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

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Summary

Background

Culture-based microbiological investigation of hospital-acquired or ventilator-associated pneumonia (HAP or VAP) is insensitive, with aetiological agents often unidentified. This can lead excess antimicrobial treatment of patients with susceptible pathogens, whilst those with resistant bacteria are treated inadequately for prolonged periods. Using PCR to seek pathogens and their resistance genes directly from clinical samples may improve therapy and stewardship.

Methods

Surplus routine lower respiratory tract samples were collected from ICU patients about to receive new or changed antibiotics for hospital-onset lower respiratory tract infections at 15 UK hospitals. Testing was performed using the BioFire FilmArray Pneumonia Panel (bioMérieux) and Unyvero Pneumonia Panel (Curetis). Concordance analysis compared machine- and routine microbiology results, while Bayesian latent class (BLC) analysis estimated the sensitivity and specificity of each test, incorporating information from both PCR panels and routine microbiology.

Findings

In 652 eligible samples; PCR identified pathogens in considerably more samples compared with routine microbiology: 60.4% and 74.2% for Unyvero and FilmArray respectively *vs.* 44.2% by routine microbiology. PCR tests also detected more pathogens per sample than routine microbiology. For common HAP/VAP pathogens, FilmArray had sensitivity of 91.7-100.0% and specificity of 87.5-99.5%; Unyvero had sensitivity of 50.0-100.0%%, and specificity of 89.4-99.0%. BLC analysis indicated that, compared with PCR, routine microbiology had low sensitivity, ranging from 27.0% to 69.4%.

Interpretation

Conventional and BLC analysis demonstrated that both platforms performed similarly and were considerably more sensitive than routine microbiology, detecting potential pathogens in patient samples reported as culture negative. The increased sensitivity of detection realised by PCR offers potential for improved antimicrobial prescribing.

Key Messages

What is the Key Question?

How do the two currently-available automated PCR-based syndromic test systems perform in the microbiological diagnosis of hospital-acquired and ventilator-associated pneumonia (HAP/VAP) in critical care?

What is the Bottom Line?

Bayesian latent class analysis demonstrated that syndromic PCR-based diagnostic tests offer considerably improved sensitivity for the microbiological diagnosis of HAP and VAP compared with standard-of-care routine microbiological culture.

Why Read On?

The improved speed and sensitivity of PCR-based diagnosis of pneumonia has potential to optimise therapy of critically ill patients and to improve antibiotic stewardship.

Introduction

Pneumonia is differentiated into its community-acquired (CAP), hospital-acquired (HAP) and ventilator-associated (VAP) forms. [1] Even pre-COVID-19, it was the most-frequently-reported infection in intensive care unit (ICU) patients,[2-4] with crude mortality estimated at 30-70% for nosocomial cases (i.e. HAP and VAP).[2] Swift effective antimicrobial therapy after clinical onset is crucial to outcome, with increased mortality among patients receiving delayed antibiotics or those that prove inactive.[5,6]

The bacteria, viruses and (rarely) fungi that cause nosocomial pneumonia cannot be distinguished from clinical symptomology. Rather, microbiological diagnosis is needed, delivering results in 48-72h and meaning that the patient must be treated empirically in the interim. EU, US and UK guidelines advocate broad-spectrum empirical antibiotics owing to the diversity of bacteria that can be responsible and the need to cover the resistances these may carry. [2,4 7,8] Aetiological investigation is by microbiological culture, hereafter termed routine microbiology , which depends upon cultivable bacteria being recoverable and fails to identify a pathogen in up to 50% of cases .[9-11] These patients nonetheless remain sick and mostly continue to receive empirical antibiotics.

The slowness and poor sensitivity of routine microbiology thus combine to promote poor stewardship and prolonged use of broad-spectrum agents, increasing the risk of side effects, including selection of resistant gut bacteria and *Clostridium difficile*.[12] A further hazard, particularly in high-resistance countries, is that the empirical agent proves ineffective against the pathogen, increasing the risk of a poor clinical outcome.

Rapid, accurate, diagnostics provide a route to improving this situation, promoting early refinement of individual patients' therapy. Commercial "sample-in, answer-out" PCR-based pneumonia tests are now available, specifically the Unyvero (Curetis) and BioFire FilmArray (bioMérieux) platforms which have both received FDA-clearance for diagnosis of pneumonia.[13] Both are substantially automated, seek prevalent pathogens and critical resistances and have turnaround times of hours instead of days.[13-16] We evaluated and compared their performance, in respect of pathogen and resistance detection using lower respiratory tract samples from patients clinically diagnosed with HAP or VAP at 15 UK ICUs. As well as providing a manufacturer-independent direct comparison, we sought to choose one test to take forward into a randomized controlled trial (RCT), evaluating outcomes compared with patient management based on routine microbiology. This is now underway (Trial ID: ISRCTN16483855).[17] Note that this study and RCT are distinct from a recently published trial for nebulised amikacin with the same name.[18]

Materials and Methods

Additional details and methods are described in supplementary data.

Patients and specimens

Between September 2016 and May 2018, surplus routine lower respiratory tract samples were collected from eligible patients with suspected HAP/VAP at the 15 participating ICUs. The sites represented a range of UK hospital types, included tertiary referral (n= 6), district general (n=7), children's (n=1) and private (n=1).

Specimens were included if they had sufficient volume (>400 μl) and were from patients hospitalized $\geq$48h about to receive a new antibiotic or change in antibiotic for suspected lower respiratory tract infection. Specimens were eligible only when collected within 12h (before or after) of antimicrobial therapy being initiated and then tested (or frozen at -80°C), within 72h of collection. All lower respiratory specimen types were accepted, whereas upper respiratory tract specimens were excluded. Second specimens from the same patient were included only when collected >14 days after the first sample.

Ethical approval

This work had study-specific approval from the UK Health Research Authority (Reference: 16/HRA/3882, IRAS ID: 201977) and the UCL DNA Infection Bank Committee, whose operation is governed by the London Fulham Research Ethics Committee (REC Reference: 17/LO/1530).

Routine microbiology

Each respiratory specimen was initially cultured locally at the laboratory serving the participating hospital. Testing was according to their standard operating procedures (SOPs), all based on the Public Health England (PHE) UK Standard.[19]

PCR Testing

Samples were transported to two central research laboratories (University of East Anglia and University College London) by courier. Upon receipt, each was promptly tested using both the Unyvero Pneumonia Panel (Curetis, Holzgerlingen, Germany) and the BioFire FilmArray Pneumonia Panel (BioFire Diagnostics, Salt Lake City, USA) according to manufacturer's instructions. The tests are described in Table 1.

Data Analysis

Analyses were carried out using Stata (v 15) and R (v 3.5 or above), and followed a pre-defined, detailed statistical plan. Results from the conventional and PCR tests were described using standard summary statistics. Agreement between results was examined by categorising each sample in terms of concordance of organisms detected by PCR and routine microbiology, then calculating overall concordance with 95% CIs. Definitions of the categories are detailed in Table 2.

Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) initially were estimated (with exact 95% CIs) for each PCR target, taking routine microbiology and routine virology as the gold standard. Owing to concerns that routine microbiology provides a poor gold standard (see Results), estimates (with 95% credible intervals) were also calculated using Bayesian latent class (BLC) models[20] incorporating results from both PCR tests, and routine microbiology. Models used non-informative priors for all parameters (except to constrain specificities above 0.15 to obtain more stable posterior distributions), and were fitted with and without assumed correlation between tests. The best-fitting models were identified based on Deviance Information Criteria.

Scoring the Overall Performance of PCR-based Diagnostic Tests

At the outset of the study, through expert consensus, a scoring system was developed to assess the suitability of each 'sample-in, answer-out' test for progression to the INHALE RCT. Tests were assessed against one essential criterion - *that the incidence of major discordances, meaning failures to detect pathogens found by routine microbiology, must be <5%*, and ten points-based 'Desirable Criteria', scoring a total of 150 (Table S1). Criteria i-iii were based on study results, criteria iv-viii on manufacturer's published information and criteria ix and x on a user questionnaire. The scale was weighted towards accurate detection of pathogens, with implementation-based criteria given a lower weighting.

Role of the Funding Source

The funder had no role in the study design, nor in the collection, analysis, and interpretation of data or in the writing of the report. The funder appointed an independent research Programme Steering Committee to provide quality assurance and oversight. Membership of the committee is listed on the study website (www.ucl.ac.uk/inhale-project/people).The corresponding author had full access to all study data and had final responsibility for the decision to submit for publication.

Results

Specimens Collected

A total of 752 samples, 652 of them eligible, were collected from the 15 participating ICUs (Figure 1). The range of eligible samples per site was 7-141, with 9 sites each providing >20 eligible samples. Most were from adults, with 72 from children; 260 were from patients with suspected HAP and 392 from patients with suspected VAP. Endotracheal aspirates (n=299) were the most numerous sample type; followed by sputa (272 samples) BALs (44 samples) and non-directed BALs (23 samples), with 14 samples in the "other" or "unknown" category. A small majority of samples (n=357) were collected before antibiotic administration.

Routine Microbiology Results

Routine microbiology was performed on all samples at the local laboratories. The median time to a result was 70.2h (interquartile range (IQR) 51.1h-92.1h), including a median of 6.1h (IQR 2.5h-15.4h) transit time from the ICU to laboratory booking-in and 55.5h (IQR 44.8h-76.5h) from sample booking to release of results. The positivity rate was 44.2%, with 35.1% recording one significant organism with 9.1% reporting two or more. The remaining 55.8% of samples were reported variously as 'normal flora', 'non-significant growth', or 'no growth'.

      Staphylococcus aureus was the most-frequently-found bacterium (Figure 2), representing 23.6% (83/352) of all organisms reported, followed by *Pseudomonas aeruginosa* (20.7%*)*; Enterobacterales collectively accounted for 38.1% of isolates, with *Klebsiella* spp. and *Escherichia coli* prominent (Figure 3a). Occasionally routine microbiology laboratories reported *Candida* spp*., Enterococcus* spp*.* and coagulase-negative staphylococci: these were excluded because there is no evidence base for their involvement in pneumonia. Table S2 lists the bacteria detected by all three methods in HAP compared with VAP patients.

      Results of standard-of care diagnostic virology were recorded if was performed within 24h of collection of the eligible bacteriology specimen. Only 113 patients, 33 of them children, had virology results meeting this criterion, and, of these, 31 (27.4%) were positive: seven had influenza A, six adenovirus and six cytomegalovirus. The study was undertaken before SARS-CoV2 began to circulate.

PCR Results

Among the 652 eligible samples, 631 had Unyvero tests and 632 had FilmArray tests within 72h of the sample's collection, or with a frozen sample (Figure 1). Among these eligible tests, 620 generated a result on the FilmArray, whilst 12 failed. Defining failure on the Unyvero is more

complex since targets are divided into eight chambers. We considered one sample where >2 chambers failed as a "total failure" along with 24 samples that failed to generate any result, leaving 606 valid results. In 32 of these 606 one or two chambers nonetheless failed. Their data were retained in the analysis, with the proviso that organisms sought by the failed chambers would have been missed. We did not note any user errors for either test; neither machine requires regular service or maintenance.

The overall positivity rate for both machines exceeded routine microbiology, at 60.4% for the Unyvero and 74.2% for the FilmArray (chi-square test: p < 0.0001). Most specimens had multiple organisms detected (Figure 2), with this proportion higher for FilmArray than Unyvero. FilmArray found only bacteria in 54.2% of samples and only viruses in 6.9% whereas 13.1% contained both. The principal species detected by PCR, and their relative prevalence were broadly similar to routine microbiology, although *E. coli* and *Klebsiella* spp. were detected relatively more frequent by PCR, whereas *S. aureus* and *P. aeruginosa* were found less frequently (Figure 3b). Among viruses detected by the FilmArray, rhinovirus was the most prominent (n=55), followed by influenza A (n=29) and B (n=25) (see Table S3); Unyvero does not seek viruses.

Performance of PCR Tests

Test performance was compared in several ways to accommodate the fact that routine microbiology is an imperfect 'gold standard' and the fact that the PCR tests seek multiple targets, more than one of which may be present in any sample, confounding simple calculation of overall sensitivity and specificity.

Overall test performance was first measured as concordance with routine microbiology, taken as a gold standard (Table 2). Both PCR tests deliver semi-quantitative outputs: the FilmArray reports bacterial targets as 10^4, 10^5, 10^6 or $\geq 10^7$ copies per ml, whereas the Unyvero reports as +, ++ or +++. In addition to detection at any concentrations, we therefore also undertook further concordance calculations, considering only targets detected at high concentration, defined as 10^6 or $\geq 10^7$ copies/ml for FilmArray and ++ or +++ for Unyvero (Table 2). Around half of the PCR results by each method demonstrated full positive or negative concordance with routine microbiology. Most of the remainder were either partially concordant or had minor discordance. Major discordance was rare, totalling only 4.6% for Unyvero and 1.8% for FilmArray. Details of results that were discordant between routine microbiology and PCR are shown in Tables S4 and S5. If PCR detections at low concentrations were excluded, full concordance increased for both tests, but major discordance increased unacceptably. A

comparison of negative results determined that there was no significant difference in the number of positive PCR detections between samples reported in routine microbiology as "no growth" and "no significant growth" compared with those reported as "normal flora" and "mixed growth" (data not shown). The number of organisms detected per sample did not vary significantly according to sample type (Table S6).

PCR assay sensitivity was >95% for most target bacteria, with NPVs > 98% (Table 3). Specificity and PPVs were lower, due to the PCR tests detecting more organisms per sample and finding more positive samples than routine microbiology. Strikingly, however, both machines often found the *same* organism as each other when routine microbiology failed to record any organism, casting doubt on routine microbiology as a gold standard. Accordingly, Table 4 shows performance estimates obtained from BLC models that make no presumption of one method being the reference. Based on this analysis, routine microbiology was the least sensitive technique, with sensitivity values for individual pathogens ranging from 27.1 % to 68.7%. In contrast, sensitivity values for the PCR tests remained high; FilmArray sensitivity ranged from 89.4% to 99.3 % versus 83.9% to 96.9% (expect *K. aerogenes*, 48.4%) for Unyvero. Specificity and PPV values for both PCR tests increased considerably compared with the values calculated using routine microbiology as a gold standard: in particular, specificity exceeded 99% for Unyvero targets and ranged from 93.9% to 99.9% for FilmArray targets. The PPV range was 62.1% to 99.3% for Unyvero and 56.1% to 96.6% for FilmArray. This BLC analysis omits data from 16S rRNA testing, also performed, as this technique could not distinguish species within several key genera, including *Streptococcus* and *Klebsiella*, reducing granularity. BLC analysis including 16S data is included as Table S9; its numbers differ slightly from Table 4 but support the same conclusions.

We further conducted sub-analyses to investigate factors that might influence the results, such as the timing of the sample in relation to antibiotic administration, fresh vs. frozen samples, or time from sample collection to testing (24h,48h or 72h). None of these factors had a significant impact on the performance of the PCR tests (Tables S10, S1 and data not shown).

Chlamydophila pneumoniae, Legionella pneumophila and *Mycoplasma pneumoniae* were excluded from analysis because they are not ordinarily sought by routine microbiology. Unyvero and FilmArray each detected *M. pneumoniae* once, in the same specimen, from participating sites where it was not sought by local microbiology. Unyvero detected two samples with *L. pneumoniae*; FilmArray and routine microbiology found none. *C. pneumophila* was not found by any method. Virology performance is shown in Table S3 for FilmArray only since

Unyvero did not seek viruses; confidence intervals are wide due to the small number of routine results available for comparison.

Antimicrobial Resistance and comprehensive culture

All routine microbiology results for antimicrobial susceptibility testing were recorded, and Table S12 shows data for antimicrobials commonly used to treat HAP and VAP against prevalent species. The PCR tests differ from routine microbiology by seeking resistance (as genes) in a whole sample, not in particular bacteria. Assessment of the machines' performance in respect of resistance gene detection is further complicated because routine microbiology often reported no organism for PCR-positive samples. In other cases, we were unable to retrieve routine isolates for genetic investigation. These isolates were supplemented with those recovered by "comprehensive culture" on a sub-set of the discrepant samples (Supplementary methods). In total, comprehensive culture detected 12 additional key resistance genes, the host bacteria of which were not isolated or reported by routine microbiology (Table 5).

Specific resistance gene detections are catalogued in Table S13. We performed concordance analysis for 'high-consequence' resistance genes only, encoding extended-spectrum β-lactamases (ESBLs), carbapenemases or methicillin-resistant *Staphylococcus aureus* (MRSA) phenotypes. Among 17 Enterobacterales with ESBL phenotypes, 12 were from specimens where Unyvero found *bla*$_{CTX-M}$ and 17 from those where FilmArray found *bla*$_{CTX-M}$. Considered from the opposite perspective, culture found ESBL producers in 12/14 cases where Unyvero found *bla*$_{CTX-M}$ and 17/32 cases where FilmArray did so. Fifteen cultured *S. aureus* isolates had an MRSA phenotype, of these 13 were from specimens where Unyvero found *mecA/C* and all 15 from those where FilmArray found *mecA/C*-MREJ. Considered from the opposite perspective, culture found MRSA in 13/25 cases where Unyvero found *mecA/C* in presence of *S. aureus* and 15/32 cases where FilmArray did so. There were only 11 detections of carbapenemase producers by Unyvero (including *Acinetobacter* OXA enzymes) and three by FilmArray, precluding review by enzyme type: culture confirmed a carbapenemase producer in 7/11 samples where Unyvero found a carbapenemase gene and 2/3 where FilmArray did so. Unyvero found a carbapenemase gene in all eight samples that grew an organism with carbapenemase phenotype, whilst FilmArray only found two carbapenamses in these isolates. (Table 5).

Overall, comprehensive culture was performed on 103 samples, from which 123 potential pathogens were grown. Routine microbiology reported 65 potential pathogens from the same

Overall Comparison of PCR Tests

Both PCR systems met the essential requirement of having <5% major discordances. Accordingly, we collated performance and implementability data in order to choose which to carry forward to the INHALE RCT. Our scoring (Table S1 and Table 6) weighted performance, but also considered ease-of-use, footprint, turnaround time and overall user experience.

FilmArray scored 105 points versus 68 for Unyvero. Unyvero was more concordant with routine microbiology but FilmArray had better sensitivity; Unyvero had a broader target panel but more failed tests. FilmArray performed better on characteristics relating to implementation, ease-of-use, turnaround time and user experience. Accordingly, we have preferred the FilmArray Pneumonia Panel for the INHALE RCT, now being undertaken across 12 UK ICUs.

Discussion

We undertook a comprehensive, independent, head-to-head comparison of the two currently available rapid tests for the microbiological investigation of pneumonia. Samples were from ICU patients for whom clinicians prescribed antimicrobials to treat pneumonia.

Both systems were considerably faster than routine microbiology and detected more organisms. This underscores the known poor sensitivity of routine microbiology in pneumonia.[9-11] Crucially, PCR tests tended to detect the *same* additional organisms in a given sample, implying that these additional detections were '*real'* and that PCR may improve microbiological diagnosis of ICU pneumonia, increasing the proportion of patients who potentially could receive targeted antimicrobials. Furthermore, we perfromed comprehensive culture on A confounder is that, unlike the molecular tests, routine microbiology was decentralised, performed across 11 different hospital laboratories, receiving specimens from the 15 ICUs. The main difference between the two PCR tests is that Unyvero seeks *S. maltophilia* whereas FilmArray seeks respiratory viruses as well as bacteria. Early detection of *S. maltophila* might lead to early tailored therapy with co-trimoxazole, whereas fast viral detection may prompt the early cessation or de-escalation of antibiotic therapy.

To analyse test performance, we initially took routine microbiology as a gold standard. Only 56.6 % of Unyvero results and 50.3 % of FilmArray results were fully concordant with routine microbiology, with the remaining partial concordances and minor discordances mostly reflecting additional organisms detected by PCR, reflecting increased sensitivity of the latter.

Per pathogen sensitivity performance was consistently good (91.7 to 100%) for FilmArray; Unyvero's performance was more variable, with sensitivity <90% for several pathogens. Cases where pathogens represented on the PCR panels were missed by these tests but found by routine microbiology were rare at 4.6% for Unyvero and 1.8% for FilmArray. Sensitivity and specificity values are similar to those reported by others in evaluations of one or other of the two PCR tests.[15,16,21-24]

We initially hoped that 16S rRNA analysis could act as an alternative, molecular, reference, but it proved less sensitive than PCR and was abandoned (See Supplementary methods and data). Instead, the widely acknowledged limitations of routine microbiology culture[25] - and the frequency with which both PCR tests detected the *same* organism that was missed by routine microbiology - led us to adopt BLC analysis. This technique, which has been used by others evaluating diagnostics in cases where traditional methods are acknowledged to be sub-optimal,[20,26,27] infers a new, unmeasurable yet underlying (i.e., latent) gold standard result, with no prior assumption about any one test being 'correct'.[28] On this basis (i) the sensitivity of routine microbiology was extremely poor and (ii) the specificity and PPV of the PCR tests were considerably higher than those calculated using routine microbiology as the "gold" standard. This suggests that both PCR tests were clearly superior to routine microbiology, and that the latter should perhaps not be considered a gold standard technique. A caveat is that it is perhaps predictable that two similar PCR tests (albeit with different primers and detection methods) should agree better with each other than with a dissimilar culture-based method. Indeed, a potential limitation of PCR-based methods is that they may detect residual nucleic acids rather than viable organisms requiring treatment. However, this argument was partly countered by the observation that comprehensive culture methodology was able to grow many viable pathogens that were not reported by routine culture. It is crucial to remember that all patients in this study were severely-ill, clinically diagnosed with respiratory infection and received contingent antibiotic treatment; it therefore seems more reasonable to consider an organism found by any one method as potentially significant rather than to dismiss those methods that most often recorded a potential pathogen in favour of one that failed to do so simply because it is the 'traditional method'.

If the molecular results are accepted, it becomes possible to identify groups of patients, e.g. those found only to have *S. aureus* pneumonia or *Haemophilus influenzae*, in whom there is wide scope to de-escalate from typical empirical therapy for HAP/VAP with e.g. piperacillin/tazobactam or a carbapenem. This supports a potential to deliver improved antimicrobial stewardship along with better targeted, personalized, treatment of pneumonia. A

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countervailing risk is that the additional organisms found by PCR instead may prompt unnecessary prescribing. Both the present systems offer semi-quantitative detection which might, in theory, assist assessment of the need for therapy. In a sub-analysis, excluding organisms detected at low concentration by PCR, we did observe increased concordance with routine microbiology, but at the price of discounting organisms confirmed by routine microbiology. Ultimately the best approach may be to combine rapid microbiology with measurement of patient biomarkers as a guide to the need for therapy.

The types and relative frequencies of organisms identified were similar for routine microbiology and both PCR tests, without any obvious bias for either approach to miss particular organisms. The species distribution resembled that reported in numerous HAP/VAP studies from Europe and North America, with *S. aureus, P. aeruginosa* and Enterobacterales predominant[7,8]. Comparison of resistance gene detection with resistance phenotypes from routine microbiology is complicated by imperfect genotype / phenotype associations and the fact that phenotypic resistance may arise from unsought mechanisms (e.g., a combination of an ESBL and impermeability may confer carbapenem resistance in Enterobacterales).[29] Moreover, except for *mecA* on the FilmArray, PCR detection of a resistance gene in a clinical sample does not indicate which bacterial species is hosting that gene. We therefore conducted independent genotypic investigation of isolates identified as resistant by routine microbiology and for further organisms recovered by comprehensive culture. Overall, despite all these caveats, 66% of Unyvero gene detections and 51% of FilmArray detections were concordant against a combination of routine microbiology and comprehensive culture results. Crucially, PCR tests identified several key high-consequence resistance genes that had been missed by routine microbiology but which were confirmed by testing bacteria recovered by comprehensive culture. Although the PCR-methods did not provide a full susceptibility profile, they do deliver a swift and sensitive predictor of critical resistance, potentially useful for early identification of patients who should be isolated or have their therapy escalated.

The run times of the machines are measured in hours rather than the days required for routine microbiology. Total turn-around will also reflect the machine's placement in the clinical pathway; this could not be measured here because the tests were run retrospectively under research conditions. However, we established that the median transport time of samples from the ICU to the laboratory was 6h, with longer times when laboratories were remote from the hospital site. If the advantages of speed are to be realised, the machine must be placed in, or near to, the ICU.

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The decision of whether to adopt a rapid diagnostic into routine clinical practice will depend not only on its performance but also on the practicalities. Here, we evaluated diagnostic accuracy as well as potential for implementation, finding the FilmArray to be more sensitive than the Unyvero, also faster, smaller and easier to use. Accordingly, we have taken the FilmArray Pneumonia panel forward into INHALE's involving an RCT where patients either receive treatment guided by results of FilmArray test, performed in the ICU, or 'standard to care', comprising empirical antibiotics, adapted once microbiology results become available. This trial will determine if the potential of PCR in ICU HAP/VAP can be realised without compromising patient safety.[30]

Transparency declarations:
DML: Advisory Boards or ad-hoc consultancy Accelerate, Allecra, Antabio, Centauri, Entasis, GSK, Meiji, Menarini, Mutabilis, Nordic, ParaPharm, Pfizer, QPEX, Roche, Shionogi, Summit, T.A.Z., VenatoRx, Wockhardt, Zambon, Paid lectures – Astellas, bioMérieux, Beckman Coulter, Cardiome, Cepheid, Hikma, Merck/MSD, Menarini, Nordic, Pfizer and Shionogi. Relevant shareholdings or options – Dechra, GSK, Merck, Perkin Elmer, Pfizer, T.A.Z, amounting to <10% of portfolio value. He also has nominated holdings in Avacta, Byotrol, Destiny, Diaceutics, Evgen, Faron, Fusion Antibodies, Genedrive, Hardide, Renalytics, Scancell and Synairgen (all with research/products pertinent to medical and diagnostic innovation) through Enterprise Investment Schemes but has no authority to trade these shares directly.

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Table 1. Features and Target Panels of the Curetis Unyvero Pneumonia Panel and the BioFire FilmArray Pneumonia Panel multiplex PCR tests.

Characteristic	Curetis Unyvero HPN Hospitalised Pneumonia Panel	BioFire FilmArray Pneumonia Panel *plus*
Technology	Automated sample preparation, multiplex PCR and microarray detection of targets	Automated sample preparation and nested PCR
Regulatory status	CE-IVD[1]	CE-IVD & FDA Cleared[2]
Hands-on preparation time	2 min, using a standard pipette to transfer sample to the sample tube. Bacteria are then lysed for 30 min in the 'Lysator' before transfer to the cartridge.	2 min, using a proprietary flock swab to transfer the sample to a sample tube, which is loaded into the test pouch with the aid of a loading station.
Run-time	5h	1h 15 min
Bacteria sought	*Acinetobacter baumannii* complex *Citrobacter freundii* *Enterobacter cloacae* complex *Escherichia coli* *Haemophilus influenzae* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Klebsiella variicola* *Moraxella catarrhalis* *Morganella morganii* *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens*	*Acinetobacter calcoaceticus-baumannii* complex *Enterobacter cloacae* complex *Escherichia coli* *Haemophilus influenzae* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Moraxella catarrhalis* *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens* *Streptococcus agalactiae* *Streptococcus pneumoniae*

	Stenotrophomonas maltophilia *Streptococcus pneumoniae*	*Streptococcus pyogenes*
Atypical organisms and Fungi sought	*Chlamydophila pneumoniae* *Legionella pneumophila* *Mycoplasma pneumoniae* *Pneumocystis jirovecii*	*Chlamydophila pneumoniae* *Legionella pneumophila* *Mycoplasma pneumoniae*
Viruses sought	None	Adenovirus Coronaviruses OD43, NL63, HKU1 and 229E Human metapneumovirus Human rhinovirus/enterovirus Influenza A Influenza B Parainfluenza virus Respiratory syncytial virus MERS Coronavirus
Antimicrobial Resistance Genes sought	*ermB* *mecA* *mecC* bla_{TEM} bla_{SHV} bla_{IMP} bla_{KPC} bla_{NDM} bla_{OXA-23} $bla_{OXA-24/40}$ bla_{OXA-48} bla_{OXA-58} bla_{VIM} *sul1* *gyrA83* *gyrA87* *mecA/C* and MREJ	bla_{KPC} bla_{NDM} $bla_{OXA-48\ like}$ bla_{VIM} bla_{IMP} bla_{CTX-M}

[1]A similar panel, featuring a reduced number of antimicrobial resistance genes, has FDA clearance.

[2]We evaluated the Research Use Only (RUO) version

Table 2. Concordance-based performance of PCR tests compared with routine microbiology

Category	Definition	All Detections		Detections reported at higher concentrations[a]	
		Unyvero (%, 95% CI)	FilmArray (%, 95% CI)	Unyvero (%, 95% CI)	FilmArray (%, 95% CI)
Full positive concordance	Organisms detected were an exact match	19.3 (16.2 - 22.4)	18.2 (15.2 - 21.3)	22.4 (19.1 - 25.8)	21.1 (17.9 - 24.3)
Full negative concordance	No organisms detected by either method	37.3 (33.4 - 41.1)	32.1 (28.4 - 35.8)	42.1 (38.1 - 46.0)	44.5 (40.6 - 48.4)
Partial concordance	PCR detected the same organism as RM plus additional organism(s)	18.2 (15.1 - 21.2)	21.0 (17.8 - 24.2)	11.6 (9.0 - 14.1)	11.8 (9.2 - 14.3)
Minor discordance	RM was negative but machine found $\geq$1 organism	20.6 (17.4 - 23.8)	26.9 (23.4 - 30.4)	15.8 (12.9 - 18.7)	14.5 (11.7 - 17.3)
Major discordance	RM found $\geq$1 organism, at least one of which was on the PCR panel, but not detected	4.6 (2.9 - 6.3)	1.8 (0.7 - 2.8)	8.1 (5.9 - 10.3)	8.1 (5.9 - 10.2)

CI - confidence interval, RM - routine microbiology

[a] Calculated based on semi-quantitative detections Reported as ++ or +++ by Unyvero or 10^6 or $\geq 10^7$ copies/ml by FilmArray

Table 3. Pathogen-specific performance of PCR tests as compared with routine microbiology as the gold standard. 95% confidence intervals are omitted to aid readability but are included in supplementary Table S7, along with frequencies of detection.

Organism	Unyvero				FilmArray			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
A. baumannii complex	100.0	99.0	45.5	100.0	100.0	99.5	66.7	100.0
C. freundii	100.0	98.7	11.1	100.0	NA**	NA	NA	NA
E. cloacae	100.0	97.5	44.4	100.0	91.7	93.4	21.6	99.8
E. coli	87.8	89.4	37.5	99.0	97.6	87.5	36.3	99.8
H. influenzae	100.0	93.7	36.2	100.0	95.2	88.1	22.0	99.8
K. aerogenes	50.0	99.5	50.0	99.5	100.0	99.2	54.5	100.0
K. oxytoca	90.9	95.0	25.0	99.8	100.0	95.2	27.5	100.0
K. pneumoniae	83.3	94.2	37.0	99.3	92.0	91.4	31.1	99.6
M. catarrhalis	100.0	98.2	26.7	100.0	100.0	96.9	17.4	100.0
M. morganii	100.0	98.3	9.1	100.0	NA	NA	NA	NA
P. aeruginosa	95.3	93.9	64.9	99.4	98.5	93.1	63.1	99.8
S. aureus	87.2	93.2	65.4	98.0	96.2	88.9	56.2	98.2
S. agalactiae	NA	NA	NA	NA	ND	96.5	0.0	100.0
S. maltophilia	92.9	94.4	28.3	99.8	NA	NA	NA	NA
S. marcescens	77.8	98.3	41.2	99.7	100.0	98.2	45.0	100.0
S. pneumoniae	100.0	97.3	27.3	100.0	100.0	94.5	15.0	100.0
S. pyogenes	NA	NA	NA	NA	100.0	98.9	22.0	100.0

*ND – not determined because routine microbiology detected no positives; **NA – not applicable; organism not on test panel

Table 4. Pathogen-specific performance of routine microbiology and PCR tests estimated using BLC models. 95% credible intervals are omitted to aid readability but are shown in supplementary table S8. Only organisms on both PCR panels are included

Organism	Routine microbiology				Unyvero				FilmArray			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
A. baumannii complex	57.5	99.9	87.4	99.4	92.6	99.5	70.9	99.0	89.4	99.9	91.3	99.8
E. cloacae	42.9	99.9	94.6	97.2	94.9	99.9	97.2	99.8	94.2	96.4	56.1	99.7
E. coli	38.8	99.7	96.1	88.5	89.6	99.7	98.6	97.8	98.9	98.7	94.2	99.8
H. influenzae	36.3	99.9	96.8	93.5	96.9	99.7	97.1	99.7	95.3	93.8	62.4	99.5
K. aerogenes	68.7	99.9	88.9	99.6	48.4	99.6	62.1	99.3	89.8	99.4	67.8	99.9
K. oxytoca	30.2	99.9	94.3	95.5	92.7	99.2	88.7	99.5	95.2	99.7	95.9	99.7
K. pneumoniae	37.8	99.5	89.3	93.5	88.9	99.8	97.6	98.8	98.1	97.7	82.2	99.8
M. catarrhalis	27.6	99.9	86.7	98.0	89.0	99.9	95.5	99.7	95.7	98.9	71.4	99.9
P. aeruginosa	64.7	99.7	97.3	93.9	95.8	99.9	99.2	99.2	99.2	99.3	96.6	99.9
S. aureus	65.2	99.2	95.2	92.5	91.1	99.8	99.3	98.0	99.3	95.6	83.9	99.8
S. marcescens	48.4	99.9	92.9	98.4	83.9	99.9	95.7	99.5	96.1	99.8	94.2	99.9
S. pneumoniae	27.1	99.9	90.0	97.0	90.8	99.9	96.7	99.6	97.1	97.1	57.9	99.9

Table 5. Concordance of antimicrobial resistance gene detection by PCR and comparator methodology

Resistance Gene	Unyvero		FilmArray	
	Concordant detections[a]/total detections by PCR	Found in cultured isolates but missed in PCR testing	Concordant detections[a]/total detections by PCR	Found in cultured isolates but missed in PCR testing
bla_{CTX-M}	12/14	3	17/32	0
Carbapenemase	8/11	0	2/3	1
mecA/mecC (+*MREJ* in FilmArray)	13/25[b]	1	15/32	0

[a]Total concordance, based on results from both routine microbiology and comprehensive culture. Each sample is only counted once in the event of both tests being positive

[b]Only includes detections where *S. aureus* as well as *mecA/C* was also reported by the Unyvero. For total detections see table S13

Table 6. Scores allocated to PCR tests based on scoring system designed to evaluate overall performance, ease of use and implementability. See table S1 for full details of the scoring system.

Criterion	Machine Score			
	Curetis Unyvero Pneumonia Panel		**BioFire FilmArray Pneumonia Panel**	
	Value	**Score**	**Value**	**Score**
Overall concordance (max 45 points)	74.8%	20	71.3%	16
Sensitivity for detection of common pathogens (max 20 points)	3 targets with better performance	6	7 targets with better performance	14
Breadth of panel (max 15 points)	244 unique detections	15	191 unique detections	12
Time to result (max 15 points)	270 min	7	75 min	14
Cost per test (max 15 points)[a]	+++	10	++	15
Failure rate (max 15 points)	9.1%[b]	0	1.9%	11
Footprint (max 5 points)	7.4 sq. ft	1	3.2 sq. ft	5
Customer service (max 5 points)	-	3	-	4
Consumable logistics (max 5 points)[c]	-	0	-	5
Ease of use (max 10 points)	-	6	-	9
Total (Max 150)	**-**	**68**	**-**	**105**

[a] Costs in the range of £150-300/test depending on local purchase conditions. Includes estimates of cost of instrument purchase and operator time.

[b] includes both total and partial failures

[c] Comprised of one point each for space required for storage, storage temperature, delivery cost, delivery timescales and shelf-life

Figure Legends

Figure 1. Schematic representation of sample eligibility

Figure 2. Number of significant organisms detected per respiratory sample by routine microbiology or PCR.

Figure 3a. Numbers and types of bacteria detected by routine microbiology culture from respiratory samples included in the study.

Figure 3b. Numbers and type of bacteria detected by PCR from respiratory samples included in the study. Unyvero, solid bars, n = 606; FilmArray, hatched bars, n = 620. Species sought by one test only are marked with an asterisk.