BioFire® FilmArray® Pneumonia (PN) Panel Results Compared to Standard Microbiologic Testing and Clinical Adjudication in Adults Hospitalized with Respiratory Illness.

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Abstract

Background: Inability to define microbial etiology of lower respiratory tract infection (LRTI) leads to unnecessary antibiotic use. Though multiplex PCR improves viral detection, bacterial LRTI diagnosis remains problematic. We evaluated the clinical utility of BioFire ® FilmArray ® Pneumonia (PN) Panel which detects 8 viruses, 3 atypical and 15 pathogenic bacteria by semi-quantitative PCR to inform bacterial diagnosis.

Methods: As part of a study to distinguish bacterial from non-bacterial LRTI in hospitalized adults, clinical, laboratory, and X-ray data were collected. Adequate sputa (<10 Epithelial & >25 PMNs) processed by Gram stain and culture were tested with FilmArray® PN. Microbial LRTI etiology (viral alone, bacterial with or without concomitant viruses, or indeterminate) was adjudicated by a four physician panel using all clinical data except bacterial PCR results. Bacterial PCR was compared to Gram stain and culture and clinical adjudication.

Results: From 737 illnesses evaluated, 423 sputa were collected and 201 deemed adequate and were tested with FilmArray® PN. Most common discharge diagnoses were pneumonia (24%), AECOPD (21%), viral illness (13%) and asthma exacerbations (9%). FilmArray® PN detected 155 typical bacteria, 9 atypical and 101 viruses. Most bacterial detections were monomicrobial (58%) often with concomitant viruses (43%). Compared to Gram stain and culture, FilmArray® PN detected more bacterial pathogens and was less affected by antibiotics. (Figure) Cases were adjudicated as viral Alone (37), bacterial (93) and indeterminate (71). Bacteria were detected by PCR in 41% of viral and 96% of bacterial cases, p=0.0001 and 76% of indeterminate cases. In cases with no bacteria detected by PCR, only 4 (9%) were adjudicated as bacterial; all deemed caused by anaerobic bacteria which are not included in the PCR panel. Comparing bacterial vs. non-bacterial (Viral + Indeterminate), FilmArray® PN bacterial PCR had 96% sensitivity, 36% specificity, 56% positive predictive value and 91% negative predictive value. Finally, sputum PCR detected 4 mycoplasma and 56 viral infections missed by standard of care testing.

Conclusions: Multiplex PCR testing of sputa for bacteria is useful to rule out bacterial infection with added value to detect viruses and atypical bacteria.

Methods

Study Period and Sites: The study was conducted at two hospitals: University of Rochester Medical Center (URMC) and Rochester General Hospital (RGH) between March 2019 and March 2022. The present analysis represents a sub-study of a study to evaluate gene-expression to distinguish bacterial and non-bacterial respiratory illnesses. Subjects who had sputum collected represent the current study population.

Subject Recruitment: Patients with signs/ symptoms of an acute cardiopulmonary illness with an admission diagnoses compatible with ARI were screened for inclusion/exclusion (>18 years old, compatible illness, ability to sign consent and absence of significant immunosuppression or antibiotics prior to admission). Subjects were enrolled within 24 hours of admission.

Acute Illness Evaluation: At enrollment demographic, clinical and laboratory information was collected from review of the medical record, and direct patient and family interviews. Signs and symptoms of the current illness were recorded.

Sample Collection: Nasal swabs (NS) for viral and atypical bacterial pathogens by PCR were collected. Blood and sputum cultures were done as part of standard of care (SOC) per the treating team with assistance by study personnel for collection of adequate samples.

Microbiological Studies: Blood and sputum cultures were processed in the clinical microbiology laboratories of each hospital using standard methods. Sputum Gram stains were interpreted by microbiology staff and quantified according to the presence of neutrophils (> or < 25 PMN/high powered field [hpf]) and epithelial cells (> or < 10/hpf) and bacterial flora (predominance of a single or mixed organisms). Specimens were judged adequate by standard criteria. *S. pneumoniae* and Legionella urine antigen testing was performed at the discretion of treating staff. NS were tested for viral and atypical bacterial by PCR using the FilmArray ® Respiratory Panel (BioFire Diagnostics, Salt Lake City, UT).

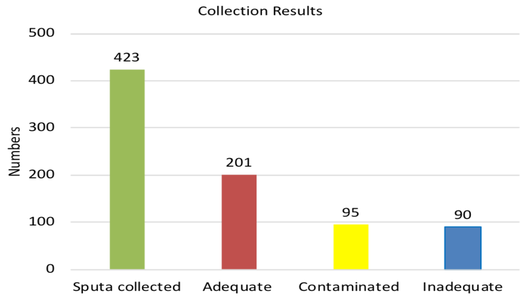
FilmArray ® PN: An aliquot of sputum was removed for testing prior to delivery to the clinical laboratory or sputa were retrieved from the clinical laboratory and transported to the research laboratory for FilmArray ® PN testing within 24-hours of collection and kept at 4°C. Only samples with > 25 WBCs per hpf by FilmArray®PN.

Clinical Adjudication: All cases were adjudicated by a panel of experts (infectious disease, pulmonary medicine and hospital medicine) and a microbiologic diagnosis assigned:

1. **Virus infection alone (V):** Respiratory sample positive for any virus. Bacterial tests all negative.
2. **Bacterial infection alone (B):** Negative viral tests and any of the following: positive blood culture, positive sputum culture for a pathogen from an adequate sample, positive urinary antigen test for *S. pneumoniae* or *L. pneumophila*, or (4) Respiratory sample PCR positive for *M. pneumoniae, C. pneumophila or B. pertussis*.
3. **Viral+ bacterial infection (V+B):** Meets definition for bacterial infection and viral infection.
4. **Indeterminate**: no positive microbiologic tests or adjudication was not unanimous.

Results

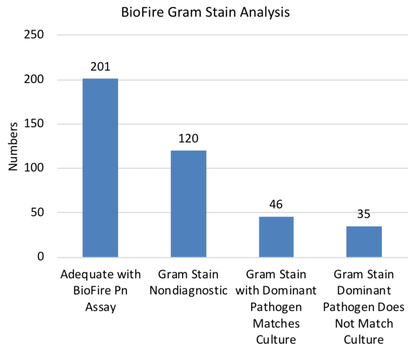
Study Population : 737 Illnesses	
Age, mean (SD) [range]	61.4(16.3)[19-98]
Female, no. (%)	396 (54)
Race, no. (%)	
White	501 (68)
Black	211(29)
Other/unknown	25 (3)
Discharge Diagnoses, no. (%)	
Pneumonia	173 (24)
COPD AE	155 (21)
Viral Infection	92 (13)
Asthma exacerbation	67 (9)
Respiratory Failure	41 (6)
Shortness of Breath	15 (2)
Congestive heart failure	11 (1.5)
Cough	11 (1.5)
Other	172 (23%)
Laboratory	
WBC, mean (SD) [range]	11.1 (5.6) [2-57.2]
Lactate,mean (SD) [range]	1.7 (1.1) [0.1 -9]
Procalcitonin, mean (SD) [range]	1.7 (8.9) [0.02 – 112.8]
CXR with infiltrates, no. (%)	313 (42%)

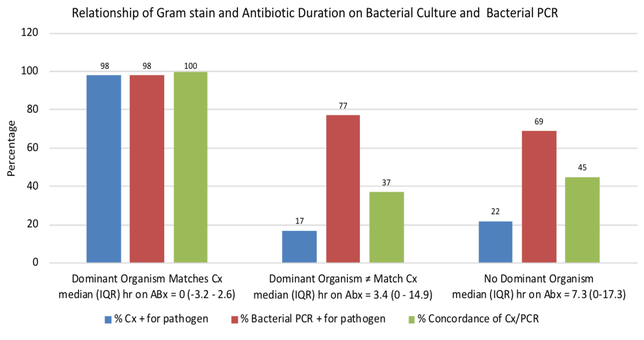


FilmArray® PN Results			
Bacteria (164)	Typical (155)	Mycoplasma (5)	Legionella (4)
No. Detections per sample (%)	Five (1%), Four (2%), Three (8%), Two (31%), One (58%)		
Viruses (101)			
No. Detections per sample (%)	Three (1%), Two (6%), One (93%)		
Microbiologic Category			
Viruses Alone	31 (15%)		
Bacteria Alone	85 (42%)		
Viruses + Bacteria	70 (35%)		
No pathogen	15 (2%)		

Gram Stain Analysis of FilmArray in Adequate Sputum Samples

- FilmArray® PN considered to match Gram stain/Culture – at least one organism with consistent morphology on stain and at least one organism grown in culture
- FilmArray® PN with zero detections matches if Gram stain showed "mixed" flora or no organisms seen (NOS) and culture reports normal flora (NF) or no growth.
- For Dominant Pathogen analysis samples noted as mixed flora were not included





Clinical Adjudication Analysis FilmArray® PN in Adequate Sputum Samples

- 130 of 201 were definitively adjudicated: 37 Viral Alone, 93 Bacterial (+/- Virus), 71 were judged as indeterminate
- Bacteria were detected by FilmArray® PN in 41% of Viral and 96% Bacterial cases, p=0.0001.
- Comparing **bacterial** (bacterial +/- virus) vs. **non-bacterial** (Viral + Indeterminate), the FilmArray® PN bacterial PCR test displayed:
  - 96% Sensitivity,
  - 36% Specificity,
  - 56% Positive predictive value
  - 91% Negative predictive value
- In cases with no bacteria detected by PCR, only 4 (9%) were adjudicated as bacterial; all deemed caused by anaerobic bacteria which are not included in the PCR panel
- Finally, sputum PCR detected 4 mycoplasma and 56 viral infections missed by standard of care testing.

Clinical Adjudication Analysis of Purulent but Contaminated Sputum Samples

- 44 of 95 were definitively adjudicated: 21 Viral Alone, 23 Bacterial (+/- Virus), 51 were judged as indeterminate
- Bacteria were detected by FilmArray® PN in 81% of Viral and 96% Bacterial cases
- Of the 22 samples with no bacteria detected by FilmArray® PN, 100% grew normal flora on standard culture
- FilmArray® PN of sputum detected 2 mycoplasma and 2 Legionella infections missed by standard of care testing.
- FilmArray® PN testing of sputum increased viral detection compared to standard of care testing

Discussion

Acute respiratory infections (ARI) occur commonly throughout life and are a leading cause of antibiotic overuse.[1] Although molecular diagnostics allow rapid diagnosis of numerous respiratory viruses, the impact on patient management and antibiotic prescription has been modest due to concern about bacterial co-infection.[2] Presently, establishing a bacterial etiology in patients with ARI is extremely difficult. Most studies of FilmArray ® PN to date have focused on the analytic sensitivity and specificity of the PN panel compared to traditional respiratory sample cultures. We evaluated FilmArray ® PN in a real life population with a variety of ARI and LRTI conditions including pneumonia, COPD AE, asthma, and CHF. FilmArray ® PN had high negative predictive value to rule out bacterial infection using clinical adjudication as the gold standard. In addition, there was significant added value in the detection of viruses and atypical bacteria compared to standard of care testing. There is increasing evidence that sputum and saliva are as good or higher yield samples than traditional nasal sampling for detection of respiratory viruses.[4,5] Thus, it might be most efficient to use sputum, even if contaminated with saliva to detect viruses with the additional benefit to rule out concomitant bacterial infection in a subset of patients.

Conclusions

- FilmArray® PN provided excellent negative predictive value to rule out non – anaerobic bacterial respiratory infections.
- FilmArray® PN detection of atypical bacterial pathogens and viruses added value to SOC testing
- FilmArray® PN of samples that were purulent but contaminated with oropharyngeal cells maintained excellent negative predictive value and added detection of atypical bacterial and viruses
- FilmArray® PN provides useful information that can be used to manage patients with respiratory illness

References

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