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Editor's Choice

ORIGINAL ARTICLE

Comparison of novel approaches for expedited pathogen identification and antimicrobial susceptibility testing against routine blood culture diagnostics

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Significance and Impact of the Study: Timely reporting of pathogen identification and antimicrobial susceptibility is crucial for patients suffering from sepsis. New approaches promising short turnaround times compared to conventional workup of blood cultures are seeking their way to routine laboratory implementation. This pilot study evaluates novel techniques for pathogen identification and antimicrobial susceptibility testing directly from positive blood cultures. The data indicate a considerable expediting in microbiological sepsis diagnosis without compromising result quality.

Keywords
antibiotic resistance, blood culture, pathogen identification, sepsis, rapid antimicrobial susceptibility testing.

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Abstract

Blood stream infections pose a major challenge for clinicians as the immediate application of an appropriate antibiotic treatment is the vital factor to safe the patients' lives. This preliminary study compares three different systems promising fast pathogen identification and susceptibility testing in comparison to conventional blood culture (BC): (i) the rapid antimicrobial susceptibility testing protocol according to EUCAST in combination with the Sepsityper® kit (sRAST), (ii) the direct inoculation method on the VITEK®2 system (dVIT) and (iii) testing with the Accelerate Pheno® system (AccPh). All methods were assessed in terms of accuracy, time to result and usability. Twenty-three BC samples obtained from patients suffering from proven sepsis were analysed in detail. Pathogen identification was successful in 95·6, 91·3 and 91·3% in sRAST, dVIT and AccPh, respectively. Categorical agreement in antimicrobial susceptibility testing was 89·5, 96 and 96·6%, respectively. Time to result from sample entry to reporting ranged from an average of 4·6 h for sRAST and 6·9 h for AccPh to 10·6 h for dVIT. These results imply a significant shortening of reporting times at considerably high agreement rates for these new diagnostic approaches.

Introduction

Despite all progress in intensive care medicine, blood stream infections (BSIs) are still associated with high mortality (Barenfanger *et al.* 1999; Rudd *et al.* 2020). In combination with other modern medical resources, the fast initialization of an appropriate antibiotic therapy is crucial in BSI, as mortality accumulates by 7% for every hour of delay (Kumar *et al.* 2006). While the survival rate in sepsis with the appropriate antibiotic therapy is 52%, it drops to 10·3% in the event of inaccurate therapy (Kumar *et al.* 2009). Moreover, the use of ineffectual antibiotics contributes to the rise of multidrug-resistant pathogens (Hicks *et al.* 2011). Since empirical antibiotic therapies often consist of combining different antibiotic groups, also the risk of adverse effects can be higher (Paul *et al.* 2006).

Blood culture (BC) currently still is the gold standard in diagnosis of BSI (Rutanga and Nyirahabimana 2016). However, this approach comprises some limitations, like a low sensitivity and a prolonged time to result (TTR) of one to three days for species identification (ID) and antimicrobial susceptibility testing (AST), respectively. Consequently, various attempts were made to shorten

TTR using modern technologies. One such method for rapid identification of the pathogen is the Sepsityper® kit (Bruker, Massachusetts), which provides a special sample preparation in order to purify and identify bacteria or yeasts directly from positive BCs. An aliquot of the BC broth is processed and can proximately be used for conventional analysis by matrix-assisted laser desorption ionization - time of flight (MALDI-TOF) mass spectrometry (Tanner *et al.* 2017). Successful ID from positive BCs can be achieved within 30 min (Jamal *et al.* 2013; Morgenthaler and Kostrzewa 2015).

With respect to AST, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has recently published an elaborate workflow for rapid AST (RAST) directly from positive BCs (Jonasson *et al.* 2020). This approach is based on the EUCAST standard disk diffusion methodology. A defined inoculum of broth from a positive BC bottle is streaked on Mueller–Hinton agar plates. After incubation of these agar plates for 4, 6 or 8 h, AST results are evaluated depending on the readability of inhibition zone diameters. EUCAST provides breakpoints for each incubation period, and thus susceptibility results could be available within 4 h at the earliest (The European Committee on Antimicrobial Susceptibility Testing 2019a). Another timesaving method involving direct testing of BC broth mixture is done on the VITEK®2 system (bioMérieux SA, Marcy l'Etoile, France). This automated approach provides both pathogen identification as well as susceptibility results (Barman *et al.* 2018). A third diagnostic approach is based on fluorescence in situ hybridization and morphokinetic cellular analysis. This is achieved by the Accelerate Pheno® device (Accelerate Diagnostics S.L., Barcelona, Spain), which was launched in 2018 and is able to identify pathogens within 90 min. It further provides minimal inhibitory concentration (MIC)-based susceptibility data within 7 h (Charnot-Katsikas *et al.* 2018).

The aim of this study was to compare the performance of three rapid identification and susceptibility testing methods: (i) the RAST protocol, which was performed in combination with the Sepsityper extraction kit (sRAST), (ii) the direct inoculation method of the VITEK®2 system (dVIT) and (iii) the analysis using the Accelerate Pheno (AccPh) system. All results were assessed against the EUCAST reference method performed routinely in our lab.

Results and discussion

This evaluation compares three different testing systems that promise to provide results on pathogen identification and AST profiles of common sepsis-causing pathogens sooner than the current standard method. Correct ID and concordance of AST results were compared individually to the results of the routine workflow consisting of MALDI-TOF-based ID and The European Committee on Antimicrobial Susceptibility Testing (2019b) used as reference methods. Figure 1 depicts the schematic workflow of the samples included in the study. Since the different methods to be evaluated feature different panels and test specifications, two criteria were defined to allow the collection of contrastable test results. Firstly, the samples to be included had to be represented in the RAST panel, which is the smallest of all three methods. So only samples containing one of the eight RAST species were included. Currently, the RAST protocol is validated for eight of the most common sepsis causing species (The European Committee on Antimicrobial Susceptibility Testing 2019a). However, taking into account that *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus* spp., *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* combined are found in approximately 73% of all BCs from septic patients, the RAST method covers the vast majority of BSI cases (Kreidl *et al.* 2019). Secondly, for analysis on the AccPh, samples must not be older than 8 h. Hence, only samples that had flagged positive within the last 8 h before test initialization were included.

In total, 23 positive BCs were analysed with all three modalities. The spectrum of pathogens tested included 17 gram-negative bacteria, i.e., *E. coli* ($n = 15$), *K. pneumoniae* ($n = 2$), and six gram-positive bacteria, i.e., *S. aureus* ($n = 4$), *E. faecium* ($n = 1$) and *E. faecalis* ($n = 1$) (see Fig. 2).

Identification of a pathogen using the Sepsityper protocol was achieved in <45 min, including preparation of the cell pellet from BC aliquots and subsequent MALDI-TOF analysis. The AccPh and dVIT methods provided ID results after 1·5 and 5·4 h (4·1–9·9 h), respectively. The accuracy rate was highest for Sepsityper (95·6%) compared to the other approaches (91·6%, each) (Fig. 2). Consequently, in spite of the time-consuming manual processing of the samples for direct MALDI analysis, the Sepsityper kit provides reliable results significantly faster than dVIT and AccPh. However, a meta-analysis by Morgenthaler and colleagues revealed a relatively low concordance of around 80% for the Sepsityper protocol compared to MALDI-TOF analysis of subcultured isolates grown on solid media (Morgenthaler and Kostrzewa 2015). As 15 out of 23 BCs in the present study contained gram-negative bacteria, the high agreement rate of 95·6% might be explained by the fact that the Sepsityper kit in general works significantly better for gram-negative than for gram-positive bacteria and yeasts (Bidart *et al.* 2015; Tanner *et al.* 2017; Fang *et al.* 2020). In this context, also MALDI-TOF threshold settings for correct species or

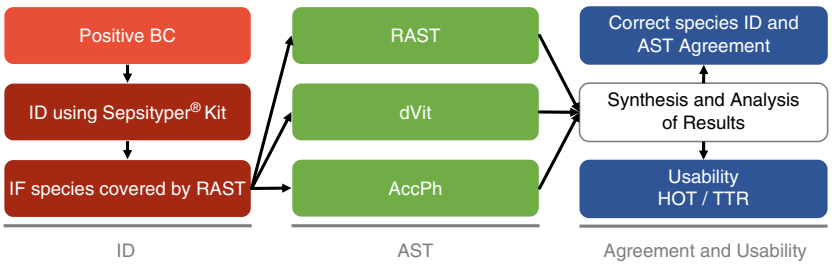

Figure 1 Evaluation workflow. Schematic representation of sample processing during the comparison of expedited pathogen identification (ID) and antibiotic susceptibility testing (AST) approaches. Positive blood culture (BC) samples were subject to Sepsityper® ID before analysis by EUCAST rapid AST (RAST), direct inoculation VITEK®2 protocol (dVIT) and analysis with the Accelerate Pheno® System (AccPh) (for details please refer to text). The data of the parallel testing were compiled and analysed in terms of correct ID and agreement of AST findings as well as usability, measured as hands-on-time (HOT) and time to result (TTR), in a real-life setting. [Colour figure can be viewed at wileyonlinelibrary.com]

	Reference Method		RAST[a]	dVIT[b]	AccPh[b]
ID	*E.coli* (n=15) *Staph. aureus* (n=4) *K. pneumoniae* (n=2) *E. faecium* (n=1) *E. faecalis* (n=1)		22/23 (95.6%)	21/23 (91.3%)	21/23 (91.3%)
AST	Complete concordance		14/23 (60.9%)	16/23 (69.6%)	11/23 (47.8%)
	Categorical agreement Σ162 combinations (pathogen/antibiotics)		145/162 (89.5%)	145/151 (96.0%)	113/117 (96.6 %)
	Very Major Error		6/162 (3.7%)	1/151 (0.7%)	2/117 (1.7%)
	Major Error		0/162 (0.0%)	3/151 (2.0%)	1/117 (0.9%)
	Minor Error		11/162 (6.8%)	2/151 (1.3%)	1/117 (0.9%)
HOT	ID	5 min (MALDI-TOF)	ST 30 min	20 min	5 min
	AST	5 min (EUCAST)	RAST 15 min		
TTR	ID	24 h (sub-culture)	ST ≤ 45 min	5.4 h (4.1–9.9 h)	1.5 h
	AST	24 h[c]/48 h (sub-culture)	RAST 4.6 h (4–8 h)	10.6 h (7.7–18.7 h)	6.9 h (6.1–7.1 h)

[a]all pathogen/antibiotic combinations compatible to EUCAST results
[b]panel of antibiotics only partially comparable to EUCAST results and drop out of individual samples and/or antibiotics
[c]preliminary AST–direct AST from positive blood culture

Figure 2 Summary of results for pathogen identification (ID), antimicrobial susceptibility testing (AST), hands-on-time (HOT) and time to result (TTR) for the 23 samples tested in this evaluation. All samples were first identified using the Sepsityper® (ST) protocol. Subsequently the rapid antimicrobial susceptibility testing (RAST), direct inoculation method on the VITEK®2 system (dVIT) and testing on the Accelerate Pheno® device (AccPh) were performed and compared to the reference method (standard EUCAST testing). Agreement rates, error rates are presented in brackets, as well as corresponding time spans. [Colour figure can be viewed at wileyonlinelibrary.com]

genus classification are definitely critical (Morgenthaler and Kostrzewa 2015). Apart from the official Sepsityper IVD protocol, there are recent publications reporting higher identification rates at even shorter manipulation times (Simon *et al*. 2019). These modified protocols could further improve the performance of the approach.

The panel of pathogens identifiable by dVIT covers a plethora of gram-positive, gram-negative, fastidious and anaerobic bacteria as well as a large number of yeasts (Hata *et al*. 2007; Nakasone *et al*. 2007; Lee *et al*. 2011). However, it is essential to determine beforehand whether the microorganism in the positive BC belongs to the *Enterobacteriaceae*, to the groups of gram-negative, fastidious or anaerobic bacteria or yeasts, as different identification cards are used for the respective pathogens. Indeed, the use of the Sepsityper protocol could supplant Gram stain and microscopy, which is mandatory for subsequent VITEK®2 AST analysis.

Contrastingly, the AccPh system is able to identify 5 gram-positive bacteria (*S. aureus*, *S. lugdunensis*, *E. faecalis*, *E. faecium* and *Streptococcus* spp.) plus the group of coagulase-negative Staphylococci, 8 gram-negative bacteria (*E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Serratia marcescens*, *P. aeruginosa*, *A. baumannii*) and the two yeasts *Candida albicans* and *C. glabrata*.

In total, 162 combinations of pathogens and antibiotics were assessed in comparison to the reference method. The sRAST, dVIT and AccPh analysis of these samples showed entirely congruent results in 14, 16 and 11 out of the 23 samples, respectively (Fig. 2). The remaining samples exhibited either one or more very major errors (VME), major errors (MAE) or minor errors (MIE) when compared to the reference method. Analysis of categorical agreement for AST results showed that the AccPh approach exhibited the highest concordance (96·6%), followed by dVIT (96·0%) and sRAST (89·5%), which is comparable with previous findings (Bazzi *et al*. 2017; Pancholi *et al*. 2018; Schneider *et al*. 2019).

In the present study, antimicrobial susceptibility data were available after a mean time of 4·6 (4–8 h), 6·9 (6·6–7·1 h) and 10·6 h (7·7–18 h) using the sRAST, AccPh and dVIT, respectively (Fig. 2). This compares with a significantly longer TTR for samples analysed using the reference method of 24 h until pathogen ID and another 24 h for obtaining antimicrobial susceptibility characteristics.

While AST results were available after almost exactly 6·9 h when using AccPh, variation regarding TTR was significantly more pronounced in RAST (4–8 h) and dVIT (7·7–18·7 h). This can—at least in part—be attributed to the different growth characteristics of gram-negative and gram-positive bacteria. Regarding RAST, readout time for gram-negative bacteria was significantly shorter (mean of 4·2 h), compared to gram-positive pathogens (mean of 5·7 h).

As TTR can be up to 48 h for conventional susceptibility testing, physicians usually administer empirical antibiotic therapies in case of a suspected BSI. Those empirical therapies normally consist of broad-spectrum antibiotics or antibiotic combinations to cover the most common sepsis-causing pathogens. Nonetheless, it has been demonstrated that empirical antibiotic therapy in BSI is occasionally inappropriate. For instance, Robineau and colleagues demonstrated that in 1952, BSI episodes evaluated only 61% of the patients received an adequate antibiotic therapy (Robineau *et al*. 2018). Another study even reported that only 51·8% of the sepsis patients received an appropriate antibiotic when treated with an empirical antibiotic regime (Herzke *et al*. 2009). French and colleagues showed that a pathogen ID on the day of BC positivity results in a clinical benefit for the around 25% of the patients by earlier evaluation of the pathogen's relevance, identification of potential site of infection or indication of appropriate antibiotics even without AST (French *et al*. 2016). For this reason, a fast and accurate pathogen ID and an expeditious availability of susceptibility data could lead to an earlier optimization of antibiotic therapy and therefore to an increased survival rate in patients suffering from sepsis. Additionally, adverse side effects of certain antibiotics could be reduced to a minimum.

For some pathogens, the antibiotics included in the AST panel of this evaluation differ considerably between the different methods. For instance, *S. aureus* is tested for susceptibility to cefoxitin, norfloxacin, gentamicin and clindamycin when using RAST protocols. The antibiotics in the AST panel of AccPh are cefoxitin, ceftarolin, trimethoprim-sulfamethoxazole, daptomycin, linezolid and vancomycin. As the latter two are so-called reserve antibiotics, the panel of the AccPh seems to be more appropriate when used for critically ill patients or in countries with high rates of multidrug-resistant pathogens. For the two pathogens *E. coli* and *K. pneumoniae*, the antibiotic panels for AST are broad in all three approaches. The RAST and AccPh protocols also offer AST for reserve antibiotics like ceftolozane-tazobactam, ceftazidime-avibactam (sRAST and AccPh) and colistin (AccPh). However, in contrast to the AccPh, sRAST as well as dVIT provide ID and AST in *S. pneumoniae*, an important sepsis-related pathogen. Remarkably, the lack of yeasts in the list of pathogens that can be diagnosed with RAST is a major shortcoming as the number of yeast-positive BCs in patients suffering from BSI is rising (Martin *et al*. 2003).

In terms of usability, all three approaches can be considered beneficial. However, absolute HOT per sample and TTR completion proved to be quite different for the

three modalities. HOT was shortest for the AccPh approach, followed by dVIT and sRAST with 5, 20 and 45 min, respectively (Fig. 2). Another important factor influencing implementation and ultimately adoption to these expedited test procedures is reporting time, equivalent to TTR measured here. It becomes clear from our preliminary evaluation that the combination of Sepsityper® and RAST protocols can substantially shorten the time to ID and AST reporting. Compared to EUCAST preliminary AST also the AccPh system provides results within a reasonable time frame. Of course, for clinicians, a 24/7 lab service would be highly desirable, but in a real-life setting, this is hardly accomplishable for most laboratories. Finally, yet importantly, the cost of the individual test systems differs tremendously. Particularly the sRAST protocol is most affordable, as no extra instrumentation is needed and MALDI-TOF analysis has become a widely used standard method. The only extra cost would be the Sepsityper® kit. Another advantage also is that even if susceptibilities cannot be inferred from the plate reads, the sample might still be processed and analysed based on conventional EUCAST workup and breakpoint criteria. The costs of the dVIT approach also seem reasonable, since the sample preparation is done with commonly available and relatively cheap reagents and equipment. ID and AST cards for the VITEK®2 device are the actual cost of the test. However, species ID and AST using the AccPh is expensive compared to conventional methods or the dVIT approach. The price of the system and the cartridges definitely represents a limitation for clinics and laboratories with limited financial resources. Nevertheless, the ease of use and the results provided could make the AccPh a useful supplement for clinic laboratories in the suitable setting of intensive-care-units with an appropriate patient population. Generally, the study shows some limitations such as the small sample number and limited comparability of the test systems, but it gives a first impression of usability of these approaches in real life laboratory setup.

Materials and methods

This evaluation used residual BC samples sent routinely to the Institute of Hygiene and Medical Microbiology for analysis between 10/01/2020 and 20/03/2020. The blood came from patients with suspected BSI and a Sequential Organ Failure Assessment (SOFA)-score of >2. It was injected into at least one set of BACTEC™ Plus Aerobic/F and BACTEC™ Lytic/10 Anaerobic/F culture vials on the wards. These BC bottles were promptly transported to the microbiological lab incubated in the compatible BACTEC FX Packaged continuous BC monitoring system (all from Becton Dickinson, Franklin Lakes, NJ) until notification of a positive growth signal. In parallel to the conventional routine testing, those positive BCs not exceeding a limit of 8 h since positivity were selected and processed for further analysis by the rapid ID and AST methods (*cf.* Fig. 1 – Evaluation workflow). Therefore, a 5-ml aliquot of culture broth was aspirated from the BC bottle and allotted to the individual tests.

Firstly, in order to determine the species, 1 ml of blood broth was used with the Sepsityper® kit (Bruker, Bremen, Germany) according to the manufacturers' instructions including the extraction sample preparation (CE-IVD) and as described previously (Kayin *et al.* 2019). Briefly, the human cells in the BC fluid are selectively lysed before the bacteria are purified by several steps of centrifugation and washing. Furthermore, the ethanol extraction including formic acid and acetonitrile is performed to guarantee high MALDI-TOF identification scores. For final analysis, 1 μl of extract is transferred onto a MALDI target plate and covered with alpha-cyano-4-hydroxycinnamonacid. The plate is transferred into the MALDI-TOF unit (Bruker) and samples analysed using MALDI Biotyper Software (ver. 3.1) Results with a score >1·7 were interpreted valid as inferred from previous studies on direct ID (Morgenthaler and Kostrzewa 2015). Secondly, after successful ID, RAST was performed according to the recommendations of EUCAST (Jonasson *et al.* 2020). In short, disk diffusion tests were performed directly from positive BCs on Mueller–Hinton agar by plating 125 μl of BC mixture from the positive BC bottle. After incubation for 4, 6 or 8 h at 37°C, results were evaluated according to the breakpoints provided by EUCAST RAST method ver. 1.1 (The European Committee on Antimicrobial Susceptibility Testing 2019a). In case of readable inhibition zone diameters, results were recorded, and the test was ended. When insufficient bacterial growth was observed after 4 h, plates were further incubated for another two hours before the next read up to a maximum incubation of 8 h.

In parallel, the pellet obtained from 1 ml of the positive BC was used for testing with the VITEK®2 60 system (bioMérieux, Marcy-l'Étoile, France). The procedure to obtain the respective pellet is described in detail by Bazzi and colleagues (2017). In short, 1 ml of BC broth is transferred to a sterile reaction tube and supplemented with 50 μl of Triton-X 10% (v/v). This mixture is vortexed, centrifuged and washed in 500-μl PCR-grade water. After a last centrifugation step, the pellet is resuspended in 100 μl of 0·45% (w/v) saline before adjustment of bacterial density to 0·5 McFarland index using the DensiCHEK device (bioMérieux). A tube containing 3 ml of the suspension is loaded into the VITEK®2 carrier station together with either the VITEK®2 GN ID or GP ID cards and the corresponding VITEK®2 AST cards for further analyses. AST

results are recorded and MIC values are automatically attributed by the system's proprietary software (ver. 8.01).

For the third approach, 500 μl of positive BC broth were processed with the AccPh according to the manufacturers' instructions. In short, the blood suspension is transferred to a dedicated sample vial, which was inserted into the reagent cartridge. The cartridge is inserted into the AccPh together with a testing cassette. Sample preparation, immobilization of the pathogens, identification by fluorescence in situ hybridization and MIC-based antimicrobial susceptibility testing is autonomously done by the automated testing system. No further manipulation is required until disposal.

Besides these rapid test workflows, 100-μl aliquots were streaked onto a MacConkey agar plate as well as a chocolate agar plate and a Columbia blood agar plate as part of the routine sample processing. These agar plates are incubated overnight at 37°C in dry air and 5% CO_2-supplemented air, respectively. In addition, a Gram stain of the sample is immediately performed and results recorded. Susceptibility testing is performed directly from culture broth (Ehren *et al.* 2020) or from subcultured isolates on the following day when following the EUCAST protocol. This is done on Mueller–Hinton agar plates by disk diffusion testing and overnight incubation at 37°C in dry air. The results from susceptibility testing are assessed on the following day according to the EUCAST guidelines (The European Committee on Antimicrobial Susceptibility Testing 2019b).

The data obtained from the different testing approaches were subsequently compiled in a spreadsheet for analysis. Comparison of AST results was performed according to the definitions and limitations specified in the work by Jasuja and colleagues (2020). In brief, a VME is defined in a case when a susceptible phenotype (S) is reported while the reference method shows a resistant phenotype (R). A MAE is defined as R instead of S in the reference method. Finally, MIE were observed when either the reference method or the compared modality displayed an intermediate phenotype (I) or was found to be in the area of technical uncertainty (ATU), while there was a clear R or S phenotype in the method to be compared.

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Conflict of Interest

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