Verification of BioFire® FilmArray Pneumonia Panel *plus* Detections by Alternate Methods

P0569
Abstract #2752

D. Judd[1], B. Graham[1], D. Sadrija[1], O. Cham[1], C. Li[1], J. Stone[1], K. Broadbent[1], C. Graue[1], M. Jones[1], J. Cloud[1], and M. Rogatcheva[1]

[1]BioFire Diagnostics, LLC, Salt Lake City, UT, USA.

Background

The BioFire FilmArray Pneumonia Panel *plus* is intended to identify pneumonia causing agents in unprocessed sputum (including endotracheal aspirate (ETA)) and bronchoalveolar lavage (BAL) specimens. The panel detects bacteria, viruses, and select antimicrobial resistance markers. Clinical performance was evaluated in a multi-center study by comparing FilmArray detections to the results of other methods used to detect pathogenic organisms.

Any discordant detections were investigated to determine the root cause of false positive (FP) and false negative (FN) discrepancies using additional molecular methods.

BioFire® FilmArray®
Pneumonia Panel *plus* Investigational Use Only
34 Targets

Bacteria
Acinetobacter calcoaceticus-baumannii complex
Serratia marcescens
Proteus spp.
Klebsiella pneumoniae group
Enterobacter aerogenes
Enterobacter cloacae complex
Escherichia coli
Haemophilus influenzae
Moraxella catarrhalis
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pneumoniae
Klebsiella oxytoca
Streptococcus pyogenes
Streptococcus agalactiae

Atypical Bacteria
Legionella pneumophila
Mycoplasma pneumoniae
Chlamydia pneumoniae

Viruses
Influenza A
Influenza B
Respiratory Syncytial Virus
Human Rhinovirus/Enterovirus
Human Metapneumovirus
Parainfluenza Virus
Adenovirus
Coronavirus
Middle East Respiratory Syndrome Coronavirus

Antimicrobial Resistance Genes
mecA/C and MREJ
KPC
NDM
OXA-48-like
CTX-M
VIM
IMP

Sample Type: Sputum, Endotracheal aspirate, Bronchoalveolar Lavage, and mini-BAL

BioFire Pneumonia Panel binning algorithm used to report semi-quantitative results

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

Materials and methods

All comparator testing was performed in a blinded manner by personnel with no knowledge of BioFire Pneumonia Panel results

Comparator Methods:
Quantitative reference culture (qRefCx) is a standard culture method that relies on plate enumeration to obtain a quantity. qRefCx was the comparator method used for semi-quantitative bacterial detections and was performed at a central reference laboratory. Results quantified at or above 10^3.5 CFU/mL were considered positive while results below the cutoff were considered negative.

Quantitative molecular method (qMol) is PCR followed by Next-Generation Sequencing (NGS). qMol was the comparator method used for AMR detections and was performed at the BioFire Laboratory.

Conventional PCR and Sanger sequencing was used in this study and utilizes 2 specific molecular assays for all atypical bacteria and viral analytes. Conventional PCR followed by Sanger sequencing was the comparator method used to confirm viral and atypical bacterial detections and was performed at the BioFire Laboratory.

Clinical samples with discordant results between FilmArray and comparator testing were selected for investigation and DNA from residual specimens was extracted and tested using molecular assays targeting genes or gene regions different from the FilmArray targets. Bi-directional sequencing of the amplified region(s) was used to confirm the identity of organisms.

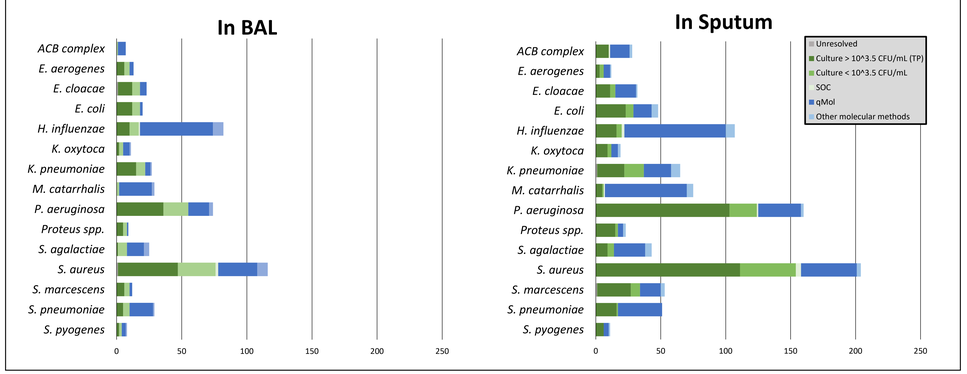
Discrepancy Methods:
Standard of Care (SOC) testing is a culture method ordered by the physician and performed at the medical clinic when a patient presents with symptoms of a lower respiratory tract infection. SOC results were used as an initial method for resolving discordant detections between FilmArray and the comparator method.

Other molecular methods refers to additional PCR based molecular assays followed by Sanger sequencing. These additional assays were designed at BioFire Diagnostics and performed in the BioFire Laboratory.

FilmArray Pneumonia Panel retests using residual sample were also used to resolve discrepancies. The retests were performed at the BioFire Laboratory and were mainly used when investigating false negative results.

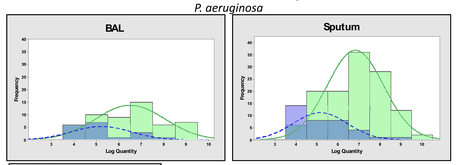
Bacteria

Confirmation of BioFire FilmArray Detections by Different Methods
Reference Method: qRefCx ≥10^3.5 CFU/mL

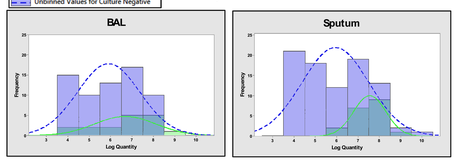


BioFire FilmArray Unbinned Values Reported
Culture Positive vs Culture Negative

P. aeruginosa

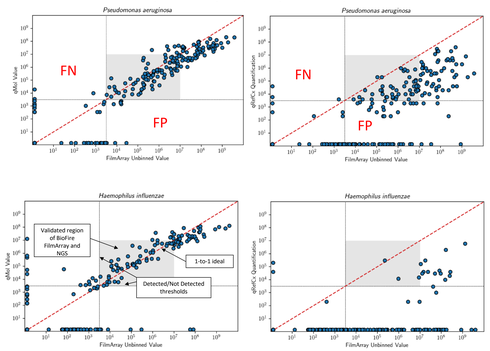


H. influenzae



Histogram distribution of BioFire FilmArray unbinned values observed in samples that were qRefCx positive compared to samples that were qRefCx negative. As expected, BioFire FilmArray is more sensitive to low level analytes than qRefCx. Unexpectedly, BioFire FilmArray also detected high titer organisms that qRefCx missed.

BioFire FilmArray Unbinned Values Compared To qMol and qRefCx in Sputum



Certain organisms are more likely to be identified by qRefCx than others. For example, *P. aeruginosa* is identified by qRefCx the majority of the time. On the other hand, the BioFire Pneumonia Panel identified *H. influenzae* more often than qRefCx.

Bacteria False Negatives

Cause of False Negative Results

Analyte	BioFire FilmArray Below Quant (low level)	Missed Detection (low level)	Misidentified
E. cloacae complex	1	1	0
E. coli	1	0	0
K. pneumoniae group	1	1	0
S. marcescens	1	0	0
S. aureus	1	1	0
E. aerogenes	0	1	1
H. influenzae	0	1	1
P. aeruginosa	1	0	2
ACB complex	0	0	1

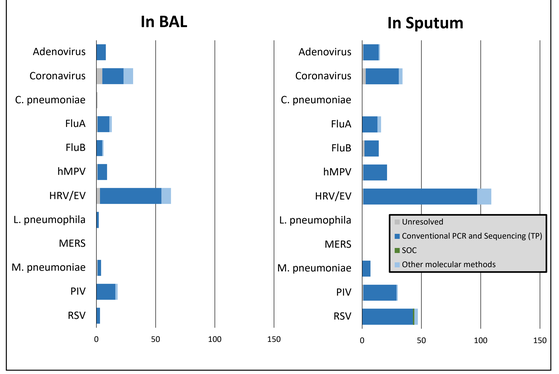
Investigation of Misidentified Organisms

Reference Lab Identification	16S Sequencing Results and ID Confidence	In House VITEK Results and ID Confidence	High Resolution Molecular Method Sequence Results and ID Confidence
ACB complex	99% *Pseudomonas* spp.	**96% *P. fluorescens***	N/A
E. aerogenes	100% *Hafnia* spp.	**99% *Hafnia alvei***	**98% *Hafnia paralvei***
H. influenzae	*100% H. influenzae* or *Haemophilus* spp.	Unidentified	**97% *H. haemolyticus***
P. aeruginosa	100 % *P. aeruginosa* or *Pseudomonas* spp.	Low Discrimintation--*Pseudomonas* spp.	**94% *P. denitrificans***
P. aeruginosa	100 % *P. aeruginosa* or *Pseudomonas* spp.	Low Discrimination--*Acinetobacter* or *Pseudomonas* spp.	**99% *P. fluorescens***

These isolates were investigated and determined to be misidentified by the reference laboratory. Resolved identifications are highlighted in green.

Viruses and Atypical Bacteria

Confirmation of BioFire FilmArray Detections by Different Methods
Reference Method: Conventional PCR and Sanger Sequencing

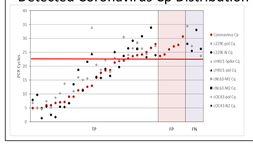


Nature of Discrepancies

Discrepancy investigation demonstrated that the majority of discordant results were a consequence of analyte levels at or below the LoD of both the BioFire FilmArray and comparator assays (which had equivalent sensitivity) and thus resulted in spurious detections.

- Nearly all discrepancies observed in the clinical trial were sequence confirmed to be present in the sample.
- When multiple replicates per specimen were tested inconsistent detections were observed for both FilmArray and comparator assays.

Detected Coronavirus Cp Distribution



Viral and Atypical Bacteria False Negatives

Analyte	Missed Detection (low level)	Unresolved
L. pneumophila	1	0
Adenovirus	4	0
Coronavirus	6	1
hMPV	1	0
HRV/EV	2	0
Influenzae B	1	0
Parainfluenza virus	3	0
M. pneumoniae	0	1

Coronavirus detections in sputum for BioFire FilmArray and comparator assays are shown in the accompanying graph. The red line depicts the level at which Coronavirus positive samples at LoD are detected. The Coronavirus false positive results are all detected later than what would be expected at LoD thus suggesting that these samples are Coronavirus positive but at a concentration below LoD. As expected, false negative results are below the LoD of the comparator assays.

Discrepancy Investigation Summary

Bacteria Discrepancy Analysis		BAL	Sputum
FN	Total Discrepant	3	13
	FilmArray below the 10^4 bin	1	5
	FilmArray negative; evidence by qMol	1	2
	Resolved--other (e.g. isolate misidentification, SOC)	1	5
	Not Resolved	**0**	**1**
FP	Total Discrepant	328	547
	qRefCx enumerated below 10^3.5 CFU/mL	105	115
	qRefCx negative; evidence by qMol	218	421
	Resolved--other (e.g. SOC)	3	9
	Not Resolved	**2**	**2**

15/16 (93.8%) of bacterial false negatives were resolved as low level or misidentification

871/875 (99.5%) of bacterial false positive results were resolved as low level true positive

Virus Discrepancy Analysis		BAL	Sputum
FN	Total Discrepant	8	10
	Evidence by independent molecular method	5	10
	Resolved--other	0	0
	Not Resolved	**3**	**0**
FP	Total Discrepant	31	33
	Evidence by independent molecular method	19	21
	Resolved--other (e.g. SOC, FilmArray retest)	2	3
	Not Resolved	**10**	**9**

15/18 (83.3%) of viral false negatives contained the analyte of interest at or below LoD

45/64 (70.3%) of viral false positive results were resolved as low level true positive

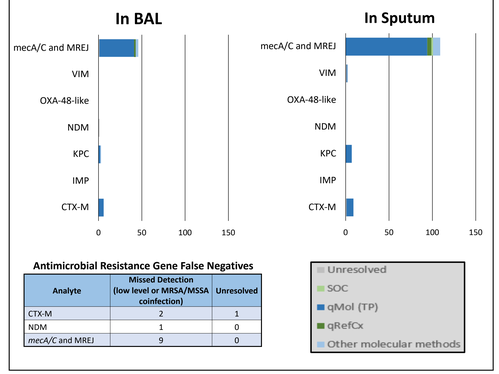
Atypical Bacteria Discrepancy Analysis		BAL	Sputum
FN	Total Discrepant	0	2
	Evidence by independent molecular method	-	1
	Resolved--other	-	0
	Not Resolved	-	**1**
FP	Total Discrepant	2	0
	Evidence by independent molecular method	0	-
	Resolved--other	0	-
	Not Resolved	**2**	-

Antimicrobial Resistance Gene Discrepancy Analysis		BAL	Sputum
FN	Total Discrepant	7	6
	Evidence by independent molecular method	7	5
	Resolved--other	0	0
	Not Resolved	**0**	**1**
FP	Total Discrepant	8	15
	Evidence by independent molecular method	6	10
	Resolved--other (e.g. qRefCx AST, SOC AST)	0	3
	Not Resolved	**2**	**2**

13/15 (86.7%) of atypical bacteria and AMR false negatives were resolved as low level
19/25 (76.0%) of atypical bacteria and AMR false positive results were resolved as low level true positive

Antimicrobial Resistance (AMR) Genes

Confirmation of BioFire FilmArray Detections by Different Methods
Reference Method: qMol



Antimicrobial Resistance Gene False Negatives

Analyte	Missed Detection (low level or MRSA/MSSA coinfection)	Unresolved
CTX-M	2	1
NDM	1	0
mecA/C and MREJ	9	0

Antimicrobial Resistance Gene Reporting Algorithm

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

AMR genes are only reported if a host bacteria is also detected above the quantification cutoff

In depth summary of MRSA detection in the BioFire FilmArray Pneumonia Panel is presented on poster P0561 by B. Graham et. al.

Conclusions

The bacterial assays in the BioFire Pneumonia Panel are highly specific and more sensitive than traditional culture methods. Across all bacterial assays for both sample types, the specificity of the panel is 96.5% compared to culture. Discrepancy investigation demonstrated that nearly all false positive detections (871/875) were correctly reported by the BioFire Pneumonia Panel. If sequence confirmed false positive detections are taken into account the specificity for all bacterial assays would be 99.9%. BioFire Pneumonia Panel exhibits higher specificity than culture due to the fundamental differences between molecular methods and culture (detects viable organisms).

The BioFire Pneumonia Panel is a sensitive and specific in vitro diagnostic device for the detection of atypical bacteria, viruses, and antimicrobial resistance genes from BAL and sputum specimens compared to molecular methods. The combined specificity across both sample types for these assays is 99.6%. Similar to what is observed with the bacterial assays, the majority of false positive detections (64/89) were correctly reported by the BioFire Pneumonia Panel. If these sequence confirmed false positive detections are taken into account the combined specificity for atypical bacteria, viral, and AMR assays would be 99.9%.

Taking discrepancy resolution into consideration, the combined specificity for all assays in the BioFire Pneumonia Panel would reach 99.9%, thus providing high confidence in detections of targeted bacteria and viruses present in lower respiratory specimens.

Data presented are from assays that have not been cleared or approved by US FDA or other regulatory agencies for diagnostic use

Contact Information:
David Judd
BioFire Diagnostics, LLC
david.judd@BioFireDx.com

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