Rapid identification of bacteria from bioMérieux BacT/ALERT blood culture bottles by MALDI-TOF MS

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ABSTRACT

Several studies have reported poor results when trying to identify microorganisms directly from the bioMérieux BacT/ALERT blood culture system using matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry. The aim of this study is to evaluate two new methods, Sepsityper and an enrichment method for direct identification of microorganisms from this system. For both methods the samples were processed using the Bruker Microflex LT mass spectrometer (Biotyper) using the Microflex Control software to obtain spectra. The results from direct analysis were compared with those obtained by subculture and subsequent identification. A total of 350 positive blood cultures were processed simultaneously by the two methods. Fifty-three cultures were polymocrobial or failed to grow any organism on subculture, and these results were not included as there was either no subculture result, or for polymicrobial cultures it was known that the Biotyper would not be able to distinguish the constituent organisms correctly. Overall, the results showed that, contrary to previous reports, it is possible to identify bacteria directly from bioMérieux blood culture bottles, as 219/297 (74%) correct identifications were obtained using the Bruker Sepsityper method and 228/297 (77%) were obtained for the enrichment method when there is only one organism was present. Although the enrichment method was simpler, the reagent costs for the Sepsityper method were approximately £4.00 per sample compared to £0.50. An even simpler and cheaper method, which was less labour-intensive and did not require further reagents, was investigated. Seventy-seven specimens from positive signalled blood cultures were analysed by inoculating prewarmed blood agar plates and analysing any growth after 1-, 2- and 4-h periods of incubation at 37°C, by either direct transfer or alcohol extraction. This method gave the highest number of correct identifications, 66/77 (86%), and was cheaper and less labour-intensive than either of the two above methods.

KEY WORDS: Bacteria.
Blood culture.
Spectrometry, mass,
matrix-assisted laser desorption-ionization.

Introduction

The bioMérieux BacT/ALERT 3D blood culture system is a rapid culture system designed to incubate and monitor blood culture specimens and signal microbial growth at the earliest possible time. The system uses bottles containing a liquid medium, in some cases with the addition of charcoal, and a specific atmosphere, either aerobic or anaerobic. Matrix–assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry examines the mass region (2000–20,000 Da) where ribosomal proteins are found within a bacterial cell.

The MALDI Biotyper (Bruker, Germany) is a software programme for analysing spectra generated by the Microflex LT mass spectrometer, which are then compared to the Biotyper database for identification and relationship analysis. The combination of MALDI-TOF and the BacT/ALERT 3D technologies can provide clinicians with reliable results to species level within hours of a signalled positive culture, thereby enhancing patient care.

Since 2010, several studies have reported the identification of bacteria directly from signal positive blood cultures systems, nearly always the BD BACTEC system.[1,2] However, Szabados and colleagues reported poor results when trying to identify microorganisms directly from positive blood cultures from the bioMérieux BacT/ALERT 3D blood culture system using MALDI-TOF.[3] Their identification rate was 78/268 (29%) of samples identified with scores of 1.7 to >2.0, which were poor regardless of the presence of charcoal.

The aim of this study is to evaluate two new methods for direct identification of microorganisms from the bioMérieux BacT/ALERT 3D blood culture system when combined with MALDI-TOF analysis. Subsequently, a rapid plate culture method is evaluated.

Materials and methods

The system used for analysis was the Bruker (MALDI-TOF) Microflex LT, using Bruker Biotyper software and database number 3740. The different bottle types used in this study were the aerobic culture bottle with charcoal (BacT/ALERT 3D FA bottle), paediatric with charcoal (BacT/ALERT 3D PF bottle), anaerobic culture bottle without charcoal (BacT/ALERT 3D SN bottle) and the aerobic culture bottle without charcoal (BacT/ALERT 3D SA bottle).

The selection of signal positive bottles analysed was random and gave a distribution of 151 FA bottles, 118 SN bottles, 27 PF bottles and one SA bottle. These had all been

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inoculated with patient blood in wards and departments of The Royal London Hospital. Eight positive bottles were selected at random and samples taken for both Sepsityper and enrichment methods. The enrichment method was performed first, followed by the Sepsityper, where there was approximately an hour between the two.

A Gram stain of the positive blood culture was obtained first, and was used to decide the choice of method to be employed. Depending on the Gram film result and the presence of charcoal, one of three methods was chosen (Fig. 1). All direct analysis methods were compared to 18- to 24-hour subculture (horse blood agar incubated in 5% CO_2 and anaerobic conditions at 35–37°C), where the subsequent colonies were analysed on the MALDI-TOF, which was our reference method.

Enrichment method for positive FA blood culture with presumptive staphylococci (method 1)

This is a specific method due to the binding of staphylococci with charcoal. The positive BacT/ALERT FA blood culture bottle was mixed by inversion, and then an 8.0 mL sample of broth was removed and dispensed into a sterile 15 mL conical tube (Falcon). The tube was placed in a U50 ultrasonic water bath (Ultrawave, Cardiff, UK) for 10 min, then vortex-mixed for 10 sec. Flocculant buffer (2 mL; 30% [w/v] dextran 70, 0.70 mol/L $CaCl_2$, 0.15 mol/L NaCl) was added to the tube, mixed by inversion and centrifuged in an Eppendorf 5417c centrifuge for 10 min at 100 x*g* with the brake off.

Supernatant (5 mL) was removed from the area immediately above the supernatant/charcoal interface without drawing up charcoal particles. This was added to a 15 mL conical tube containing 5 mL wash buffer (0.005% [w/v] Brij-97 in deionised water), then vortex-mixed briefly and centrifuged for 10 min at 3000 x*g* using an Eppendorf 5417C centrifuge with a swing arm (Eppendorf AG, Germany Type A-8-11) rotor.

The supernatant was discarded and 1 mL wash buffer was used to resuspend the pellet. The suspension was layered over 500 µL density cushion (24% [w/v] CsCl, 0.005% [w/v] Brij-97, 10 mmol/L HEPES [pH 7.4]), preloaded into a clean 1.5 mL microfuge tube. This was centrifuged at 10,000 x*g* for 2 min, with the centrifuge set to soft acceleration and braking.

The supernatant was discarded and the pellet washed again with 1-mL wash buffer, vortex-mixed for 5 sec and centrifuged at 10,000 x*g* for 2 min. The supernatant was discarded again and the pellet resuspended in 50 µL 70% formic acid, ensuring a minimum contact time of 2 min between the formic acid and sample prior to adding 50 µL acetonitrile. The tubes were centrifuged at 10,000 x*g* for 2 min.

Extract supernatant (1 µL) was pipetted onto each of two MALDI target spots. A 1 in 10 dilution of the supernatant was also processed (diluted with a 50:50 mix of 70% formic acid and acetonitrile). HCCA matrix (1 µL) was pipetted over the dried protein spots and left to air dry before analysis.

Enrichment method for use with positive FA blood cultures excluding staphylococci (method 2)

The positive FA bottle was mixed and vented as in method 1. Broth (5.0 mL) was removed and dispensed into a sterile 15-mL conical tube. The tube was vortex-mixed for 5 sec then centrifuged at 50 x*g* for 5 min with the brake off. Using a pipette, the tip was placed 1–2 mm below the surface and 500 µL broth was withdrawn. The broth was carefully layered over 500 µL Density Cushion as in the previous method. The tubes were centrifuged at 10,000 x*g* in a swinging bucket rotor for 2 min with the centrifuge set to soft acceleration and braking.

The supernatant was discarded, removing as much liquid as possible from the charcoal-microorganism pellet. The pellet was resuspended with 1-mL wash buffer by pipetting until the pellet was uniformly dispersed. The tube was vortex-mixed for 5 sec, and then centrifuged at 10,000 x*g* for 2 min. The supernatant was discarded as before and resuspended in 50 µL 70% formic acid, ensuring a minimum contact time of 2 min between the formic acid and sample prior to adding 50 µL acetonitrile then mixed as before. The tubes were centrifuged at 10,000 x*g* for 2 min. Extracts were prepared for Microflex analysis as in method one.

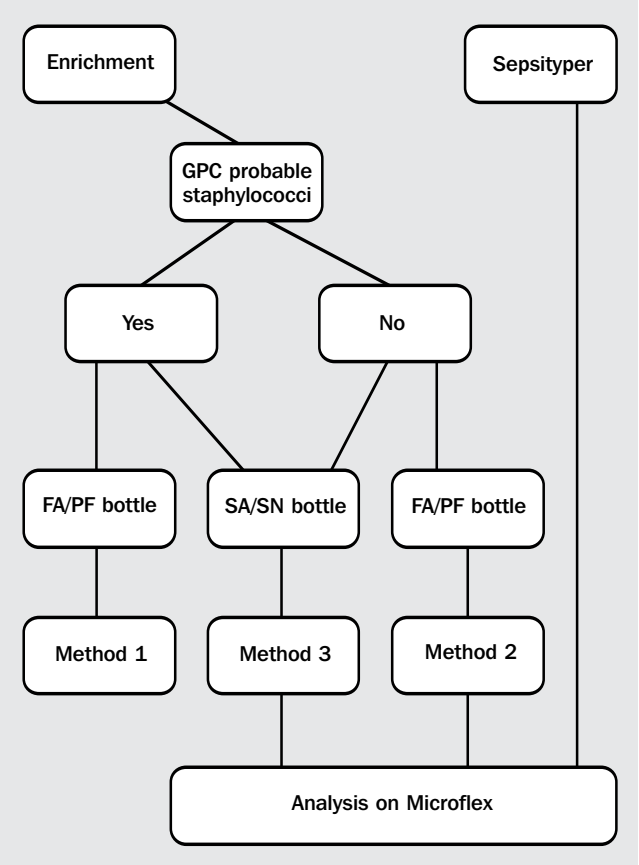

Fig. 1. Selection of different methods to process positive blood cultures depending on Gram stain and media type.

Enrichment method for positive SA/SN blood cultures (method 3)

Broth (700 µL) was removed from the positive bottle and added to a 1.5 mL conical centrifuge tube. Lysis buffer (350 µL) was added to the tube and vortex-mixed for 5 sec, and then allowed to lyse for 1 min. The lysate was slowly layered on 500 µL of Density Cushion in a separate 1.5 mL conical centrifuge tube. The tube was centrifuged at 10,000 x*g* in a swinging bucket rotor for 2 min with the centrifuge set to soft acceleration. The supernatant was discarded as before, and the pellet was resuspended with 1.0 mL saline

Table 1. Summary of results obtained from two direct blood culture analysis methods.

			Sepsityper method				Enrichment method			
Organism type	Total (%)	Charcoal containing bottles (%)	Correctly identified (%)	Unable to identify (%)	Incorrect ID (%)*	ID failure from bottles containing charcoal (%)	Correctly identified (%)	Unable to identify (%)	Incorrect ID (%)*	ID failure from bottles containing charcoal (%)
GNB	122 (41)	67 (55)	111 (91)	11 (9)	0 (0)	9 (82)	107 (88)	15 (12)	0 (0)	12 (80)
All staphylococci	127 (43)	88 (69)	84 (66)	43 (34)	2 (2)	38 (88)	102 (80)	25 (20)	3 (2)	22 (88)
S. aureus	22 (7)	12 (55)	15 (68)	7 (31)	0 (0)	5 (83)	20 (91)	2 (9)	0 (0)	1 (50)
CNS	105 (83)	76 (72)	69 (66)	36 (34)	2 (2)	32 (89)	82 (78)	23 (22)	3 (2)	21 (91)
All streptococci	27 (9)	16 (59)	19 (70)	8 (30)	1 (4)	7 (88)	17 (63)	10 (37)	1 (4)	7 (70)
GPB	14 (4)	3 (21)	5 (36)	9 (64)	0	2 (22)	2 (14)	12 (86)	0 (0)	3 (25)
Yeast	6 (2)	NA	0 (0)	6 (100)	0	NA	0 (0)	6 (100)	0 (0)	NA
GNC	1(<1)	NA	0 (0)	1 (100)	0	NA	0 (0)	1 (100)	0 (0)	NA
TOTAL	297		219 (74)	78 (26)	3 (1)		228 (77)	69 (23)	4 (1)	

GNB: Gram-negative bacilli; CNS: coagulase-negative staphylococci; GPB: Gram-positive bacilli; GNC:Gram-negative cocci
*See Table 2.

solution. The tube was vortexed for 5 seconds and then centrifuged at 10,000 g for 2 minutes. The supernatant was discarded as before and the deposit resuspended by adding 50-µL (25-µL if the pellet was <10 µL) 70% formic acid. An equal volume of acetonitrile was added to maintain a 1:1 ratio, and then centrifuged at 10,000 x*g* for 2 min. Extracts were prepared for Microflex analysis as in method 1.

Sepsityper method

Broth (1 mL) was removed from the positive blood culture bottle, as described previously, and put into an Eppendorf 1.5 mL centrifuge tube. Solution 1 (200 µL) was added and vortex-mixed. The total volume was pipetted into a SigmaPrep (Sigma-Aldrich, Poole, Dorset, UK) spin column and centrifuged at 420 x*g* for 2 min. The filtrate was transferred to a centrifuge tube and centrifuged at 17,900 x*g* for 2 min. The supernatant was discarded and the pellet resuspended in Solution 2 (1 mL). This was then centrifuged at 17,900 x*g* for 2 min and the supernatant discarded.

Table 2. Cases where identification differed from the direct and subculture ID.

Sepsityper ID	ID obtained after culture
Staphylococcus warneri	*Staphylococcus epidermidis*
Staphylococcus epidermidis	*Staphylococcus hominis*
*Abiotrophia defectiva**	*Rothia mucilaginosa*
Enrichment ID	**ID obtained after culture**
Staphylococcus warneri	*Staphylococcus epidermidis*
Staphylococcus epidermidis	*Staphylococcus hominis*
*Staphylococcus hominis**	*Staphylococcus epidermidis*
Enterococcus avium	*Enterococcus raffinosus*

*These IDs were produced only after manual spectra modification, without which there was previously no reliable ID.

The pellet was resuspended in 300-µL deionised water, and then 900-µL 100% ethanol (HPLC grade) was added and mixed. The tube was centrifuged at 17,900 x*g* for 2 min, supernatant and the deposit resuspended in 5–25 µL 70% formic acid, depending upon the deposit size. An equal volume of acetonitrile was added and the tube was flicked to mix, then centrifuged at 17,900 x*g* for 2 min. Supernatant (1 µL) was pipetted onto the target plate and allowed to air-dry. Matrix (HCCA; 1 µL) was pipetted onto the dried protein spot and also allowed to air-dry.

Bruker's identification parameters state that scores of 1.700–1.999 are acceptable to the genus and that scores of 2.000–3.000 are acceptable to species level. We accepted scores of 1.700–3.000 as identification to the species level. Our experience using the MALDI-TOF instrument routinely had not shown any results to contradict these parameters. A dilution of 1 in 10 was performed with a 50:50 solution of 70% formic acid and acetonitrile.

Manual spectrum manipulation

This was achieved using the Bruker Flexanalysis (spectra analysis) software. In this programme, the sum spectra were manipulated, removing the lower molecular weight range of 2000–3000 Da. This new spectra was then reanalysed in the Biotyper software and compared to the database (Table 3).

Dilution of formic extract

When a read of 'No Peaks' was resulted for the Sepsityper method, the formic acid extract was diluted first by 1 in 10 and then further by 1 in 100 if a 'No Peaks' result was obtained for a second time. This was achieved by adding 10 µL original extract to 90 µL of a 50:50 solution of 70% formic acid and acetonitrile (or 1 µL to 99 µL, respectively) mixing and spotting 1-µL on the target plate and then covering with 1-µL matrix when air dry.

Direct plating culture with short incubation method

Horse blood agar plates (Oxoid, Basingstoke, UK) were prewarmed before inoculation with the positive blood

culture broth. The plates were placed in a 37°C incubator for 1 h. Ten drops of broth were removed from the blood culture bottle using a sterile airway needle (bioMèrieux, Basingstoke, UK) and plated onto the prewarmed blood agar plates.

The positive blood culture broths were Gram stained as in the previous methods. If from the Gram stain morphology it was suspected that the organism was a *Haemophilus* species a chocolate agar (Oxoid, Basingstoke, UK) plate was used instead of blood agar.

Plates were inoculated with the positive blood culture broth and then incubated in a 5% CO_2 atmosphere at 37°C for 1 h in the case of Gram-negative bacilli, and 2 h in the case of Gram-positive cocci, Gram-positive bacilli and Gram-negative cocci. After the relevant incubation period, growth was harvested from the agar plate using a 10 µL inoculation loop or a 'cocktail' stick when directly inoculating the target plate. At this incubation time it was not possible to see colonies; however, it was possible to see a haze of growth on the agar, either on, or leading from, the primary inoculum.

Growth was transferred as above into an Eppendorf centrifuge tube (1.5 mL) containing 1-mL sterile deionised water. Only the tubes for charcoal-containing bottles were vortex-mixed on maximum for several seconds and then centrifuged at 400 xg for 2 min. The supernatant was carefully removed to another Eppendorf, making sure that the maximum amount of fluid was removed without disturbing the charcoal pellet. These samples were then added to any non-charcoal samples and centrifuged together at 17,900 x*g* for 2 min. The supernatant was discarded and the pellet resuspended in 300-µL deionised water, to which 900 µL 100% ethanol was added.

The tube was inverted several times and then centrifuged at 17,900 x*g* for 2 min. All the ethanol solution was removed by tipping off into a discard pot, recapped and pulse spun for 20 sec, with any remaining ethanol solution pipetted away, leaving the deposit intact.

The deposits were then treated following the Bruker ethanol/formic acid extraction protocol (Section 3.3, MALDI Biotyper 2.0 manual) used in the previous methods. The resultant extraction product was pipetted onto an MSP 96 steel target plate as in the previous methods.

Results

Comparison of results obtained by enrichment and Sepsityper methods

A total of 350 positive blood cultures were processed simultaneously both by enrichment and Sepsityper methods. Thirty-seven cultures yielded more than one organism and 16 failed to grow any organism upon subculture. Of the 16 that failed to grow upon subculture, six were 'No organisms seen', two were GPR, four were probable GPC strep, two were GPC staph and two were possible GPC but unable to say staph- or strep-like. These were removed from the following analysis, which left 297 for comparison. Overall, the two methods gave similar results, with 219/297 (74%) correct identifications obtained with the Bruker Sepsityper method and 228/297 (77%) for the enrichment method (Table 1).

Gram-negative bacilli (GNB) were correctly identified in 111/122 (91%) and 107/122 (88%) of cases, respectively. Gram-positive bacilli (GPB) proved to be the most difficult organisms to identify, with only 2/14 (14%) correctly identified by the enrichment method compared to 5/14 (36%) with the Sepsityper system. There was only one occasion when the Gram stain result adversely affected the method choice for that sample; this was where the Gram result was GPC ?streptococci, and upon subculture the organism proved to be *Staphylococcus haemolyticus*. The staphylococcal protocol was not employed and there was no direct result for either Sepsityper or enrichment method. There were no significant differences between the results obtained by either method for any type of organism (χ^2 test with Yates correction: $P>0.5$ in all cases; Table 1).

The enrichment method employed double spotting of the formic extract. At no time was there a discordant result; had there been then the sample would have been repeated. The same was true of the neat and diluted extracts. The presence of charcoal in the blood culture medium had an impact on the ability of both methods to provide good identification. Of the 11/122 (9%) GNB that failed to give a valid identification with the Sepsityper method, 9/11 (82%) were isolates from charcoal bottles. Similarly, 15/122 (12%) of GNB with the enrichment method failed to ID, of which 12/15 (80%) contained charcoal.

No yeasts were identified by either method; however, there were only six such isolates out of the 297 specimens sampled. Dilution of the extract was beneficial, as 60/297 (20%) additional IDs were achieved with both methods when this was employed.

Table 3. All cases where manual spectra modification was required to obtain a reliable ID by both direct analysis methods.

Organism ID	
Sepsityper method	**Enrichment method**
Abiotrophia defectiva[*]	*Enterobacter cloacae*
Acinetobacter baumanii (2)	*Klebsiella pneumoniae* (2)
Corynebacterium striatum	*Propionibacterium acnes*
Enterococcus faecium	*Pseudomonas aeruginosa* (2)
Enterococcus raffinosus	*Salmonella* sp.
Escherichia coli (3)	*Staphylococcus aureus* (2)
Klebsiella oxytoca	*Staphylococcus epidermidis* (2)
Klebsiella pneumoniae (5)	*Staphylococcus hominis*[†]
Propionibacterium acnes	*Staphylococcus hominis*
Proteus mirabilis	*Streptococcus pneumoniae*
Pseudomonas aeruginosa (4)	
Salmonella sp.	
Staphylococcus aureus (3)	
Staphylococcus epidermidis (11)	
Staphylococcus hominis (2)	
Streptococcus agalactiae	
Streptococcus gallolyticus	
Streptococcus pneumoniae	
Streptococcus pyogenes	

[*]Misidentified. Subculture result *Rothia mucilaginosa*

[†]Misidentified. Subculture result *Staphylococcus epidermidis*

Table 4. Summary of results obtained by plating positive blood culture samples on prewarmed plates and incubating for up to four hours.

			Incubation					
			1 hour		2 hours		4 hours	
Organism type	Total (%)	Correct ID	Direct	Extract	Direct	Extract	Direct	Extract
GNB	26 (39)	25 (96)	19 (73)	24 (92)	24 (92)	25 (96)	24 (92)	25 (96)
Staphylococci	20 (30)	19 (95)	NT	NT	7 (35)	19 (95)	11 (55)	19 (95)
Streptococci	12 (18)	11 (92)	NT	NT	6 (50)	10 (83)	11 (92)	11 (92)
GPB	2 (<1)	1 (50)	NT	NT	0	0	0	1 (50)
Yeast	4 (5)	1 (25)	NT	NT	0	0	0	1 (25)

NT: Not tested

There were 30 mixed cultures in total. The Sepsityper method gave a correct ID for one of the constituent organisms in 10 of these, but three were incorrect when compared to the result obtained from subculture. The enrichment methods gave a similar result of 14 single organism IDs out of 30, two of which were incorrect.

Positive blood culture bottles that failed to grow an organism upon subculture were excluded from the analysis as there was no culture result to use for comparison. It was not possible with the current software to identify multiple organisms within a mixture. Therefore, mixtures were also excluded from the main analysis. Organisms identified by the Sepsityper method from samples that failed to grow upon subculture were single cases of *Propionibacterium acnes*, *Staphylococcus hominis*, *Streptococcus pneumoniae* and *Corynebacterium aurimucosum*; these were not identified by the enrichment method.

Direct plating method (Table 4)

Eighty positive blood culture broths were examined by the direct plating method. After 18- to 24-hours' culture, eight samples proved to be polymicrobial and six were 'no growth'. These were removed from the analysis.

Presumptive Enterobacteriaceae plates were examined after 1-h incubation, which yielded a 92% (24/26) identification rate when extracted. Similarly, staphylococci had an identification rate of 95% (19/20) after 2-h incubation when extracted. Streptococci required 4-h incubation to give the highest identification rate for these organisms (100%; 12/12). At these short incubation times GPB and yeasts were much more difficult to identify.

Overall for GNB, staphylococci and streptococci, the identification rate was 95% (55/58) at two hours and 97% (56/58) at four hours by extraction. The direct analysis worked almost as well for this group, with an overall identification rate of 67% (39/58) and 97% (56/58) at two and four hours, respectively. Only formic acid extraction after 4-h incubation yielded any result for GPB and yeast.

Discussion

Both direct broth methods gave similar results overall. Two sets of results were prominent; the first being the better performance of the enrichment method with staphylococcal-like organisms, and the second where the Sepsityper method identified 3/14 (21%) more GPB isolates. It is likely that the better performance of the enrichment method in the identification of staphylococci was due to the additional steps in the protocol. These additional steps were designed to detach bound cocci from charcoal particles via sonication and the use of a flocculant buffer. We were following protocols that were not devised by ourselves, and therefore we did not want to deviate from them. It is unclear why the Sepsityper method worked better with regard to GPB.

Positive signalled bottles that failed to grow upon subculture (Table 5) gave an identification by Sepsityper for four out of the 16, and six were 'No Organisms Seen' (NOS). Positive bottles with NOS were explained by the presence of a greater than usual number of white blood cells in the sample. The six samples that had a positive Gram stain but failed to grow and failed direct ID were explained by the presence of stain deposit. Of the four samples that were

Table 5. Positive blood culture bottles that were 'No Growth' on subculture

Gram stain result	Sepsityper result	Enrichment result
GPC ?staph	*Staphylococcus hominis*	NRI
GPC	No peaks	NRI
GPC ?staph	NRI	NRI
GPC ?staph	NRI	NRI
GPC ?strep	NRI	NRI
GPC ?strep	No peaks	NRI
GPC ?strep	*Streptococcus pneumoniae*	NRI
GPC ?strep	NRI	NRI
GPR	*Corynebacterium aurimucosum*	NRI
GPR	*Propionibacterium acnes*	NRI
No orgs seen	NRI	NRI
No orgs seen	NRI	NRI
No orgs seen	NRI	NRI
No orgs seen	NRI	NRI
No orgs seen	NRI	NRI
No orgs seen	NRI	NRI

NRI: No reliable identification.

Gram-positive and Sepsityper identified, one was a *Streptococcus pneumoniae* that had autolysed in the blood culture bottle. The other three produced a small amount of growth in the bottle and routine culture plates were not given extended incubation.

The direct plating method achieved an overall higher percentage ID, though this was a much smaller sample number, and these samples were not the same as those run in the other two methods.

The presence of charcoal had a significant effect on identification of GNB, staphylococci and streptococci, but it did not reduce the number of identifications to a level that would make it an unsuitable method of identification in the diagnostic laboratory. Charcoal had the greatest effect upon the correct identification of staphylococci by the Sepsityper method. As this method did not employ any steps to detach staphylococci from the charcoal particles, it is not surprising that the identification rate was lower. The presence of charcoal in the final extract inhibited identification; therefore, it was necessary to use the spin column filter to reduce the amount of charcoal coming through to the final stage. However, removing the majority of the charcoal also reduced the number of staphylococci identified. This was due to the binding that occurred between staphylococci and the charcoal particles. Relative to GPC and GNB, the GPB and yeasts had very low identification rates, which may be due to there often being low numbers of these organisms seen in the Gram film when the blood culture bottle flagged positive. There were also far fewer of these types of organism in the comparison.

Dilution of the formic extract increased identification by 22% with the enrichment method and 13% with the Sepsityper; a result consistent with the observation that ineffective matrix binding can be caused by the availability of too much target protein.

Manual modification of spectra was only performed when no identification was made initially, and only recorded when a sample was identified (Table 3). However, this did not have a significant effect on enhancing identification for either method, although the ID rate was most improved with the Sepsityper method (42/272; 15%). However, as shown in Table 2, this method could lead to misidentification and is not recommended.

Three isolates were misidentified by Sepsityper and four by enrichment. These were mainly staphylococci, where only the genus was correct. One isolate was identified as an *Enterococcus* by the enrichment method, which again was incorrect at species level, and one isolate by the Sepsityper method was incorrect at the genus level. This constitutes approximately 1% of the blood cultures studied. Thus, it may be prudent to treat results from the direct blood culture analysis as provisional in the clinical setting, and wait for conformation from plate culture before sending out a final report.

The Sepsityper method had far fewer steps and was therefore quicker and easier to follow. The lysis element of the enrichment method meant it was not possible in practice to process more than four samples at a time. The first two had to be lysed initially, followed by the second two once they were being centrifuged; therefore, samples had to be processed in sets of fours. We feel that the complexity of the enrichment method would make it an unlikely prospect for the routine diagnostic laboratory.

Overall, the results show that, contrary to previous reports, it is possible to identify bacteria directly from bioMérieux blood cultures in approximately three-quarters of cases when only one organism is present, and, provided the correct procedures are in place within the laboratory, this method would provide valuable data to enhance empirical prescribing regimes.

The study by Szabados *et al*. showed a much lower percentage of successful identifications. This was probably due to the sample preparation, which was much simpler than that employed in this study. The use of Vaccuette gel tubes (Greiner) and performing a simple wash step with deionised water proved insufficient to purify and concentrate the target microorganisms.

Recently, several workers have reported the effect of 'homemade' reagents to extract positive blood cultures.[4,5] These perform as well as the more expensive commercial methods; however, they still require considerable labour time to process the specimens, and, to be of value, this has to commence as soon as possible when a positive blood culture is detected.

Owing to the complexity and cost of the direct methods, we subsequently evaluated a rapid plate culture method. This yielded a higher overall percentage identification rate (86%) across all organism types, incubation times and methods tested, with a 97% ID rate for Enterobacteriaciae (Table 4), and much higher ID rates for staphylococci (87%) and streptococci (92%). The fact that the direct transfer method yielded excellent results suggests that this would be a simple and quick method for the diagnostic laboratory. It is worth noting that this method produced higher identification rates with less operator time and reagent outlay, and, compared to the enrichment method, is much easier. This method has the advantage that it will work irrespective of the type of blood culture system employed or future changes to these culture system components. Its disadvantage is that even if cultures are incubated anaerobically, obligate anaerobes do not grow quickly enough to be analysed by this method.

Recently, Klein *et al*. suggested that it would be best practice to ID presumptive staphylococci by a polymerase chain reaction (PCR) method only, and to process other Gram-positive cocci both by PCR and MALDI-TOF. Using the rapid plate method, accurate results can be achieved in a similar time frame to that of a PCR method, and with the use of a commercial penicillin binding protein kit test (e.g., Clearview Exact PBP2a; Alere, Stockport UK), which can be run at the same time as the extraction, rapid identification of methicillin-resistant *Staphylococcus aureus*, if present, is possible.

The addition of these steps to the rapid plate method would achieve similar results to those described by Klein *et al*, but at lower cost. Furthermore, the rapid plate method has been in use in the diagnostic laboratory at The Royal London Hospital for over two years and has proved reliable. □

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