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Direct identification of bacteria from BacT/ALERT® blood cultures using the VITEK® MS MALDI TOF Mass Spectrometry system.

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Objectives

Blood cultures are critical clinical samples processed by a clinical microbiology laboratory. Rapid identification and reporting of organisms present in positive blood cultures has the potential to positively impact on patient management. The rapid identification of organisms present allows earlier targeted antimicrobial therapy and allows clinicians to manage care pathways more effectively.

Here, we evaluated the performance of a new lysis - filtration method[1] in conjunction with the bioMérieux VITEK® MS MALDI TOF mass spectrometry system for the direct identification of blood culture isolates obtained from samples of non-charcoal containing positive BacT/ALERT® blood cultures.

Methods

Blood was inoculated into non-charcoal containing BacT/ALERT® SA and/or SN bottles (bioMérieux, France) and incubated on a BacT/ALERT® automated blood culture system (bioMérieux, France). When bottles flagged as positive, a Gram's stain was performed and bottles were cultured for routine identification alongside the trial protocol[1] described below:.

A 2mL aliquot of positive blood culture was treated for 2 minutes with 1mL lysis buffer (0.6% w/v Brij 97 in 0.4M CAPS), the lysate was added to a 0.45µm membrane filter (Millipore, United Kingdom) under vacuum.

The bacterial film was washed 3 times in wash buffer (20 mM sodium phosphate, 0.05% w/v Brij 97, 0.45% w/v NaCl) and then further washed using deionized water. The bacterial film was harvested using a Texwipe CleanTips® Swab , transferred to a disposable target slide (bioMérieux, France) and air dried.

Bacterial isolates were overlaid with 1µL of a-cyano-4-hydroxycinnamic acid (C.H.C.A.) matrix (bioMérieux, France). Fungal isolates were overlaid with 0.5µL Formic Acid (bioMérieux, France), air dried and overlaid with matrix.

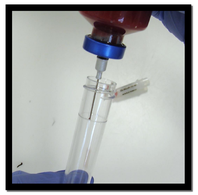
The isolates were tested in a VITEK® MS mass spectrometry system and resulting spectra were analysed in MYLA® software. (bioMérieux, France).

Identifications obtained were compared to the identifications obtained from colonies recovered on sub-culture by routine methods in use within the laboratory at City Hospital, Birmingham.

Discordant identifications and isolates which were unable to be identified using routine methods were characterized using 16s rDNA sequencing. 216 bottles from 146 patients aged 18-98 years (mean = 66y) were analysed using the described protocol.

Methods (Continued)

Figure 1. Pictorial overview of method.

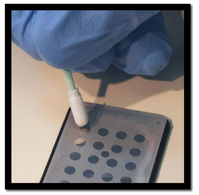

1. Aspirate 2mL of blood.

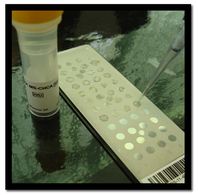

2. Add 1mL of lysis buffer

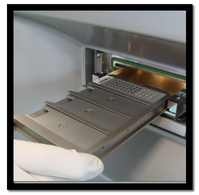

3. Filter lysate


4. Add wash buffer.


5. Harvest microbial cells


6. Inoculate target slide


7. Add 1uL of Matrix


8. Analyse in VITEK MS


9. Obtain identification

Time from unloading bottle to bacterial identification <30 minutes.

Results

In 162/197 (82.2%) mono-microbic bottles, the direct identification obtained matched the recovered isolate.

In 19 multi-microbic bottles, the direct identification obtained matched one or more of the recovered isolates in 15 bottles (78.9%) .

In total, 177/216 bottles (81.9%) gave an identification which proved correct and matched an organism recovered on subculture.

No identification was obtained for 35 bottles, either due to the absence of isolates in the database or insufficient spectra generated for analysis. In only 4 of the 216 bottles was direct identification incorrect.

Identification of one or more of the recovered isolates was obtained in 83.4% of aerobic bottles (SA) and in 80.3% of anaerobic (SN) bottles.

Results (Continued)

Table 1. Organisms identified directly from blood cultures using lysis-filtration method.

Species Identified	Total (n=177)
Staphylococcus species (epidermidis/hominis/capitis/haemolyticus)	57
Escherichia coli	40
Enterobacter species (cloacae,gergoviae,asburiae)	18
Staphylococcus aureus	11
Proteus mirabilis	9
Klebsiella species (pneumonia/oxytoca)	8
Streptococcus species (salivarius/mitis/oralis/parasanguinis)	8
Enterococcus faecium	6
Fusobacterium species (necrophorum/nucleatum)	3
Pseudomonas aeruginosa	3
Streptococcus agalactiae	3
Granulicatella adiacens	2
Micrococcus species (luteus/lylae)	2
Streptococcus dysgalactiae	2
Acinetobacter baumanii	1
Bifidobacterium species	1
Corynebacterium aurimucosum	1
Kocuria kristinae	1
Stenotrophomonas maltophilia	1

When only isolates within the database were considered, direct identification of one or more of the isolated bacteria was obtained in 177/207 (85.5%) bottles.

Conclusions

The VITEK MS MALDI-TOF Mass spectrometry system combined with the described lysis - filtration method is a rapid and accurate method for direct identification of pathogens from positive blood cultures.

The time to identification of organisms present in positive blood cultures is significantly reduced from overnight to under 30 minutes. Importantly, this method is very simple to perform.

Although no yeast species were isolated during the trial period, subsequent use of the protocol has shown excellent concordance when a yeast species is present in blood.

References

Fothergill A et. al. 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. J. Clin. Microbiol. March 2013 ; 51:3 805-809