Sputum	48	23/24	95.8	787/812	96.9
Haemophilus influenzae	BAL	82	10/10	100	764/836	91.4
	Sputum	107	16/18	88.9	727/818	88.9
Klebsiella oxytoca	BAL	11	2/2	100	835/844	98.9
	Sputum	19	9/9	100	817/827	98.8
***Klebsiella pneumoniae* group**	BAL	27	15/15	100	819/831	98.6
	Sputum	65	21/23	91.3	769/813	94.6
Moraxella catarrhalis	BAL	29	0/0	-	817/846	96.6
	Sputum	75	5/5	100	761/831	91.6
***Proteus* spp.**	BAL	9	5/5	100	837/841	99.5
	Sputum	23	15/15	100	813/821	99.0
Pseudomonas aeruginosa	BAL	74	36/36	100	772/810	95.3
	Sputum	160	103/106	97.2	673/730	92.2
Serratia marcescens	BAL	12	6/6	100	834/840	99.3
	Sputum	53	26/27	96.3	782/809	96.7
Staphylococcus aureus	BAL	116	46/47	97.9	729/799	91.2
	Sputum	204	111/112	99.1	631/724	87.2
Streptococcus agalactiae	BAL	25	1/1	100	821/845	97.2
	Sputum	43	9/9	100	793/827	95.9
Streptococcus pneumoniae	BAL	29	5/5	100	817/841	97.1
	Sputum	51	16/16	100	785/820	95.7
Streptococcus pyogenes	BAL	8	2/2	100	838/844	99.3
	Sputum	11	6/6	100	825/830	99.4

Discrepancy investigation summary: Evidence of analyte presence was observed in 2/3 FN BAL, 7/13 FN sputum, 326/328 FP BAL, and 545/547 FP sputum specimens by various methods. FN in 5 sputum specimens were due to misidentification of the organism by the comparator method, and 1 FN sputum was due to a specimen swap.

Performance: AMR genes compared to conventional PCR + seq (from specimen) when BioFire Pneumonia Panel *plus* had applicable detection

Analyte	Specimen Type	Panel Det.	Sensitivity/PPA TP/(TP+FN)	Sensitivity/PPA %	Specificity/NPA TN/(TN+FP)	Specificity/NPA %
CTX-M	BAL	6	6/7	85.7	144/144	100
	Sputum	9	8/10	80.0	280/281	99.6
IMP	BAL	0	0/0	-	151/151	100
	Sputum	0	0/0	-	291/291	100
KPC	BAL	3	2/2	100	148/149	99.3
	Sputum	7	7/7	100	284/284	100
***mecA/C* and MREJ**	BAL	46	40/45	88.9	64/70	91.4
	Sputum	107	94/98	95.9	91/104	87.5
NDM	BAL	0	0/1	0	149/150	99.3
	Sputum	0	0/0	-	291/291	100
OXA-48-like	BAL	0	0/0	-	151/151	100
	Sputum	0	0/0	-	291/291	100
VIM	BAL	0	0/0	-	151/151	100
	Sputum	2	1/1	100	289/290	99.7

Discrepancy investigation summary: Evidence of AMR gene presence was observed in 7/7 FN BAL, 5/6 FN sputum, 6/8 FP BAL, and 13/15 FP sputum specimens by various methods.

Performance: Atypical Bacteria compared to conventional PCR + seq

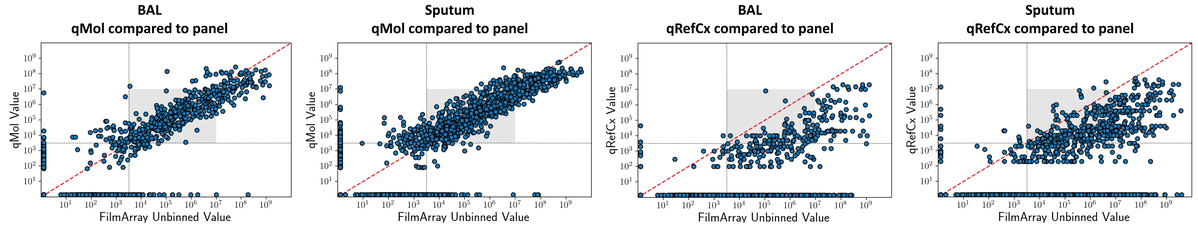
Analyte	Specimen Type	Panel Det.	Sensitivity/PPA TP/(TP+FN)	Sensitivity/PPA %	Specificity/NPA TN/(TN+FP)	Specificity/NPA %
Chlamydia pneumoniae	BAL	1	0/0	-	844/845	99.9
	Sputum	0	0/0	-	835/835	100
Legionella pneumophila	BAL	2	2/2	100	833/833	100
	Sputum	0	0/1	0	826/826	100
Mycoplasma pneumoniae	BAL	4	3/3	100	841/842	99.9
	Sputum	7	7/8	87.5	827/827	100

Discrepancy investigation summary: *L. pneumophila* was observed by an independent molecular method in the single FN sputum specimen.

BioFire Pneumonia Panel *plus* Raw Value vs Quantitative Comparators

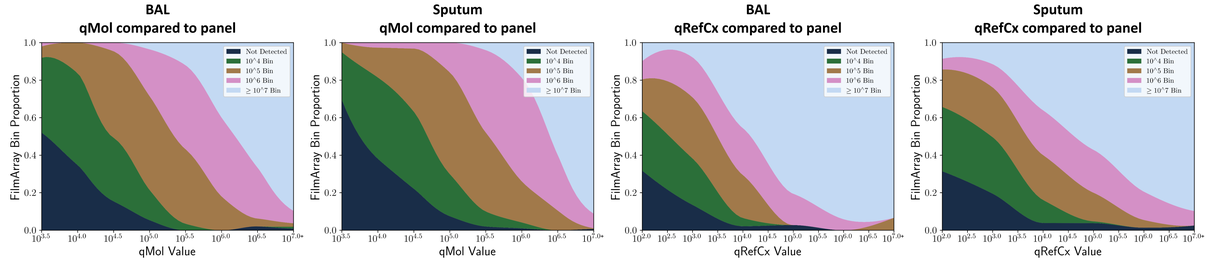
qMol was used to measure the quantity of bacteria present in a specimen. Individual specimen positions are determined by the qMol value on the y-axis and the BioFire Pneumonia Panel *plus* unbinned (raw) value on the x-axis. The region shaded in grey represents the range for values that span the 10^3.5 Detected/Not Detected threshold and the highest value (10^7.0) validated by both the BioFire Pneumonia Panel *plus* and qMol comparator.

BioFire Pneumonia Panel *plus* unbinned values reported relative to the qRefCx are also presented to demonstrate how the BioFire FilmArray Pneumonia Panel result compares to a relevant gold standard.



BioFire Pneumonia Panel *plus* Bin vs Quantitative Comparators

The stacked area charts below demonstrate the proportion of each reported BioFire Pneumonia Panel *plus* bin result over the range of qMol and qRefCx values.



Bin Performance

BioFire Pneumonia Panel *plus* bin performance compared to qMol is shown for BAL and sputum. The qMol cells are broken into one-log ranges. A BioFire Pneumonia Panel *plus* bin result is considered concordant if the qMol value is within 0.5 log of the bin boundary. For example, the 10^5 BioFire Pneumonia Panel *plus* bin (10^4.5-10^5.5) is concordant with the qMol range of 10^4 to <10^6. The top line (bold) in the table shows the qMol range broken down by one-log increments. The second line (in parentheses) shows the BioFire Pneumonia Panel *plus* bins that are concordant with that qMol range.

BAL Bin Performance							
qMol Range (Concordant Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (ND or 10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
Panel Bin	ND	12025	25	15	0	2	0
	10^4	47	28	36	5	0	1
	10^5	5	6	47	45	5	1
	10^6	3	1	7	48	27	2
	≥10^7	2	0	0	17	74	66
% concordant		**12025/12082 (99.5%)**	**53/60 (88.3%)**	**83/103 (79.0%)**	**93/115 (80.9%)**	**101/108 (93.5%)**	**66/70 (94.3%)**
			396/458 (86.5%)				

Sputum Bin Performance							
qMol Range (Concordant Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (ND or 10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
Panel Bin	ND	11392	51	47	6	1	1
	10^4	79	31	84	18	2	0
	10^5	12	9	75	80	20	1
	10^6	2	1	11	80	65	15
	≥10^7	4	0	0	13	113	207
% concordant		**11392/11489 (99.2%)**	**82/92 (89.1%)**	**159/217 (73.3%)**	**160/197 (81.2%)**	**178/201 (88.6%)**	**207/224 (92.4%)**
			786/931 (84.4%)				

Conclusions

- The BioFire Pneumonia Panel *plus* is a specific test for the detection of bacteria, atypical bacteria, viruses, and antimicrobial resistance genes from BAL and sputum specimens compared to molecular methods. Specificity of bacterial assays relative to culture was observed to be as low as 88% due to the fundamental differences between the molecular method used by the BioFire Pneumonia Panel *plus* (measuring target genomic copies) and culture (counting viable organisms), particularly for fastidious organisms.
- The BioFire Pneumonia Panel *plus* is a sensitive test for the detection of atypical bacteria, viruses, and antimicrobial resistance genes from BAL and sputum specimens. Sensitivity of bacterial assays relative to reference culture was excellent; five out of 16 total FN results in both specimen types were due to misidentifications made by the reference method, and one additional FN was attributed to a specimen swap.
- The binned bacterial result assigned by the BioFire Pneumonia Panel *plus* is highly concordant with an independent quantitative molecular method. However, the test consistently reports bacteria in sputum and BAL specimens at higher levels than reference culture. This is attributed to the fundamental differences between the molecular method used by the BioFire Pneumonia Panel *plus* (measuring target DNA copies) and culture (counting viable organisms), particularly for fastidious organisms.

Data presented are from assays that are Investigational Use Only (IUO) and have not been cleared or approved for diagnostic use. Contact Information: scott.kerr@biofiredx.com