

Performance evaluation of the Verigene® (Nanosphere) and FilmArray® (BioFire®) molecular assays for identification of causative organisms in bacterial bloodstream infections

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Abstract Molecular assays designed to provide bacterial identification and detection of resistance genes directly from positive blood cultures can significantly reduce the time to definitive results. This has the potential to improve patient management and antimicrobial stewardship. However, the extent of such an impact is yet to be fully assessed. We tested two such assays, the Verigene® System Bloodstream Infection Tests (Nanosphere, Inc., Northbrook, IL, USA) (both Gram-positive and Gram-negative cartridges) and the FilmArray® Blood Culture Identification Panel (BioFire® Diagnostics, Inc., Salt Lake City, UT, USA). We compared their accuracy and speed of organism and resistance gene identification to conventional culture-based methods for 173 positive blood cultures. We also retrospectively determined, for organisms deemed not to be contaminants, the potential impact on antimicrobial prescribing. Both the Verigene® and FilmArray® assays accurately identified organisms, on average, 27.95 and 29.17 h earlier than conventional methods, respectively. There were a significant number of false-positives for *Pseudomonas aeruginosa* with the FilmArray® assay, which may have been related to contamination of the bioMérieux BacT standard anaerobic blood culture bottles, which the manufacturer has acknowledged. Both panels provided results significantly faster than conventional methods. In our setting, the extent of the potential positive impact on antimicrobial prescribing

was modest (9 out of 173 samples). However, this may be an underestimation, since probable contaminants were not included in this analysis. In conclusion, both panels gave accurate results with significantly improved turnaround times.

Introduction

Despite advances in its management, sepsis remains a leading cause of morbidity and mortality. Between 32 and 47 thousand deaths per year are attributed to sepsis, accounting for up to 7.7 % of all deaths in England [1]. Twenty-seven percent of intensive care admissions in England and Wales are for severe sepsis and almost half of these patients die in hospital [2]. Outcomes can be improved by the early administration of effective antimicrobials [3, 4]. Increasing antibiotic resistance has led to both a broadening of the spectrum of activity of agents chosen to empirically treat septic patients and a critical need for improved antimicrobial stewardship [3, 5]. The tension between these important goals is exacerbated by the slow speed with which culture-based techniques provide diagnostic information (12–72 h) [6].

Conventional microbiological methods for identification of micro-organisms from blood cultures (such as agar-based culture techniques) are slow and sometimes insensitive. The introduction of automated techniques and, more recently, mass spectrometry have improved accuracy and turnaround times [7, 8]. Further reductions in the time to identification of organisms and provision of antimicrobial susceptibility data are now being explored using molecular techniques and automated technologies [9–23].

We assessed two automated molecular assays; the Verigene® Blood Culture Gram-Positive (BC-GP) and Gram-Negative (BC-GN) tests (Nanosphere, Inc., Northbrook, IL, USA) (cartridges) and the FilmArray® Blood Culture Identification Panel (BioFire® Diagnostics,

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Inc., Salt Lake City, UT, USA). Both assays are designed to provide bacterial identification and detection of resistance genes directly from pre-incubated positive blood cultures. Our aims were to compare the diagnostic accuracy of each assay against conventional culture-based laboratory methods and estimate the average time to definitive results. Although results of the molecular panels were not disclosed to physicians, we retrospectively reviewed antimicrobial prescribing data to determine whether the earlier provision of information could have impacted upon clinical management.

Materials and methods

The study was conducted in a large tertiary academic hospital in central London. Clinicians routinely investigate all patients with fever or suspected systemic infection by obtaining blood cultures (up to 10 mL each taken into separate bioMérieux BacT/ALERT® standard aerobic and anaerobic bottles; paediatric patients have only aerobic samples taken). Aliquots of all positive blood cultures between 18th June and 22nd August 2013 were removed from blood culture bottles as close to the time of identification of positive growth as possible (within 10 h) and stored frozen at -20°C until they could be tested (within 7 days). After testing by conventional methods, frozen aliquots were thawed and tested using each of the molecular platforms (see below for details). Neither manufacturer recommends freezing samples before testing, but this allowed more convenient retrospective testing. Only residual anonymised specimens collected for standard-of-care purposes were used in the analysis, samples were included as a performance evaluation and patient consent was not sought.

Conventional methods

During ‘working hours’ (between 0900 h and 1600 h Monday to Friday and 0900 and 1400 on Saturday and Sunday), positive blood culture bottles were processed immediately. Samples were inoculated directly onto appropriate agar plates determined by the Gram stain result. Outside of working hours, positive bottles were removed in batches at midnight and 0400 h, inoculated directly onto blood, chocolate and fastidious anaerobic agar plates (Oxoid, Basingstoke, UK), and a Gram stain prepared.

For identification, at 0900 h each day, all agar plates from the previous working day and from subculture at midnight and 0400 h were examined for growth. All morphologically distinct colonies were identified to the species level using matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) (Bruker Daltonics, Bremen, Germany) and, where necessary, biochemical identification (basic bench-side biochemical tests and/or VITEK 2, bioMérieux, Marcy l’Etoile,

France). Antimicrobial sensitivity testing was performed by automated microdilution using the appropriate testing card for VITEK 2.

Molecular methods

Separate aliquots of positive blood culture samples were tested using Verigene® Blood Culture Gram-positive and/or Gram-negative cartridges (according to the preliminary Gram stain results) and the FilmArray® Blood Culture Identification Panel. All assays were performed according to the manufacturers’ instructions.

The Verigene® Gram-positive assay detects nine species (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Streptococcus anginosus* group, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Enterococcus faecium*) and four genera (*Staphylococcus* spp., *Streptococcus* spp., *Micrococcus* spp., *Listeria* spp.), along with three antibiotic resistance genes (*mecA* for methicillin, *vanA* and *vanB* for vancomycin) in approximately 2.5 h. The Verigene® Gram-negative assay detects five species (*Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Serratia marcescens*) and four genera (*Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *Proteus* spp.), along with six antibiotic resistance genes (*CTX-M* for the detection of extended-spectrum beta-lactamases, *IMP*, *KPC*, *NDM*, *OXA* and *VIM* for the detection of carbapenemases) in approximately 2 h. Both assays run on the Verigene® Processor and Reader platforms, which extract and purify nucleic acids, followed by hybridisation to specific oligonucleotide-labelled gold nanospheres on a microarray.

The FilmArray® assay detects 24 pathogens (*Enterococcus* spp., *Listeria monocytogenes*, *Staphylococcus* spp., *S. aureus*, *Streptococcus* spp., *S. agalactiae*, *S. pneumoniae*, *S. pyogenes*, *Acinetobacter baumannii*, *Haemophilus influenzae*, *Neisseria meningitidis*, *P. aeruginosa*, Enterobacteriaceae, *Enterobacter cloacae* complex, *E. coli*, *K. oxytoca*, *K. pneumoniae*, *Proteus* spp., *S. marcescens*, *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*) and three antibiotic resistance genes (*mecA* for methicillin, *vanA/B* for vancomycin and *KPC* for carbapenem resistance) in approximately 1 h. The system performs extraction and purification, nested polymerase chain reaction (PCR) and detection using end-point melt curve data.

Analysis

Duplicate blood cultures were excluded from analysis. For each sample, the transportation time (from sample collection to loading onto the automated blood culture system), bottle

culture time (from loading onto the automated blood culture system to identified positive growth), Gram stain time (from identified positive growth to available Gram stain result), species-level identification time (from available Gram stain result to available species identification) and antimicrobial susceptibility testing time (from available Gram stain result to available antimicrobial susceptibility results) for conventional methods were calculated. For molecular techniques, the total turnaround time was calculated by adding the time taken to complete the molecular assay to the sum of the transportation, bottle culture and Gram stain times. Whilst the FilmArray[®] assay does not require a Gram stain, this time was included as it largely represented the standard workflow in our laboratory.

The conventional method for species identification and antimicrobial sensitivity testing was considered to be the gold standard against which both molecular methods were compared. Physicians were informed of all Gram stain results from positive blood cultures by a microbiology or infectious diseases physician. Advice given, including currently prescribed antimicrobials and any recommended changes, was documented in the electronic patient record. These data were used to determine the antimicrobials each patient was prescribed at the time the Gram stain result was known and to estimate the frequency with which the molecular techniques would have facilitated an earlier change to therapy. Samples with organisms that were considered to be contaminants were not included in the clinical utility analysis.

Results

Between 18th June and 22nd August 2013, there were 191 positive blood cultures. Eighteen of these samples were excluded from analysis, as they were duplicate samples. This left 173 samples from 159 patients with a median age of 53 years (interquartile range 21 to 70 years), 67 of whom were female. Ninety-seven (56 %) of the 173 samples were taken within 48 h of the patient's admission to hospital and 25 (15.7 %) patients died within 30 days of the blood culture being taken (all-cause mortality).

Turnaround times

The mean time taken for each step of the laboratory process is shown in Table 1. Since the molecular assays were performed retrospectively at convenience, we assumed that these assays were run immediately following the Gram stain result. In reality, the total turnaround time may be considerably longer than this, depending on when the samples are actually processed. The mean total turnaround time from sample collection to species-level identification for conventional culture-based

methods was 57.29 h. Both molecular panels were significantly faster, with mean turnaround times of 41.44 and 40.22 h for the Verigene[®] and FilmArray[®] assays, respectively. The conventional culture-based method took an additional mean of 12.1 h to obtain antimicrobial susceptibility results. The Verigene[®] assay produced six failed tests, which were repeated with success. No failed tests were reported by the FilmArray[®] assay.

Identification of micro-organisms

A total of 180 organisms were identified by conventional culture-based methods, including those where two or more pathogens were identified in the same sample. These are shown in Table 2. The most common pathogens identified were *S. epidermidis* (25.6 %), *E. coli* (16.8 %), coagulase-negative staphylococci (CNS) (15 %) and *S. aureus* (9.8 %). The molecular panels were not able to identify all organisms isolated by conventional techniques. There were 17 organisms belonging to genera not featured on the Verigene[®] panel and which produced a 'not detected' result. Eleven of these were considered to be contaminants (*Propionibacterium* spp., 5; *Corynebacterium* spp., 4; *Paenibacillus macerans*, 1; and *Brevibacterium casei*, 1) and six were significant isolates (*Haemophilus parainfluenzae*, 2; *Bacteroides fragilis*, 1; *Fusobacterium necrophorum*, 1; *Prevotella denticola*, 1; and *Aeromonas* spp., 1).

There were 23 organisms belonging to genera not featured on the FilmArray[®] panel and which produced a 'not detected' result. Seventeen of these were considered to be contaminants (as above, with the addition of six samples with *Micrococcus* spp.) and six significant isolates, identical to those for the Verigene[®] assay. In total, the Verigene[®] and FilmArray[®] assays detected 90.6 % and 87.2 %, respectively, of all organisms detected by conventional techniques.

Organism-specific sensitivity, specificity, and positive and negative predictive values for the molecular assays are shown in Tables 3 (Verigene[®]) and 4 (FilmArray[®]). These should be interpreted with caution, as there were small numbers for any individual pathogen, resulting in large confidence intervals.

The Verigene[®] assay produced occasional false-negative results (three for *Streptococcus* spp. and one each for *Staphylococcus* species, *Micrococcus* spp., *Enterococcus faecalis* and *Acinetobacter* spp.). There were also misidentifications for *Streptococcus pneumoniae* (two cases where *Streptococcus viridans* was identified by conventional methods), *Staphylococcus epidermidis* (one case where *Staphylococcus hominis* was identified by conventional methods), *Enterococcus faecium* (one case where *Enterococcus avium* was identified by conventional methods), *Streptococcus agalactiae* (one case where *S. aureus* was identified by conventional methods but was also detected by Verigene[®]) and *Citrobacter* spp.

Table 1 Mean time in hours taken for species-level identification and antimicrobial sensitivity testing

Task	Conventional method (h)	Verigene® (h) Mean for BC-GP and BC-GN	FilmArray® (h)
Sample transportation	3.7	3.7	3.7
Bottle culture	27.91	27.91	27.91
Gram stain	7.5	7.5	7.5
Species identification*	18.18	2.3	1.07
Antimicrobial sensitivity testing*	30.28	2.3	1.07
Total time from sample collection to species-level identification	57.29	41.44	40.22
Total time from sample collection to antimicrobial sensitivity testing	69.39	41.44	40.22

*For conventional culture techniques, both species identification and antimicrobial sensitivity testing were commenced in parallel, resulting in differing times to species-level identification and antimicrobial sensitivity results

(one case where *Enterococcus faecalis* was identified by conventional methods).

The FilmArray® assay produced only one false-negative result, for *Klebsiella oxytoca*. There were 25 presumed false-positive results for *Pseudomonas aeruginosa*; these were repeated but produced the same result. In all cases, conventional methods failed to yield any *Pseudomonas* species, but did isolate an alternative organism. Additionally, testing by the Verigene® assay did not produce any positive results for *Pseudomonas* for any of these samples. In 22 of the samples with presumed false-positive results, the correct organism was also identified by the FilmArray® assay. The remaining three samples produced positive results for *Pseudomonas* in addition to the correctly identified organism (positive for more than one target). The details of all samples with discrepant results are shown in Table 5.

There were seven samples where conventional techniques identified two pathogens. The Verigene® assay failed to detect one of the pathogens in two samples (a Group C/G *Streptococcus* and an *E. faecalis*) and the FilmArray® assay failed to detect a *Klebsiella* in one sample. Full details of results for the seven samples that had two pathogens detected are shown in Table 6.

Detection of resistance markers

The results for the detection of antimicrobial resistance genes are listed in Table 7. On most occasions where an *mecA* gene was identified, it was in a coagulase-negative *Staphylococcus*. All four isolates of *S. aureus* that were phenotypically resistant to meticillin were identified as having an *mecA* gene by both the Verigene® and FilmArray® assays. One *S. aureus* isolate was identified by both Verigene® and FilmArray® assays as having an *mecA* gene, but was phenotypically meticillin-sensitive; however, no further testing was undertaken to resolve this result. This particular patient was known to be colonised at several sites with meticillin-resistant *S. aureus* (MRSA).

For the remaining CNS samples, the Verigene® assay produced two false-negatives and three false-positives, and

Table 2 Organisms isolated by conventional methods and frequency of isolation

Species	Number (%) of samples containing species
<i>Staphylococcus epidermidis</i>	46 (25.6)
<i>Escherichia coli</i>	29 (16.8)
Other staphylococci	26 (15)
<i>Staphylococcus aureus</i>	17 (9.8)
<i>Streptococcus</i> other	8 (4.6)
<i>Micrococcus</i> spp.	6 (3.5)
<i>Enterococcus faecium</i>	5 (2.9)
<i>Propionibacterium</i> spp.	5 (2.9)
<i>Klebsiella pneumoniae</i>	4 (2.3)
<i>Enterobacter</i> spp.	4 (2.3)
<i>Corynebacterium</i> spp.	4 (2.3)
<i>Streptococcus agalactiae</i>	4 (2.3)
<i>Citrobacter</i> spp.	3 (1.7)
<i>Pseudomonas aeruginosa</i>	2 (1.2)
<i>Enterococcus faecalis</i>	2 (1.2)
<i>Klebsiella oxytoca</i>	2 (1.2)
<i>Acinetobacter</i> spp.	2 (1.2)
<i>Haemophilus parainfluenzae</i>	2 (1.2)
<i>Streptococcus pneumoniae</i>	1 (0.6)
<i>Enterococcus</i> other	1 (0.6)
<i>Aeromonas</i> spp.	1 (0.6)
<i>Bacteroides fragilis</i>	1 (0.6)
<i>Brevibacterium casei</i>	1 (0.6)
<i>Fusobacterium necrophorum</i>	1 (0.6)
<i>Morganella morganii</i>	1 (0.6)
<i>Paenibacillus macerans</i>	1 (0.6)
<i>Prevotella denticola</i>	1 (0.6)
Total	180

Table 3 Results for Nanosphere Verigene® by organism

	True-positive	True-negative	False-positive	False-negative	Sensitivity % (95 % CI)	Specificity % (95 % CI)	PPV % (95 % CI)	NPV % (95 % CI)	Kappa (95 % CI)
<i>Staphylococcus epidermidis</i> 44	128	1	0	0	100 (92–100)	99.2 (95.8–100)	97.8 (88.2–99.9)	100 (97.2–100)	0.98 (0.96–1.0)
<i>Staphylococcus aureus</i> 17	156	0	0	0	100 (80.5–100)	100 (97.7–100)	100 (80.5–100)	100 (97.7–100)	1.0
<i>Staphylococcus</i> spp. 23	149	0	1	1	95.8 (78.9–99.9)	100 (97.6–100)	100 (85.2–100)	99.3 (96.3–100)	0.98 (0.93–1.0)
<i>Micrococcus</i> 5	167	0	1	1	83.3 (35.9–99.6)	100 (97.8–100)	100 (47.8–100)	99.4 (96.7–100)	0.91 (0.72–1.0)
<i>Streptococcus pneumoniae</i> 1	170	2	0	0	100 (2.5–100)	98.8 (95.9–99.9)	33.3 (0.8–90.6)	100 (97.9–100)	0.50 (–0.1–1.0)
<i>Enterococcus faecium</i> 5	167	1	1	0	100 (47.8–100)	99.4 (96.7–99.9)	83.3 (35.9–99.6)	100 (97.8–100)	0.91 (0.72–1.0)
<i>Enterococcus faecalis</i> 1	171	0	1	1	50 (1.3–98.7)	100 (97.9–100)	100 (2.5–100)	99.4 (96.8–100)	0.66 (0.05–1.0)
<i>Streptococcus agalactiae</i> 3	169	1	1	0	100 (29.2–100)	99.4 (96.8–100)	75 (19.4–99.4)	100 (94.8–99.6)	0.85 (0.57–1.0)
<i>Streptococcus</i> spp. 8	162	0	3	3	72.7 (39–94)	100 (97.7–100)	100 (63.1–100)	98.2 (96.4–99.9)	0.83 (0.65–1.0)
<i>E. coli</i> 29	144	0	0	0	100 (88.1–100)	100 (97.5–100)	100 (88.1–100)	100 (97.5–100)	1.0
<i>Pseudomonas aeruginosa</i> 2	171	0	0	0	100 (15.8–100)	100 (97.9–100)	100 (15.8–100)	100 (97.9–100)	1.0
<i>Klebsiella pneumoniae</i> 4	169	0	0	0	100 (39.8–100)	100 (97.8–100)	100 (39.8–100)	100 (97.8–100)	1.0
<i>Klebsiella oxytoca</i> 2	171	0	0	0	100 (15.8–100)	100 (97.9–100)	100 (15.8–100)	100 (97.9–100)	1.0
<i>Citrobacter</i> spp. 2	170	1	1	0	100 (15.8–100)	99.4 (96.8–100)	66.7 (9.4–99.2)	100 (97.9–100)	1.0
<i>Enterobacter</i> spp. 4	169	0	0	0	100 (39.8–100)	100 (97.8–100)	100 (39.8–100)	100 (97.8–100)	1.0
<i>Acinetobacter</i> spp. 1	171	0	1	1	50 (1.3–98.7)	100 (97.9–100)	100 (2.5–100)	99.4 (96.8–100)	0.66 (0.05–1.0)

PPV=positive predictive value, NPV=negative predictive value

Table 4 Results for BioFire® FilmArray® by organism

	True-positive (n)	True-negative (n)	False-positive (n)	False-negative (n)	Sensitivity % (95 % CI)	Specificity % (95 % CI)	PPV % (95 % CI)	NPV % (95 % CI)	Kappa (95 % CI)
<i>Staphylococcus aureus</i>	17	156	0	0	100 (80.5–100)	100 (97.7–100)	100 (80.5–100)	100 (97.7–100)	1.0
<i>Staphylococcus</i> spp.	69	104	0	0	100 (94.8–100)	100 (96.5–100)	100 (94.8–100)	100 (96.5–100)	1.0
<i>Streptococcus pneumoniae</i> 1	172	172	0	0	100 (2.5–100)	100 (97.9–100)	100 (2.5–100)	100 (97.9–100)	1.0
<i>Enterococcus</i> spp.	8	165	0	0	100 (63.1–100)	100 (97.8–100)	100 (63.1–100)	100 (97.8–100)	1.0
<i>Streptococcus agalactiae</i> 3	169	169	1	0	100 (29.2–100)	99.4 (94.7–99.6)	78.6 (49.2–95.3)	100 (97.7–100)	0.87 (0.73–1.0)
<i>Streptococcus</i> spp.	11	159	3	0	100 (71.5–100)	98.1 (96.7–99.9)	87.5 (49.8–98)	100 (97.7–100)	0.93 (0.79–1.0)
<i>E. coli</i>	29	144	0	0	100 (88.1–100)	100 (97.5–100)	100 (88.1–100)	100 (97.5–100)	1.0
<i>Pseudomonas aeruginosa</i> 2	146	146	25	0	100 (15.8–100)	85.4 (79.2–90.3)	7.4 (0.9–24.3)	100 (97.5–100)	0.12 (–0.03–0.27)
<i>Klebsiella pneumoniae</i> 4	168	168	1	0	100 (39.8–100)	99.4 (96.7–100)	80 (28.4–99.5)	100 (97.8–100)	1.0
<i>Klebsiella oxytoca</i>	1	171	0	1	50 (1.3–98.7)	100 (97.9–100)	100 (2.5–100)	99.4 (96.8–100)	0.66 (0.05–1.0)
<i>Enterobacter</i> spp.	4	169	0	0	100 (39.8–100)	100 (97.8–100)	100 (39.8–100)	100 (97.8–100)	1.0
Enterobacteriaceae	2	171	0	0	100 (15.8–100)	100 (97.9–100)	100 (15.8–100)	100 (97.9–100)	1.0

PPV= positive predictive value, NPV= negative predictive value

the FilmArray® assay produced nine false-negatives and five false-positives. The Verigene® assay only tests for *mecA* in *S. aureus* and *S. epidermidis*, so the total number of evaluable samples was lower.

vanA/B was detected in only one sample (an *Enterococcus faecium*) by both Verigene® and FilmArray® assays, with compatible phenotypic results. *CTX-M* was detected in five samples by the Verigene® assay (four cases with *E. coli* and one case with *Enterobacter cloacae*). In all cases, the phenotypic susceptibility testing was compatible, showing resistance to amoxicillin, co-amoxiclav, cefuroxime, ceftazidime, cefpodoxime, cefotaxime and cephalothin. There were no samples testing positive for any of the carbapenemase genes. The results for resistance markers are shown in Table 7.

Clinical utility

We assessed the potential impact that earlier species and resistance mechanism identification could have had on patient management and antimicrobial stewardship, had the tests been performed prospectively and the results released to physicians. The difference in the mean total time to the final identification of species and antibiotic resistance between standard and molecular methods was 27.95 h for the Verigene® assay and 29.17 h for the FilmArray® assay. This time period represented the window of opportunity in which earlier identification by the molecular panels could potentially benefit patient management.

Organisms were separated into those where the species would have indicated a need to broaden the spectrum of antimicrobial coverage (*Enterococcus faecium*, *Pseudomonas aeruginosa* and Enterobacteriaceae with predictable chromosomal resistance to beta-lactams), those where the combination of organism and resistance gene indicated a need to broaden antimicrobial therapy, and those where the species would indicate a change from empirical therapy (usually co-amoxiclav) to a more targeted antimicrobial was possible (e.g. *S. aureus* and flucloxacillin, *E. faecalis* and amoxicillin, and streptococci and penicillin). For each sample in these categories, the antimicrobial that the patient was receiving when the Gram stain was performed was noted and an assessment made as to whether knowledge of the species or resistance gene could have changed this choice.

There were 13 (7.5 %) samples where species identification suggested a need to broaden the spectrum of antimicrobial coverage. Of these, two patients were not prescribed antimicrobials that would have covered the identified organism at the point of Gram stain. The other 11 patients were prescribed antimicrobials that deviated from hospital guidelines due to previous isolation of resistant organisms or their clinical condition (unwell on the intensive care unit or neutropaenic).

There were 11 (6.4 %) samples which had an identified resistance gene and, of these, nine had already received

Table 5 Details of all discrepant samples

Conventional methods identification	Verigene® identification	FilmArray® identification	Number of occurrences
<i>Staphylococcus epidermidis</i>	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus epidermidis</i> and <i>Pseudomonas aeruginosa</i>	9
<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i> and <i>Pseudomonas aeruginosa</i>	4
<i>Staphylococcus hominis</i>	<i>Staphylococcus</i> spp.	<i>Staphylococcus</i> spp. and <i>Pseudomonas aeruginosa</i>	2
<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> and <i>Pseudomonas aeruginosa</i>	
<i>Klebsiella oxytoca</i>	<i>Klebsiella oxytoca</i>	<i>Klebsiella oxytoca</i> and <i>Pseudomonas aeruginosa</i>	
<i>Streptococcus bovis</i>	<i>Streptococcus</i> spp.	<i>Streptococcus</i> spp. and <i>Pseudomonas aeruginosa</i>	
<i>Citrobacter braakii</i> and <i>Klebsiella oxytoca</i>	<i>Citrobacter</i> spp. and <i>Klebsiella oxytoca</i>	<i>Enterobacter</i> spp. and <i>Pseudomonas aeruginosa</i>	
Group C/G <i>Streptococcus</i>	<i>Streptococcus</i> spp.	<i>Streptococcus</i> spp. and <i>Pseudomonas aeruginosa</i>	
<i>Enterobacter cloacae</i>	<i>Enterobacter</i> spp.	<i>Enterobacter cloacae</i> and <i>Pseudomonas aeruginosa</i>	
<i>Enterococcus faecium</i>	<i>Enterococcus faecium</i>	<i>Enterococcus</i> spp. and <i>Pseudomonas aeruginosa</i>	
<i>Propionibacterium acnes</i>	ND	<i>Pseudomonas aeruginosa</i>	
<i>Micrococcus luteus</i>	<i>Micrococcus</i> spp.	ND	6
<i>Corynebacterium</i> spp.	ND	ND	4
<i>Propionibacterium acnes</i>	ND	ND	3
<i>Haemophilus parainfluenzae</i>	ND	ND	2
<i>Brevibacterium casei</i>	ND	ND	
Unidentified Gram-positive rod	ND	ND	
<i>Aeromonas hydrophila</i>	ND	ND	
<i>Bacteroids fragilis</i>	ND	ND	
<i>Paenibacillus macerans</i>	ND	ND	
<i>Acinetobacter lwoffii</i>	ND	ND	
<i>Prevotella denticola</i>	ND	ND	
<i>Morganella morganii</i>	ND	ND	
<i>Acinetobacter ursingii</i>	<i>Acinetobacter</i> spp.	ND	
<i>Staphylococcus hominis</i>	<i>Staphylococcus</i> spp.	<i>Staphylococcus</i> spp. and <i>Klebsiella pneumoniae</i>	
<i>Staphylococcus aureus</i> and Group C/G <i>Streptococcus</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i> and <i>Streptococcus</i> spp.	
<i>Streptococcus viridans</i>	<i>Streptococcus pneumoniae</i>	<i>Streptococcus</i> spp.	
<i>Fusobacterium necrophorum</i>	ND	<i>Pseudomonas aeruginosa</i>	
<i>Streptococcus viridans</i>	<i>Streptococcus pneumoniae</i>	<i>Streptococcus</i> spp.	
<i>Enterococcus avium</i>	<i>Enterococcus faecium</i>	<i>Enterococcus</i> spp.	
<i>Enterococcus faecalis</i>	<i>Enterococcus faecalis</i> and <i>Staphylococcus</i> spp.	<i>Staphylococcus</i> spp. and <i>Enterococcus</i> spp.	
<i>Enterococcus faecalis</i> and <i>Citrobacter freundii</i>	<i>Citrobacter</i> spp.	<i>Enterococcus</i> spp. and <i>Enterobacter</i> spp.	

appropriate antimicrobials by the time the blood culture Gram stain was available and one had died prior to the blood culture becoming positive. Again, the reason for deviation from hospital antimicrobial guidelines was due to prior knowledge of resistant organisms isolated from other sites on the same patient.

Thirty-one (17.9 %) samples isolated a *Streptococcus*, meticillin-sensitive *S. aureus* (MSSA) or *E. faecalis*. When clinical details were reviewed, six patients could have had their antimicrobials changed sooner if the molecular result had

been available. The other patients were either already on an appropriate antimicrobial, were suspected to have a mixed infection or were thought to have a contaminant in the blood culture.

Discussion

A total of 173 bacteraemic episodes were assessed in 159 patients, covering a broad age range and divided

Table 6 Results for polymicrobial samples

Results for conventional testing		Results for the Verigene® assay		Results for the FilmArray® assay	
<i>S. aureus</i>	Group C/G <i>Streptococcus</i>	<i>S. aureus</i>	ND	<i>S. aureus</i>	<i>Streptococcus</i> spp.
<i>S. epidermidis</i>	<i>Streptococcus parasanguinis</i>	<i>S. epidermidis</i>	<i>Streptococcus</i> spp.	<i>Staphylococcus</i> spp.	<i>Streptococcus</i> spp.
<i>Citrobacter braakii</i>	<i>Klebsiella oxytoca</i>	<i>Citrobacter</i> spp.	<i>Klebsiella oxytoca</i>	Enterobacteriaceae	<i>Pseudomonas aeruginosa</i>
<i>Citrobacter freundii</i>	<i>Enterococcus faecalis</i>	<i>Citrobacter</i> spp.	ND	<i>Enterococcus</i> spp.	Enterobacteriaceae
<i>S. aureus</i>	<i>Streptococcus agalactiae</i>	<i>S. aureus</i>	<i>Streptococcus agalactiae</i>	<i>S. aureus</i>	<i>Streptococcus agalactiae</i>
<i>S. haemolyticus</i>	<i>S. parasanguinis</i>	<i>Staphylococcus</i> spp.	<i>Streptococcus</i> spp.	<i>Staphylococcus</i> spp.	<i>Streptococcus</i> spp.
<i>Citrobacter braakii</i>	<i>S. aureus</i>	<i>Citrobacter</i> spp.	<i>S. aureus</i>	<i>Citrobacter</i> spp.	<i>S. aureus</i>

approximately equally between community- and hospital-acquired infection. Reflecting the high morbidity associated with bacteraemia, 16 % of patients died within 30 days of microbiological confirmation of bloodstream infection.

Both molecular assays correctly identified targeted organisms with high sensitivity and specificity. Samples were frozen, which is not recommended by either manufacturer and we are unable to assess the effect this might have had on the performance characteristics. The FilmArray® assay identified *P. aeruginosa* in 25 samples where this organism had not been found by conventional testing or using the Verigene® panel. On one occasion, where the true organism was *Fusobacterium necrophorum*, this may have resulted in the use of inappropriate antimicrobials had the physician acted on the result.

False-positive tests resulting in pseudo-outbreaks due to contamination of blood culture bottles have previously been described [24, 25], as has contamination of molecular reagents [26]. Evaluation by other authors of the FilmArray® Blood Culture Identification Panel have not identified this issue [27]. However, in May 2014, BioFire® released an advisory note which detailed the risk of false-positive results for *P. aeruginosa* and *Enterococcus* spp. when using bioMérieux BacT standard anaerobic bottles. This is thought to be related to the presence of nucleic acid from non-viable organisms in these bottles, and the company advises that any positive results for these organisms should be confirmed by another method prior to reporting the test results.

The Verigene® assay produced occasional false-positive results (6/173); however, two of these were *S. pneumoniae*, which has previously been noted to give false-positives for this organism [15, 16]. It is difficult to comment on the presumed false-positive results, since we performed no further analysis of these samples (e.g. by sequencing). The clinical significance of these findings are unknown; however, in all cases, an alternative pathogen was identified by both conventional techniques and the molecular assays.

The average total time to organism identification for both molecular assays was significantly shorter than for the conventional methods. Had these assays been used to inform clinical practice, then the final results (including antimicrobial susceptibility data) would, on average, have been available

27.95 h earlier for the Verigene® assay and 29.17 h earlier for the FilmArray® assay. This is broadly similar to previously published studies [13, 17, 18]. The potential for an earlier result to positively impact upon clinical management in our setting was relatively modest (9 out of 173 samples, 5.2 %); however, we did not include samples that were thought to have contaminants only in the analysis. Three patients could have benefited from an earlier change from ineffective to effective antimicrobials. Antimicrobial stewardship could have been improved in a further six patients whose antimicrobial spectrum could have been narrowed at an earlier stage. Previous studies have examined the use of the Verigene® assay for improving antimicrobial prescribing for *S. aureus* and *Enterococcus* spp. [20, 21]. These studies found a greater potential benefit, but had much higher frequencies of resistant organisms.

Most patients are prescribed antimicrobials because the attending physician is concerned that they have a bacterial infection. Knowledge of a contaminant in the blood culture might not be an indication for changing or stopping antimicrobial therapy. We excluded samples that isolated contaminant organisms only from the prescribed antimicrobial analysis; however, this knowledge could feasibly affect antimicrobial prescribing behaviour and would have likely led to an underestimation of the clinical utility of these tests. On these occasions, earlier knowledge of species would expedite cessation of antimicrobials.

Although the molecular assays are rapid and generally performed well, they have yet to establish a clear role in day-to-day clinical practice. They are a tool to augment rather than replace conventional methods, as samples still require an initial blood culture incubation step and the assays do not detect all organisms and resistance mechanisms. Consequently, they are likely to result in a significant additional cost for laboratories. In our setting, where extensive data were available for local resistance rates and previous microbiology results were frequently available, the impact of earlier organism identification on antimicrobial therapy was limited. The low rates of antimicrobial resistance locally also reduced the opportunity for the assays to identify occasions where broadening or narrowing initial empirical

Table 7 Resistance genes identified for both Nanosphere Verigene® and BioFire® FilmArray® molecular assays

Resistance gene	Number of positive samples	Results for Nanosphere Verigene®						Results for BioFire® FilmArray®					
		Sensitivity % (95 % CI)	Specificity % (95 % CI)	PPV % (95 % CI)	NPV % (95 % CI)	Kappa (95 % CI)		Sensitivity % (95 % CI)	Specificity % (95 % CI)	PPV % (95 % CI)	NPV % (95 % CI)	Kappa (95 % CI)	
<i>mecA</i>	32/46*	93.8 (79.9–98.3)	92.5 (80.1–97.4)	90.0 (77–96.8)	94.9 (82.8–98.6)	0.86 (0.71–0.98)		80.4 (66.8–89.3)	85.7 (70.6–93.7)	88.1 (76.5–94.4)	76.9 (64.6–85.9)	0.65 (0.49–0.82)	
<i>vanA/B</i>	1	100 (20.7–100)	100 (64.6–100)	100	100	1.0		100 (20.7–100)	100 (64.6–100)	100	100	1.0	
<i>CTX-M</i>	5	83.3 (43.6–97.0)	100 (89.8–100)	100	97.1 (85–99.5)	0.9 (0.69–1.0)		N/A	N/A	N/A	N/A	N/A	

PPV=positive predictive value, NPV=negative predictive value

*The Verigene® assay gives an *mecA* result only for *S. aureus* and *S. epidermidis*, so the total number of evaluable samples is lower ($n=32$) than for the FilmArray® assay ($n=46$)

antimicrobials was appropriate. A final consideration is that each molecular assay can process only one sample at a time. In order to gain maximum benefit from timely results, samples should be processed in real time using multiple platforms in parallel, providing staff are available to operate them.

There were a number of limitations to our study. The use of frozen samples (outside of the manufacturers’ recommended protocol) may have resulted in degradation and decreased the sensitivity of the molecular assays. The number of samples tested, although comparable to previous studies [12, 20], was relatively small. This resulted in large confidence intervals around performance characteristics for less frequently detected organisms and resistance mechanisms. We did not review the full antimicrobial prescribing data for patients; instead, we determined which antimicrobials were prescribed at the time clinicians were notified of the Gram stain, together with the initial microbiology advice given. It is reasonable to assume that these are the antimicrobials and decisions the molecular assays could have an impact upon; however, it is an incomplete analysis and excluded contaminants such as CNS. The study was performed retrospectively, introducing potential bias, as the assessor was not blinded to both conventional and molecular assay results.

In conclusion, both the Verigene® and FilmArray® molecular assays for positive blood cultures provided accurate results more rapidly than conventional culture-based methods. However, in our setting, where antimicrobial resistance is low and there is good knowledge of resistance patterns at both population and individual levels, the impact of earlier results on antimicrobial choices was limited. These assays are likely to have the greatest impact in settings with higher levels of antimicrobial resistance and where there are no prior microbiological samples available for patients.

Conflict of interest CW reports receiving an educational grant from Nanosphere to attend ECCMID 2014. All other authors report no conflicts of interest relevant to this article.

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