A streamlined method for the fast and cost-effective detection of bacterial pathogens from positive blood cultures for the BacT/ALERT blood culture system using the Vitek MS mass spectrometer

Johannes Forster1*, Britta Kohlmorgen1, Julian Haas3, Philipp Weis4, Lukas Breunig5, Doris Turnwald1, Boris Mizaikoff3,6, Christoph Schoen1

1 Institute for Hygiene and Microbiology, University of Würzburg, Würzburg, Germany, 2 Institute of Hygiene and Environmental Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany, 3 Hahn-Schickard-Society for Applied Research, Ulm, Germany, 4 Department of Internal Medicine I, Caritas-Krankenhaus Bad Mergentheim, Bad Mergentheim, Germany, 5 Department of Internal Medicine – Cardiology, DRK Klinikum Berlin Westend, Berlin, Germany, 6 Institute of Analytical and Bioanalytical Chemistry, Ulm University, Ulm, Germany

* forster_j1@ukw.de

Abstract

Background and objective
Prompt pathogen identification of blood stream infections is essential to provide appropriate antibiotic treatment. Therefore, the objective of this prospective single centre study was to establish an inexpensive, fast and accurate protocol for bacterial species identification with SDS protein-extraction directly from BacT/Alert® blood culture (BC) bottles by VitekMS®.

Results
Correct species identification was obtained for 198/266 (74.4%, 95%-CI = [68.8%, 79.6%]) of pathogens. The protocol was more successful in identifying 87/96 (91.4%, 95%-CI = [83.8%, 93.2%]) gram-negative bacteria than 110/167 (65.9%, 95%-CI = [58.1%, 73.0%]) gram-positive bacteria. The hands-on time for sample preparation and measurement was about 15 min for up to five samples. This is shorter than for most other protocols using a similar lysis-centrifugation approach for the combination of BacT/Alert® BC bottles and the Vitek® MS mass spectrometer. The estimated costs per sample were approx. 1.80€ which is much cheaper than for commercial kits.

Conclusion
This optimized protocol allows for accurate identification of bacteria directly from blood culture bottles for laboratories equipped with BacT/Alert® blood culture bottles and VitekMS® mass spectrometer.
**Introduction**

Bloodstream infections (BSI) are one of the leading causes of death worldwide with fatality rates being as high as one fifth of patients [1,2]. Fast identification of the causative pathogen is essential to select appropriate treatment [3]. Therefore, rapid diagnostic test (RDT) may have positive impacts on appropriate antibiotic treatment, clinical outcomes and health care costs [4–7].

Different DNA-based methods are commercially available to shorten the time to identify pathogens in positive blood cultures (BCs) [8,9] but are expensive in acquisition and/or expendables. Due to its accuracy, very short time-to-result and low running costs, mass spectrometry (MS) became the standard method of bacterial identification in many clinical laboratories [10]. While different commercial mass spectrometers are in use, BioTyper® (Bruker Daltonics, Bremen, Germany) and Vitek MS® (bioMérieux, Marcy l’Étoile, France) are the most common systems for clinical use worldwide.

To date, various groups reported successful identification of bacteria directly from positive BCs using MS and commercial preparation kits for both systems (Sepsityper Kit, Bruker Daltonics, and Vitek® MS Blood Culture Kit, bioMérieux) are available. In addition, numerous in-house protocols with non-inferior or improved performance have been established. While separation and extraction method vary, most of the protocols have in common that lysis of blood cells is followed by centrifugation (lysis-centrifugation method) or filtration (lysis-filtration method) and several washing steps [11–16].

The effect on the results of RDT of the blood culture bottles widely used (Bact/ALERT® [bioMérieux] and BD BACTEC [BectonDickinson, Franklin Lakes, NJ, USA]) been studied and discussed previously [17] Most data on the performance of different RDT protocols is available for the combination of BD BACTEC (BectonDickinson) BC bottles and BioTyper® MS (Bruker) [18].

In order to have an impact on routine clinical management a RDT for the direct identification of bacteria from positive BCs using MS and commercial preparation kits for both systems (Sepsityper Kit, Bruker Daltonics, and Vitek® MS Blood Culture Kit, bioMérieux) are available. In addition, numerous in-house protocols with non-inferior or improved performance have been established. While separation and extraction method vary, most of the protocols have in common that lysis of blood cells is followed by centrifugation (lysis-centrifugation method) or filtration (lysis-filtration method) and several washing steps [11–16].

The effect on the results of RDT of the blood culture bottles widely used (Bact/ALERT® [bioMérieux] and BD BACTEC [BectonDickinson, Franklin Lakes, NJ, USA]) been studied and discussed previously [17] Most data on the performance of different RDT protocols is available for the combination of BD BACTEC (BectonDickinson) BC bottles and BioTyper® MS (Bruker) [18].

In order to have an impact on routine clinical management a RDT for the direct identification of bacteria from positive BCs has to be cheap, fast, reproducible, accurate, without any specialized equipment needed and easy to integrate into the standard laboratory workflow.

In this brief research report, we present and validate such a novel lysis-centrifugation protocol for laboratories equipped with Bact/ALERT® (bioMérieux) and Vitek MS® (bioMérieux) based on a simplified protocol first published by Foster [19].

**Materials and methods**

**Routine identification of bacteria grown on solid media (standard of care, SOC)**

Positive BCs were Gram stained and sub-cultured overnight on Columbia blood (bioMérieux) and chocolate agar (BD, Heidelberg, Germany) at 37°C and 5% CO₂. The next day, a single bacterial colony was transferred from the agar plate to a single well of a Vitek® MS-DS target slide (bioMérieux). One microliter of matrix (Vitek® MS-CHCA, [3.1g/100μl alpha-Cyano-4-hydroxycinnamic acid]) (bioMérieux) was added and the mixture was air dried. The slide was analyzed in the Vitek® MS mass spectrometer (bioMérieux, [337 nm nitrogen laser, 19.9 kV, maximum pulse rate 50 Hz, mass range 2000–20000 Da]) and only results with confidence level > 99% were used for identification. If no identification could be obtained, biochemical identification using the Vitek® 2 (bioMérieux) and/or the 16S rDNA sequencing [20] were performed.
Preparation of bacterial pellets from positive BacT/ALERT® blood culture bottles

Seven hundred microliter of BC broth were mixed with 0.3 ml wash buffer 1 (0.5ml 1.5% SDS in 2.5 ml sterile distilled water) in a 1.5 ml Eppendorf tube and centrifuged for 2 min at 3,000 × g (Mikro 200, Hettich, Tuttingen, Germany). The supernatant was removed, the pellet was resuspended in 1 ml of wash buffer 2 (0.5 ml 1.5% SDS in 9.5ml sterile distilled water) and the suspension was centrifuged for 2 min at 3,000 × g. The same procedure was then followed with wash buffer 3 (1 ml of sterile distilled water containing 1% (w/v) N-acetyl-l-cysteine (N-ACC)) and with 1 ml of sterile distilled water. The supernatant was removed and the remaining pellet was used for identification with the Vitek® MS.

Direct mass spectrometric identification of bacteria grown in blood bottles using the Vitek® MS

One microliter of the pellet was each placed in triplicate on a Vitek® MS-DS target slide (bioMérieux), 0.5 μl formic acid (Vitek® MS-FA) were added to each well, and the slide was air dried. To each well 1 μl of matrix (Vitek® MS-CHCA) were added. The slide was air dried and analyzed in the Vitek® MS mass spectrometer (bioMérieux). A confidence level of ≥ 99% in one of the triplicates was considered an acceptable identification. No identification result was assumed if no identification was given for all triplicates. A misidentification by the RDT was assumed if it did not match the identification given by the SOC.

Validation of the fast identification protocol using clinical samples

To assess the performance of the modified protocol, we used a convenience sample of positive BCs that were taken for routine diagnostics from patients at the University Hospital of Würzburg in two time periods, comprising 1250 positive BCs sampled between March 2017 and September 2017 and 1308 positive BCs between August 2018 and February 2019, respectively. Blood samples were inoculated into BacT/Alert FA (aerobic culture) (bioMérieux) and/or FN (anaerobic culture) (bioMérieux) blood culture bottles and incubated at 37˚C using automated BACT/ALERT® 3D system (bioMérieux) for 7 days until flagged positive. Prior inclusion to the study, Gram stain was performed and samples of the same appearance in Gram stain from the same patient within two weeks were excluded to avoid copy strains. In addition, microscopically polymicrobial samples were excluded. The resulting 266 positive samples were processed according to the SOC as outlined above. Authors had access to information that could identify individual participants during data collection.

Statistical methods

Concordance was calculated as number of correctly identified species by RDT divided by the number of identified species by the SOC. 95%-Confidence intervals (95%-CI) were obtained by a procedure given by Clopper and Pearson [21] as implemented in the “binom.test” function of the R stats package version 4.0.3 [22].

Ethics approval and consent to participate

This study was approved by the Ethics committee of the Medical Faculty of the University of Würzburg, Germany (reference number: 2021072801) and a waiver for informed consent was granted. All methods were performed in accordance with the relevant guidelines and regulations.
After removing positive BCs with either copy strains or no visible bacterial growth in gram microscopy, 2309 positive BCs were eligible for validation (Fig 1). Of these, a convenience sample of 301 positive BCs was used, including 35 positive BCs that were excluded from further analysis due to polymicrobial growth on solid media. Bacteria identified by the SOC in the remaining 266 positive BCs (200 aerobic and 66 anaerobic) comprised 164 aerobic gram-positive bacteria, 93 aerobic gram-negative bacteria, 6 anaerobes and 3 yeast belonging to 42 species and 24 genera, respectively (Table 1). As further shown in Table 2, the distribution of the bacteria over the different groups in the convenience sample as identified by SOC was representative for all the isolates from positive BCs in the year 2017 at our laboratory (Pearson’s $\chi^2$ test, $\chi^2 = 13.09$, df = 11, p = 0.29). This suggests that there was no bias in the composition of the convenience sample with respect to a certain group of pathogen.

On the species level concordant results between the SOC and the rapid identification protocol were obtained for 198 positive BCs (74.4%, 95%-CI = [68.8%, 79.6%]) (Fig 1 and Table 1). The rapid identification protocol failed to provide a species identification in 64 and gave a wrong species identification in only 4 BCs, respectively. It was thus correct for 98.0% (95-CI = [95.0%, 99.5%]) of the samples with an identification and in turn failed to give an identification in 24.1% of the tested clinical samples (95-CI = [19.1%, 29.7%]).

One-hundred ten of the 167 BCs with gram-positive bacteria identified by the SOC were correctly identified at the species level also by the rapid identification protocol (65.9%, 95%-CI = [58.1%, 73.0%]). There was however a significant heterogeneity of the concordance values between the SOC and the rapid identification protocol among the gram-positive bacteria at the genus level ($\chi^2 = 24.1$, df = 2, p-value < 0.00001, 3-sample test for equality of proportions
Table 1. Sensitivity and concordance of the in-house protocol on clinical samples by condition.

| Identification by reference method | Any identification by rapid protocol | Correct identification by rapid protocol | Concordance (%) |
|-----------------------------------|-------------------------------------|----------------------------------------|-----------------|
| (n)                               | (n)                                 | (n)                                    |                 |
| **Gram-positive bacteria**        |                                     |                                        |                 |
| Any identification by rapid       |                                     |                                        |                 |
| protocol                          |                                     |                                        |                 |
| **Staphylococci**                 |                                     |                                        |                 |
| Any identification by rapid       |                                     |                                        |                 |
| protocol                          |                                     |                                        |                 |
| **Staphylococcus aureus**         | 26                                  | 24                                     | 92.31%          |
| **Staphylococcus capitis**        | 5                                   | 4                                      | 80.00%          |
| **Staphylococcus haemolyticus**   | 8                                   | 6                                      | 75.00%          |
| **Staphylococcus hominis**        | 15                                  | 13                                     | 86.67%          |
| **Staphylococcus epidermidis**    | 67                                  | 46                                     | 68.66%          |
| **Staphylococcus warneri**        | 2                                   | 2                                      | 100.00%         |
| **Staphylococcus lugdanensis**    | 1                                   | 1                                      | NA              |
| **Staphylococcus saccharolyticus**| 1                                   | 0                                      | NA              |
| **Streptococci**                  |                                     |                                        |                 |
| Any identification by rapid       |                                     |                                        |                 |
| protocol                          |                                     |                                        |                 |
| **Streptococcus parasanguinis**   | 1                                   | 0                                      | NA              |
| **Streptococcus anginosus**       | 1                                   | 0                                      | NA              |
| **Streptococcus mitis/ Streptococci** | 4                           | 1                                      | 25.00%          |
| **Streptococcus pneumoniae**      | 3                                   | 0                                      | 0.00%           |
| **Streptococcus agalactiae**      | 1                                   | 1                                      | NA              |
| **beta- haemolytic streptococcus**| 1                                   | 0                                      | NA              |
| **Group C**                       |                                     |                                        |                 |
| **Enterococci**                   |                                     |                                        |                 |
| Any identification by rapid       |                                     |                                        |                 |
| protocol                          |                                     |                                        |                 |
| **Enterococcus faecalis**         | 9                                   | 8                                      | 66.67%          |
| **Enterococcus faecium**          | 8                                   | 1                                      | 12.50%          |
| **Enterococcus gallinarium**      | 1                                   | 0                                      | NA              |
| **Gram-positive anaerobes**       |                                     |                                        |                 |
| Any identification by rapid       |                                     |                                        |                 |
| protocol                          |                                     |                                        |                 |
| **Brevibacterium**                |                                     |                                        |                 |
| **B. luteolum**                   | 1                                   | 1                                      | 33.33%          |
| **Clostridium**                   |                                     |                                        |                 |
| **tettantium**                    | 1                                   | 1                                      | NA              |
| **Parvimonas**                    | 1                                   | 0                                      | NA              |
| **Other Gram-positive**           |                                     |                                        |                 |
| Any identification by rapid       |                                     |                                        |                 |
| protocol                          |                                     |                                        |                 |
| **Bacillus cereus group**         | 1                                   | 1                                      | NA              |
| **Corynebacterium**               |                                     |                                        |                 |
| **glucuronolyticum**              | 1                                   | 0                                      | NA              |
| **Gemella haemolytica**           | 2                                   | 1                                      | 50.00%          |
| **Gemella morbillorium**          | 1                                   | 0                                      | NA              |
| **Micrococcus**                   | 5                                   | 2                                      | 40.00%          |
| **luteus**                        | 1                                   | 0                                      | NA              |
| **Gram-negative bacteria**        |                                     |                                        |                 |
| Any identification by rapid       |                                     |                                        |                 |
| protocol                          |                                     |                                        |                 |
| **Enterobacterales**              |                                     |                                        |                 |
| Any identification by rapid       |                                     |                                        |                 |
| protocol                          |                                     |                                        |                 |
| **Escherichia coli**              | 47                                  | 44                                     | 93.62%          |
| **Citrobacter**                   |                                     |                                        |                 |
| **koseri**                        | 3                                   | 3                                      | 100.00%         |
| **Enterobacter**                  | 5                                   | 4                                      | 80.00%          |
| **complex**                       | 2                                   | 2                                      | 100.00%         |
| **Klebsiella**                    | 12                                  | 11                                     | 91.67%          |
| **pneumoniae**                    | 12                                  | 10                                     | 76.9%           |
| **Proteus mirabilis**             | 7                                   | 8                                      | 100.00%         |
| **Salmonella**                    | 2                                   | 2                                      | 100.00%         |
| **Serratia liquefaciens**         | 1                                   | 1                                      | NA              |
| **Serratia marcescens**           | 1                                   | 1                                      | NA              |
| **Nonfermenter**                  | 13                                  | 10                                     | 76.9%           |
| **Pseudomonas aeruginosa**        | 12                                  | 10                                     | 83.33%          |

(Continued)
without continuity correction), ranging from 76.8% (95%-CI = [68.4%, 83.9%]) for staphylococci to 18.2% (95%-CI = [2.2%, 51.8%]) for streptococci. Of the 26 Staphylococcus aureus isolates 24 were correctly identified to the species level by the rapid identification protocol (92.3%, 95%-CI = [74.9%, 99.1%]) and 72 of the 99 coagulase-negative Staphylococcus (72.7%, 95%-CI = [62.9%, 81.2%]). Among the enterococci the rapid identification protocol successfully identified 6 of 9 Enterococcus faecalis to the species level but only 1 of 8 E. faecium.

Of the 96 gram-negative pathogens detected in the BCs by the SOC, 87 were correctly identified by the rapid identification protocol (91.4%, 95%-CI = [83.8%, 93.2%]) with an excellent identification rate of 75 out of 80 for Enterobacterales (93.7%, 95%-CI = [86.0%, 97.9%]). Ten of the 13 non-fermenting Gram-negative rods identified by the SOC were also correctly

Table 1. (Continued)

| Identification by reference method (n) | Any identification by rapid protocol (n) | Correct identification by rapid protocol (n) | Concordance (%) |
|----------------------------------------|----------------------------------------|---------------------------------------------|-----------------|
| Stenotrophomonas maltophilia 1         | 0                                      | 0                                           | NA              |
| Gram-negative anaerobes 3              | 2                                      | 2                                           | 66.6%           |
| Bacteroides fragilis 1                 | 1                                      | 1                                           | NA              |
| Bacteroides thetaiotaomicron 1         | 1                                      | 1                                           | NA              |
| Eggerthella lenta 1                   | 0                                      | 0                                           | NA              |
| Other                                  |                                        |                                             |                 |
| Vibrio fluvialis 0                    | 1                                      | 0                                           | NA              |
| Yeast 3                                | 1                                      | 1                                           | 33.33%          |
| Candida albicans 1                    | 1                                      | 0                                           | NA              |
| Candida krusei 1                      | 1                                      | 1                                           | NA              |
| Candida glabrata 1                    | 1                                      | 0                                           | NA              |
| Total                                  | 266                                    | 202                                         | 74.44%          |

Table 2. Frequency distribution of bacteria and yeasts in the convenience sample and in all positive blood cultures for the reference year 2017.

| Group                                                        | Convenience sample | Total 2017 |
|--------------------------------------------------------------|--------------------|------------|
| Potential contaminants (skin or environmental)\(^{(1)}\)      | 109                | 1025       |
| Staphylococcus aureus                                       | 26                 | 215        |
| α-haemolytic streptococci                                   | 6                  | 94         |
| β-haemolytic streptococci                                   | 2                  | 33         |
| Streptococcus pneumonia                                    | 3                  | 22         |
| Enterococcus spp.                                            | 18                 | 171        |
| Enterobacterales                                            | 80                 | 585        |
| Pseudomonas aeruginosa                                      | 12                 | 74         |
| Non-fermenters (excl. P. aeruginosa)                        | 1                  | 19         |
| Anaerobes (excl. Propionibacterium spp.)                    | 6                  | 39         |
| Yeasts                                                      | 3                  | 76         |
| Others                                                      | 0                  | 11         |
| Total                                                       | 266                | 2364       |

\(^{(1)}\) Coagulase-negative staphylococci, spore-forming aerobes except Bacillus anthracis, non-sporeforming rods except Listeria spec. and Corynebacterium diphtheriae, Brevibacterium spec., Micrococcus spec., Propionibacterium (Cutibacterium) spec.

https://doi.org/10.1371/journal.pone.0267669.t002
identified by the rapid identification protocol (76.9%, 95%-CI = [46.2%, 95.0%]), which was not significantly different yet from the respective value for the Enterobacterales ($\chi^2 = 2.17$, df = 1, p > 0.1).

For anaerobes correct species identification was achieved in 3 of 6 samples and in 1 of 3 samples for yeasts.

Four pathogens were misidentified (all with a confidence level of 99.9%): One Streptococcus pneumoniae and one Streptococcus mitis/oralis were both misidentified as E. faecalis, one Parvimonas micra was misidentified as Vibrio fluvialis and one Escherichia coli was misidentified as Proteus mirabilis.

### Discussion

The present study evaluates the accuracy of the Vitek$^{\text{R}}$ MS for the direct identification of bacteria from positive BacT/Alert$^{\text{R}}$ BC bottles using a simplified version of a protocol first described by Foster [19]. The main aim was to reduce additional equipment and hands-on time to a minimum to allow easy integration into the routine laboratory work-flow at minimal extra cost. By making only a single attempt to identify the microorganism and by reducing the sample volume given in the original protocol[19] we could reduce the hands-on time for sample preparation and measurement from 30–45 min to about 15 min for even up to five samples. This is shorter than for most other protocols using a similar lysis-centrifugation approach for the combination of BacT/Alert$^{\text{R}}$ BC bottles and the Vitek$^{\text{R}}$ MS mass spectrometer [16,24–29].

The estimated costs per sample were 0.65 € for pellet extraction plus 1.15 € for the VitekMS$^{\text{R}}$ consumables, adding up to approx. 1.80 € per sample which is much cheaper than, e.g., the commercial Vitek$^{\text{R}}$ MS Blood Culture Kit (bioMérieux) with about 8€ per sample.

Despite its lower costs, the overall performance of our in-house protocol was yet comparable to the commercial Vitek$^{\text{R}}$ MS Blood Culture Kit (bioMérieux) with a reported 77.8% correct identification rate at the species level (95%-CI = [72.2%, 82.7%]) [27].

In line with previous studies using a similar approach [16,24–30] it showed in particular excellent performance for gram-negative bacteria, in particular Enterobacterales, P. aeruginos and S. aureus but failed so for streptococci and enterococci.

Previous studies using the combination of BacT/Alert$^{\text{R}}$ BC bottles and the Vitek$^{\text{R}}$ MS mass spectrometer consistently reported a higher rate of correct identifications for gram-negative bacteria (average 90.4%, 95%-CI = [76.5%, 98.5%]) than for gram-positive bacteria (74.9%, 95%-CI = [53.2%, 95.0%]) [16,24–30]. The thicker peptidoglycan layer of the gram-positive cell wall renders these bacteria more resistant to cleavage. Degrading of bacterial cell wall is an essential preparation step to achieve valid spectra by mass spectrometry. Therefore, optimized protocols for species identification of gram-positive bacteria using lysozyme or formic acid have been proposed for conventional mass spectrometry [31,32]. Albeit rapid detection protocols vary considerably, they all make no difference in the treatment of gram-positive and gram-negative bacteria. Given the altered composition of the cell walls of these bacteria, accounting for such difference is thus likely to result in further improvements yet.

During the course of this study two similar lysis-centrifugation protocols for the combination of BacT/Alert$^{\text{R}}$ BCs with the Vitek$^{\text{R}}$ MS of were published that showed overall identification rates of 83.9% (95%-CI = [80.6%, 86.9%]) [29] and 84.9% (95%-CI = [80.5%, 88.7%]) [30], respectively. They differ from the presented method mainly in the use of different detergents used for protein extraction (Triton (30) and saponin (29) respectively, instead of SDS), indicating that optimization of the extraction chemistry is likely to further improve the performance of rapid identification methods.
Limitations

Our study has several limitations. First, for direct ID from positive BC bottles, poly-microbial infections were excluded and, second, due to its relatively small size, only a limited number of some important organisms such as in particular yeasts and anaerobes were included. Third, we tested convenience samples as workforce availability in the routine diagnostic laboratory allowed for. However, positive BC bottles were tested in a routine diagnostic setting and the spectrum of organisms tested is representative of the mixture of BC isolates at our center. Last, our SOC was considered as a benchmark to study the performance of the optimized protocol. The SOC is continuously monitored by internal and external quality assessment (DAkkS). Therefore, we consider it unlikely that generated false bacterial species identification. However, we clearly cannot exclude misidentifications by the SOC.

Acknowledgments

We thank our technicians Barbara Conrad and Waltraud Flederer for their expert technical assistance.

Author Contributions

Conceptualization: Christoph Schoen.

Data curation: Johannes Forster, Britta Kohlmorgen, Doris Turnwald, Christoph Schoen.

Formal analysis: Johannes Forster, Christoph Schoen.

Investigation: Johannes Forster, Britta Kohlmorgen.

Methodology: Britta Kohlmorgen, Julian Haas, Philipp Weis, Lukas Breunig, Boris Mizaikoff, Christoph Schoen.

Project administration: Christoph Schoen.

Resources: Doris Turnwald, Christoph Schoen.

Supervision: Christoph Schoen.

Validation: Britta Kohlmorgen.

Visualization: Johannes Forster.

Writing – original draft: Johannes Forster, Christoph Schoen.

Writing – review & editing: Johannes Forster, Britta Kohlmorgen, Julian Haas, Philipp Weis, Lukas Breunig, Doris Turnwald, Boris Mizaikoff, Christoph Schoen.

References

1. Adhikari NK, Fowler RA, Bhagwanjee S, Rubenfeld GD. Critical care and the global burden of critical illness in adults. Lancet. 2010; 376(9749):1339–46. https://doi.org/10.1016/S0140-6736(10)60446-1 PMID: 20934212

2. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). Jama. 2016; 315(8):801–10. https://doi.org/10.1001/jama.2016.0287 PMID: 26903338

3. Buehler SS, Madison B, Snyder SR, Derzon JH, Cornish NE, Saubolle MA, et al. Effectiveness of Practices To Increase Timeliness of Providing Targeted Therapy for Inpatients with Bloodstream Infections: a Laboratory Medicine Best Practices Systematic Review and Meta-analysis. Clinical microbiology reviews. 2016; 29(1):69–103. https://doi.org/10.1128/CMR.00053-14 PMID: 26596385

4. Brozanski BS, Jones JG, Krohn MJ, Jordan JA. Use of polymerase chain reaction as a diagnostic tool for neonatal sepsis can result in a decrease in use of antibiotics and total neonatal intensive care unit
length of stay. Journal of Perinatology. 2006; 26(11):688–92. https://doi.org/10.1038/sj.jp.7211597 PMID: 17024143

5. Brown J, Paladino JA. Impact of Rapid Methicillin-Resistant Staphylococcus aureus Polymerase Chain Reaction Testing on Mortality and Cost Effectiveness in Hospitalized Patients with Bacteremia. Pharmacoeconomics. 2010; 28(7):567–75. https://doi.org/10.2165/11533020-00000000-00000 PMID: 20550222

6. Viek AL, Bonten MJ, Boel CH. Direct matrix-assisted laser desorption ionization time-of-flight mass spectrometry improves appropriateness of antibiotic treatment of bacteremia. PLoS One. 2012; 7(3): e32589. https://doi.org/10.1371/journal.pone.0032589 PMID: 22438880

7. Mizrahi A, Amzalag J, Couzigou C, Pean De Ponfily G, Pilmis B, Le Monnier A. Clinical impact of rapid bacterial identification by MALDI-TOF MS combined with the beta-LACTA test on early antibiotic adaptation by an antimicrobial stewardship team in bloodstream infections. Infectious diseases (London, England). 2018; 50(9):668–77.

8. Wellinghausen N, Wirths B, Franz AR, Karolyi L, Marre R, Reischl U. Algorithm for the identification of bacterial pathogens in positive blood cultures by real-time LightCycler polymerase chain reaction (PCR) with sequence-specific probes. Diagn Microbiol Infect Dis. 2004; 48(4):229–41. https://doi.org/10.1016/j.diagmicrobio.2003.11.005 PMID: 15062914

9. Peters RP, Savelkoul PH, Simoons-Smit AM, Danner SA, Vande nbroek e CM, van Agtmael MA. Faster identification of pathogens in positive blood cultures by fluorescence in situ hybridization in routine practice. J Clin Microbiol. 2006; 44(1):119–23. https://doi.org/10.1128/JCM.44.1.119-123.2006 PMID: 16390958

10. Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clinical microbiology reviews. 2013; 26(3):547–603. https://doi.org/10.1128/CMR.00072-12 PMID: 23824373

11. Loonen AJ, Jansz AR, Stalpers J, Wolffs PF, van den Brule AJ. An evaluation of three processing methods and the effect of reduced culture times for faster direct identification of pathogens from BacT/ALERT blood cultures by MALDI-TOF MS. European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology. 2012; 31(7):1575–83. https://doi.org/10.1007/s10096-011-1480-y PMID: 22080416

12. Meex C, Neuville F, Descy J, Huynen P, Hayette MP, De Mol P, et al. Direct identification of bacteria from BacT/ALERT anaerobic positive blood cultures by MALDI-TOF MS: MALDI Sepsityper kit versus an in-house saponin method for bacterial extraction. Journal of medical microbiology. 2012; 61(Pt 11):1511–6. https://doi.org/10.1099/jmm.0.044750-0 PMID: 22837218

13. Prod’hom G, Bizzini A, Durussel C, Bille J, Greub G. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for direct bacterial identification from positive blood culture pellets. Journal of clinical microbiology. 2010; 48(4):1481–3. https://doi.org/10.1128/JCM.01780-09 PMID: 20164269

14. Simon L, Ughetto E, Gaudart A, Degand N, Lotte R, Ruimy R. Direct identification of 80% of bacteria from blood culture bottles by MALDI-TOF MS using a 10-minute extraction protocol. Journal of clinical microbiology. 2018.

15. Ferroni A, Suarez S, Beretti JL, Dauphin B, Bille E, Meyer J, et al. Real-time identification of bacteria and Candida species in positive blood culture broth by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2010; 48(5):1542–8. https://doi.org/10.1128/JCM.02485-09 PMID: 20237092

16. Fothe r 1111, Kasinathan V, Hyman J, Walsh J, Drake T, Wang YF. Rapid identification of bacteria and yeasts from positive-blood-culture bottles by using a lysis-filtration method and matrix-assisted laser desorption ionization-time of flight mass spectrum analysis with the SARAMIS database. Journal of clinical microbiology. 2013; 51(8):805–9. https://doi.org/10.1128/JCM.02326-12 PMID: 23254131

17. Romero-Gomez MP, Mingorance J. The effect of the blood culture bottle type in the rate of direct identification from positive cultures by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. The Journal of infection. 2011; 62(3):251–3. https://doi.org/10.1016/j.jinf.2010.12.008 PMID: 21187113

18. Ruiz-Aragón J, Ballester o-Téllez M, Gutiérrez-Gutiérrez B, de Cueto M, Rodríguez-Baño J, Pascual Á. Direct bacterial identification from positive blood cultures using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry: A systematic review and meta-analysis. Enferm Infecc Microbiol Clin (Engl Ed). 2018; 36(8):484–92. https://doi.org/10.1016/j.eimc.2017.08.012 PMID: 29110928

19. Foster AG. Rapid Identification of microbes in positive blood cultures by use of the vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system. Journal of clinical microbiology. 2013; 51(11):3717–9. https://doi.org/10.1128/JCM.01679-13 PMID: 23985920
20. Liese JG, Schoen C, van der Linden M, Lehmann L, Goettler D, Keller S, et al. Changes in the incidence and bacterial aetiology of paediatric parapneumonic pleural effusions/empyema in Germany, 2010–2017: a nationwide surveillance study. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2019; 25(7):857–64. https://doi.org/10.1016/j.cmi.2018.10.020 PMID: 30395932

21. Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika. 1934; 26(4):404–13.

22. Team RC. R: A Language and Environment for Statistical Computing. Vienna, Austria. 2016.

23. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: The STARD Initiative. Ann Intern Med. 2003; 138(1):40–4. https://doi.org/10.7326/0003-4819-138-1-200301070-00010 PMID: 12513043

24. Broyer P, Perrot N, Rostaing H, Blaise J, Pinston F, Gervasi G, et al. An Automated Sample Preparation Instrument to Accelerate Positive Blood Cultures Microbial Identification by MALDI-TOF Mass Spectrometry (Vitek®MS). Front Microbiol. 2018; 9:911. https://doi.org/10.3389/fmicb.2018.00911 PMID: 29867822

25. Farina C, Arena F, Casprini P, Cichero P, Clementi M, Cosentino M, et al. Direct identification of microorganisms from positive blood cultures using the lysis-filtration technique and matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS): a multicentre study. New Microbiol. 2015; 38(2):245–50. PMID: 25938749

26. Machen A, Drake T, Wang YF. Same day identification and full panel antimicrobial susceptibility testing of bacteria from positive blood culture bottles made possible by a combined lysis-filtration method with MALDI-TOF VITEK mass spectrometry and the VITEK2 system. PLoS One. 2014; 9(2):e87870. https://doi.org/10.1371/journal.pone.0087870 PMID: 24551067

27. Randazzo A, Simon M, Goffinet P, Classen JF, Hougardy N, Pierre P, et al. Optimal turnaround time for direct identification of microorganisms by mass spectrometry in blood culture. J Microbiol Methods. 2016; 130:1–5. https://doi.org/10.1016/j.mimet.2016.08.019 PMID: 27558618

28. Wattal C, Oberoi JK. Microbial identification and automated antibiotic susceptibility testing directly from positive blood cultures using MALDI-TOF MS and VITEK 2. European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology. 2016; 35(1):75–82. https://doi.org/10.1007/s10096-015-2510-y PMID: 26597941

29. Barberino MG, Silva MO, Arraes ACP, Correia LC, Mendes AV. Direct identification from positive blood broth culture by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS). Braz J Infect Dis. 2017; 21(3):339–42. https://doi.org/10.1016/j.bjid.2017.03.007 PMID: 28399425

30. Samarawayake W, Dempsey S, Howard-Jones AR, Outhred AC, Kesson AM. Rapid direct identification of positive paediatric blood cultures by MALDI-TOF MS technology and its clinical impact in the paediatric hospital setting. BMC Res Notes. 2020; 13(1):12. https://doi.org/10.1186/s13104-019-4861-4 PMID: 31907060

31. McElvania Tekippe E, Shuey S, Winkler DW, Butler MA, Burnham C-AD. Optimizing identification of clinically relevant Gram-positive organisms by use of the Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry system. Journal of clinical microbiology. 2013; 51(5):1421–7. https://doi.org/10.1128/JCM.02680-12 PMID: 23426925

32. Smole SC, King LA, Leopold PE, Artbeit RD. Sample preparation of Gram-positive bacteria for identification by matrix assisted laser desorption/ionization time-of-flight. J Microbiol Methods. 2002; 48(2–3):107–15. https://doi.org/10.1016/s0167-7012(01)00315-3 PMID: 11777561