Multinational evaluation of the BioFire® FilmArray® Pneumonia plus Panel as compared to standard of care testing

Christine C. Ginocchio 1,2, Carolina Garcia-Mondragon 3, Barbara Mauerhofer 3, Cory Rindlisbacher 1, and the EME Evaluation Program Collaborative

Received: 2 December 2020 / Accepted: 9 February 2021 / Published online: 2 March 2021 © The Author(s) 2021

Abstract
This study compared standard of care testing (SOC) to BioFire® FilmArray® Pneumonia plus Panel (PN plus). PN plus detects 15 bacteria with semiquantitative log bin values, 7 antibiotic resistance markers, three atypical bacteria (AB), and eight viral classes directly from bronchoalveolar lavage-like specimens (BLS) and sputum-like specimens (SLS). Fifty-two laboratories from 13 European countries and Israel tested 1234 BLS and 1242 SLS with PN plus and SOC. Detection rates and number of pathogens/samples were compared for PN plus pathogens. PN plus bin values and SOC quantities were compared. Three thousand two hundred sixty-two bacteria in PN plus were detected by PN plus and/or SOC. SOC detected 57.1% compared to 95.8% for PN plus (p ≤ 0.0001). PN plus semiquantitative bin values were less than SOC, equal to SOC, or greater than SOC in 5.1%, 25.4%, and 69.6% of results, respectively. PN plus bin values were on average ≥ 1 log than SOC values (58.5% 1–2 logs; 11.0% 3–4 logs). PN plus identified 98.2% of MRSA and SOC 55.6%. SOC detected 73/103 AB (70.9%) and 134/631 viruses (21.2%). PN plus detected 93/103 AB (90.3%) and 618/631 viruses (97.9%) (p ≤ 0.0001). PN plus and SOC mean number of pathogens/samples were 1.99 and 1.44, respectively. All gram-negative resistance markers were detected. PN plus and SOC results were fully or partially concordant for 49.1% and 26.4% of specimens, respectively. PN plus was highly sensitive and detected more potential pneumonia pathogens than SOC. Semiquantification may assist in understanding pathogen significance. As PN plus generates results in approximately 1 h, PN plus has potential to direct antimicrobial therapy in near real time and improve antimicrobial stewardship and patient outcomes.

Keywords Pneumonia · CAP · HAP · VAP · Diagnosis · BioFire Pneumonia plus Panel

Introduction
Determining the etiology of community-acquired pneumonia (CAP), hospital-associated pneumonia (HAP), and ventilator-associated pneumonia (VAP) can be complicated [1]. Based on traditional test methods such as Gram stain and culture, diagnostic yield can be low [2–4]. Additionally, poor specimen quality may yield inconclusive or difficult to interpret results, sampling may require an invasive procedure, and/or patients may be on empiric therapy prior to specimen collection, reducing diagnostic yield [5]. Although international guidelines from the Infectious Diseases Society of America (IDSA)/American Thoracic Society (ATS) and the European Respiratory Society (ERS) recommend diagnostic testing for moderate to severe CAP [6, 7], actual compliance with guidelines can be low (16.7% and 23.9%, respectively) and differs by geography and disease severity [8]. Aside from influenza A/B, often no viral testing is performed, despite evidence that other viruses are a significant cause of CAP in all age patients [9–11]. Consequently, broad use of empiric antibiotic treatment for undiagnosed viral infections has been associated with increased antibiotic resistance globally [12–14].

In HAP and VAP, empiric therapy often includes broad-spectrum antibiotics for both gram-positive and gram-negative bacteria due to the risk of infection with multidrug-resistant pathogens [15, 16]. Identification of specific pathogen(s) is of increasing importance to allow prompt initiation of
targeted and effective therapy and should have a positive impact on antimicrobial resistance and consequently on healthcare expenditure, while reducing risks for adverse events such as renal impairment and development of Clostridium difficile disease [17–19].

To address these unmet diagnostic needs and to provide a solution for prompt initiation of targeted therapy, the BioFire® FilmArray® Pneumonia (PN)/Pneumonia plus (PNplus) Panels (BioFire Diagnostics, LLC, Salt Lake City, UT) were developed. BioFire PN/PNplus Panels are US Food and Drug Administration (FDA) cleared and CE-IVD marked highly multiplexed nested polymerase chain reaction tests that identify the common causes of CAP, HAP, and VAP [20]. BioFire PN/PNplus Panels contain identical test reagents; however, results for Middle East respiratory syndrome coronavirus (MERS-CoV) are masked by the software for BioFire PN Panel. BioFire PN/PNplus Panels detect 15 bacteria with a semiquantitative log value (10^4, 10^5, 10^6, ≥ 10^7), three atypical bacteria (Legionella pneumophila [all serotypes], Mycoplasma pneumoniae, Chlamydia pneumoniae), viruses reported as eight groups (adenovirus, coronaviruses [OC43, NL63, HKU-1, 229E], MERS-CoV [BioFire PNplus Panel], human metapneumovirus, human rhinovirus/enterovirus [HRV/EV], influenza A, influenza B, parainfluenza viruses, respiratory syncytial virus), and 7 genetic markers of antibiotic resistance (mecA/C and MREI, blaCTX-M, blaKPC, blavIM, blaoXA-48-like, blatIM, blaNDM). BioFire PN/PNplus Panels are intended for use in persons of all ages and in various settings, including outpatient, emergency department, and hospitalized. The tests are validated for lower respiratory tract specimens, including sputum-like specimens (SLS) (induced, expectorated sputa, endotracheal aspirates [ETA]) and bronchoalveolar lavage (BAL)-like specimens (BLS) (BAL, mini-BAL). Specimen preprocessing is not required, test setup is approximately 5–10 min, and time to results is approximately 1 h.

This multicenter study assessed BioFire PNplus Panel performance as compared to standard of care testing (SOC) performed at a variety of institutions with variable test utilization and laboratory practices.

**Materials and methods**

**Clinical sites, specimens, and SOC testing** Forty-eight academic medical center laboratories and four independent medical laboratories from 13 countries (Austria, Belgium, Denmark, Israel, Italy, France, Germany, Netherlands, Portugal, Spain, Sweden, Switzerland, and the UK) tested 2476 unique specimens (1234 BLS; 1242 SLS) from adult and pediatric patients suspected of pneumonia (Supplemental Table 7). Specimen selection was at discretion of study site. SOC was performed per institutional policies and healthcare provider prescription. SOC varied by site and all included bacterial culture and phenotypic susceptibility testing when indicated. Additional test methods were at the discretion of the laboratory and may have included additional cultures (fungal, viral, mycobacterial cultures) as needed, urinary antigen testing (Streptococcus pneumoniae, L. pneumophila), immunofluorescence tests, and nucleic acid amplification tests (NAATs) for selected bacteria, viruses, and antibiotic resistance markers. Methicillin-susceptible Staphylococcus aureus (MSSA) was differentiated from methicillin-resistant S. aureus (MRSA) using phenotypic methods, mecA NAAT, and/or PBP2a latex agglutination at the discretion of the laboratory. The investigators were instructed to perform the BioFire PNplus Panel in accordance with the manufacturer’s instruction for use. Specimens were split and BioFire PNplus Panel testing was performed using either fresh specimens or from a frozen aliquot. Data was deidentified, no protected health information was provided, and participation was in accordance with local institutional ethical guidelines.

BioFire PNplus and SOC results were compared for the following:

1. **Detection of BioFire PNplus Panel pathogens by BioFire PNplus Panel and SOC:** Results were evaluated for all specimens and by specimen types. SOC detected or not detected was determined in consideration of all test results reported. SOC C. pneumoniae, L. pneumophila, M. pneumoniae, and viral results were counted as not detected when a negative or no result was provided as patterns of testing varied extensively by institution. All SOC results were considered true positive. Results of BioFire PNplus Panel were considered true positive or true negative based on performance data established in US FDA clinical studies [20]. Mean numbers of BioFire PNplus Panel pathogens per specimen, rate, and composition of coinfections were compared to SOC. No discordant analyses were performed due to large numbers of isolates that would have been referred to a supplemental PCR. Additionally, most laboratories would not have another culture-independent method to confirm or refute results and the study was designed to mimic real-life interpretation of data.

2. **Number and type of microorganisms reported by SOC not on BioFire PNplus Panel:** Organisms considered always as normal flora (ex viridans streptococci) were excluded from analysis, and remaining organisms were classified as potential pathogens and questionable pathogens.

3. **BioFire PNplus Panel and SOC results were evaluated for overall concordance.** For common bacteria identified by routine culture, positive percent agreement (PPA) and negative percent agreement (NPA) for on-panel pathogens were calculated. The negative predictive value (NPV) for BioFire PNplus on panel pathogens and off panel pathogens was calculated.
4. Comparison of semiquantitative BioFire PN plus Panel and SOC values for bacteria: SOC reporting varied, including no quantification, semiquantitative descriptive (rare, few, moderate, many, etc.), semiquantitative numerical (1+, 2+, 3+, 4+), and quantitative culture in log values (10^2–10^8 CFU/mL). To equate SOC to BioFire PN plus Panel bin values, standardized reference values (SRV) were established as defined in Supplemental Table 8. Data was evaluated for all SOC and BioFire PN plus Panel comparisons (n = 1297) and for a subset of comparisons where quantitative numerical culture values were provided (n = 903).

**Statistical analysis** p values were calculated using a 2-specimen test for equality of proportions with a continuity correction. BioFire PN plus Panel PPA, NPA, and NPV for the common bacterial pathogens were calculated using the Clopper-Pearson method.

**Results**

**Detection of BioFire PN plus Panel pathogens by BioFire PN plus and SOC** Of the 2476 specimens tested with BioFire PN plus, 13 specimens (0.53%) gave invalid results (5 SLS; 8 BLS) leaving 2463 specimens (1237 SLS, 1226 BLS) evaluable. BioFire PN plus Panel detected one or more pathogens in 1875 specimens (76.13%) and SOC in 1380 specimens (56.03%) (p ≤ 0.0001) (Fig. 1). In total, 3893 bacteria and viruses were detected by at least one method (Table 1). BioFire PN plus Panel detected 3743/3893 pathogens (96.15%) compared to 1995/3893 pathogens (51.25%) for SOC (p ≤ 0.0001). BioFire PN plus Panel and SOC concordance for all pathogens detected was 47.39%. Sensitivities of BioFire PN plus Panel detections ranged from 8.89 to 100%.

**Statistical analysis** p values were calculated using a 2-specimen test for equality of proportions with a continuity correction. BioFire PN plus Panel PPA, NPA, and NPV for the common bacterial pathogens were calculated using the Clopper-Pearson method.

**Results**

**Detection of BioFire PN plus Panel pathogens by BioFire PN plus and SOC** Of the 2476 specimens tested with BioFire PN plus, 13 specimens (0.53%) gave invalid results (5 SLS; 8 BLS) leaving 2463 specimens (1237 SLS, 1226 BLS) evaluable. BioFire PN plus Panel detected one or more pathogens in 1875 specimens (76.13%) and SOC in 1380 specimens (56.03%) (p ≤ 0.0001) (Fig. 1). In total, 3893 bacteria and viruses were detected by at least one method (Table 1). BioFire PN plus Panel detected 3743/3893 pathogens (96.15%) compared to 1995/3893 pathogens (51.25%) for SOC (p ≤ 0.0001). BioFire PN plus Panel and SOC concordance for all pathogens detected was 47.39%. Sensitivities of BioFire PN plus Panel detections ranged from 8.89 to 100%, and sensitivities of SOC detections ranged from 8.89 to 100%.

For all specimen types, 3262 bacteria were detected by at least one method (Table 1). BioFire PN plus Panel detected 3125/3262 bacteria (95.77%) and SOC 1861/3262 (57.05%) (p ≤ 0.0001). BioFire PN plus Panel and SOC concordance was 52.85%. The most frequently detected bacteria per sample by BioFire PN plus Panel were *S. aureus* (21.15%), *Haemophilus influenzae* (19.69%), and *Pseudomonas aeruginosa* (15.63%) and by SOC were *P. aeruginosa* (12.83%), *S. aureus* (12.71%), and *H. influenzae* (8.65%).

For BLS, 1358 bacteria were detected by at least one method (Supplemental Table 9). BioFire PN plus Panel identified 1288/1358 bacteria (94.85%) compared to 841/1358 (61.93%) for SOC (p ≤ 0.0001). BioFire PN plus Panel and SOC concordance was 56.77%. For SLS, 1904 bacteria were detected by at least one method (Supplemental Table 10). BioFire PN plus identified 1836/1904 (96.43%) bacteria and SOC 1020/1904 (53.57%) (p ≤ 0.0001). BioFire PN plus Panel and SOC concordance was 50%. PPAs for the individual PN plus common bacterial pathogens ranged from 68.42 to 100% (mean 92.90%; 95% confidence interval [CI] 91.61 to 94.04%) for all specimens combined; 50.0 to 100% (mean 91.77%; 95% CI 89.66 to 93.56%) for BLS; and 80.95 to 100% (mean 93.74%; 95% CI 92.03 to 95.18%) for SLS.

NPAs for the individual BioFire PN plus Panel common bacterial pathogens ranged from 87.07 to 99.31% (mean 96.10%; 95% CI 95.89 to 96.30%) for all specimens combined; 90.18 to 99.43% (mean 97.14%; 95% CI 96.89 to 97.39%) for BLS; and 83.86 to 99.19% (mean 94.98%; 95% CI 94.73 to 95.37%) for SLS. NPVs for the individual BioFire PN plus Panel common bacterial pathogens ranged from 99.04 to 99.96% (mean 99.63%; 95% CI 99.56 to 99.68%) for all specimens combined; 99.0 to 99.92% (mean 99.61%; 95% CI 99.51 to 99.69%) for BLS; and 99.15 to 99.94% (mean 99.64%; 95% CI 99.54 to 99.71%) for SLS. This evaluation did not consider the quantity of the common bacterial pathogens reported and the NPA and NPV may in practice be higher based on laboratory sample type and pathogen reporting thresholds. Overall for specimens negative by BioFire PN plus Panel but positive by SOC culture for which either a semiquantitative or quantitative result was provided, 45.1% had quantities reported by SOC (e.g., 1+, rare, 1 colony, < 10^3) that would be considered below the level of detection of the BioFire PN plus Panel.

*C. pneumoniae, M. pneumoniae, and L. pneumophila* were detected in 0.28%, 1.66%, and 2.23% of all specimens, respectively. Sensitivities of *C. pneumoniae, M. pneumoniae, and L. pneumophila* detections by BioFire PN plus Panel were 100% (7/7), 90.24% (37/41), and 89.09% (49/55) respectively, and SOC were 57.14% (4/7), 48.78% (20/41), and 89.09% (49/55), respectively (Table 1). Detection of *M. pneumoniae* was significantly better by BioFire PN plus Panel than SOC (p = 0.0001). BioFire PN plus Panel and SOC detection rates for atypical bacteria combined were 90.29% (93/103) and 70.87% (73/103), respectively (p = .0008). BioFire PN plus Panel detected 97.94% (618/631) of the total viral detections and SOC 21.24% (134/631) (p ≤ 0.0001). RV/EV was the predominant virus group detected (58.32%), followed by PIVs (10.78%) and the non-SARS-CoVs (8.24%).

**Single and codetections for BioFire PN plus Panel pathogens identified by BioFire PN plus and SOC** Distribution of negative and positive results for all specimens and breakdown of composition for BioFire PN plus Panel and SOC are shown in Figs. 2 and 1. Majority of BioFire PN plus Panel results (70.57%) and SOC results (55.75%) contained at least one bacteria, and at least one virus was detected in 20.49% and 2.93% of specimens, respectively. Mean number of bacteria and viruses detected by BioFire PN plus Panel in all specimens, BLS, and SLS were 1.99, 1.8, and 2.15, respectively, compared to 1.44,
| Pathogen                          | Number SOC (+) | Number PNplus (+) | Number SOC (−) | Number PNplus (−) | Number total (+) | Percentage (%) detected PNplus | Percentage (%) detected SOC | p value*                  |
|----------------------------------|----------------|------------------|----------------|------------------|------------------|-------------------------------|----------------------------|---------------------------|
| Acinetobacter calcoaceticus-baumannii complex | 78             | 45               | 2              | 125              | 123              | 80                           | 98.40                      | 64.00                     | < 0.0001*                 |
| Adenovirus                       | 3              | 41               | 1              | 45               | 44               | 4                            | 97.78                      | 8.89                      | < 0.0001*                 |
| Chlamydia pneumoniae             | 4              | 3                | 0              | 7                | 7                | 4                            | 100.00                     | 57.14                     | 0.19                      |
| Coronavirus                       | 5              | 46               | 1              | 52               | 51               | 6                            | 98.08                      | 11.54                     | < 0.0001*                 |
| Enterobacter cloacae complex     | 102            | 99               | 10             | 211              | 201              | 112                          | 95.26                      | 53.08                     | < 0.0001*                 |
| Escherichia coli                 | 150            | 113              | 19             | 282              | 263              | 169                          | 93.26                      | 59.93                     | < 0.0001*                 |
| Haemophilus influenzae           | 194            | 291              | 19             | 504              | 485              | 213                          | 96.23                      | 42.26                     | < 0.0001*                 |
| Human metapneumovirus            | 5              | 5                | 1              | 11               | 10               | 6                            | 90.91                      | 54.55                     | 0.15                      |
| Rhinovirus/enterovirus           | 57             | 308              | 3              | 368              | 365              | 60                           | 99.18                      | 16.30                     | < 0.0001*                 |
| Influenza A                      | 24             | 20               | 1              | 45               | 44               | 25                           | 97.78                      | 55.56                     | < 0.0001*                 |
| Influenza B                      | 2              | 0                | 0              | 2                | 2                | 2                            | 100                        | 100                       | 1.00                      |
| Klebsiella aerogenes             | 29             | 33               | 8              | 70               | 62               | 37                           | 88.57                      | 52.86                     | < 0.0001*                 |
| Klebsiella oxytoca               | 26             | 45               | 12             | 83               | 71               | 38                           | 85.54                      | 45.78                     | < 0.0001*                 |
| Klebsiella pneumoniae group      | 179            | 76               | 15             | 270              | 255              | 194                          | 94.44                      | 71.85                     | < 0.0001*                 |
| Legionella pneumophila           | 43             | 6                | 6              | 55               | 49               | 49                           | 89.09                      | 89.09                     | 1.00                      |
| Moraxella catarrhalis            | 48             | 90               | 3              | 141              | 138              | 51                           | 97.87                      | 36.17                     | < 0.0001*                 |
| Mycoplasma pneumoniae            | 16             | 21               | 4              | 41               | 37               | 20                           | 90.24                      | 48.78                     | 0.0001*                   |
| Parainfluenza viruses            | 14             | 49               | 5              | 68               | 63               | 19                           | 92.65                      | 27.94                     | < 0.0001*                 |
| Proteus spp.                     | 43             | 35               | 3              | 81               | 78               | 46                           | 96.30                      | 56.79                     | < 0.0001*                 |
| Pseudomonas aeruginosa           | 305            | 80               | 11             | 396              | 385              | 316                          | 97.22                      | 79.80                     | < 0.0001*                 |
| Respiratory syncytial virus      | 11             | 28               | 1              | 40               | 39               | 12                           | 97.50                      | 30.00                     | < 0.0001*                 |
| Serratia marcescens              | 58             | 42               | 6              | 106              | 100              | 64                           | 94.34                      | 60.38                     | < 0.0001*                 |
| Staphylococcus aureus            | 303            | 218              | 10             | 531              | 521              | 313                          | 98.12                      | 58.95                     | < 0.0001*                 |
| Streptococcus agalactiae         | 7              | 43               | 2              | 52               | 50               | 9                            | 96.15                      | 17.31                     | < 0.0001*                 |
| Streptococcus pneumonia          | 134            | 144              | 7              | 285              | 278              | 141                          | 97.54                      | 49.47                     | < 0.0001*                 |
| Streptococcus pyogenes           | 5              | 17               | 0              | 22               | 22               | 5                            | 100                        | 22.73                     | < 0.0001*                 |
| Total                            | 1845           | 1898             | 150            | 3893             | 3743             | 1995                         | 96.15                      | 51.25                     | < 0.0001*                 |

Abbreviations: (+): positive; (−): negative

* Significant p value ≤ 0.05

**Coronaviruses: total includes OC43, NL63, 229E, and HKU-1

**Parainfluenza viruses: total includes types 1, 2, 3, and 4
Fusobacterium anaerobes including three Enterococcus gram-positive cocci of questionable significance including 24 Aspergillus fumigatus 28 specimens, including 11 Pneumocystis era, were reported less than 10 times. Yeasts were reported for another 28 gram-negative bacterial spp., representing 18 gen-

Among the 1027 BioFire PN plus Panel specimens with codetections, 63.0% (647/1027) had only multiple bacteria (2 to 8); 35.44% (364/1027) had both bacteria (1 to 7) and virus(es) (1 to 3); and 1.33% (16/1207) had only 2 viruses detected. Among the 476 SOC specimens with codetections, 86.34% (411/476) had only multiple bacteria (2 to 5); 13.03% (62/476) had both bacteria (1 to 5) and virus(es) (1 to 2); and 0.63% (3/476) had 3 viruses only detected.

**Pathogens detected by SOC not included in BioFire PN plus Panel** Excluding normal oral flora, 649 additional potential pathogens were detected by SOC including 230 gram-negative bacteria, the most prevalent being Stenotrophomonas maltophilia (n = 70), Citrobacter spp. (n = 31), Acinetobacter spp. (n = 28), Haemophilus parainfluenzae (n = 22), Morganella morganii (n = 15), and Hafnia alvei (n = 11). Another 28 gram-negative bacterial spp., representing 18 genera, were reported less than 10 times. Yeasts were reported for 283 specimens, Candida spp. (n = 123), Candida albicans (n = 113), C. glabrata (n = 27), C. parapsilosis (n = 6), C. krusei (n = 5), C. tropicalis (n = 5), C. kefyr (n = 2), and C. lusitaniae (n = 2). Pneumocystis sp. was reported for 11 specimens, molds for 28 specimens, including 11 Aspergillus fumigatus, and five anaerobes including three Fusobacterium spp. There were 42 gram-positive cocci of questionable significance including 24 Enterococcus spp. and seven Streptococcus C/G groups. There were 24 gram-positive rods, including 15 Corynebacterium spp. and 1 Nocardia sp. and four Mycobacterium (two M. tuberculosis, one M. avium, and one M. fortuitum). Additional viral targets (n = 22) included cytomegalovirus (n = 10), herpes simplex virus (n = 5), Epstein-Barr virus (n = 4), human herpes virus 6 (n = 2), and varicella zoster virus (n = 1).

Including BioFire PN plus Panel pathogens and additional SOC pathogens not in BioFire PN plus Panel, the number of potential pathogens for all specimens totaled 4542, of which BioFire PN plus Panel detected 82.41% (3743/4542) and SOC detected 58.21% (2644/4542) (p ≤ 0.0001). Excluding pathogens of questionable significance that may be considered colonizers (yeasts, gram-positive cocci, gram-positive rods [excluding Mycobacteria spp., Nocardia spp.], molds [due to difficulty in interpretation even in immunocompromised persons]), the total number of potential pathogens was 4274 of which BioFire PN plus Panel detected 87.58% (3743/4274) and SOC detected 55.59% (2376/4274) (p ≤ 0.0001). The NPVs for individual pathogens not included in the BioFire PN plus Panel ranged from 97.16% for S. maltophilia to 99.96% for organisms reported only once. The composite NPV of the BioFire PN plus Panel for all off-panel pathogens minus those of questionable significance was 89.12%. This evaluation did not consider the quantity of the off-panel pathogens reported and the NPV may in practice be higher based on laboratory sample type and pathogen reporting thresholds.

**Concordance of BioFire PN plus Panel and SOC results** Overall, 1209/2463 specimens (49.10%) demonstrated total concordance between BioFire PN plus Panel and SOC results (Table 3). This included positive concordance (27.0%), negative concordance (5.36%) for BioFire PN plus Panel bacteria and viruses, and 16.73% for SOC specimens positive for non-BioFire PN plus Panel bacteria and viruses. 26.43% (651/2463) of specimens demonstrated partial concordance and 24.48% (603/2463) no concordance. Positive, partial, and
negative concordances for BLS were 56.85%, 20.55%, and 22.59%, respectively, and 41.13%, 32.34%, and 26.35%, respectively, for SLS.

**Bacterial quantification by BioFire PNplus Panel and SOC**

Semiquantitative results for BioFire PNplus Panel and SOC bacteria were compared for 1297 matched detections (Table 4). Mean differences between all three levels of SOC SRVs and BioFire PNplus Panel SRVs for all bacteria, BLS bacteria, and SLS bacteria were 1.06 (range 0.96–1.14), 1.34 (range 1.19 to 1.52), and 0.90 (range 0.71 to 1.14), respectively. For a subset of specimens \( n = 903 \) with SOC quantitative culture results, mean difference between SOC SRV and BioFire PNplus Panel SRV for bacteria was 1.18. For specimens BioFire PNplus Panel positive and SOC negative, mean BioFire PNplus Panel SRVs for all bacteria, BLS bacteria, and SLS bacteria were 3.21, 3.10, and 3.27, respectively. Using this subset of BioFire PNplus Panel positive/SOC negative results, if a threshold for BLS reporting was set at BioFire PNplus Panel bin values of \( 10^4 \), \( 10^5 \), \( 10^6 \), or \( \geq 10^7 \), then 482, 290, 158, and 77 BioFire PNplus Panel bacteria results, respectively, would be reported as positive and

**Table 2** Number of pathogens (contained in the BioFire PNplus Panel) detected by BioFire Pneumonia plus (PNplus) Panel versus standard of care (SOC)

| Sample type       | Method   | Number positive | 1 pathogen, number (%) | 2 pathogens, number (%) | 3 pathogens, number (%) | 4 pathogens, number (%) | 5 pathogens, number (%) | 6+ pathogens, number (%) | Mean number per sample |
|-------------------|----------|-----------------|------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-----------------------|
| All specimens     | PNplus   | 1875            | 848 (45.22)            | 536 (28.59)             | 275 (14.67)             | 130 (6.93)              | 60 (3.20)               | 26 (1.39)               | 1.99                  |
|                   | SOC      | 1380            | 904 (65.51)            | 370 (26.81)             | 85 (6.16)               | 15 (1.09)               | 4 (0.29)                | 2 (0.14)                | 1.44                  |
| BAL-like specimens| PNplus   | 855             | 446 (52.16)            | 239 (27.95)             | 100 (11.70)             | 43 (5.03)               | 22 (2.57)               | 5 (0.59)                | 1.8                   |
|                   | SOC      | 640             | 432 (67.50)            | 200 (31.25)             | 4 (0.78)                | 3 (0.47)                | 0 (0.0)                 | 0 (0.0)                 | 1.44                  |
| Sputum-like specimens | PNplus | 1020           | 402 (39.41)            | 297 (29.12)             | 175 (17.16)             | 87 (8.53)               | 38 (3.73)               | 21 (2.06)               | 2.15                  |
|                   | SOC      | 740             | 472 (63.78)            | 199 (26.89)             | 56 (7.57)               | 10 (1.35)               | 1 (0.14)                | 2 (0.27)                | 1.8                   |

*BAL-like* bronchoalveolar lavage-like
discordant with SOC. If a threshold for SLS reporting was set at BioFire PNplusPanel bin values of $10^4, 10^5, 10^6,$ or $\geq 10^7$, then 834, 540, 336, and 177 bacteria results, respectively, would be reported as discordant with SOC. Therefore, increasing the BioFire PNplusPanel bin threshold for reporting bacteria as detected would result in increased concordance with SOC reported as culture negative. Establishment of reporting guidelines in consideration of both BioFire PNplusPanel bin values and the inherent log value differences between BioFire PNplusPanel results and SOC results improve overall concordance of bacteria reporting.

SOC SRVs and BioFire PNplusPanel SRVs were equivalent for 25.37% of all bacteria, 19.38% of BLS bacteria, and 31.92% of SLS bacteria (Table 5). BioFire PNplusPanel SRVs were greater than SOC SRVs for 69.55% of all bacteria, 76.28% of BLS bacteria, and for 62.88% of SLS bacteria, with majority of BioFire PNplusPanel SRVs 1–2 logs greater than SOC SRVs. SOC SRVs were greater than BioFire PNplusPanel SRVs for 5.09% of all bacteria, 4.34% of BLS bacteria, and 5.83% of SLS bacteria, with majority demonstrating a one log difference.

### Detection of MSSA and MRSA
A total of 531 specimens contained S. aureus, 97.93% (520/531) detected by BioFire PNplusPanel and 58.95% (313/531) detected by SOC ($p \leq 0.0001$) with a concordance of 56.87% (Table 6). BioFire PNplusPanel did not detect 11 S. aureus (2.07%, all MSSA). A total of 24 specimens were reported to contain MSSA by SOC but were reported to contain MRSA by BioFire PNplusPanel and two specimens reported to contain MRSA by SOC were reported as MSSA by BioFire PNplusPanel. Mean SRVs for S. aureus detected by SOC, BioFire PNplusPanel, and detected only by BioFire PNplusPanel were 3.14, 4.18, and 2.92, respectively.

### Detection of gram-negative resistance markers
BioFire PNplusPanel detected in 1537 specimen bacteria (Acinetobacter baumannii-calcoaceticus complex, Enterobacter cloacae complex, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae group, Proteus spp., P. aeruginosa, Serratia marcescens) for which ESBL and/or carbapenemase resistance genes would be reported. Of these, 185 (12.04%) had a total of 229 resistance genes ($bla_{CTX-M}$ [$n = 133$], $bla_{KPC}$ [$n = 67$], $bla_{IMP}$ [$n = 1$], $bla_{SHV}$ [$n = 56$], $bla_{NDM}$ [$n = 27$], $bla_{OXY}$ [$n = 10$], $bla_{GIM}$ [$n = 2$].

### Table 3: Concordance of BioFire PNplus (PNplus) Panel and standard of care (SOC) results for all specimens, BAL-like specimens, and sputum-like specimens

|                        | All specimens | BAL-like specimens | Sputum-like specimens |
|------------------------|---------------|--------------------|-----------------------|
|                        | Total number  | Percentage of total| Total number          | Percentage of total| Total number       | Percentage of total |
| Overall concordance     | 1209 (2463)   | 49.10              | 697 (1226)            | 56.85              | 511 (1237)         | 41.31               |
| Overall concordant positive | 665 (2463)   | 27.00              | 353 (1226)            | 28.79              | 311 (1237)         | 25.15               |
| Concordant bacteria     | 601 (2463)   | 24.40              | 320 (1226)            | 26.10              | 280 (1237)         | 22.64               |
| Concordant bacteria and virus | 25 (2463) | 1.02                | 6 (1226)               | 0.49                | 19 (1237)           | 1.54                |
| Concordant virus        | 39 (2463)    | 1.58                | 27 (1226)             | 2.20                | 12 (1237)          | 0.97                |
| Concordant negative for any pathogen | 412 (2463) | 16.73              | 257 (1226)            | 20.96              | 155 (1237)         | 12.53               |
| Concordant negative for PNplus pathogens | 132 (2463) | 5.36               | 87 (1226)             | 7.10                | 45 (1237)          | 3.64                |
| Partial concordance     | 651 (2463)   | 26.43              | 252 (1226)            | 20.55              | 400 (1237)         | 32.34               |
| No concordance          | 603 (2463)   | 24.48              | 277 (1226)            | 22.59              | 326 (1237)         | 26.35               |

---

*a Overall concordance: samples fully concordant for analytes detected by PNplus Panel and SOC and for samples reported as negative by both methods

*b Concordant positive: PNplus Panel and SOC in agreement for all pathogens detected

*c Concordant bacteria: PNplus Panel and SOC in agreement for all bacteria detected

*d Concordant bacteria and virus: PNplus Panel and SOC in agreement for all bacteria and viruses

*e Concordant virus: PNplus Panel and SOC in agreement for all viruses detected

*f Concordant negative for any pathogen: PNplus Panel and SOC both reported as negative for any pathogen

*g Concordant negative for PNplus Panel analytes: PNplus Panel and SOC negative for pathogens on PNplus Panel (other pathogens may have been detected by SOC)

|h Partial concordance: PNplus Panel and SOC in agreement for some pathogens detected

|i No concordance: PNplus Panel and SOC not in agreement for pathogens in PNplus Panel

BAL-like specimens bronchoalveolar lavage-like specimens
Table 4 Comparison of standard of care (SOC) bacteria standard reference values (SRV) and BioFire Pneumonia plus (PNplus) Panel bacteria standard reference values

|                      | SOC level 1 | SOC level 2 | SOC level 3 | PNplus value | Mean delta, PNplus/SOC | PNplus value, SOC ND |
|----------------------|-------------|-------------|-------------|--------------|------------------------|----------------------|
| All detections (N = 1297) | Mean SRV\(\text{a}\) | 2.94        | 3.19        | 3.32         | 4.27                   | 3.21                 |
|                      | Log delta\(\text{a}\) | 1.14        | 1.09        | 0.96         | 1.06                   |                      |
| BAL-like detections (N = 645) | Mean SRV | 2.64        | 2.85        | 2.97         | 4.16                   | 3.10                 |
|                      | Log delta  | 1.52        | 1.31        | 1.19         | 1.34                   |                      |
| Sputum-like detections (N = 652) | Mean SRV | 3.25        | 3.53        | 3.68         | 4.39                   | 3.27                 |
|                      | Log delta  | 1.14        | 0.86        | 0.71         | 0.90                   |                      |

The standardized reference values (SRV) (1–5) were derived from three different interpretation levels (defined in Supplemental Table 6)

\(\text{a}\) Combined mean difference between the BioFire PNplus SRVs and SOC SRVs for all 3 interpretation levels

\(\text{b}\) Mean SRV for bacteria detected by BioFire PNplus Panel but not detected by SOC

\(\text{c}\) Mean SRV for all samples per interpretation level 1, 2, or 3

\(\text{d}\) Difference between BioFire PNplus Panel SRV and SOC SRV (descriptive, numerical, and quantitative) per interpretation level

**Discussion**

This multinational study is the largest to date that compared BioFire PNplus Panel to SOC testing, which varied extensively by site and physician prescribing practices. BioFire PNplus Panel identified significantly more positive specimens (76.13%) than SOC (56.03%) \((p \leq 0.0001)\) and more potential pathogens than SOC \((p \leq 0.0001)\) independent of specimen type. Largest discrepancies for bacterial detections were for fastidious pathogens, which may be concealed by overgrowth of normal flora, or may be non-viable, including Streptococcus pyogenes (77.27%), Streptococcus agalactiae (78.85%), Moraxella catarrhalis (61.7%), H. influenzae (53.97%), and S. pneumoniae (48.07%). Despite detection differences, the three most common bacteria identified by BioFire PNplus Panel and SOC were similar, S. aureus, H. influenzae, and P. aeruginosa. Lower SOC bacteria detection may relate to local reporting guidelines and testing of specimens from patients on antimicrobials. The most prevalent pathogen not included on BioFire PNplus Panel detected by SOC was S. maltophilia; however, the incidence was low (2.84%), similarly with other various gram-negative rods (6.5%). Despite the low prevalence of pathogens not detected by BioFire PNplus Panel, it is essential to perform culture or other ancillary testing to detect off-panel pathogens and to provide susceptibility results.

Our rate of bacteria detection (95.77%) as compared to culture was consistent with Mitton et al. (92.0%) [21] and Yoo et al. (99.3%) [22]. A study by Webber et al. demonstrated that the BioFire PNplus Panel identified most bacteria (98.4%) detected by SOC and additionally 92 bacteria including more S. aureus (23.9%) and H. influenza (27.2%) [23]. Murphy et al. identified potential pathogens in 48.82% of BLS and in 72.01% of SLS with BioFire PN Panel, detecting more S. aureus, H. influenzae, M. catarrhalis, and P. aeruginosa [20]. In this study, the overall BioFire PNplus Panel PPA and NPA for common bacteria were 92.90% and 96.10%, respectively, and similar to other studies. Clinical studies for FDA clearance demonstrated a PPA of 96.2% and NPA of 98.3% for BLS and a PPA of 96.3% and NPA of 97.2% for SLS after discordant resolution using a molecular comparator. Lee et al. demonstrated a PPA of 90% and NPA of 97.4% as compared to SOC bacteria detection, with BioFire PN Panel identifying a pathogen in 47.4% of BLS and in 60% of SLS for an overall positivity rate of 55.93% [24]. Edin demonstrated for on-panel pathogens a PPA of 100% and an NPA of 73.2% [25], and Gastl demonstrated a PPA of 94.4% and an NPA of 96.0% as compared to culture [26]. Similarly, a VAP study using a research use only version of BioFire PNplus Panel demonstrated for bacteria an 89.0% PPA and 95.9% NPA with SOC [27]. BioFire PNplus Panel reflects high performance compared to SOC, yielding additional clinically actionable results that may be missed by SOC.

Prevalence of atypical pathogens in this specimen set was low (4.17%), with L. pneumophila the most frequently detected. Low SOC percent detections of M. pneumoniae (48.78%) and C. pneumoniae (57.14%) compared to BioFire PNplus

\(\text{bla}_{\text{NDM}}(n = 2), \text{bla}_{\text{OXA-48-like}}(n = 26)\). Six \(\text{bla}_{\text{OXA-48-like}}\) genes were detected in 1029 specimens (0.58%) positive by BioFire PNplus Panel for one or more of the following: E. cloacae complex, E. coli, K. aerogenes, K. oxytoca, K. pneumoniae group, Proteus spp., or S. marcescens. One resistance gene was detected in 76.76% (142/185) of specimens, two in 20.0% (37/185), and three in 3.24% (6/185). Due to large variations and inconsistencies in reporting of SOC phenotypic/genetic susceptibility data, no comparisons could be made.
Panel (90.24% and 100%, respectively) were mainly due to lack of testing and therefore a missed opportunity in CAP to either limit treatment to a macrolide or fluoroquinolone or stop treatment if tested negative. This missed opportunity for applying antimicrobial stewardship principles and streamlining therapy is of particular importance due to adverse effects of fluoroquinolones [28]. Of the 41 patients *M. pneumoniae* positive, age was available for 31 (range 7 to 88 years), including 12 (range 23 to 80 years) with no SOC result. Lack of testing may be a study artifact or be indicative of local testing practices. Adults may not be tested as *M. pneumoniae* is often viewed as an illness of school age children, adolescents, and young adults, although studies demonstrate infections in all aged adults, with up to a 15% prevalence in persons aged 56 or older [29]. Conversely, percentage of *L. pneumophila* detected by BioFire PNplus Panel and SOC were similar (89.09%). There were only 6/55 specimens *L. pneumophila* positive with no SOC result, indicating a higher awareness compared to *M. pneumoniae* and *C. pneumoniae*.

BioFire PNplus Panel detections were limited for certain viruses (example, influenza A, influenza B) due to seasonality and time of specimen collection. SOC viral detections were additionally limited by lack of testing. Webber et al. compared standard viral testing with BioFire PNplus Panel and demonstrated a 99.2% correlation [23]. Hughes et al. found an 87% PPA and 100% NPA for BioFire PNplus Panel viral detections compared to BioFire® FilmArray® Respiratory 2 (RP2) Panel (BioFire Diagnostics, LLC) [30]. Virus detection in absence of a bacterial pathogen and in conjunction with clinical presentation, chest radiograph, and other diagnostic tests, such as a low procalcitonin, could support antimicrobial stewardship and discontinuation of antibiotics in the setting of CAP [31].

BioFire PNplus Panel identified more codetections (41.85%) compared to SOC (19.21%), which was mainly influenced by lack of SOC viral testing. Codetections were commonly identified by Webber et al. (25%) [23], Murphy et al. (29.49%) [20], and Lee et al. (42.3%) [24]. All three studies found the majority of codetections contained 2 pathogens, but could rarely contain 5 to 6+ pathogens, similar to our results. In this study, BioFire PNplus Panel on average identified more potential pathogens (1.99) per specimen than SOC (1.44). Detection of multiple pathogens raises interpretation questions that need to be viewed in light of clinical parameters, pathogens detected, and abundances.

Use of BioFire PNplus Panel has led to concerns that identification of more bacteria than SOC, which may be colonizers, could lead to antibiotic overtreatment. Specimen types should be considered. SLS are prone to more oropharyngeal contamination compared to BLS. *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* can be normal flora, and hospitalized or ventilated patients may be colonized with gram-negative bacilli and *S. aureus*. Although laboratory
reporting varies depending on specimen type, patient populations, and clinical need, clinical guidelines recommend using different reporting thresholds for different specimen types. Generally, SLS bacteria are considered, in light of other variables, significant at $\geq 10^6$ or $\geq 10^7$ (moderate, numerous, 3+, 4+) and in BLS $\geq 10^3$ or $\geq 10^4$ (few, moderate, 2+ or greater). For this reason, the BioFire PNplus Panel lower limit of reporting for bacteria was set at $10^{3.5}$ genomes/mL. The bin values may allow for different interpretations based on specimen type. For example, SOC and BioFire PNplus Panel concordance for bacteria in SLS was 51.23% but when only evaluating BioFire PN Panel results reported at $10^6$ and $\geq 10^7$, correlation increased to 66.8%. Additionally, more BioFire PNplus Panel negative/SOC negative results would be obtained when higher bin thresholds for reporting BioFire PNplus Panel bacteria are used. Overall, the mean SRVs for SOC were consistently $\sim 1$ log lower than BioFire PNplus Panel SRV when using three different interpretation schemes. Mean SRV for specimen culture negative, BioFire PN Panel positive, was 3.21 and would potentially equate to SOC SRV of 2.21, which likely is below the limit of detection or limit of culture reporting. Similarly, SOC Staphylococcus aureus-negative specimens but BioFire PNplus positive had a SRV of 2.92.

Murphy et al. demonstrated that BioFire PN Panel bin values were accurate and reproducible within $\pm 0.5 \log_{10}$ copies/mL and correlated with another quantitative molecular method [20]. Despite low concordance with quantitative culture, particularly when values were $< 10^6$ (3.1–38.9%), concordance improved to 90.9–100% when quantitative culture values were $> 10^6$. There were few instances when BioFire PN Panel did not detect a bacterium or reported values lower than quantitative culture, which is similar to what we report in this study. Lee et al. also demonstrated an overestimation of quantification by BioFire PN Panel [24]. Buchan et al. demonstrated that PN values were frequently higher than culture values, resulting in semiquantitative agreement (within the same log$_{10}$ value) of 43.6% [32]. Gastli et al. reported that 90.1% of organisms with a BioFire PN Panel result of $\geq 10^6$ grew significantly in culture [26] and Yoo et al. reported that 86% of bacteria considered significant by culture (moderate or many quantities) yielded BioFire PN Panel results of $\geq 10^7$ [22 not 26] 22.

Although BioFire PNplus Panel does not make claims as to the significance of the bin value, knowing the relative bacteria abundance may be helpful in understanding coinfections and differentiating colonization versus infection. BioFire PN Panel bin values should be interpreted in consideration of specimen type, Gram stain, types and bin values for other pathogens detected, type of pneumonia, presence of resistance markers, biomarkers, and clinical risk factors. Laboratories should establish reporting guidelines and provide physician education in conjunction with a multidisciplinary team consisting of ID, critical care specialists, pulmonologists, stewardship committee, and infection control practitioners.

Major limitations of this study were a lack of clinical information and comprehensive gram-negative phenotypic antibiotic susceptibility data, such as that described by Murphy et al. [20], needed to better understand the relevance of resistance marker results and impact on patient care. Discordant analyses were not performed due to the large number of participating sites and the number of specimens tested. Discordant analyses would have required some type of confirmatory testing for 150 culture isolates (BioFire PN Panel negative) and 1898 individual PCRs or direct sample sequencing for the confirmation of pathogens only detected by BioFire PN Panel (SOC negative). However, this study therefore does highlight the need for microbiologists and clinicians to address issues relating to discordant results and test interpretation. Additionally, detailed information regarding testing for viral or atypical bacterial pathogens for each sample was not available so no direct performance comparison could be made. Gram stains were not systematically reported and it is not known if a quality score was a testing requirement. However, considering the high sensitivity and specificity of BioFire PN Panel as demonstrated in the US FDA clinical studies [20], in combination with detection of key gram-

---

### Table 6 Detection of methicillin sensitive Staphylococcus aureus (MSSA) and methicillin resistant S. aureus (MRSA) by BioFire Pneumonia plus (PNplus) Panel and standard of care (SOC)

|                    | Total (+) number (%) | PNplus (+) number (%) | SOC (+) number (%) | SOC (+) PNplus number (%) | SOC (-) PNplus number (%) | SOC (+) PNplus number (%) |
|--------------------|----------------------|-----------------------|-------------------|---------------------------|---------------------------|---------------------------|
| Staphylococcus aureus (total) | 531 (100)           | 520 (97.93)           | 313 (58.95)       | 302 (56.87)               | 218 (41.05)               | 11 (2.07)                 |
| Staphylococcus aureus (MSSA)    |                      |                       |                   |                           |                           |                           |
| Staphylococcus aureus (MRSA)    |                      |                       |                   |                           |                           |                           |
| Mean standard SVRc            | 3.14                 | 4.18                  | 2.92              |                           |                           |                           |

(+) S. aureus detected; (−) S. aureus not detected

a Twenty-four samples reported to contain MRSA by SOC were reported to contain MSSA by BioFire PN Panel

b Twenty-four samples reported to contain just MSSA by SOC were reported to contain MRSA by BioFire PN Panel

c SVR: standard reference value based on interpretation level 2 (refer to Supplemental Table 6)
positive and gram-negative resistance genes, BioFire PNplus Panel may facilitate decisions to optimize therapy, including discontinuation, de-escalation, and escalation, which should be evaluated on a patient-by-patient basis. Depending on local epidemiology, negative results for drug-resistant pathogens may yield a high negative predictive value and may allow for therapy de-escalation in the right clinical context, an approach endorsed by IDSA CAP guidelines and in CDC Core Elements of Hospital Antibiotic Stewardship Programs [6, 33]. Similarly, several hypothetical therapeutic reviews based on BioFire PN Panel results as compared to actual treatment revealed potential for antibiotic adjustment. Buchan et al. found, based on BioFire PN Panel results, 70.7% of patients could have had an antibiotic adjustment, including discontinuation or de-escalation in 48.2% resulting in an average savings of 6.2 antibiotic days/patient [32]. Lee et al. identified a potential for BioFire PN Panel to alter antibiotic prescription in 40.7% of patients [24]. A study by Monard et al. demonstrated that a multidisciplinary committee proposed modifications of empiric therapy in 77% of pneumonia episodes, including de-escalation (40%) and escalation (22%), and in microbiologic documented cases, the BioFire PN Panel increased appropriateness of therapy in 87% of cases as compared to 77% in routine care [34]. This data provides an early indication that proper use and interpretation of BioFire PN Panel could lead to targeted therapy not an increase in inappropriate antimicrobial usage. Prospective intervention studies in progress will provide data on interpretation of bin values, detection of resistance genes, and clinical impact of a rapid diagnosis. Finally, the BioFire PN Panel bin comparison to SOC reporting was difficult to standardize as culture reporting can vary from technologist to technologist and laboratory to laboratory, which could lead to interpretive error. However, despite using 3 different interpretative criteria, results did not significantly differ. Strengths of the study include the large specimen size, the even distribution of specimen types, and geographical diversity of testing sites with differences in both ordering practices and results reporting.

The clinical laboratory plays a vital role in diagnosis of CAP, HAP, and VAP but faces numerous challenges due to testing complexity [1, 5, 8-10]. Often poor-quality sputum specimens are submitted and without quality rejection screening by Gram stain, culture results can be misleading or negative. HAP and VAP patients pose a different dilemma since these patients quickly become colonized with *S. aureus* and various gram-negative bacilli which may lead to pneumonia with multidrug-resistant strains [15, 16]. Specimens can contain a diversity of pathogens including bacteria, viruses, and fungi [4, 6-10, 34-38]. Time to traditional bacterial detection is 24–72 h and antibiotic susceptibility data takes an additional 24–48 h. Comprehensive viral diagnostics are often not performed aside from influenza A/B testing, may not be performed 24/7, or require referral to a reference laboratory delaying time to results. Urinary antigen tests for *S. pneumoniae* provide results in < 30 min but can be false negative and false positive, particularly in children [39]. Urinary antigen tests for *L. pneumophila* are restricted in serotype detection and can have sensitivities of < 50% [40, 41]. Serology can be difficult to interpret and may require an acute and convalescent serum collected weeks apart. NAATs are the gold standard for the detection of the atypical bacteria but may not be routinely performed. Consequently, testing imitations lead to empiric HAP/VAP treatment with broad-spectrum antibiotics, especially in regions with high antimicrobial resistance rates. The switch to targeted therapy can take days, increasing risk of antimicrobial resistance and adverse events such as acute kidney injury and *C. difficile* disease [18]. Finally, if specimens are obtained after the start of antibiotic treatment, results may be altered or negative, without identifying the etiologic agent [5].

In conclusion, the BioFire PN Panel meets the challenges associated with routine test methods including poor pathogen recovery, lack of diagnostic comprehensiveness, and delayed time to result [42]. However, several factors need to be considered including the lack of a specimen quality marker and the inability to report the presence or absence of normal flora [43]. Although a Gram stain is not required prior to testing, good laboratory practice should still be followed to insure sample quality [43]. Pretreatment or dilution of samples would affect both the sensitivity of the assay and semiquantitative results and therefore is not recommended in the manufacturer’s instructions for use. Interpretation challenges include understanding the increased detection rates, significance of the bin value, the differentiation between colonization and infection, and the presence of gram-negative resistance markers without direct linkage to a specific pathogen. However, approved for use with BLS and SLS, BioFire PN Panel allows for easy specimen testing for CAP [6, 7] and meets IDSA/ATS recommendations [13] for non-invasive diagnostic testing as a preferred method for VAP and ERS guidelines to test distal quantitative specimens [16]. Additionally, studies that used BioFire PN Panel in COVID-19 patients demonstrated not only improved diagnosis of bacterial coinfections but enhanced options for appropriate therapy [35, 44, 45]. Verroken et al. demonstrated that BioFire PN Panel speeded up therapeutic changes in 46.9% of COVID-19 patients, five patients having antibiotics stopped and one third remained antibiotic free [35]. BioFire PN Panel is rapid, simple to perform, and highly robust, with only 0.53% of the specimens in this study yielding invalid results. Detection of pathogens and antibiotic resistance markers can be used to inform immediate treatment decisions and improve patient outcomes.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10096-021-04195-5.
Acknowledgements The members of the EME Evaluation Program Collaborative for their support and contribution to this study: Hospital Universitario Central de Asturias, Asturias, Spain, Forcellado L, Fernández J; ADMED Laboratoires - Analyses et Diagnostics Médicaux, La Chaux-de-Fond, Switzerland, Lienhard R; Analyse Biolab GmbH, Linz, Austria, Kerschner H; AOUC Azienda Ospedaliero Universitaria Careggi, Florence, Italy, Rossolini GM; AP-HP Nord Université de Paris, Hôpital Bichat, Paris, France, Armand-Lefèvre L, d’Humières C; APHP-Hôpital Lariboisière-Fernand Widal, Université de Paris, Paris, France, Cambau E, Bemmansour H; Azienda Ospedaliero Universitaria San Giovanni Battista Molinette, Torino, Italy, Cavallo R; Bioanalytical, Lucerne, Switzerland, Altweg M, Berlinger L; Centre Hospitalier Universitaire de Clermont-Ferrand, Clermont, France, Bonnet R, Saint-Sardos P; Centre Hospitalier Universitaire de Liège - Sart Tiňam, Liège, Belgium, Meex C; Centre Hospitalier Universitaire de Nîmes, Nîmes, France, Lavigne JP; Centre Hospitalier Universitaire de Poitiers, Poitiers, France, Leveque N, Brouix L; Centre Hospitalier Universitaire de Rennes, Rennes, France, Cattoir V, Auger G; Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal, Pereira H; Clinical Microbiology Lab, Meir Medical Center, Kfar Saba, Israel, and Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Israel, Paitan Y; Cliniques Universitaires Saint-Luc - UCL, Louvain, Belgium, Verroken A; Département de Biologie des Agents Infectieux , UF de bactériologie, CHU Angers, Angers, France, Paillolies H, Lemaré C; Ente Ospedaliero Cantonale - Servizio di Microbiologia EOLAB, Bellinzona, Switzerland, Martinetti-Lucchini G, Frigerio Malossa S; Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma, Italy, Sanguinetti M, Spanu T; Hôpitaux Civils de Lyon, Lyon, France, Vandenesch F; Hôpital Cochin Paris, Paris, France, Poyant C, Loubaix J, Mira JP; Hôpital Robert-Debré AP-HP, Paris, France, Bonacorsi S, Coindreau A; Hospital General Universitario Gregorio Marañón, Madrid, Spain, Muñoz P, Kestler M; Hospital Sant Joan de Déu, Barcelona, Spain, Esteva C; Hospital Universitari de Girona, Doctor Josep Trueta, Girona, Spain, Queralt X; Hospital Universitario La Paz, Madrid, Spain, García-Rodriguez J; Hospital Universitario y Politécnico La Fe, Valencia, Spain, Gómez MD, López-Hontlangas J; Laboratory of Microbiology and Virology, Ospedale Amedeo di Savoia, Torino, Italy, Ghisetti V, Burdino E; Max von Pettenkofer Institute of Hygiene and Medical Microbiology, Faculty of Medicine, LMU Munich, Germany, Schubert S; Medical Microbiology, Department of Medicine, University of Perugia, Perugia, Italy and Microbiology, Perugia General Hospital, Perugia, Italy, Mencacci A, Allegrucci F; Noordwest Ziekenhuisgroep, Alkmaar, The Netherlands, Rozemeijer W, Paternotte N; Norlands Universitetssjukhus, Umeå, Sweden, Allard A; Operative Unit of Clinical Microbiology, Policlinico Sant’Orsola Malpighi, Bologna, Italy, Re MC, Ambretti S; O.U.H Odense Universitetshospital, Odense, Denmark, Skov M, Agergaard CN; Royal Bolton Hospital, Bolton, England, Subudhi P; Universitäts Klinikum Frankfurt, Frankfurt, Germany, Wichelhaus TA; University Hospital Basel, Basel, Switzerland, Egli A, Hinic V; University Hospitals of North Midlands, Stoke-on-Trent, England, Alcock A, Banavathi K; UOC Microbiologia e Virologia, P.O. Cotugno, Azienda Ospedaliera Specialistica dei Colli, Napoli, Italy, Tiberio C, Ruocco G, Attipaldi L.

Code availability Not applicable.

Author contribution CCG, CG-M, and BM contributed to the study conception and design. Material preparation and data collection were performed by CCG, CG-M, BM, and the EME Evaluation Program Collaborative. All members of the EME Evaluation Program Collaborative contributed equally to the study. Data analysis was performed by CCG, CG-M, and CR. The manuscript was written by CCG and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. All members of the EME Evaluation Program Collaborative reviewed and approved the manuscript.

Funding BioFire Pneumonia plus Panels were provided free of charge by bioMérieux, France. No other compensation was provided to the test sites.

Data availability The datasets generated during and/or analyzed during the current study are not publicly available due to restrictions by individual contributors but are available as composite data from the corresponding author on reasonable request.

Declarations

Competing interests Christine C. Ginocchio, Carolina Garcia-Mondragón, and Barbara Maurerhofer are employees of bioMérieux. Cory Rindlisbacher is an employee of BioFire Diagnostics. EME Evaluation Program Collaborative sites did not receive any other resources to perform this study other than the test panels. The study design, authors, data analysis, interpretation of data, and writing of report were performed by bioMérieux employees as per author contribution listed above. Data was reviewed and approved by the EME Evaluation Program Collaborative sites.

Ethics approval Ethical approval was in accordance with individual institution requirements.

Consent to participate Not applicable.

Consent for publication All authors and contributors have read and approved the manuscript.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Campbell S, Forbes BA (2011) The clinical microbiology laboratory in the diagnosis of lower respiratory tract infections. J Clin Microbiol 49(9 Suppl):S30–S33. https://doi.org/10.1128/JCM.00789-11
2. Bartlett JG (2011) Diagnostic tests for agents of community-acquired pneumonia. Clin Infect Dis 52(Suppl 4):S296–S304
3. Feikin DR, Hammitt LL, Mwerkdo DR, O’Brien KL, Scott AG (2017) The enduring challenges of determining pneumonia etiology in children. Consideration for future research priorities. Clin Infect Dis 64(S3):5188–5196
4. Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, Bramley AM, Reed C, Grijalva CG, Anderson EJ, Courtney DM, Chappell JD, Qi C, Hart EM, Carroll F, Trabue C, Donnelly HK, Williams DJ, Zhu Y, Arnold SR, Ampofo K, Waterer GW, Levine M, Lindstrom S, Winchell JM, Katz JM, Erdman D, Schneider E, Hicks LA, McCullers JA, Pavia AT, Edwards KM, L, Finelli L for the CDC
30. Hughes AEO, Webber DM, Wallace MA, Johnson C, Bumham C-A D, Neil W, Anderson NW (2020) Comparable detection of viral pathogens in lower respiratory tract specimens with the BioFire Respiratory Panel 2 and BioFire Pneumonia Panel. J Clin Microbiol 58(6):e00254–e00220. https://doi.org/10.1128/JCM.00254-20

31. Schuetz P, Wirz Y, Sager R et al (2017) Procalcitonin to initiate or discontinue antibiotics in acute respiratory tract infections. Cochrane Database Syst Rev 10:CD007498

32. Buchan BW, Windham S, Balada-Llasat J-M, et al. (2020) 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. Posted Online 29 April 2020 J. Clin. Microbiol. doi:https://doi.org/10.1128/JCM.00135-20.

33. Centers for Disease Control and Prevention https://www.cdc.gov/antibiotic-use/core-elements/hospital.html.2019

34. Monard C, Pehlivan J, Auger G, Alviset S, Dinh AT, Duquaire P, Gastli N, d’Humières C, Maamar A, Boibieux A, Baldeyrou M, Loubìnouin J, Dauwalder O, Cattoir V, Armand-Lefèvre L, Kerméis S, and the ADAPT study group (2020) Multicenter evaluation of a syndromic rapid multiplex PCR test for early adaptation of antimicrobial therapy in adult patients with pneumonia. Crit Care 24:434. https://doi.org/10.1016/s1355-0242(20)30114-7

35. Verroken A, Scohy A, Gérard L, Wittebole X, Collienne C, Laterre P-F (2020) Co-infections in COVID-19 critically ill and antibiotic management: a prospective cohort analysis. Crit Care 24:410. https://doi.org/10.1016/s1355-0242(20)30135-7

36. Falsey AR, Becker KL, Swinburne AJ, Nylen ES, Formica MA, Hennessey PA, Criddle MM, Peterson DR, Baran A (2013) Walsh EE (2013) Bacterial complications of respiratory tract viral illness: a comprehensive evaluation. J Infect Dis 208:432–441

37. Jain S, Williams DJ, Arnold SR, for the Centers for Disease Control and Prevention (CDC) Etiology of Pneumonia in the Community (EPIC) Study Team et al (2015) Community-Acquired Pneumonia Requiring Hospitalization Among U.S. Children. N Engl J Med 372(9):835–845. https://doi.org/10.1056/NEJMoal405870

38. Vos LM, Bruning AHL, Reitsma JB, Schuurman R, Riezebos-Brilman A, Hoepelman AIM, Oosterheert JJ (2019) Rapid molecular tests for influenza, respiratory syncytial virus, and other respiratory viruses: a systematic review of diagnostic accuracy and clinical impact studies. Clin Infect Dis 69:1243–1253

39. Molinos L, Zalacain R, Menéndez R, Reyes S, Capelastegui A, Cillóñiz C, Rajas O, Borderías L, Martin-Villaseñor JJ, Bello S, Añafagéne I (2015) Sensitivity, specificity, and positivity predictors of the pneumococcal urinary antigen test in community-acquired pneumonia. Ann Am Thorac Soc 12(10):1482–1489

40. Svørrer CW, Lück C, Elverdal PL, Uldum S (2012) Immunochromatic kits Xpect Legionella and BinaxNOW Legionella for detection of Legionella pneumophila urinary antigen have low sensitivities for the diagnosis of Legionnaires’ disease. J Med Microbiol 61:213–221

41. Pierre DM, Baron J, Yu VL, Stout JE (2017) Diagnostic testing for Legionnaires’ disease. Ann Clin Microbiol Antimicrob 16:59. https://doi.org/10.1186/s12941-017-0229-6

42. Jain S, Pavia AT (2016) The modern quest for the “holy grail” of pneumonia etiology. Clin Infect Dis 62:826–828

43. Alby K, Mitchell SL (2018) Lower respiratory multiplex panels for the detection of bacterial and viral infections. Clin Microbiol News 40(16):131–136. https://doi.org/10.1016/j.clinmicnews.2018.07.005

44. Dhesi Z, Enne VI, Brealey D, Livermore DM, High J, Russell C, Colles A, Kandil H, Mack D, Martin D, Page V, Parker R, Roultson K, Singh S, Wey E, Swart AM, Stirling S, Barber JA, O’Grady J, Gant V. Organisms causing secondary pneumonias in COVID-19 patients at 5 UK ICUs as detected with the FilmArray test. medRxiv preprint doi: https://doi.org/10.1101/2020.06.22.20131573

45. Kreitmann L, Monard C, Dauwalder O, Simon M (2020) Argaud L (2020) Early bacterial co-infection in ARDS related to COVID-19. Intensive Care Med 13:1–3. https://doi.org/10.1007/s00134-020-06165-5

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.