

Rapid Microbiological Tests In Pneumonia

Christie George Joseph

Overview

- Pneumonia and its diagnostics
- Need for rapid testing
- Multi-plexed PCRs
- BioFire[®] Pneumonia Panel – working, analysis of use
- Application

Pneumonia- Disease burden






- India : 23 per cent of global pneumonia burden and 36 per cent WHO regional burden in patients under five years
- ATS -2005 -The incidence of HAP varies from 5 to 10 cases/1,000 admitted patients, increasing 6–20 folds in mechanically ventilated patients

Farooqui H, Jit M, Heymann DL, Zodpey S. Burden of Severe Pneumonia, Pneumococcal Pneumonia and Pneumonia Deaths in Indian States: Modelling Based Estimates. *PLoS One*. 2015 Jun 18;10(6):e0129191.

Guidelines for the Management of Adults with Hospital-acquired, Ventilator-associated, and Healthcare-associated Pneumonia." *American Journal of Respiratory and Critical Care Medicine*, 171(4), pp. 388–416

Original article

Economic burden analysis of nosocomial infections in tertiary care hospital in western India: A prospective evidence-based study

Pranali Patil ^a , Jignesh Shah ^b , Amol Muthal ^a , Asavari Raut ^c  

Among all the different types HAIs, VAP contributed to significantly increase in costs followed by CAUTI, SSI and CRBSI. This finding is in adherence to the study by Tiwari et al.²⁷ VAP patients require more resources, including mechanical ventilation, which is a significant cost driver. The need for prolonged mechanical ventilation not only increases the direct costs of care but also raises the risk of further complications, leading to additional interventions and extended ICU stays.²⁸

The length of ICU stay was statistically found to be the only factor which is responsible for the increase cost of treatment among patients with HAIs. This is supported by the observations of Mathai et al.²⁹ This may be due to the direct expenses of prolonged hospitalization, the need for more intensive treatment, and the increased risk of secondary infections. Reducing HAIs and shortening ICU stays are crucial for controlling costs and improving patient outcomes.^{30,31}

Table 1. Empirical treatment of CAP, HAP, VAP according to the most recent guidelines.

		IDSA-ATS 2019		NICE 2019	
CAP	Mild	Without risk factors for MDR	Outpatients: amoxicillin or doxycycline or macrolide; in patients with comorbidities: combination with amoxicillin/clavulanate or cephalosporin and macrolide or doxycycline or monotherapy with respiratory fluoroquinolone Inpatients: beta-lactam + macrolide or fluoroquinolone	Oral amoxicillin (add macrolide if atypical pneumonia suspected) If penicillin allergy/atypical pneumonia suspected: macrolide or doxycycline	
		With risk factors for MDR	Obtain cultures/nasal PCR and add MRSA and <i>P. aeruginosa</i> coverage only if positive Prior respiratory isolation of MRSA/ <i>P.aeruginosa</i> : add antibiotic coverage and obtain cultures/nasal PCR to allow deescalation or confirmation of therapy		
	Moderate-severe	Without risk factors for MDR	Beta-lactams + macrolide or beta-lactam + fluoroquinolone	Amoxicillin or amoxicillin/clavulanate + macrolide If penicillin allergy: macrolide or levofloxacin	
		With risk factors for MDR	Add MRSA/ <i>P. aeruginosa</i> coverage and obtain cultures/nasal PCR to allow deescalation or confirmation of therapy		

Severe CAP and Nosocomial infections – HAP, VAP

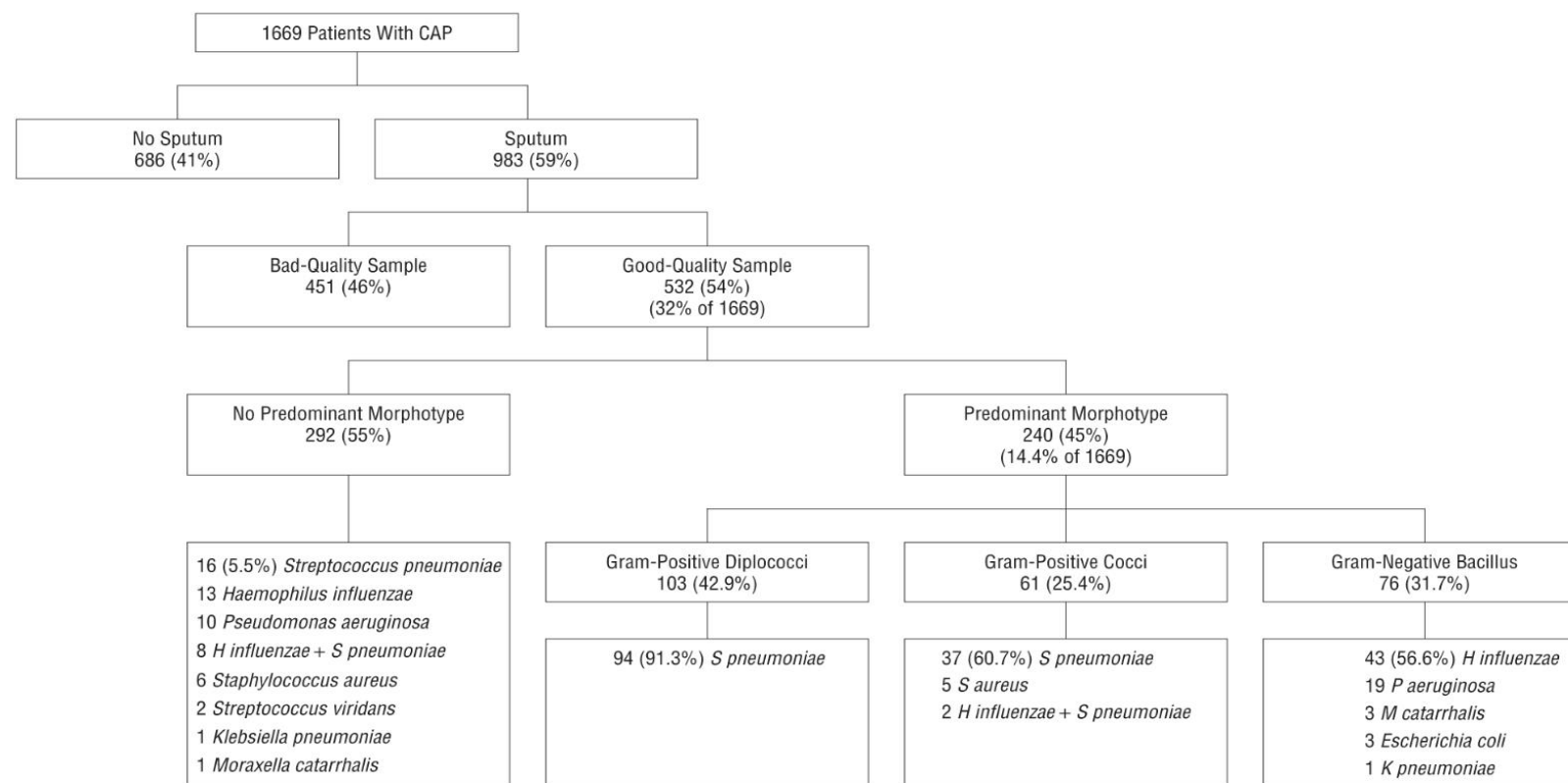
- Definitive diagnosis : isolating the organism
- Gold standard: Respiratory sample microscopy -----→ culture of specimens
- a causative pathogen can be detected in less than 40% of adults with CAP

Microbiological culture of organisms

- low sensitivity : ~ 60% ¹, further affected by prior antibiotic use, sampling technique, (14% good quality sputum ¹) storage and transport conditions, sampling site etc.
- long turnaround time: the definitive results – gap of 24 to 72 h.
- Diagnostic delay - necessitates the initiation of empiric broad-spectrum antibiotic therapy - may not always be appropriate,
 - encourages multi-drug resistance
 - unfavourable outcomes

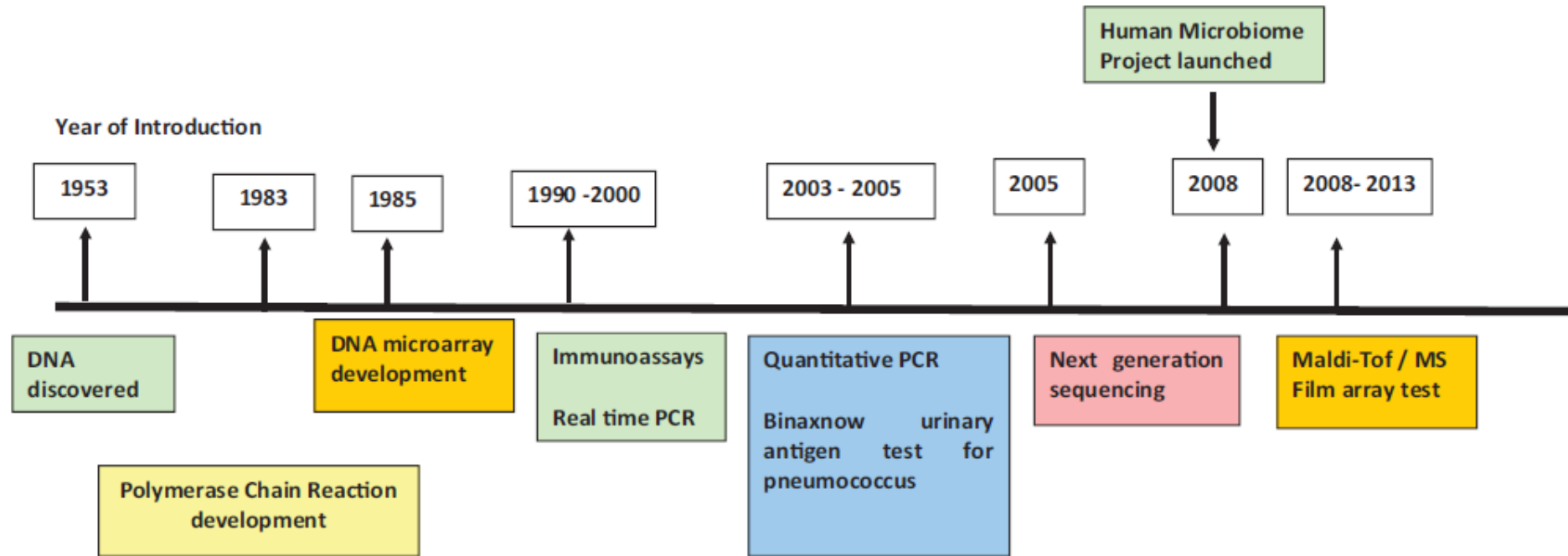
¹ García-Vázquez E, Marcos MA, Mensa J, de Roux A, Puig J, Font C, Francisco G, Torres A. Assessment of the usefulness of sputum culture for diagnosis of community-acquired pneumonia using the PORT predictive scoring system. Arch Intern Med. 2004 Sep 13;164(16):1807-11. doi: 10.1001/archinte.164.16.1807. PMID: 15364677.

Sputum samples were obtained from 983 patients (59%). Of the 532 samples (54%) that were of good quality, 240 (45%, ie, representing 14.4% of the initial 1669 patients) showed a PM. There were 61 gram-positive cocci, 76 gram-negative bacilli, and 103 gram-positive diplococci. Sputum culture yielded a causative organism in 207 (86%) of the 240 samples with a PM and in 57 (19.5%) of the 292 good-quality samples with no PM ($P<.05$). *Streptococcus pneumoniae* was the microorganism cultured in 133 (81%) of the 164 samples with a gram-positive PM and in 24 (8%) of the 292 samples with no PM ($P<.05$) (Figure 1).

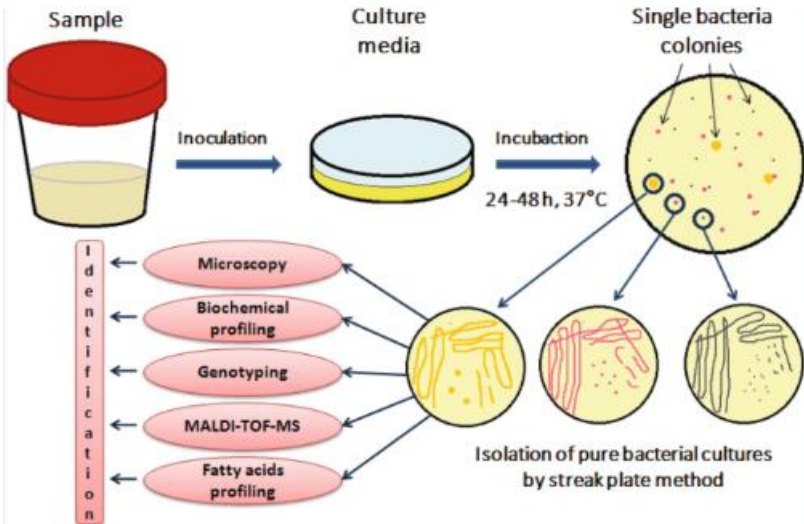


Rapid tests

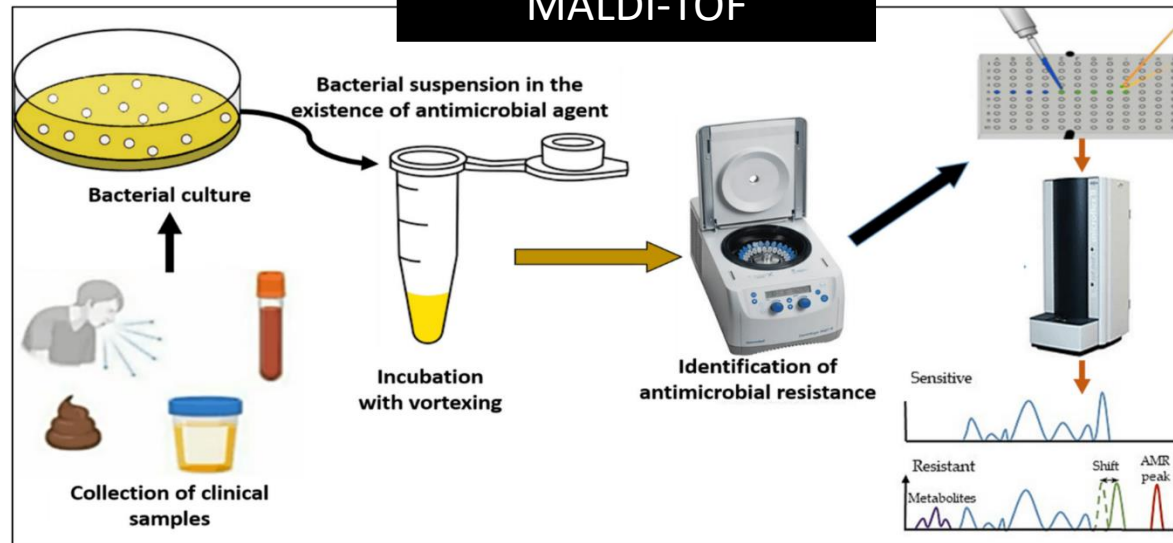
- The search for microbial antigens by rapid immunoassays or for microbial nucleic acids using amplification tests (NAATs).



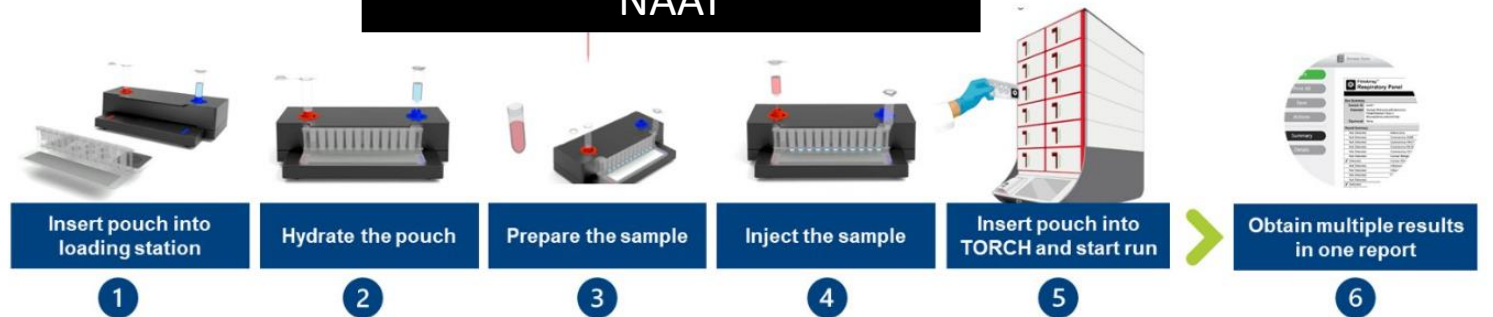
Conventional Culture



MALDI-TOF



NAAT



ERS/ESICM/ESCMID/ALAT guidelines for the management of severe community-acquired pneumonia

Ignacio Martin-Loeches  | Antoni Torres  | Blin Nagavci [Show More](#) 

European Respiratory Journal 2023 61(4): 2200735; DOI: <https://doi.org/10.1183/13993003.00735-2022>

Question 1: In patients with sCAP, should rapid microbiological techniques be added to current testing of blood and respiratory tract samples?

Recommendations

If the technology is available, we suggest sending a lower respiratory tract sample (either sputum or endotracheal aspirates) for multiplex PCR testing (virus and/or bacterial detection) whenever non-standard sCAP antibiotics are prescribed or considered (conditional recommendation, very low quality of evidence).

Based on 1 systematic review (of 28 observational studies) and 1 RCT

RCT – ResPOC trial – LRTI pts to POC viral molecular test or SOC

- Did not reduce proportion of pts treated with antibiotics, no redn in duration, reduced duration of stay +

Systematic review –

- For Respiratory viral pathogens identified in adults with community-acquired pneumonia in Europe

PCR detected bacteria at low NA loads

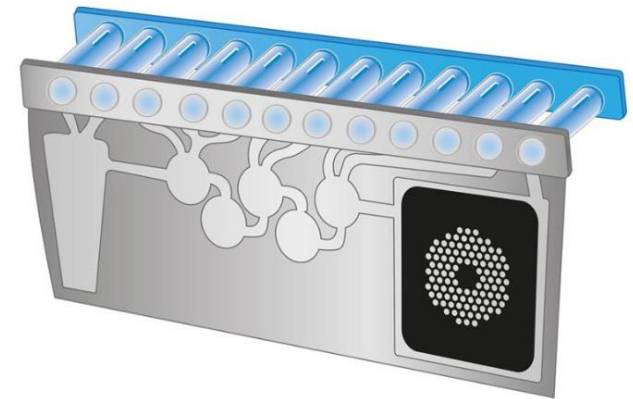
ELISA, Serology, IF- modalities used

Commercially available
PCR Platforms

Proprietary Name	Parent Company	Characteristics	Sensitivity and Specificity		
BioFire® FA Pneumonia Panel Plus	BioMérieux, France	<ul style="list-style-type: none"> - FDA cleared and CE-IVD marked test - -Detects 18 bacteria (3 atypical), 9 viruses, and 7 antimicrobial resistance-associated genes 	<p>Declared sensitivity of 75–100% (sputum) 85.7–100% (BAL)</p> <p>Specificity of 87.2–99.4% (sputum) 91.2–99.5% (BAL)</p>		<p>Semi-quantitative results for 15 pathogens</p> <p>TAT 75 mins</p>
VERIGENE® Respiratory Pathogens Flex Nucleic Acid Test	Luminex Molecular Diagnostics, Inc., Canada	<ul style="list-style-type: none"> - FDA cleared and CE-IVD marked test - - 13 viral and 3 bacterial targets 	Declared sensitivity of 79.2–100% and specificity of 97.1–100%, depending on target.	<i>Designed for CAP, BUT SARS-CoV -2, S. pneumoniae, H. influenzae, Mycoplasma pneumoniae, and Legionella pneumophila- NOT INCLUDED</i>	<p>ONLY qualitative results.</p> <p>TAT 2 hours</p>
Unyvero system	Curetis GmbH, Germany	<ul style="list-style-type: none"> - FDA Cleared , and CE-IVD marked - - 20 bacterial targets, 1 fungal target, and 17 antibiotic resistance targets, but it lacks viral targets 	Declared sensitivity 80–100% and specificity 98– 100%	<i>Resistance detection – suboptimal- poor consistency between resistance marker detection and organisms’ antibiograms, (predecessor panel Unyvero P55 : AMR detection (18%))</i>	<p>Qualitative results</p> <p>TAT of about 4– 5 hours</p>

BIOFIRE® FILMARRAY® Pneumonia Panel plus (BIOFIRE Pneumonia Panel plus)

- multiplexed nucleic acid test
- used with BIOFIRE® FILMARRAY® 2.0 (BIOFIRE 2.0) or BIOFIRE® FILMARRAY® TORCH (BIOFIRE TORCH) systems





Nucleic acid purification

cDNA (RT) synthesis

Multiplex PCR to amplify pathogen nucleic acids

Detection of amplicons to confirm targets



BIOFIRE TORCH Module

FilmArray Torch Duplex
Module enclosure; houses two FilmArray Torch Modules

FilmArray Torch Module
Interacts with the reagent pouch to purify and amplify targeted nucleic acid sequences using nmPCR; includes pouch slot and LED status light

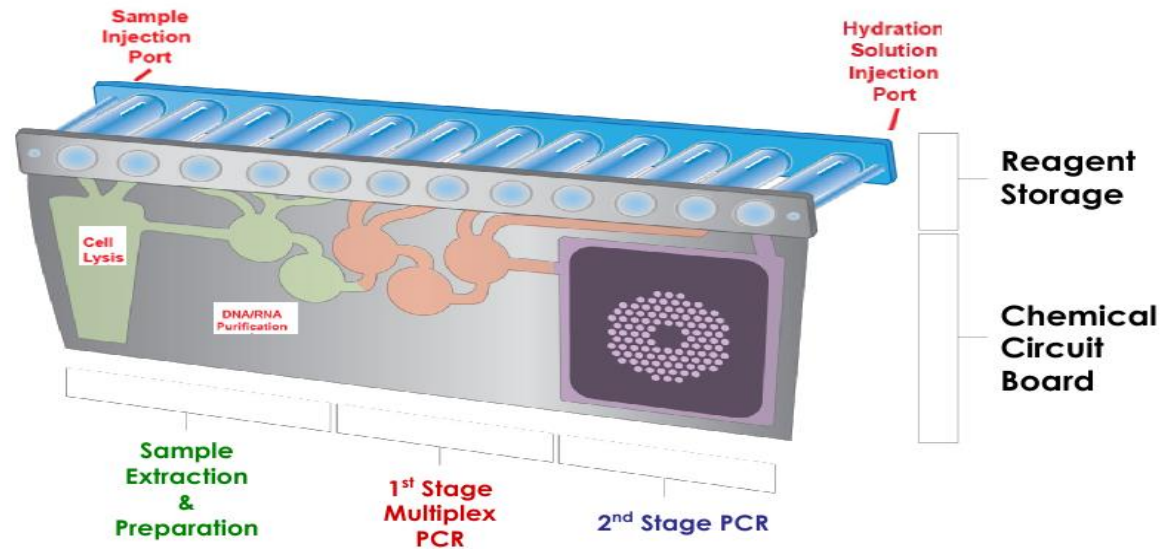
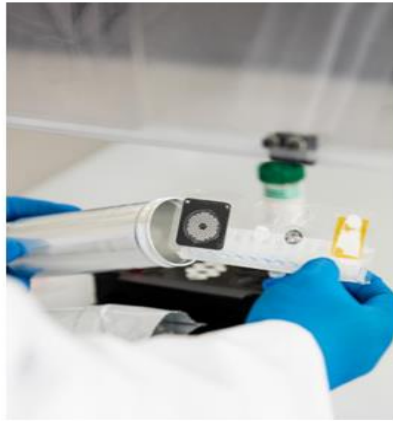
FilmArray Torch System Base
Includes barcode scanner, touch screen, and USB ports



Resulting PCR products evaluated by DNA melting analysis

The FILMARRAY TORCH software automatically determines results – provides report

BIOFIRE FILMARRAY CHEMISTRY



Nested Multiplex PCR

nmPCR: 2 stages of PCR

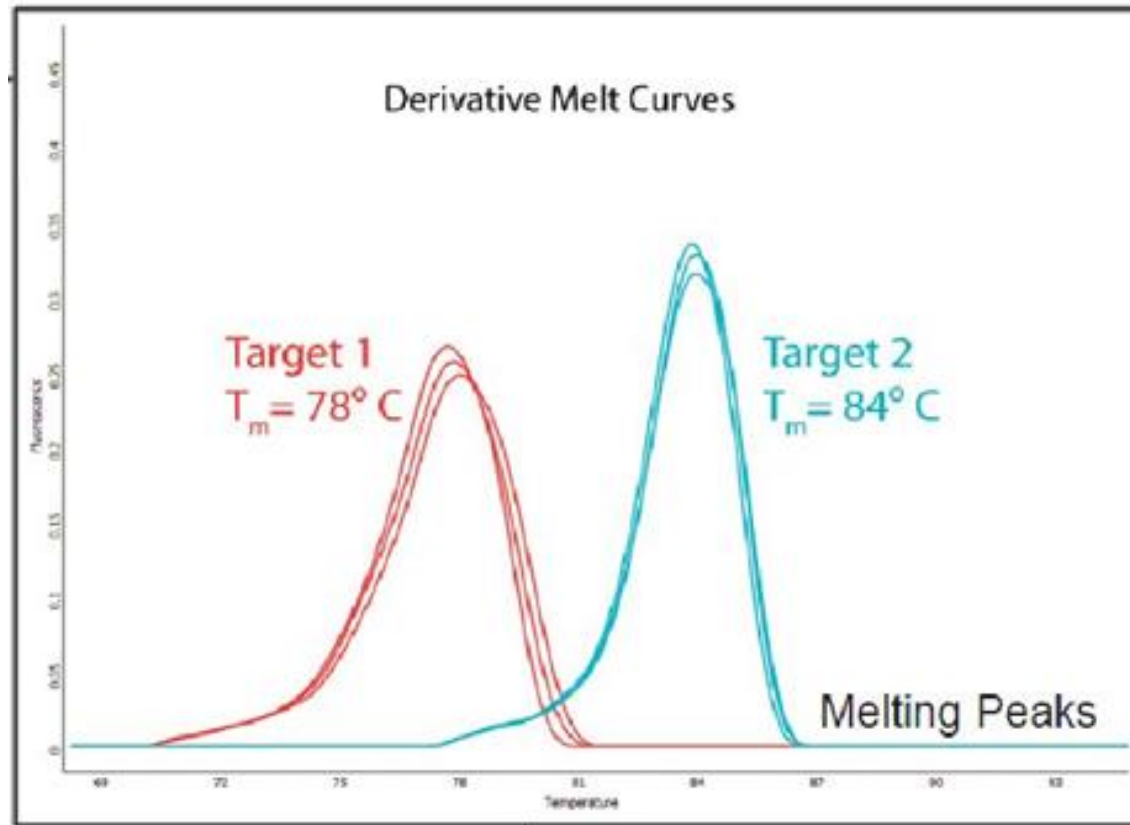
1st Stage – multiple “outer primers” used to perform multiplex PCR on the target templates

2nd Stage – performed in a singleplex format to amplify DNA copies generated during 1st stage

High resolution melting analysis : Optics and Imaging

- To identify targets from positive PCR reactions, DNA melting curve analysis is performed
- Fluorescence emitted by the LCGreen[®] Plus dye is imaged by a camera
- DNA melting curves captured by slowly inc temperature of PCR array and capturing fluorescent signal
- Temperature increases and copies of double-stranded DNA melt, LCGreen Plus dye is released and reduction in fluorescence is detected
- Images are processed automatically by the System Base, and the data is analyzed

Melting point analysis



- **Derivative calculation (dF/dT)** – The negative derivative of fluorescence (F) vs. temperature (T) is plotted: Peaks in the **negative derivative plot ($-dF/dT$ vs. T)** indicate **T_m values** where DNA strands denature.
- **Each target has a distinct T_m** , allowing differentiation of multiple pathogens.
- Since the sequence and T_m of amplicon from a specific target is known & consistent, pathogen specific PCR product can be identified as being copied from that target.
- Non-specific PCR products with different T_m s are excluded

Reporting

- 15 bacteria reported semi-quantitatively : a \log_{10} binned value of 10^4 , 10^5 , 10^6 , or $>10^7$ genomic copies/ml; Targets quantified at $<10^{3.5}$ copies/ml are reported as “not detected.”

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

- Qualitative reporting of :

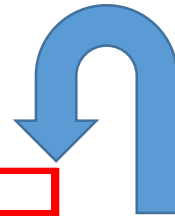
Atypical Bacteria		
<i>Chlamydia pneumoniae</i>	<i>Legionella pneumophila</i>	<i>Mycoplasma pneumoniae</i>
Viruses		
Middle East respiratory syndrome coronavirus (MERS-CoV)		
Adenovirus	Human rhinovirus/enterovirus	Parainfluenza virus
Coronavirus	Influenza A	Respiratory syncytial Virus
Human metapneumovirus	Influenza B	
Antimicrobial Resistance Genes		
CTX-M	NDM	<i>mecA/C</i> and MREJ (MRSA)
IMP	OXA-48-like	
KPC	VIM	

- ✓ Viral and atypical bacterial targets, a qualitative result of either “detected” or “not detected” is reported.
- ✓ Genetic markers of antimicrobial resistance are reported qualitatively as “detected” or “not detected” only if a commensurate bacterial target is detected and reported

Specimens:

The **BIOFIRE PN and PN*plus* Panels** identify over 30 clinically relevant targets from sputum-like (sputum or endotracheal aspirate) or bronchoalveolar lavage (BAL)-like (BAL or mini-BAL) specimens. For 15 bacteria, the panels provide semi-quantitative results, which may help determine whether an organism is a colonizer or a pathogen. The fast and comprehensive panels are run on the [BIOFIRE®FILMARRAY® TORCH System](#), which integrates sample preparation, amplification, detection, and analysis.


- ✓ **Simple:** 2 minutes hands-on time
- ✓ **Fast:** Turnaround time of about 1 hour
- ✓ **Comprehensive:**
 - ✓ **BIOFIRE PN Panel:** 33 targets (18 bacteria, 8 viruses, 7 antimicrobial resistance genes)
 - ✓ **BIOFIRE PN*plus* Panel:** 34 targets (18 bacteria, 9 viruses, 7 antimicrobial resistance genes)
- ✓ **Accurate:**
 - ✓ **BAL-like:** 96.2% sensitivity and 98.3% specificity (PN*plus*: 98.4%)¹
 - ✓ **Sputum-like:** 96.3% sensitivity and 97.3% specificity (PN*plus*: 97.4%)¹



Internal data

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Practical Comparison of the BioFire FilmArray Pneumonia Panel to Routine Diagnostic Methods and Potential Impact on Antimicrobial Stewardship in Adult Hospitalized Patients with Lower Respiratory Tract Infections

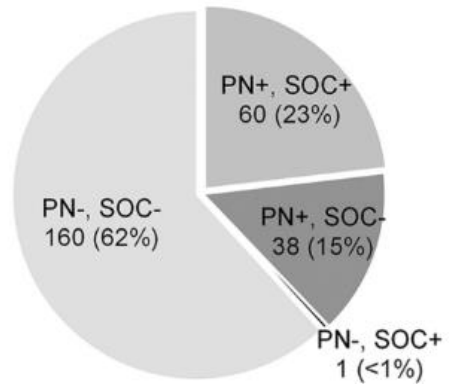
Blake W. Buchan ^a, Sam Windham^a, Joan-Miquel Balada-Llaser^b, Amy Leber^c, Amanda Harrington^d, Ryan Relich^e, Caitlin Murphy^f, Jennifer Dien Bard^g, Samia Naccache^g, Shira Ronen^a, Amanda Hopp^a, Derya Mahmutoglu^a, Matthew L. Faron^a, Nathan A. Ledebor^a, Amanda Carroll^b, Hannah Stone^b, Oluseun Akerele^b, Kathy Everhart^c, Andrew Bonwit^d, Christina Kwong^d, Rebecca Buckner^e, Del Warren^e, Randal Fowler^f, Sukantha Chandrasekaran^h, Holly Huse^h, Shelley Campeau^{h,*}, Romney Humphries^{h,*}, Corrin Graueⁱ, Angela Huang^{a,j}

- Retrospective analysis of 259 residual BAL ($n = 237$) or mini-BAL ($n = 22$) specimens from the clinical trial for regulatory clearance of the PN panel
- at eight U.S. clinical centers between October 2016 and July 2017
- analysis of this subset specimens
 - a) Comparing results reported using routine SOC methods to those obtained using the PN panel and
 - b) assessing the potential impact of the PN panel results on antibiotic utilization in these patients.

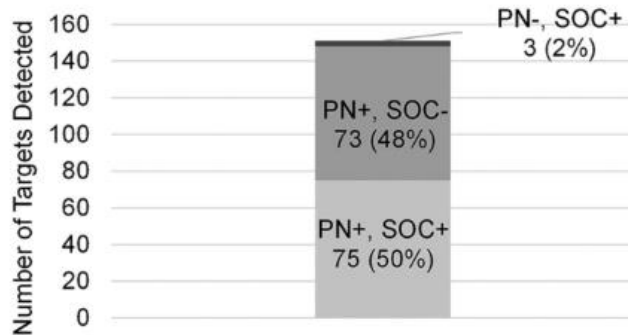
... might also have been beneficial in managing care.

This study did have limitations. First, it was assumed that all specimens were obtained from patients with clinical symptoms concerning for pneumonia. It is possible that some of the specimens could have been collected as part of surveillance protocols from asymptomatic patients who had recently undergone transplant procedures. Sec-

A Total number of BAL Specimens (n=259) with Bacterial Target(s) Detected



B Number of Bacterial Targets (n=151) Detected in all BAL Specimens



(i) SAMPLE POSITIVITY

and 3 on-panel targets were detected by culture alone (Fig. 1B). These data demonstrate a 63.3% increase in the number of BAL specimens reported as positive and a 94.8% increase in individual on-panel targets reported when the PN panel was compared to routine bacterial culture. Off-panel bacterial targets were reported by culture in 30 (11.6%) specimens. These included *Haemophilus parainfluenzae* ($n = 1$), *Burkholderia cepacia* ($n = 1$), *Morganella morganii* ($n = 1$), *Providencia stuartii* ($n = 1$), *Lactobacillus* sp. ($n = 1$), *Corynebacterium* spp. ($n = 2$), *Achromobacter* spp. ($n = 2$), viridans group streptococci ($n = 3$), *Enterococcus* spp. ($n = 3$), "beta-hemolytic *Streptococcus* not type A" ($n = 3$), coagulase-negative staphylococci ($n = 3$), and other bacteria that were not definitively identified by the reporting clinical laboratory ($n = 9$). Many of these targets are not considered respiratory pathogens unless they are in pure cultures or predominant; however, 17/30 (56.6%) were quantified at $\geq 10^4$ CFU/ml and may have been clinically significant.

FIG 1 The BioFire PN panel (PN) identified at least one bacterial target in 63% standard-of-care culture (SOC) (A) and identified nearly twice as many total bacterial targets (B).

- 1) 63% (38/60) - but only additional 38 samples
- 2) Targets detected - ?less important from clinician view compared to yield from each sample.
?Multiple targets in each sample increases this number

(ii) Concordance for predominant bacteria detected

TABLE 4 Correlation of predominant bacterial target detected in 59 culture-positive BAL specimens

No. of targets detected with the PN panel	Detection rate ^a for number of targets detected by culture (%)			
	1	2	3	4
1	29/29 (100)			
2	11/13 ^b (84.6)	8/9 ^c (88.9)	1/1 (100)	
3	1/1 (100)	2/2 (100)		
4	1/1 (100)	1/1 (100)		0/1 ^d (0)
5				
6	1/1 (100)			

^aValues are given as (number of BAL specimens with the same bacterial target reported as predominant by PN panel and culture)/(number of BAL specimens with the indicated number of bacterial and PN panel detections).

^bOne specimen was reported as 10⁶ *S. aureus* and 10⁵ *E. cloacae* by the PN panel with only 10⁵ *E. cloacae* reported in culture, and one specimen was reported as 10⁵ *S. aureus* and 10⁴ *E. cloacae* by the PN panel with only 10⁴ *E. cloacae* reported in culture.

^cOne specimen was reported as 10⁷ *S. pneumoniae* and 10⁵ *P. aeruginosa* by the PN panel while culture reported 10⁴ *P. aeruginosa* and "few" *S. pneumoniae*.

^dOne specimen was reported as 10⁵ *S. aureus* and 10⁴ *K. oxytoca*, *S. marcescens*, and *P. aeruginosa* by the PN panel while culture reported 10⁴ *K. oxytoca*, *S. marcescens*, and *P. aeruginosa* and only 10³ *S. aureus*.

(iii) Quantitative agreement b/w SOC and PN

TABLE 3 Quantitative agreement of bacterial targets (Table view)

PN panel result (copies/ml)	No. of samples ^a with SOC culture result (CFU/ml)			
	Not detected	10 ³	10 ⁴	≥10 ⁵
Not detected	3,734	3	0	0
10 ⁴	24 (8)	6	4	0
10 ⁵	27 (17)	3	4	1
10 ⁶	9 (4)	2	12	3
≥10 ⁷	13 (7)	2	15	23
% concordant ^b	98.1 (3,734/3,807)	18.8 (3/16)	11.4 (4/35)	100 (27/27)

^a Numbers in parentheses are the numbers of culture-negative results obtained for specimens from patients who received antibiotics with potential activity against the given bacterial target detected within 72 h preceding specimen collection. One laboratory reported bacterial culture quantitation (11 isolates) as “few,” “moderate,” or “many”; these were categorized as 10³, 10⁴, and ≥10⁵ CFU/ml, respectively. Shading indicates concordance between the BioFire PN panel and routine culture quantitation.

^b Concordance between the PN panel and culture quantitation among all positive cultures was 43.6% (34/78).

For BAL: 10⁴
 All 10⁴ in SOC :
 detected at 10⁴ and
 above in PN
 But 81% 10³ colonies
 (SOC) reported as >10⁴
 copies in PN

- Among positive cultures, concordance between the PN panel and culture quantitation was 43.6% (34/78).
- Concordance was poorest at low bacterial culture CFU; 18.8% (3/16) for 10³ CFU/ml and 11.4% (4/35) for 10⁴ CFU/ml in culture
- In all discordant cases, the PN panel result was higher than that of culture – among these, 77.2% (34/44) exceeded culture quantitation by >1 log.
- All >10⁵ CFU/ml in culture were reported as 10⁵, 10⁶, or ≥10⁷ genomic copies/ml by the PN panel (100% concordance)

Comparison of AMR detection

TABLE 5 Comparison of BioFire PN panel and phenotypic results for detection of carbapenemase and ESBL-producing organisms^a

Specimen	PN panel bacterial target detected	PN panel resistance marker detected	Culture result	Phenotypic susceptibility (method)
B-08-008	<i>A. baumannii</i> , 10 ⁶ <i>K. pneumoniae</i> , 10 ⁴	<i>bla</i> _{KPC}	<i>A. baumannii</i> , "few" ND	Carbapenem resistant (MIC) NA
B-03-008	<i>E. cloacae</i> , 10 ⁶ <i>P. aeruginosa</i> , ≥10 ⁷	<i>bla</i> _{KPC}	<i>E. cloacae</i> , ≥10 ⁵ <i>P. aeruginosa</i> , ≥10 ⁵	Carbapenem resistant (MIC), ESBL positive (Etest ESBL) Phenotypic tests not performed
B-01-015	<i>E. cloacae</i> , 10 ⁴	<i>bla</i> _{NDM}	<i>E. cloacae</i> , 10 ³	Carbapenem susceptible (MIC), ESBL negative (BMD)
B-08-056	<i>P. aeruginosa</i> , 10 ⁶	<i>bla</i> _{CTX-M}	<i>P. aeruginosa</i> , "moderate"	Carbapenem resistant (MIC)
B-01-040	<i>K. pneumoniae</i> , 10 ⁶	ND	<i>K. pneumoniae</i> , 10 ⁴	Carbapenem susceptible (MIC), ESBL positive (BMD)
B-05-012	<i>E. cloacae</i> , 10 ⁵	ND	<i>E. cloacae</i> , ≥10 ⁵	Carbapenem not tested, ESBL positive (KB)
B-08-024	<i>P. aeruginosa</i> , ≥10 ⁷	ND	<i>P. aeruginosa</i> , "few"	Carbapenem resistant (MIC)
B-08-027	<i>K. oxytoca</i> , 10 ⁴	ND	<i>K. oxytoca</i> , "rare"	Carbapenem susceptible (MIC), ESBL positive (MIC)
B-08-029	<i>P. aeruginosa</i> , ≥10 ⁷	ND	<i>P. aeruginosa</i> , "few"	Carbapenem resistant (MIC)
B-08-053	<i>P. aeruginosa</i> , ≥10 ⁷	ND	<i>P. aeruginosa</i> , "moderate"	Carbapenem resistant (MIC)

^aND, not detected; KB, Kirby-Bauer disk diffusion test; BMD, broth microdilution test; Etest ESBL, Etest ESBL test strip.

- a combined 20% (2/10) concordance between the identification of genetic resistance markers by the PN panel and the phenotypic characterization of Gram-negative isolates recovered from these specimens.

Decision on antibiotics

- Complete medical chart data were available for 253/269 (94.1%) patients
- Potential antibiotic adjustments were based on comparison between the PN panel and routine culture results.
- Actual antibiotic prescription and modification based on the SOC results were determined by medical chart review
- Potential antibiotic adjustments based on the PN panel results were considered appropriate only if PN panel and SOC results were in positive or negative agreement.
- In these cases, it was assumed that the same appropriate adjustment could have been made at the time of PN panel result.

TABLE 7 Potential impact of the BioFire PN panel result on antibiotic utilization

Potential modification	No. of antimicrobials	No. (%) of patients	No. of hrs
Appropriate de-escalation/discontinuation	206	122 (48.2)	18,284.07
Appropriate escalation/initiation	11	11 (4.3)	184.66
Inappropriate de-escalation/discontinuation	4	4 (1.6)	
Inappropriate escalation/continuation	42	42 (16.6)	
No change		74 (29.2)	
Unable to assess ^a		16	

^aNo stop date was listed for antimicrobials, concomitant infection was present, or antimicrobials were used for longer durations than would be used for a lower respiratory tract infection (>30 days).

Appropriate Antibiotic De-escalation/Discontinuation

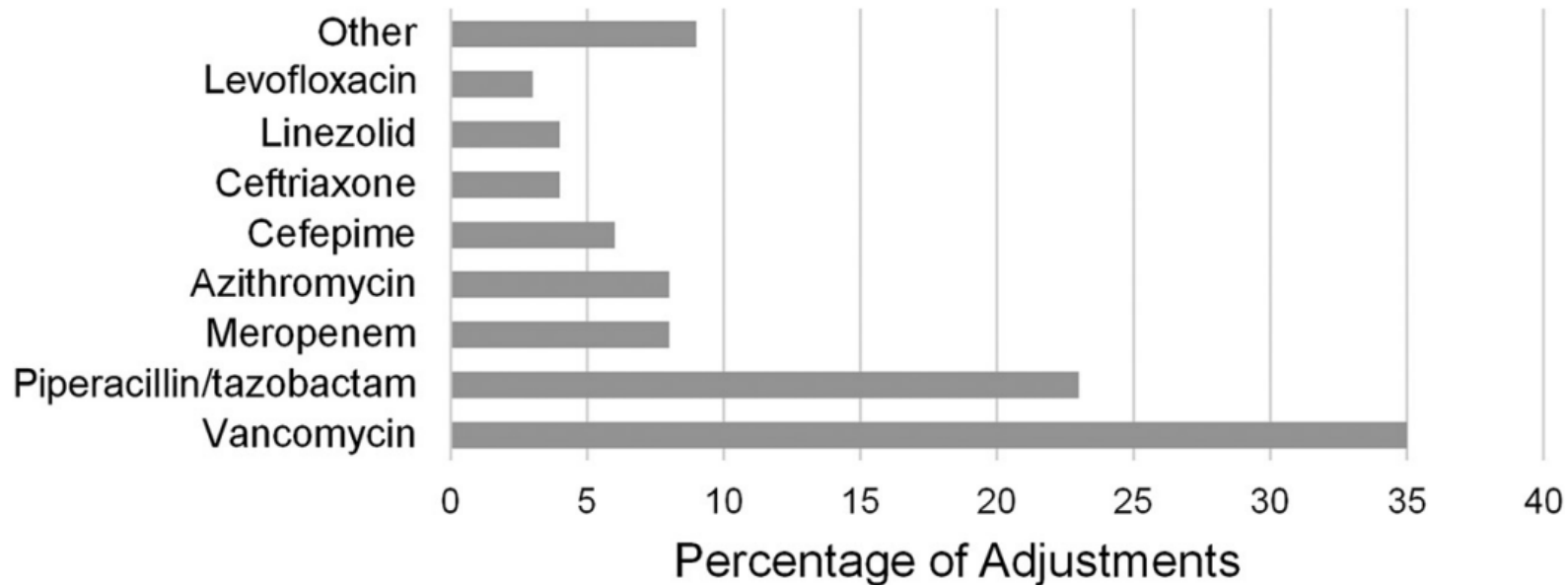
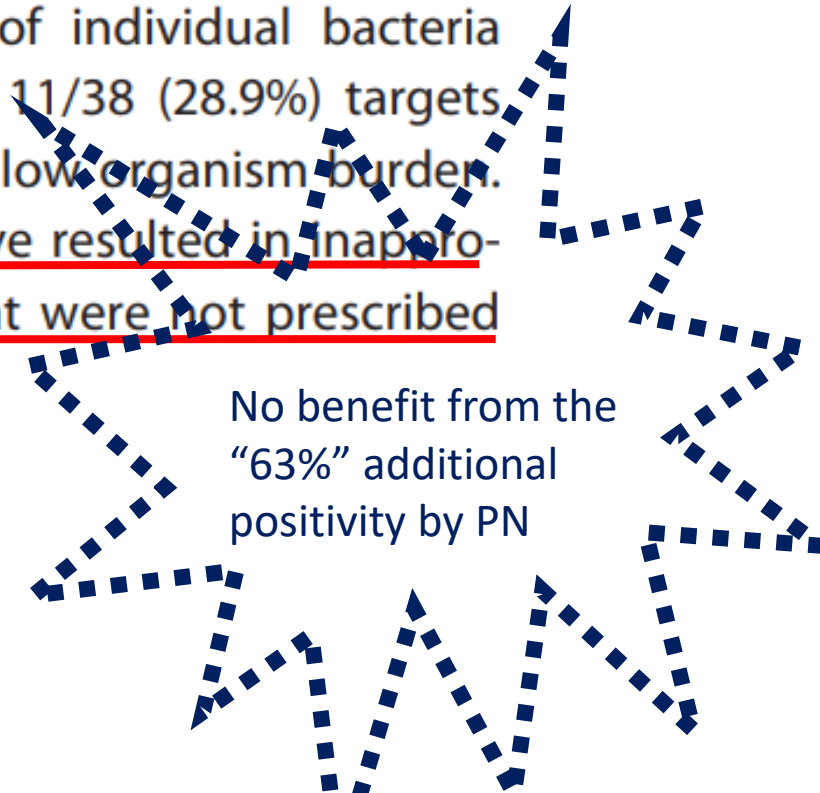


FIG 4 Percentage of total antibiotic de-escalations and discontinuations among 122 patients with negative agreement between SOC and PN panel results. Vancomycin and piperacillin-tazobactam accounted for 58% of total antimicrobial de-escalations and discontinuations based on negative results for MRSA and *Enterobacteriaceae*, respectively.

On a per-patient basis, this equated to an average potential of 6.2 fewer total antibiotic days per patient or 3.7 days per antibiotic.

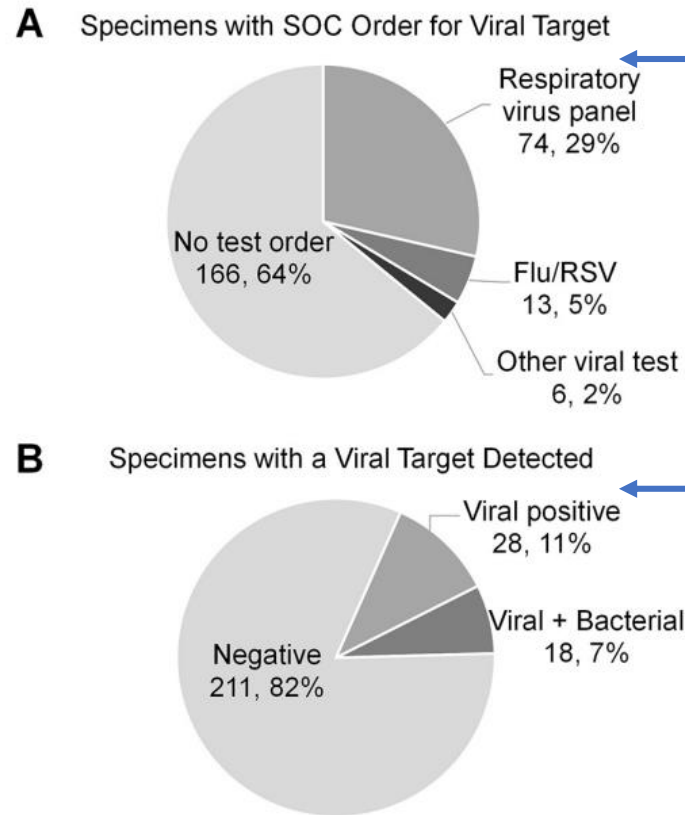
Decision on antibiotics

There were 38 culture-negative specimens with at least one bacterial target detected by the PN panel (31 with single targets and 7 with multiple targets). In the majority of these cases (23/38; 60.5%), SOC reported "normal oral flora." Due to laboratory-specific culture reporting protocols, identification of individual bacteria present in these specimens was not completed. Furthermore, 11/38 (28.9%) targets were quantified at 10^4 copies/ml by the PN panel, indicating a low organism burden. In these specimens, utilization of the PN panel result might have resulted in inappropriate initiation or escalation/continuation of antimicrobials that were not prescribed based on culture results.



No benefit from the
"63%" additional
positivity by PN

Viral detection



- ✓ The SOC positivity rate was 14/93 (15.1%) among specimens with a clinical order for viral-pathogen testing
- ✓ At least one viral target was detected by the PN panel in 46/259 (17.7%) BAL specimens.
- ✓ Among these, 18/46 (39.1%) were also positive for at least one bacterial target by the PN panel
- ✓ Among these, the PN panel demonstrated 96.7% (87/90) agreement with the SOC result, with no false-positive detections

Only 11/46 (23.9%) specimens with a positive viral detection by the PN panel had a clinician-ordered molecular test for viral pathogens.


 Significance of this positivity

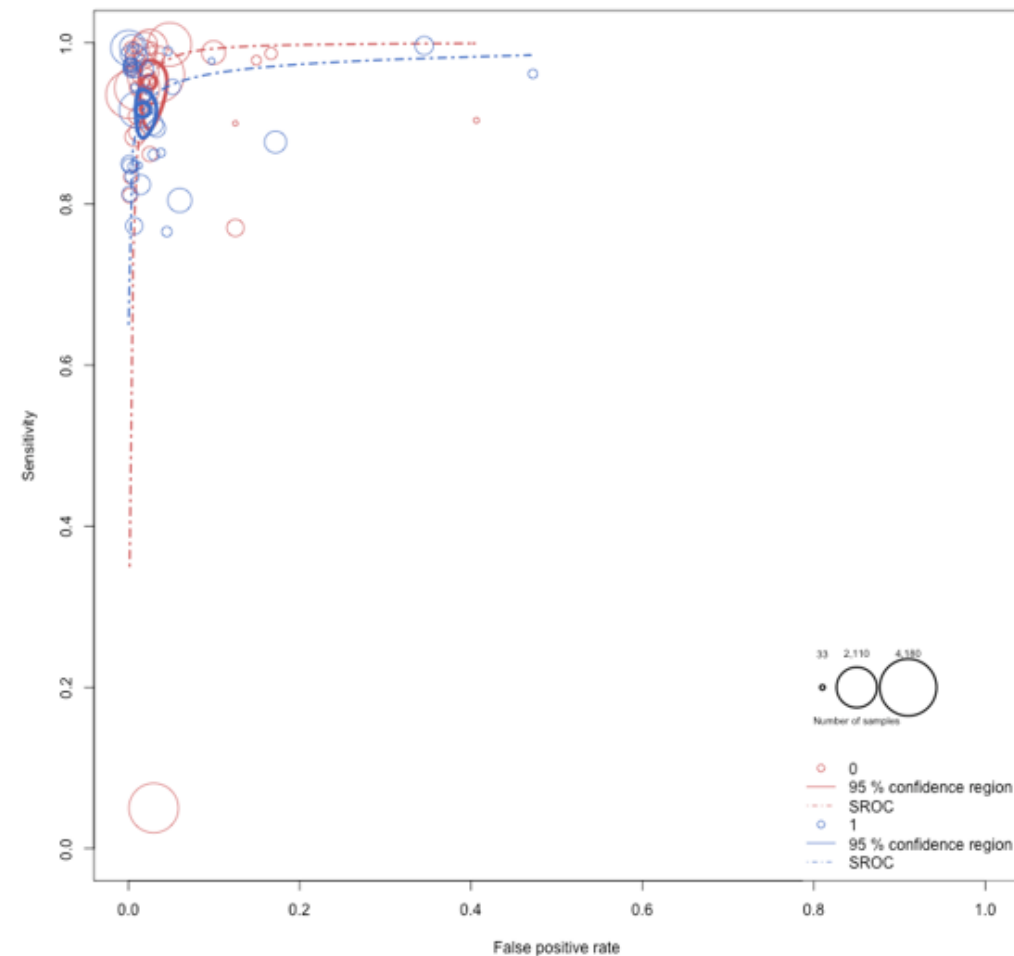
FIG 2 Molecular tests for viral pathogens were clinically ordered for only 93/259 (35.9%) BAL specimens submitted for bacterial culture and included primarily multiplexed respiratory panel tests (A). At least one viral target was detected by the PN panel in 46/259 (17.7%) BAL specimens, either alone or in addition to bacterial targets (B). Only 11/46 (23.9%) specimens with a positive viral detection by the PN panel had a clinician-ordered molecular test for viral pathogens.

Diagnostic accuracy of point-of-care tests in acute community-acquired lower respiratory tract infections. A systematic review and meta-analysis

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- 46 studies
- Sensitivity of multiplex PCR (91.1% CI 88-94) for diagnosis of influenza lower than that of stand alone PCR (95.1% CI 88-97) with similar sensitivity of 99%

Figure S27. SROC for the diagnostic accuracy of multiplex vs standalone PCR for the detection of Influenza A/B



PCR, multiplex vs standalone (N. studies, population)	Sen (%)	95% CI	Spc (%)	95% CI
Standalone (30, 25027)	95.1	89.3-97.8	97.5	95.5-98.7
Multiplex (37, 14032)	91.7	88.2-94.2	98.3	96.7-99.1

Sample : Sputum or BAL

- Based on the full clinical trial set,

Sputum and ET aspirate:

*3 as likely as BAL or mini-BAL specimens to have ≥ 3 targets detected (51 BAL and 162 sputum specimens)

* 2 likely to have bacterial targets that were not recovered by specialized reference culture methods (328 BAL and 547 sputum specimens)

Evaluation of the clinical relevance of the Biofire[®] FilmArray pneumonia panel among hospitalized patients

[Kirstine K Søgaard](#)^{1,2,3}, [Vladimira Hinic](#)^{1,4}, [Daniel Goldenberger](#)¹, [Alexander Gensch](#)^{1,2}, [Michael Schweitzer](#)^{1,2},
[Veronika Bättig](#)⁵, [Martin Siegemund](#)⁶, [Stefano Bassetti](#)⁷, [Roland Bingisser](#)⁸, [Michael Tamm](#)⁹, [Manuel Battegay](#)⁵,
[Maja Weisser](#)⁵, [Daiana Stolz](#)^{9,#}, [Nina Khanna](#)^{5,#}, [Adrian Egli](#)^{1,2,4,✉,#}

- a retrospective observational study
- 1078 BAL samples analysed by both culture (standard of care and reference standard) and Biofire FilmArray[®] Pneumonia (PN) panel
- Indications for BAL were either diagnostics of interstitial lung disease or diagnosis of acute or chronic infection.
- Stratified as pneumonia +/- : physician diagnosis

- Among 840 patients, 1078 BAL samples were examined by both PN-panel and culture.
- The PN-panel detected bacterial pathogens in 506 (47%) samples, BUT *growth of these same pathogens was reported in only 185 (17%) samples after culture.*
- PN-panel detected additional 346 *bacterial targets* (including 6 atypical pneumonia bacteria and 153 viral pathogens) among 114 patients
- Culture detected 19 additional bacteria (included in the PN panel, but not detected) and *171 other bacteria or fungi which could represent pathogens or colonizing flora (not included in the PN panel).*

	Pneumonia (232 BALs from 175 patients)				No pneumonia (846 BAL among 665 patients)			
	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Bacterial species								
<i>A. calcoaceticus-baumannii</i> complex	–	100 (98–100)	–	100 (98–100*)	–	100 (99–100)	–	100 (100–100*)
<i>E. cloacae</i> complex	100 (40–100*)	100 (98–100)	80 (28–99)	100 (98–100*)	100 (40–100*)	99 (98–100)	36 (11–69)	100 (100–100*)
<i>E. coli</i>	89 (52–100)	98 (95–100)	67 (35–90)	100 (97–100)	92 (62–100)	98 (96–99)	35 (19–55)	100 (99–100)
<i>H. influenzae</i>	67 (22–96)	90 (86–94)	15 (4–35)	99 (97–100)	100 (72–100*)	87 (85–89)	9 (5–16)	100 (99–100*)
<i>K. aerogenes</i>	–	100 (98–100)	–	99 (97–100)	–	100 (100–100*)	–	100 (100–100*)
<i>K. oxytoca</i>	–	100 (98–100*)	–	100 (98–100*)	–	100 (99–100)	–	100 (100–100*)
<i>K. pneumoniae</i> -group	–	99 (96–100)	25 (1–81)	100 (98–100)	88 (47–100)	99 (98–100)	47 (21–73)	100 (99–100)
<i>M. catarrhalis</i>	100 (3–100*)	98 (96–100)	20 (1–72)	100 (98–100*)	100 (29–100*)	98 (96–99)	13 (3–34)	100 (100–100*)
<i>Proteus</i> spp.	100 (16–100*)	98 (96–100)	33 (4–78)	100 (98–100*)	100 (48–100*)	100 (100–100*)	100 (48–100*)	100 (100–100*)
<i>P. aeruginosa</i>	93 (66–100)	96 (93–98)	62 (38–82)	100 (97–100)	92 (74–99)	99 (98–99)	70 (51–84)	100 (99–100)
<i>S. marcescens</i>	–	100 (98–100)	–	100 (98–100*)	100 (16–100*)	99 (99–100)	29 (4–71)	100 (100–100*)
<i>S. aureus</i>	91 (59–100)	96 (92–98)	53 (29–76)	99 (97–100)	89 (75–97)	94 (92–96)	42 (31–53)	99 (99–100)
<i>S. agalactiae</i>	100 (3–100*)	98 (95–99)	20 (1–72)	100 (98–100*)	100 (3–100*)	98 (97–99)	7 (0–34)	100 (100–100*)
<i>S. pneumoniae</i>	80 (28–100)	96 (92–98)	29 (8–58)	100 (97–100)	89 (65–99)	97 (96–98)	40 (25–57)	100 (99–100)
<i>S. pyogenes</i>	–	99 (97–100)	–	100 (98–100*)	–	100 (99–100)	–	100 (100–100*)

Crude odds ratios of bacterial pneumonia among 840 unique hospitalized patients including 175 with pneumonia, according to microbiological test results and presence of chronic pulmonary disease

A positive PN-panel was a predictor of pneumonia only in patients without chronic pulmonary disease, whereas the odds ratio for pneumonia was increased in those with positive culture regardless of underlying chronic pulmonary disease.

Result interpretation - in combination with the clinical condition, otherwise it could lead to inappropriate use of antibiotics

	Odds ratio (95% CI)
PN-panel positive	1.4 (1.0–1.9)
Chronic pulmonary disease	1.2 (0.6–2.2)
No chronic pulmonary disease	1.6 (1.0–2.3)
PN-panel positive only	1.1 (0.7–1.6)
Chronic pulmonary disease	0.6 (0.3–1.4)
No chronic pulmonary disease	1.3 (0.8–2.2)
Culture positive	1.9 (1.3–2.8)
Chronic pulmonary disease	2.3 (1.2–4.4)
No chronic pulmonary disease	1.8 (1.1–3.0)
Culture positive only	2.6 (1.3–5.3)
Chronic pulmonary disease	2.9 (0.8–10.2)
No chronic pulmonary disease	2.5 (1.0–5.9)
PN-panel and culture positive	1.6 (1.0–2.4)
Chronic pulmonary disease	1.9 (1.0–3.8)
No chronic pulmonary disease	1.5 (0.9–2.6)

Real-life Assessment of BioFire FilmArray Pneumonia Panel in Adults Hospitalized With Respiratory Illness

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Table 1. Demographic and Clinical Characteristics of Study Population (N = 298)

	No. (%)
Age, y ^a	63.1 ± 16.0 (19–98)
Female	151 (50.1)
Race	
White	209 (70.1)
Black	76 (25.6)
Mixed/unknown	11 (3.7)
Ethnicity	
Non-Hispanic	269 (90.3)
Hispanic	28 (9.4)
Unknown	1 (0.3)
Underlying pulmonary conditions	
Asthma	91 (30.5)
COPD	140 (47.0)
Primary discharge diagnosis	
Respiratory failure	24 (8.1)
Acute exacerbation of COPD	60 (20.1)
Asthma	22 (7.4)
Acute bronchitis	9 (3.0)
Community-acquired pneumonia	95 (31.9)
Congestive heart failure	3 (1.0)
Viral syndrome	24 (8.1)
Other	61 (20.5)
Required ICU	59 (19.8)
Mechanically ventilated	13 (4.4)
Laboratory ^b	
WBC, 10 ³ /μL	10.9 (2.3–37.9)
Lactate, mmol/L	1.4 (0.6–6.8)
PCT, ng/mL	0.13 (0.04–100)

Abbreviations: COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; PCT, procalcitonin; WBC, white blood cell.

^aMean ± SD (range).

^bMedian (range).

- ✓ Patients with acute resp infections (symptoms/clinical) recruited in 24 hr
- ✓ Adjudicated by a panel of 4 physicians and classified into - viral infection alone, bacterial infection alone, or bacterial-viral coinfection.
- ✓ Adjudicators were blinded to the BioFire PN bacterial PCR results but not to atypical bacterial and viral results.

298 good and moderate quality sputum samples BioFire PN

- detected 350 bacteria and 16 atypical bacteria - an average of 1.23 potential bacterial pathogens per sample.
- 225 (75.5%) samples had typical bacteria detected.

SOC:

- 144 organisms (143 bacteria and 1 atypical bacteria) averaging 0.48 organisms per sample
- 126 samples (42.3%) grew a potential pathogen

Table 2. Concordance of BioFire PN With Sputum Culture According to Use of Antibiotics in Good Quality Sputum Samples

	Total (n = 203)	Antibiotics		P Value
		No (n = 85)	Yes (n = 118)	
A: Concordance of culture to BioFire PN				
Partially concordant	43 (21)	25 (29)	18 (15)	.02
Fully concordant	75 (37)	37 (44)	38 (32)	.11
Partially or fully concordant	119 (59)	62 (73)	56 (47)	.0003
Discordant	85 (42)	23 (27)	62 (53)	.0003
B: Culture				
Monomicrobial	82 (40)	48 (57)	34 (29)	.0001
Polymicrobial	10 (5)	5 (6)	5 (4)	.74
No growth/normal flora	111 (55)	32 (38)	79 (67)	.0001
C: BioFire PN				
Monomicrobial	91 (45)	33 (39)	58 (49)	.16
Polymicrobial	64 (32)	31 (37)	33 (28)	.22
No growth/normal flora	48 (23)	21 (25)	27 (23)	.87

Data are presented as No. (%).

Abbreviation: BioFire PN, BioFire FilmArray Pneumonia Panel.

Table 5. Comparison of Clinical Markers of Inflammation and Clinical Adjudication by Bacterial Abundance

Variable	Quantity, Copies/mL		P Value
	10 ⁴ –10 ⁵ (n = 40)	10 ⁶ to ≥10 ⁷ (n = 109)	
Median (IQR)			
WBC, 10 ³ /μL	12.35 (8.1–14.5)	11.5 (9.4–15.3)	.23
PCT, ng/mL ^a	0.12 (0.06–0.21)	0.16 (0.06–0.92)	.16
No. (%)			
PCT >0.25 ng/mL ^a	8/39 (21)	44/108 (41)	.03
Adjudicated as bacterial ^b	11/40 (28)	69/109 (63)	<.0001

Results of good quality samples with quantifiable standard bacteria detected (n = 149) were analyzed by low and high genomic copy number. Samples with atypical bacteria detected were excluded because testing was qualitative.

Abbreviations: PCT, procalcitonin; WBC, white blood cell.

^aPCT testing was missing in 1 participant in each group.

^bBacterial = bacterial alone or bacteria + virus.



Multicentre evaluation of two multiplex PCR platforms for the rapid microbiological investigation of nosocomial pneumonia in UK ICUs: the INHALE WP1 study

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- PCR identified more pathogens :
BioFire:74.2% , Unyvero: 60.4%, Routine microbiology: 44%

- Surplus routine lower respiratory tract samples - from intensive care unit patients about to receive new or changed antibiotics for hospital-onset lower respiratory tract infections
- 652 samples from 15 UK hospitals.
- Testing was performed using the BioFire FilmArray Pneumonia Panel (bioMérieux) and Unyvero Pneumonia Panel (Curetis).
- Concordance analysis compared machine and routine microbiology results

	Sensitivity	Specificity
BioFire	91.7%–100%	87.5%–99.5%
Unyvero	50.0%–100%	89.4%–99.%

Utility of BioFire:

1) Utilising a positive report:

Pre-test probability

Log Bin value – more confident diagnosis at higher levels, to correlate with culture

Other markers ?

Resistance gene (?expressed) vs other phenotypic methods of resistance

2) Cost

3) Novel pathogens

A limited panel of genetic markers for bacterial/viral/fungal identification and/ or resistance mechanisms - novel or rare pathogens may be missed. *Affects negative predictive value.*

4) Be cautious about pathogens not included:

Eg: *S. maltophilia* in ICU patients

Aspergillus fumigatus and other moulds in immunocompromised patients or ICU patients

Recommendation :

- To be used in patients where a non-infective focus is more likely, and negative infective work-up aids therapeutics

Eg: before immunosuppression

(caveat – fungal/tubercular)

- In patients with suspected infective focus : can be accepted at face value only in the context of clinical setting

- Thank You