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Multicentric evaluation of BioFire FilmArray Pneumonia Panel for rapid bacteriological documentation of pneumonia

Running title: FA-PP for pneumonia diagnosis

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Abstract

Objectives: To evaluate performances of the rapid multiplex PCR assay BioFire FilmArray Pneumonia Panel (FA-PP) for detection of bacterial pathogens and antibiotic resistance genes in sputum, endotracheal aspirate (ETA) and bronchoalveolar lavage (BAL) specimens.

Methods: This prospective observational study was conducted in 11 French university hospitals (July to December 2018) and assessed performance of FA-PP by comparison with routine conventional methods.

Results: A total of 515 respiratory specimens were studied, including 58 sputa, 217 ETA and 240 BAL. The FA-PP detected at least one pathogen in 384 specimens, yielding an overall positivity rate of 74.6% (384/515). Of them, 353 (68.5%) specimens were positive for typical bacteria while 8 atypical bacteria and 42 resistance genes were found. While identifying most bacterial pathogens isolated by culture (374/396, 94.4%), the FA-PP detected 294 additional species in 37.7% (194/515) of specimens. The FA-PP demonstrated positive percentage agreement and negative percentage agreement values of 94.4% (95% CI, 91.7-96.5%) and 96.0% (95% CI, 95.5-96.4%), respectively, when compared to culture. Of FA-PP false-negative results, 67.6% (46/68) corresponded to bacterial species not included in the panel. At the same semi-quantification level (in DNA copies /mL for FA-PP *versus* in CFU/mL for culture), the concordance rate was 43.4% (142/327) for culture-positive specimens with FA-PP reporting higher semi-quantification of $\geq 1 \log_{10}$ in 48.6% (159/327) of cases. Interestingly, 90.1% of detected bacteria with $\geq 10^6$ DNA copies/mL grew significantly in culture.

Conclusions: FA-PP is a simple and rapid molecular test that could complement RCM for improvement of diagnosis accuracy of pneumonia.

Introduction

Rapid detection of bacterial pathogen and determination of antimicrobial susceptibility profile is crucial since timely administration of appropriate antibiotics is a key element of care in patients with pneumonia [1-3]. However, the gold standard for bacteriological documentation of bacterial pneumonia still relies mainly on conventional, slow and insensitive culture-based methods [4]. Indeed, these techniques require at least 24-72 h to produce results and fail to detect many clinically-relevant pathogens, especially due to prior empirical antibiotic therapy, failures during preanalytical process (transport, storage) or suboptimal growth conditions [5].

Whereas there is a multitude of rapid syndromic molecular tests developed for the diagnosis of pneumonia caused by viruses and atypical bacteria [6], only two FDA-approved, CE-marked are currently commercialized for the detection of common typical bacteria and resistance genes: the FilmArray Pneumonia panel [FA-PP] (BioFire diagnostics LLC, Salt Lake City, USA) and the Unyvero Hospitalised Pneumonia panel (Curetis GmbH, Holzgerlingen, Germany) [4]. The FA-PP is a fully-automated microbiological diagnostic assay based on nested multiplex PCR analysis that allows the detection of 34 markers (see below) from sputum, endotracheal aspirate (ETA) and bronchoalveolar lavage (BAL) specimens [7]. To date, very little data is available since only three small-sized monocentric studies have been published on FA-PP performances in routine care [8-10].

The aim of this large prospective observational multicentric study was to evaluate analytical performances of the FA-PP for the detection of bacteria and resistance genes by comparison with findings obtained by routine conventional methods (RCM).

Materials and Methods

Study design and clinical specimens

This prospective observational study was performed from July to December 2018 in 11 university hospitals distributed in 8 of the 13 regions of metropolitan France (Table 1). All respiratory specimens were sampled from consecutive patients (no exclusion criteria) with suspected pneumonia defined according to IDSA guidelines [2]. Very few clinical data were collected (age and ward). Note that the type of pneumonia was collected for patients hospitalized in four centers (Paris, Cochin; Paris, Bichat; Rennes; Hospices Civils de Lyon). Different types of respiratory samples (sputum, ETA and BAL) were included but the protected-specimen brush was excluded since it is not validated for this assay. Each respiratory sample (either fresh or frozen) was tested with the “investigational-use-only” (IUO) version of the FA-PP (identical to the final FDA-cleared and CE-marked version) and RCM.

Microbiological techniques

Bacterial culture of respiratory specimens was performed by semi-quantitative method using serial dilutions according to the French Standard Guidelines in Medical Microbiology (www.sfm-microbiologie.org). Bacterial identification was performed using the Vitek2 system (bioMérieux, Marcy l’Etoile, France) and/or the MALDI-TOF mass spectrometry (Microflex LT, Bruker Daltonics, Bremen, Germany; or Vitek MS, bioMérieux, Marcy l’Etoile, France) depending on the hospital laboratory. *In vitro* antimicrobial susceptibility testing was carried out either by disk diffusion or using the Vitek2 automated system (bioMérieux) depending on each laboratory procedures in the hospitals and/or the species identified, and results were interpreted following EUCAST recommendations (www.eucast.org).

For atypical bacteria, specific PCR assays were used for detection of *L. pneumophila*, *M. pneumoniae* and *C. pneumoniae*. A viral test was performed for a very limited number of samples, and then no evaluation of FA-PP performance was possible for viruses.

FA-PP testing

Note that the test was performed on remnant clinical specimens and not in real time while results were never communicated to the physician in charge of the patient. The FA-PP is a closed, pouch-based, syndrome-specific multiplex PCR test intended for use with the FilmArray 1.5, 2.0 and Torch automated instruments. From approximately 200 µL of specimen, it includes all steps (nucleic acid extraction, nested multiplex PCR and melting curve analysis) and provides results in around 1 hour and 15 min. The panel allows the detection of 15 typical bacteria (*A. calcoaceticus-baumannii* complex, *E. cloacae* complex, *E. coli*, *H. influenzae*, *Klebsiella aerogenes*, *Klebsiella oxytoca*, *K. pneumoniae* group, *Moraxella catarrhalis*, *Proteus* spp., *P. aeruginosa*, *Serratia marcescens*, *S. aureus*, *Streptococcus agalactiae*, *S. pneumoniae* and *Streptococcus pyogenes*), 3 atypical bacteria (*C. pneumoniae*, *L. pneumophila* and *M. pneumoniae*), 7 resistance genes (methicillin resistance [*mecA/C* and MREJ], carbapenemases [*bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48-like}, *bla*_{VIM} and *bla*_{IMP}] and extended-spectrum β-lactamases (ESBLs) [*bla*_{CTX-M}]) and 8 viruses (adenovirus, coronavirus [except SARS-CoV-2], human metapneumovirus, human rhinovirus/enterovirus, influenza virus A & B, parainfluenza virus, respiratory syncytial virus [RSV]). The 15 typical bacteria are automatically reported to the nearest whole log as DNA copies/mL (10⁴, 10⁵, 10⁶ or ≥10⁷). The results of the antimicrobial resistance genes are reported qualitatively and conditionally if the potential microorganism of the gene is also detected in the sample. Note that the detected resistance markers cannot be linked to the detected microorganisms.

Data analysis

Results from RCM and FA-PP were compared for detection of typical and atypical bacteria as well for antibiotic resistance. Culture was considered negative for samples that grew only bacterial commensals of upper respiratory tract microbiota.

For each micro-organism identification, a result was considered as true positive (TP) or as true negative (TN) if results of FA-PP and conventional techniques were concordant. In the first analysis, conventional techniques were defined as the gold standard, meaning that a micro-organism identified only by the FA-PP and not by conventional techniques was considered as a false positive (FP) and conversely a target found by conventional methods and not by the FA-PP was considered as a false negative (FN). Secondly, agreement between the two methods was assessed by calculating the positive percentage agreement (PPA), negative percentage agreement (NPA) and the Cohen's kappa coefficient (κ). PPA was calculated as $[TP / (TP + FN)]$ and NPA as $[TN / (TN + FP)]$. The positive predictive value (PPV) and the negative predictive value (NPV) were calculated as $100*[TP / (TP + FP)]$ and $100*[TN / (TN + FN)]$, respectively. The κ coefficient was defined as $[(Po - Pe) / (1 - Pe)]$ where Po is the proportion of observed concordant ratings and Pe is the expected proportion of concordant ratings due to chance alone [11]. Assuming that observations are mutually independent, Po is estimated by $[(TN + TP)/N]$ and Pe by $[(TN+FN) (TN+FP) + (FN + TP) (FP + TP)]/N^2$. Agreement between methods was considered very strong for κ between 0.81 and 1, strong for κ between 0.61 and 0.80 or insufficient for $\kappa < 0.61$.

For the semi-quantification evaluation, we compared results for typical bacteria numerated by the FA-PP (in DNA copies/mL) and culture (in CFU/mL) and calculated the difference of \log_{10} quantification between both techniques. A significant culture was defined as at least one pathogen load $\geq 10^4$ CFU/mL for BAL, $\geq 10^5$ CFU/mL for ETA, and $\geq 10^7$ CFU/mL for sputum

samples. Results for which we could not report the difference between FA-PP and culture semi-quantification were excluded from the analysis.

Ethics statement

This study is in compliance with the Helsinki Declaration (Ethical Principles for Medical Research Involving Human Subjects) and was conducted in accordance with the guidelines of research boards at each study site. All patient data were anonymously reported, with no possibility of connecting specimens and isolates to individual patients. Because specimens used in this study were part of the routine patient management without any additional sampling, this study did not need to be examined by an ethical committee and patients' informed consent was not required. For every patient hospitalized in each of the participating hospitals, a document mentioning that health data could be reused for research, studies and evaluation was signed by the patient.

Results

Study population and samples

A total of 515 respiratory specimens from 515 patients were included in this study. The large majority (n= 482, 93.6%) of patients were adults, mainly hospitalized in ICUs (n = 452, 87.8%). The respiratory specimens comprised 240 BAL (47%), 217 ETA (42%) and 58 sputa (11%) (Table 1).

Overall FA-PP findings

The FA-PP detected at least one pathogen in 384/515 of tested specimens, yielding an overall positivity rate of 74.6%. Of them, 353 (68.5%) specimens were positive for typical bacterial targets. The most frequently detected typical bacteria ($\geq 15\%$) were *S. aureus* (n=146, 28.3%), *H. influenzae* (n=106, 20.6%) and *E. coli* (n=78, 15.1%) (Figure 1). The FA-PP identified one, two or ≥ 3 pathogens (up to 6) in 173 (33.6%), 95 (18.4%) and 85 (16.5%) of the 515 respiratory samples, respectively.

A total of 8 atypical bacteria (8/515, 1.6%) were also found, including *M. pneumoniae* (n = 4) and *L. pneumophila* (n = 4), all of them being confirmed by specific PCR assays (Table 1 and Figure 1). The FA-PP also detected a total of 84 viruses in 79/515 (15.3%) samples (Table 1), with a concomitant bacteria found in 57/79 (72.2%) of these cases. Finally, a total of 42 resistance genes were detected in 42 specimens, including *bla*_{CTX-M} (n = 23), *mecA/C* and MREJ (n = 17) and *bla*_{VIM} (n = 2).

Performances of FA-PP for bacteria detection

Conventional culture-based methods detected one, two or ≥ 3 (up to 5) pathogens in 181 (35.1%), 81 (15.7%) and 32 (6.2%) of the 515 respiratory samples, respectively. The FA-PP identified most bacterial pathogens isolated by culture (374/396, 94.4%) and detected 294

additional species in 194/515 (37.7%) specimens, mainly *H. influenzae* (n = 63), *S. aureus* (n = 48) and *E. coli* (n = 36) (Figure 1 and Table S1).

The FA-PP demonstrated PPA and NPA values of 94.4% (95% CI, 91.7-96.5%) and 96.0% (95% CI, 95.5-96.4%), respectively, when compared to culture (Table S1). Since many bacterial strains were not found by culture, PPV values were quite low, especially for *S. pyogenes*, *Proteus* spp. and *K. oxytoca* (Table S1). By contrast, NPV values were very high ($\geq 99.2\%$; 95% CIs between 97.8 and 100%) for all the typical bacteria targets (Table S1). The overall kappa coefficient of agreement between FA-PP and standard techniques was of 0.68 (95% CI, 0.65-0.71) (Table S1). The strongest agreement was reported with *P. aeruginosa*, *K. pneumoniae* group, *K. aerogenes* and *S. aureus*. Note that performances of FA-PP (evaluated on 159 patients from 4 centers) were not significantly different depending on the type of pneumonia (Table S2).

Several false-negative results were obtained with the FA-PP. A total of 46 pathogens, species not included in the panel, were isolated by culture: *Citrobacter* spp. (n=10), *Stenotrophomonas maltophilia* (n=9), *Morganella morganii* (n=9), *Hafnia alvei* (n=9), *Achromobacter* spp. (n=4), *Raoultella ornithinolytica* (n=2), *Acinetobacter* spp. (n=2) and *Ochrobactrum* spp. (n=1) (Figure 1). Moreover, the FA-PP did not detect 22 pathogens corresponding to bacterial species normally covered by the panel, mostly *Enterobacteriaceae* (n=15; 68%) including *Klebsiella* spp. (n=9; 41%) (Table 2).

Out of the 42 resistance genes detected by the FA-PP, 24 markers were confirmed by routine AST methods: ESBL-producing *Enterobacteriaceae* (n = 17) and methicillin-resistant *S. aureus* (n = 7). In addition, FA-PP detected *bla*_{CTX-M} (n=6) and *bla*_{VIM} (n=2) targets in 8 samples with no Gram-negative rods in culture. Conversely, one strain of ESBL-producing *Citrobacter freundii* and another of *M. morganii*, both not included in the FA-PP, were only reported by culture. The highest rate (10/17, 58.8%) of discrepancies was related to methicillin resistance where

detection of *mecA/C* and MREJ was either discordant with routine AST (n = 3) or reported in *S. aureus*-culture-free samples (n = 7).

Comparison of semi-quantitative bacterial results

The comparison between FA-PP (in DNA copies/mL) and culture (in CFU/mL) was possible on 327 typical bacterial semi-quantification results sorted from sputa (n=26, 8%), ETA (n=170, 52%) and BAL (n=131, 40%) (Figure 2). Bacterial targets reported at the same semi-quantification level with both FA-PP and culture accounted for 65.4% (17/26) in sputum, 50.6% (86/170) in ETA and 29.8% (39/131) in BAL samples, leading to a concordance rate of 43.4% (142/327) for culture-positive specimens. FA-PP reported higher semi-quantification of at least 1 log₁₀ (up to a difference of 4 log₁₀) in 48.6% (159/327) of cases: 5/26 sputum, 72/170 ETA and 82/131 BAL (Figure 2), regardless of the bacterial species (data not shown). Interestingly, 90.1% (231/254) of detected bacteria with $\geq 10^6$ DNA copies/mL grew significantly in culture.

Discussion

We report here high performances of the FA-PP for pathogen detection, similar to those reported by initial multicentric evaluation studies for other Biofire panels for the detection of main pathogens involved in meningitis/meningoencephalitis (PPA, 85.7-100%; NPA, 99.5-100%) [12], bacteraemia (PPA, 92.2-100%; NPA, 98.3-100%) [13], upper respiratory infections (PPA, 66.7-100%; NPA, 93.5-100%) [14] and gastroenteritis (PPA, 94.5-100%; NPA, 97.1-100%) [15].

The overall performance of FA-PP in our study (PPA, 94.4%; NPA, 96.0%) was comparable to that reported in the three small-sized monocentric studies previously published. In the first study, 117 BAL specimens from a clinical trial [16] were retrospectively used to evaluate the FA-PP and the authors found an overall PPA of 93.1% and NPA of 98.2% after discrepancy resolution [8]. In the second publication, Lee *et al.* studied 59 ETA and BAL specimens and reported an overall PPA of 90.0% and NPA of 97.7% with a concordance rate of 53.6% for semi-quantitative results [9]. In the third study, Yoo *et al.* included 99 respiratory samples (sputa and ETA) and reported an overall sensitivity of 98.5% and specificity of 76.5% [10]. Like in these studies, we report a much higher proportion of samples positive for pathogens with the FA-PP than by culture (especially for *H. influenzae*). Indeed, the positivity rate in our study was 74.6%, similar to the previously published findings of Yoo *et al.* (72.7%) but higher than those of Lee *et al.* (55.9%) in smaller cohorts [9,10]. The number of false-negative results with the FA-PP was relatively low in our study (n = 22), which might be explained by point mutations affecting PCR. It is mainly related to Gram-negative bacteria (especially *Klebsiella* spp.), knowing that some false-negative results have been reported elsewhere for *K. aerogenes*, *K. oxytoca*, *K. pneumoniae* group and *P. aeruginosa* [9,10]. In these two previous studies, there were substantial discrepancies in the detection of resistance genes as demonstrated here, especially for *bla*_{CTX-M} and *mecA/C*-MREJ [9,10].

Regarding the semi-quantification, the concordance rate of FA-PP results with those obtained by culture was not so high for the culture-positive specimens (43.4%) and was similar to that previously reported by Lee *et al.* (53.6%) [9]. The overestimation of bacterial load by the FA-PP is likely attributed to the detection of dead or non-cultivable viable bacteria. Also, we have to keep in mind that FA-PP provides results in DNA copies per mL (and culture in CFU/mL) and is less impacted by prior exposure to antibiotics. This improvement of pathogen detection by PCR in antimicrobial-exposed patients was recently demonstrated in patients with community-acquired pneumonia [17]. Finally, even though the distinction between an actual pathogen and a colonizer in a respiratory specimen remains a challenge, FA-PP semi-quantitative results may help for the interpretation as do culture results. Indeed, the performances of quantitative cultures for accurate diagnosis of nosocomial pneumonia (particularly ventilator-associated pneumonia) can vary significantly depending on the sampling or a previous antimicrobial therapy for instance [18-21] and then results can also be difficult to interpret. In addition, approaches for diagnosis of nosocomial pneumonia may be different between countries according to applied guidelines [22].

Some limitations could also be addressed. First, clinical data were only available for 4 centers, a situation that is close to most situations for clinical microbiologists in routine conditions. Second, the interpretation of false-positive FA-PP results was difficult since previous antibiotic exposure, significantly reducing yield of cultural methods, was not assessed during this study. Also, fastidious organisms are more difficult to grow under usual growth conditions and this may explain why *H. influenzae* and *S. pneumoniae* were most often detected by the molecular approach. Third, the study was not conducted over a 12-month period, and then the seasonal pattern of respiratory viruses was not taken into account. Lastly, FA-PP performance could not

be evaluated for virus detection since standard molecular methods were not performed for all positive specimens.

In addition, the FA-PP presents few limitations, of which the most notable is that the panel of targets does not include some clinically-relevant *Enterobacteriaceae* (especially *Citrobacter* spp., *H. alvei* and *M. morgani*) and some opportunistic non-fermentative Gram-negative bacteria species (such as *S. maltophilia* and *Achromobacter* spp.) or fungi (e.g., *Aspergillus* spp., *Pneumocystis jirovecii*) potentially responsible for nosocomial pneumonia. Indeed, these pathogens were only found by culture in our study. Like other molecular methods, the detection of DNA does not imply the presence of a viable pathogen and the lack of detection of resistance gene is not synonymous of susceptibility to the corresponding antibiotic(s). Finally, even if the FA-PP could be used on sputa, the quality of these specimens must be warranted before processing.

As with all syndromic molecular tests, interpretation of FA-PP reports with bacterial, antibiotic resistance and viral targets might also be challenging. The quantitative bacterial detection specific to the FA-PP is important to consider the relative contribution of each bacterium for a more accurate diagnosis but its interpretation must always be considered in the clinical context and the local epidemiology.

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Author's contribution

GN, JL, SK and VC conceived the study and wrote the first draft. All authors commented on, edited drafts and approved the final version of the manuscript.

Transparency declaration

bioMérieux provided the test cartridges used in this study. No other economical support or other benefits were received by the authors from the company.

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Appendix

The French FA-PP study group includes the following investigators: Sophie Alviset (Paris Cochin), Laurence Armand-Lefèvre (Paris Bichat), Marion Baldeyrou (Rennes), Agathe Becker (Hospices Civils de Lyon), André Boibieux (Hospices Civils de Lyon), Stéphane Bonacorsi (Paris, Robert-Debré), Christophe Burucoa (Poitiers), Emmanuelle Cambau (Paris Lariboisière), Jean-Sébastien Casalegno (Hospices Civils de Lyon), Aurélie Cointe (Paris, Robert-Debré), Julie Cremniter (Poitiers), Grégory Destras (Hospices Civils de Lyon), Paul Duquaire (Hospices Civils de Lyon), Guillaume Geslain (Paris, Robert-Debré), Claude-Alexandre Gustave (Hospices Civils de Lyon), Hervé Jacquier (Paris Lariboisière), Achille Kouatchet (Angers), Emmanuel Lecorche (Paris Lariboisière), Manon Lejeune (Paris Bichat), Bruno Lina (Hospices Civils de Lyon), Rafaël Mahieu (Angers), Adel Maamar (Rennes), Anthony Michaud (Poitiers), Céline Monnard (Hospices Civils de Lyon), Philippe Montravers (Paris Bichat), Catherine Neuwirth (Dijon),

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References

- [1] Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2007;44 Suppl 2:S27-72.
- [2] Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin Infect Dis* 2016;63:e61-e111.
- [3] Torres A, Niederman MS, Chastre J, Ewig S, Fernandez-Vandellos P, Hanberger H, et al. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia: Guidelines for the management of hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) of the European Respiratory Society (ERS), European Society of Intensive Care Medicine (ESICM), European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Asociacion Latinoamericana del Torax (ALAT). *Eur Respir J* 2017;50.
- [4] Poole S, Clark TW. Rapid syndromic molecular testing in pneumonia: The current landscape and future potential. *J Infect* 2020;80:1-7.
- [5] Torres A, Lee N, Cilloniz C, Vila J, Van der Eerden M. Laboratory diagnosis of pneumonia in the molecular age. *Eur Respir J* 2016;48:1764-78.
- [6] Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, Patel R. Syndromic panel-based testing in clinical microbiology. *Clin Microbiol Rev* 2018;31.
- [7] Poritz MA, Blaschke AJ, Byington CL, Meyers L, Nilsson K, Jones DE, et al. FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. *PLoS One* 2011;6:e26047.
- [8] Yugueros-Marcos J, Barraud O, Iannello A, Ploy MC, Ginocchio C, Rogatcheva M, et al. New molecular semi-quantification tool provides reliable microbiological evidence for pulmonary infection. *Intensive Care Med* 2018;44:2302-4.
- [9] Lee SH, Ruan SY, Pan SC, Lee TF, Chien JY, Hsueh PR. Performance of a multiplex PCR pneumonia panel for the identification of respiratory pathogens and the main determinants of resistance from the lower respiratory tract specimens of adult patients in intensive care units. *J Microbiol Immunol Infect* 2019;52:920-8.
- [10] Yoo IY, Huh K, Shim HJ, Yun SA, Chung YN, Kang OK, et al. Evaluation of the BioFire(R) FilmArray(R) Pneumonia Panel for rapid detection of respiratory bacterial pathogens and antibiotic resistance genes in sputum and endotracheal aspirate specimens. *Int J Infect Dis* 2020.
- [11] Cohen J. A coefficient of agreement for nominal scales. *Educ Psychol Meas* 1960;20:27-46.
- [12] Leber AL, Everhart K, Balada-Llasat JM, Cullison J, Daly J, Holt S, et al. Multicenter evaluation of BioFire FilmArray Meningitis/Encephalitis Panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. *J Clin Microbiol* 2016;54:2251-61.
- [13] Salimnia H, Fairfax MR, Lephart PR, Schreckenberger P, DesJarlais SM, Johnson JK, et al. Evaluation of the FilmArray Blood Culture Identification Panel: Results of a multicenter controlled trial. *J Clin Microbiol* 2016;54:687-98.
- [14] Leber AL, Everhart K, Daly JA, Hopper A, Harrington A, Schreckenberger P, et al. Multicenter evaluation of BioFire FilmArray Respiratory Panel 2 for detection of viruses and bacteria in nasopharyngeal swab samples. *J Clin Microbiol* 2018;56.
- [15] Buss SN, Leber A, Chapin K, Fey PD, Bankowski MJ, Jones MK, et al. Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. *J Clin Microbiol* 2015;53:915-25.
- [16] Clavel M, Barraud O, Moucadel V, Meynier F, Karam E, Ploy MC, et al. Molecular quantification of bacteria from respiratory samples in patients with suspected ventilator-associated pneumonia. *Clin Microbiol Infect* 2016;22:812 e1- e7.
- [17] Gadsby NJ, Russell CD, McHugh MP, Mark H, Conway Morris A, Laurenson IF, et al. Comprehensive molecular testing for respiratory pathogens in community-acquired pneumonia. *Clin Infect Dis* 2016;62:817-23.
- [18] Chastre J, Fagon JY, Soler P, Bornet M, Domart Y, Trouillet JL, et al. Diagnosis of nosocomial bacterial pneumonia in intubated patients undergoing ventilation: comparison of the usefulness of bronchoalveolar lavage and the protected specimen brush. *Am J Med* 1988;85:499-506.
- [19] Torres A, Puig de la Bellacasa J, Xaubet A, Gonzalez J, Rodríguez-Roisin R, Jiménez de Anta MT, et al. Diagnostic value of quantitative cultures of bronchoalveolar lavage and telescoping plugged catheters in mechanically ventilated patients with bacterial pneumonia. *Am Rev Respir Dis* 1989;140:306-10.

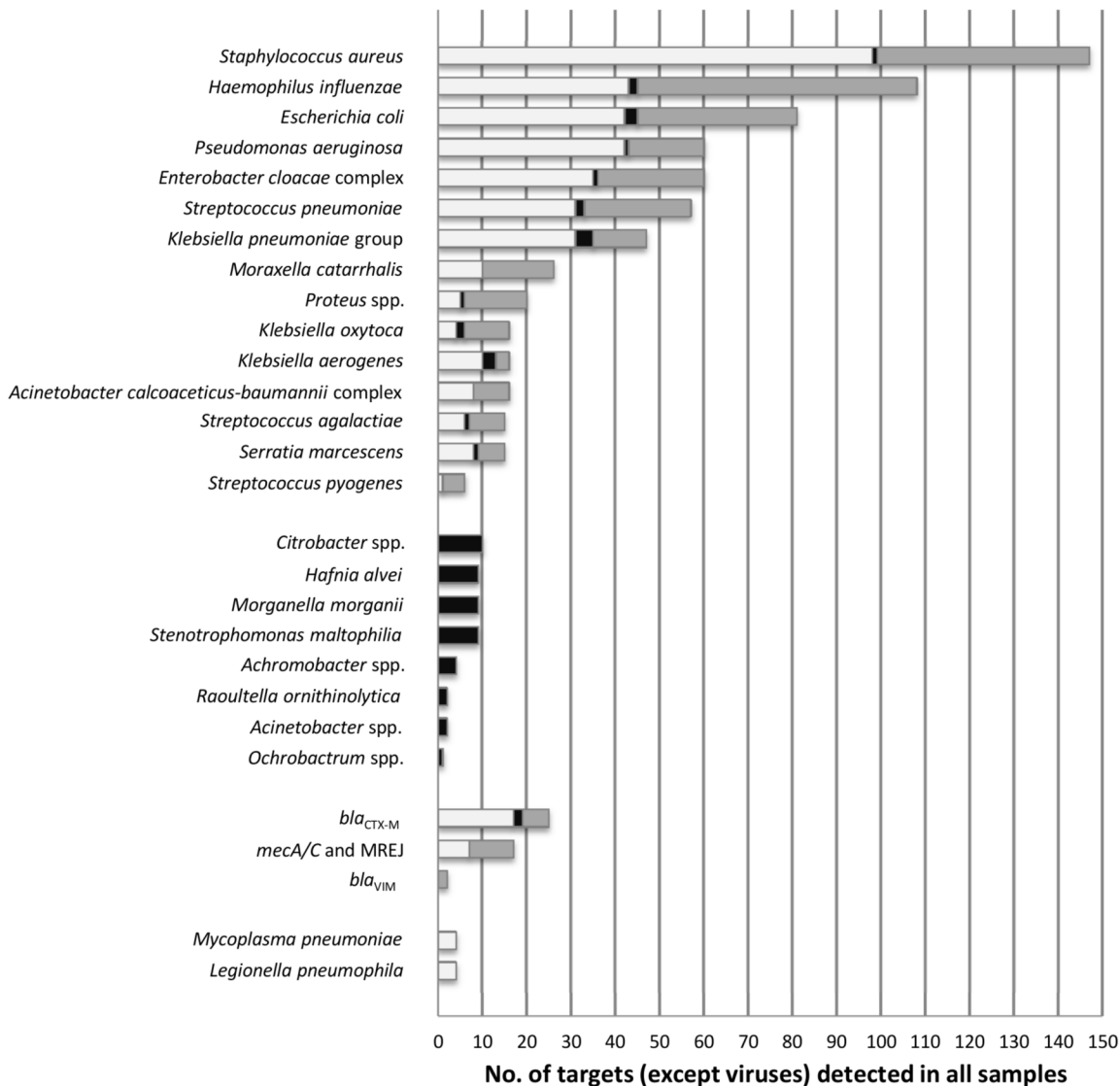
- [20] Jourdain B, Novara A, Joly-Guillou ML, Dombret MC, Calvat S, Trouillet JL, et al. Role of quantitative cultures of endotracheal aspirates in the diagnosis of nosocomial pneumonia. *Am J Respir Crit Care Med* 1995;152:241-6.
- [21] Souweine B, Veber B, Bedos JP, Gachot B, Dombret MC, Regnier B, Wolff M. Diagnostic accuracy of protected specimen brush and bronchoalveolar lavage in nosocomial pneumonia: impact of previous antimicrobial treatments. *Crit Care Med* 1998;26:236-44.
- [22] Martin-Loeches I, Rodriguez AH, Torres A. New guidelines for hospital-acquired pneumonia/ventilator-associated pneumonia: USA vs. Europe. *Curr Opin Crit Care* 2018;24:347-52.

Legends of the figures

Figure 1. Performance of the FilmArray Pneumonia Panel compared to standard of care techniques. FA-PP, FilmArray Pneumonia Panel; RCM, routine conventional methods.

Figure 2. Comparison between FA-PP and culture for semi-quantification of 327 typical bacterial targets sorted from sputa (n=26, 8%), ETA (n=170, 52%) and BAL (n=131, 40%). The X-axis represents semi-quantification results obtained by culture. The Y-axis presents the difference (in \log_{10}) between FA-PP and culture quantification. The size of the circles is proportional to the number of targets in different categories (that is indicated inside each circle). Culture results are expressed in CFU/mL and FA-PP results in DNA copies/mL. A significant culture was defined as at least one pathogen load $\geq 10^4$ CFU/mL for BAL, $\geq 10^5$ CFU/mL for ETA, and $\geq 10^7$ CFU/mL for sputum samples.

□ RCM+/FA-PP+ ■ RCM+/FA-PP- ▒ RCM-/FA-PP+



No. of targets (except viruses) detected in all samples

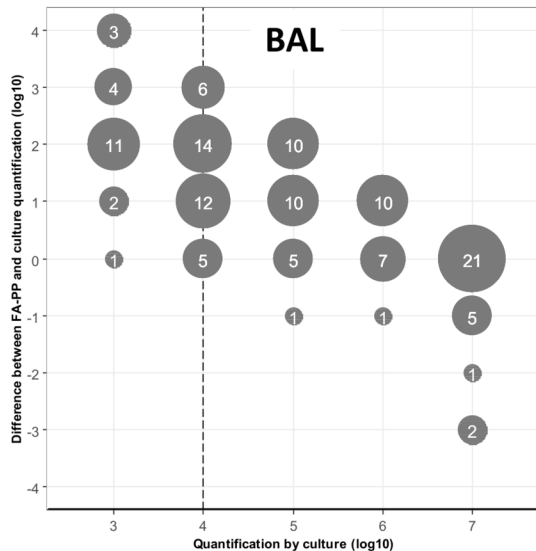
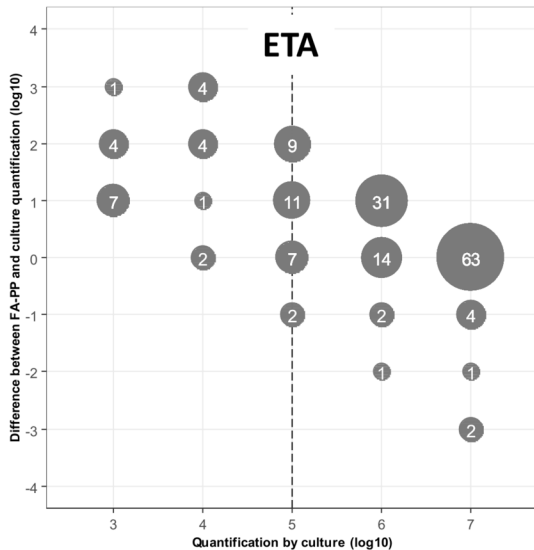
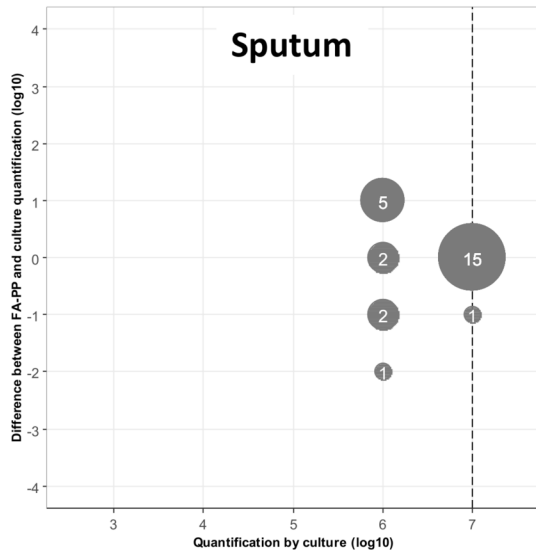


Table 1. Overview of testing of the 515 respiratory specimens.

Hospital	Respiratory samples ^a (n= 515)				Typical bacterial targets (no. of positive samples)		Atypical bacterial targets (no. of positive samples)		Viral targets (no. of positive samples)
	Sputum	ETA	BAL	TOTAL	RCM ^b (n=515)	FA-PP ^c (n=515)	RCM ^b (n=515)	FA-PP ^c (n=515)	FA-PP ^c (n=515)
Paris, Cochin	27	36	0	63	41	44	2	2	11
Dijon	0	50	10	60	29	38	1	1	15
Nimes	0	0	59	59	24	27	0	0	2
Clermont-Ferrand	5	13	40	58	22	37	2	2	7
Angers	1	15	41	57	36	40	1	1	9
Paris, Lariboisière	9	10	38	57	28	41	0	0	7
Paris, Bichat	5	4	30	39	29	28	1	1	8
Poitiers	5	32	1	38	37	37	0	0	3
Rennes	1	15	15	31	23	23	0	0	4
Hospices Civils de Lyon	4	20	5	29	9	20	1	1	8
Paris, Robert-Debré	1	22	1	24	16	18	0	0	5
TOTAL (%)	58 (11.3)	217 (42.1)	240 (46.6)	515	294 (57.1)	353 (68.5)	8 (1.6)	8 (1.6)	79 (15.3)

^aBAL, bronchoalveolar lavage; ETA, endotracheal aspirate.

^bRCM, routine conventional methods.

^cFA-PP, FilmArray Pneumonia panel.

Table 2. FA-PP false-negative results (indicated in bold).

No.	Respiratory sample ^a	Culture semi-quantitative results (CFU/mL) ^b	FA-PP semi-quantitative results (DNA copies/mL) ^b
1	ETA	<i>S. pneumoniae</i> >10 ⁶ <i>E. cloacae</i> <10⁵ <i>E. coli</i> <10⁵	<i>S. pneumoniae</i> ≥10 ⁷ - -
2	ETA	<i>S. aureus</i> <10 ⁵ <i>E. cloacae</i> <10 ⁵ <i>K. pneumoniae</i> <10 ⁵ <i>E. coli</i> <10⁵ -	<i>S. aureus</i> 10 ⁶ <i>E. cloacae</i> complex 10 ⁵ <i>K. pneumoniae</i> group 10 ⁴ - <i>H. influenzae</i> 10 ⁴
3	Sputum	<i>S. aureus</i> 10 ⁷ <i>H. influenzae</i> 10⁶	<i>S. aureus</i> ≥10 ⁷ -
4	BAL	<i>S. aureus</i> 10 ⁵ <i>H. influenzae</i> 10⁵ -	<i>S. aureus</i> 10 ⁶ - <i>Proteus</i> spp. 10 ⁴
5	ETA	<i>K. aerogenes</i> 10⁴	-
6	BAL	<i>H. influenzae</i> >10 ⁶ <i>K. aerogenes</i> 10²	<i>H. influenzae</i> ≥10 ⁷ -
7	ETA	<i>S. aureus</i> 10 ⁷ <i>K. aerogenes</i> 10⁶ <i>E. coli</i> 10⁴	<i>S. aureus</i> ≥10 ⁷ - -
8	BAL	<i>H. influenzae</i> 10 ⁵ <i>K. oxytoca</i> 10³	<i>H. influenzae</i> ≥10 ⁷ -
9	BAL	<i>S. aureus</i> >10 ⁶ <i>A. baumannii</i> 10 ⁵ <i>K. oxytoca</i> 10⁵ -	<i>S. aureus</i> 10 ⁵ <i>A. calcoaceticus-baumannii</i> complex 10 ⁵ - <i>H. influenzae</i> 10 ⁴ <i>S. agalactiae</i> 10 ⁴
10	Sputum	<i>K. pneumoniae</i> 10⁷	-
11	ETA	<i>K. pneumoniae</i> 10⁵	-
12	BAL	<i>P. aeruginosa</i> >10 ⁴ <i>K. pneumoniae</i> 10⁴	<i>P. aeruginosa</i> 10 ⁶ -
13	BAL	<i>K. pneumoniae</i> 10³	-
14	ETA	<i>P. penneri</i> 10⁷ -	<i>S. pneumoniae</i> 10 ⁶
15	ETA	<i>S. pneumoniae</i> 10 ⁵ <i>P. aeruginosa</i> 10³	<i>S. pneumoniae</i> 10 ⁵ -
16	ETA	<i>S. marcescens</i> 10⁶	-
17	BAL	<i>S. pneumoniae</i> 10⁷ <i>S. pneumoniae</i> 10⁵	- -
18	ETA	- -	<i>S. aureus</i> 10 ⁴ <i>S. agalactiae</i> 10 ⁶
19	ETA	<i>S. aureus</i> 10⁶	-
20	BAL	<i>H. influenzae</i> 10 ⁴ <i>S. agalactiae</i> 10⁴ -	<i>H. influenzae</i> 10 ⁵ - <i>S. aureus</i> 10 ⁴

^aBAL, bronchoalveolar lavage; ETA, endotracheal aspirate.

^b-, negative.