

# 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

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In cases of bacteraemia, a rapid species identification of the causal agent directly from positive blood culture broths could assist clinicians in the timely targeting of empirical antimicrobial therapy. For this purpose, we evaluated the direct identification of micro-organisms from BacT/ALERT (bioMérieux) anaerobic positive blood cultures without charcoal using the Microflex matrix-assisted laser desorption/ionization (MALDI) time of flight MS (Bruker), after bacterial extraction by using two different methods: the MALDI Sepsityper kit (Bruker) and an in-house saponin lysis method. Bruker's recommended criteria for identification were expanded in this study, with acceptance of the species identification when the first three results with the best matches with the MALDI Biotyper database were identical, whatever the scores were. In total, 107 monobacterial cultures and six polymicrobial cultures from 77 different patients were included in this study. Among monomicrobial cultures, we identified up to the species level 67 and 66% of bacteria with the MALDI Sepsityper kit and the saponin method, respectively. There was no significant difference between the two extraction methods. The direct species identification was particularly inconclusive for Gram-positive bacteria, as only 58 and 52% of them were identified to the species level with the MALDI Sepsityper kit and the saponin method, respectively. Results for Gram-negative bacilli were better, with 82.5 and 90% of correct identification to the species level with the MALDI Sepsityper kit and the saponin method, respectively. No misidentifications were given by the direct procedures when compared with identifications provided by the conventional method. Concerning the six polymicrobial blood cultures, whatever the extraction method used, a correct direct identification was only provided for one of the isolated bacteria on solid medium in all cases. The analysis of the time-to-result demonstrated a reduction in the turnaround time for identification ranging from 1 h 06 min to 24 h 44 min, when performing the blood culture direct identification in comparison with the conventional method, whatever the extraction method.

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## INTRODUCTION

Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) MS is a well implemented technology in microbiological laboratories for the identification of bacteria and yeast from fresh colonies grown on agar plates. The efficiency and the rapidity of this technique are now well demonstrated (Descy *et al.*, 2010; Bizzini & Greub, 2010; Neville *et al.*, 2011). Furthermore, the cost of consumables is very low (Gaillot *et al.*, 2011). Therefore, its use has expanded among many laboratories performing

numerous identifications per day. Beyond identification from colonies, there are other suggested uses as direct identification from urine or positive blood culture broths.

In the clinical setting, it is well known that bacteraemia and sepsis are associated with high rates of morbidity and mortality. The early initiation of treatment with the appropriate antibiotic is a key criterion for a reduction in the morbidity and mortality rates (Kumar *et al.*, 2009), together with a reduction in the length of stay in hospital and the associated spending. Hence, direct bacterial identification from positive blood cultures is a major objective for a clinical laboratory. Indeed, rapid species identification of the causal agent directly from positive

Abbreviation: MALDI-TOF, matrix-assisted laser desorption/ionization time of flight.

A supplementary table is available with the online version of this paper.

blood culture broth could assist clinicians in the timely targeting of empirical antimicrobial therapy.

The aim of this study was to evaluate the direct identification of micro-organisms from BacT/ALERT (bioMérieux) anaerobic positive blood cultures using the Microflex MALDI-TOF MS (Bruker) and its database. For the preceding bacterial extraction, two different extraction methods were compared: the MALDI Sepsityper kit (Bruker) and an in-house saponin lysis method.

## METHODS

**Clinical samples and blood culture system.** The blood culture bottles currently in use in the University Hospital of Liège are BacT/ALERT FA (FAN aerobic) bottles with charcoal and SN (standard anaerobic) bottles without charcoal (bioMérieux) for aerobic and anaerobic cultures, respectively. As charcoal particles are a well-described hindrance to good and reproducible extraction of bacteria, direct MALDI identifications were performed from positive-culture SN bottles, from which most aerobic pathogens are usually recovered with the exception of non-fermenting Gram-negative bacilli.

Among blood cultures collected from patients and submitted to the medical microbiology laboratory of the University Hospital of Liège, we analysed the 113 anaerobic bottles that were positive by the BacT/ALERT 3D system (bioMérieux) during the week days in March and April 2011, collected from 77 patients.

For the anaerobic bottles that were detected as positive before or at the same time as the aerobic bottles, the time-to-identification obtained by direct MALDI-TOF analysis was compared with the time required by the conventional method.

**Conventional identification.** Positive anaerobic bottles were plated onto two Columbia agar plates with 5% sheep blood (Becton Dickinson), one was incubated at 35 °C in air and the other one was incubated at 35 °C in anaerobic conditions. After overnight incubation, isolated colonies were directly spotted on a MALDI sample target, overlaid with 1.5 µl  $\alpha$ -cyano-4-hydroxycinnamic acid (HCCA) matrix, dried in air and further analysed by the Microflex MALDI-TOF MS (Bruker).

**Bacterial extraction for direct identification from positive blood culture broth.** Two different procedures were performed for the separation of bacteria from blood cells and the protein extraction: the procedure using the MALDI Sepsityper kit and an in-house saponin method.

The MALDI Sepsityper kit was used according to the manufacturer's instructions: 1 ml positive blood culture broth was added to 200 µl lysis buffer and the mixture was further centrifuged at 16 600 g for 1 min. The supernatant was discarded and the pellet was washed with 1 ml washing buffer and the protein extraction was performed according to the Bruker ethanol/formic acid protocol (Barbuddhe *et al.*, 2008).

An in-house procedure for bacterial separation from blood culture broth using saponin was developed, based on a published protocol (Ferroni *et al.*, 2010). Different volumes and ratios of broth and saponin lysis agent were tested. The combination giving the most effective lysis and the best reproducibility, obtained with 500 µl blood culture broth and 400 µl 5% saponin, was used in this study. This mix was vortexed for 10 s and centrifuged at 16 600 g for 1 min. The pellet was washed with 1 ml water for MS (Fluka Analytical) and the next step of ethanol/formic acid extraction was performed on the pellet, as recommended by Bruker. Two microlitres of each extract was spotted

twice on a target plate, overlaid with 1.5 µl HCCA matrix and air-dried at room temperature.

**MS.** All mass spectra were acquired using a Microflex MALDI-TOF MS and integrated using the flexControl 3.0 software. Identifications were obtained after comparison with the reference database for identification (MALDI Biotyper DB Update v3.1.2.0) using the MALDI Biotyper 2.0 software. Pairing scores, according to the analogy with reference spectra, were generated for each identification. Bruker's recommended cut-off values for identification from agar plates are 'an acceptable identification to the species level' if the score is  $\geq 2.0$  and an 'acceptable identification to the genus level' if the score is  $\geq 1.7$ . These are the cut-off values that were employed in this study for conventional identification. When working directly from the blood culture broth, Bruker's recommended criteria are lowered with an 'acceptable identification to the species level' if the score is  $\geq 1.8$ . In this study, criteria for acceptance of direct identification from blood culture broth were adapted after a first step evaluation was performed with the positive blood culture bottles used for the development of the in-house saponin method (data not shown). Different from Bruker's recommended criteria for identification, we accepted species identification when the first three results having the best matches with the MALDI Biotyper database were identical, whatever the scores were. All the direct identifications accepted according to these modified criteria were compared with results obtained by the conventional identification method, which was considered as the reference method in this study.

**Statistics.** We used Student's *t*-test and McNemar test for the statistical calculations using Statistica software (Statsoft).

## RESULTS

The 113 anaerobic blood culture bottles that were positive were all confirmed positive by subculture and identified by the conventional method as 107 monomicrobial cultures and six polymicrobial cultures.

### Direct identification of monomicrobial cultures

The results for monomicrobial cultures are shown in Table 1. Overall, 67 and 66% of bacteria were identified to the species level directly from positive broth with the MALDI Sepsityper kit and the saponin method, respectively. There was no significant difference between the two extraction methods. No misidentifications were given by the direct procedures when compared with identifications provided by the conventional method. There was a clear difference between Gram-negative and Gram-positive bacteria, as 82.5 and 90% of Gram-negative bacilli were correctly identified with the MALDI Sepsityper kit and the saponin method, respectively, against 58 and 52% identification of Gram-positive bacteria. No significant difference was observed between the two methods. Among Gram-negative bacilli, the saponin method only failed in the detection of the two *Campylobacter fetus* isolates and the *Pseudomonas aeruginosa* isolate, while the Sepsityper kit did not lead to the identification of the *Bacteroides vulgatus* isolate, of one *Proteus mirabilis* isolate out of four and of one *C. fetus* isolate out of two. *Salmonella paratyphi* B was identified only to the genus level after extraction with the two methods,

**Table 1.** Results for 107 monomicrobial blood cultures according to the species and to the extraction method used prior to MS testing

NA, Not applicable.

Species (identified by reference method)	No.	Extraction method			
		MALDI Sepsityper kit		Saponin lysis	
		Direct identification <i>n</i> (%)	Mean scores (min–max)	Direct identification <i>n</i> (%)	Mean scores (min–max)
<b>Gram-negative</b>					
<i>Escherichia coli</i>	26	23 (88.46)	2.291* (1.426–2.437)	26 (100)	1.902* (1.515–2.176)
<i>Proteus mirabilis</i>	4	3 (75)	2.345* (2.044–2.518)	4 (100)	1.805* (1.454–2.081)
<i>Klebsiella pneumoniae</i>	4	4 (100)	2.310* (2.137–2.386)	4 (100)	1.917* (1.876–2.191)
<i>Klebsiella oxytoca</i>	1	1 (100)	2.133* (2.133)	1 (100)	1.830* (1.830)
<i>Bacteroides vulgatus</i>	1	0 (0)	NA	1 (100)	1.860* (1.860)
<i>Pseudomonas aeruginosa</i>	1	1 (100)	2.236* (2.236)	0 (0)	NA
<i>Campylobacter fetus</i>	2	1 (50)	2.208* (2.208)	0 (0)	NA
<i>Salmonella paratyphi B</i>	1	0 (0) (1 to genus level)	2.214* (2.214)	0 (0%) (1 to genus level)	1.843* (1.843)
<b>Subtotal</b>	40	33 (82.5)†	NA	36 (90)†	NA
<b>Gram-positive</b>					
<i>Staphylococcus aureus</i>	15	12 (80)	2.161‡ (1.726–2.485)	12 (80)	1.863‡ (1.678–2.125)
<i>Staphylococcus hominis</i>	8	6 (75)	2.508‡ (2.041–2.309)	4 (50)	1.570‡ (1.320–1.777)
<i>Staphylococcus epidermidis</i>	27	15 (55.56)	1.858‡ (1.414–2.051)	10 (37.04)	1.587‡ (1.017–1.931)
<i>Staphylococcus capitis</i>	2	1 (50)	2.216‡ (2.216)	0 (0)	NA
<i>Staphylococcus haemolyticus</i>	2	1 (50)	1.857‡ (1.482–2.231)	1 (50)	2.159‡ (2.159)
<i>Streptococcus agalactiae</i>	2	1 (50)	2.273‡ (2.273)	1 (50)	1.628‡ (1.628)
<i>Streptococcus dysgalactiae</i>	1	0 (0)	NA	1 (100)	1.361‡ (1.361)
<i>Streptococcus pneumoniae</i>	5	1 (20)	2.092‡ (2.092)	4 (80)	1.521‡ (1.148–1.811)
<i>Enterococcus faecalis</i>	2	1 (50)	1.610‡ (1.610)	2 (100)	1.801‡ (1.729–1.873)
<i>Lactobacillus</i> sp.	2	0 (0)	NA	0 (0)	NA
<i>Actinomyces odontolyticus</i>	1	1 (100)	1.573‡ (1.573)	0 (0)	NA
<b>Subtotal</b>	67	39 (58.21)§	NA	35 (52.24)§	NA
<b>Total</b>	107	72 (67.29)	NA	71 (66.36)	NA

\* $P=0.0005$ † $P=0.4497$ ‡ $P=0.1086$ § $P=0.5563$ || $P=1.000$ 

but the non-discrimination of the *Salmonella* serotype is a well-known limitation of MALDI-TOF MS identification. Among Gram-positive bacteria, we observed particularly inconclusive results for *Staphylococcus epidermidis*, other coagulase-negative staphylococci and also for less frequently detected micro-organisms such as *Lactobacillus* with the two methods. Results for *Streptococcus pneumoniae* were better with the saponin method but this advantage is limited since confirmation by an alternative method is always needed for *Streptococcus pneumoniae*, because of its spectrum homology with other viridans streptococci.

### Direct identification of polymicrobial cultures

Concerning the six polymicrobial blood cultures, whatever the extraction method used, a correct direct identification

was always provided only for one of the isolated bacteria on solid medium for each specimen (Table S1, available in JMM Online). It is interesting to note that the Gram staining performed directly on the positive blood culture broth did not suggest the presence of more than one pathogen.

### Matching scores and types of results

Looking at the matching scores, those obtained after use of the MALDI Sepsityper kit for bacterial extraction were significantly higher ( $P=0.0005$ ) than those obtained by the saponin method for Gram-negative bacilli, but were not significantly different ( $P=0.1086$ ) for Gram-positive bacteria. With the saponin method, scores were sometimes very low (1.0–1.1) but were associated with a repeated and likely result for identification, which was accepted.

In the absence of direct identification, the results given by the MALDI Biotyper software were of two types: a non-reliable result (no reproducible species identification after software analysis) or a 'no-peak-found' result (absence of measurable spectra after analysis in the Microflex). Using the MALDI Sepsityper kit, among the 107 monomicrobial samples, 18 % gave inconclusive results and 14 % obtained a 'no-peak-found' result, especially for blood cultures with staphylococci, as identified by the conventional method. These 'no-peak-found' results were mainly obtained when no bacterial pellet was observed after blood cell lysis. Nevertheless, knowing that pellets are sometimes very small, the whole procedure was performed for these samples. With the saponin method, all of the unidentified samples were linked to inconclusive results.

### Reduction of the time-to-result

The time-to-result needed for identification of the micro-organism by each method was measured for 47 positive blood cultures. The time saved, when a direct extraction either with the MALDI Sepsityper kit or by using the saponin method was performed, ranged from 1 h 06 min to 24 h 44 min, by comparison with conventional methods. This wide range of variation could be explained, in part, by the bacterial species itself: the rapidity of growth on agar plates of some bacteria allowed identification by the conventional method a few hours after the positive blood culture result. On the other hand, the time at which the blood cultures became positive also mattered: when it became positive at the end of the day, the direct identification was directly performed while the conventional identification was automatically postponed to the next day.

## DISCUSSION

In this study we evaluated two methods for the bacterial extraction from BacT/ALERT SN anaerobic positive blood culture bottles, prior to carrying out MALDI-TOF analysis: the MALDI Sepsityper kit method and an in-house saponin method. The criteria defined in this study for accepting a proposed identification were having the same identification for the first three results with the best matches, whatever the MALDI scores were. When using these criteria, the observed rate of species identification was very good for Gram-negative bacilli, with 82.5 and 90 % correct identification to the species level with the MALDI Sepsityper kit and the saponin method, respectively. Almost all the *Enterobacteriaceae* were correctly identified with excellent scores after direct extraction by either of the two methods. Few data are available in this study for non-fermenting Gram-negative bacilli, as only the anaerobic positive blood culture bottles were included for direct identification. Whatever the extraction method used, the rate of direct species identification was low for Gram-positive bacteria, especially for coagulase-negative staphylococci, as only 58

and 52 % of Gram-positive bacteria were identified to the species level with the MALDI Sepsityper kit and the saponin method, respectively.

Recently, several studies have been published describing results obtained by using the MALDI Sepsityper kit for bacterial extraction from blood culture bottles, followed by analysis on the Microflex. In one of these, Buchan *et al.* (2012) obtained, overall, a rate of identification of 85.5 % in monomicrobial cultures. They worked from BACTEC aerobic and anaerobic bottles and used a cut-off value of  $\geq 1.7$  as an acceptable species identification score. Their results for Gram-positive bacteria were superior to ours, with 80 % of acceptable identifications. Nevertheless, they mentioned lower scores for *Staphylococcus epidermidis*, which was consistent with our results.

Other studies, similar to ours, comparing results obtained with the Sepsityper kit and other extraction methods have also been published; Loonen *et al.* (2012) performed a study using BacT/ALERT SA aerobic bottles. Overall, their results, also using a cut-off score for identification to the species level of  $\geq 1.7$ , correctly identified 78 % of isolates using the Sepsityper kit: when their results for Gram-negative bacilli reached 96 % of correct identifications, results among Gram-positive bacteria were lower, with 64 % of correct identifications. The results they obtained by using alternative extraction methods [commercial MolYsis Basic method (Molzym) or an in-house differential centrifugation method] were lower than results obtained by using the Sepsityper kit. The authors also mentioned that they finally did not include the BacT/ALERT SN anaerobic bottles in their study because of frequent unreliable data in their preliminary experiments. Working from BACTEC bottles, Juiz *et al.* (2012) also obtained better results with the Sepsityper kit by comparison with an in-house centrifugation method, using a cut-off score for identification to the species level of 2.0. Schubert *et al.* (2011) also demonstrated the superiority of the Sepsityper extraction method by comparison with a differential centrifugation protocol on BACTEC bottles and highlighted the possibility of accepting species identifications with low scores ( $\geq 1.5$ ) if the first three proposed results were identical. Recently, some studies have demonstrated, similar to our study, that alternative extraction methods could give identical or better results than the Sepsityper kit and that using lower cut-offs could improve the results. From BACTEC bottles, Saffert *et al.* (2012) demonstrated equivalent results when performing bacterial extraction by differential centrifugation or blood lysis using 10 % SDS or the Sepsityper kit prior to MALDI-TOF MS analysis, with improved results when accepting species identification when the score was  $\geq 1.7$  instead of  $\geq 2.0$ . Similar to our study, Martiny *et al.* (2012) published a study comparing direct identification from BACTEC positive blood culture bottles after bacterial extraction with an in-house saponin method, slightly different from ours, and with the Sepsityper kit. Overall, when using lower cut-off values (1.4 and 1.6 for correct genus and species identifications,

**Table 2.** Percentage of correct identifications based upon the acceptance criteria defined in this study or those criteria proposed by Bruker for the direct identification by MALDI–TOF MS among 107 monomicrobial blood cultures

Identified bacteria were Gram-negative (40) or Gram-positive (67).

Accepted species identification with:	Gram-negative		Gram-positive	
	MALDI Sepsityper kit <i>n</i> (%)	Saponin <i>n</i> (%)	MALDI Sepsityper kit <i>n</i> (%)	Saponin <i>n</i> (%)
Criteria used in this study	33 (82.5)*	36 (90)†	39 (58.2)‡	35 (52.2)§
Bruker's criteria (score $\geq 1.8$ )	32 (80)*	29 (72.5)†	27 (40.3)‡	14 (20.9)§

\* $P=1.000$

† $P=0.0233$

‡ $P=0.0060$

§ $P=0.0000$

respectively), the proportions of identification to the species level they obtained were similar with their in-house saponin method and the Sepsityper kit, with 74 and 68 % of correct identifications to the species level.

These various results led to the conclusion that the composition of the blood culture broth, the incubation atmosphere and the bacterial extraction method play a role in the quality of further direct identification (Szabados *et al.*, 2011), together with the defined criteria for accepting identification. Nevertheless, whatever the method used, direct identification of Gram-positive bacteria is always inferior to direct identification of Gram-negative bacteria, with no apparent explanation described until now. Similarly, results from BACTEC bottles always seem better than those obtained from BacT/ALERT bottles without charcoal, with no detailed explanation, except a different composition of broth.

Currently, few studies have been published describing the results of direct identification when working from BacT/ALERT bottles without charcoal. Moreover, no studies focused particularly on anaerobic bottles, while, as demonstrated in this study, they become frequently positive before or at the same time as aerobic bottles. To our knowledge, this study is the first one evaluating the MALDI Sepsityper kit for the extraction of micro-organisms from anaerobic BacT/ALERT blood culture bottles. If using our acceptance criteria, three repeated identical identifications no matter the score, the observed results do not demonstrate a superiority of this extraction procedure by comparison with the saponin method used in this study. Scores observed after extraction with the saponin method were sometimes very low and the resulting identification would not have been accepted using the Bruker criteria for direct identification from blood culture broths (acceptable species identification if score  $\geq 1.8$ ). So, 75 against 93 % of the Gram-negative cultures and 21 against 52 % of the Gram-positive cultures would have been identified after extraction with the saponin method using the Bruker criteria. This difference is significant. Using the MALDI

Sepsityper kit, identification rates with the Bruker criteria would have been 83 against 85 % for Gram-negative cultures and 40 against 58 % for Gram-positive cultures. Although less remarkable, this difference remains significant for Gram-positive cultures (Table 2). These results suggest that, whatever the extraction method used, in this application to BacT/ALERT anaerobic blood culture bottles, the Bruker criteria could be expanded to avoid the exclusion of a significant percentage of correct identifications, mainly among Gram-positive bacteria.

In this study, when referring to our decision criteria for accepting an identification provided by the Bruker database after analysis with the Microflex MALDI-TOF MS, we have observed no misidentification. Moreover, the saponin method has given similar results to those obtained by using the MALDI Sepsityper kit extraction method and is as easy to perform and less expensive.

Further validations should be conducted using BacT/ALERT aerobic blood culture bottles without charcoal to complete the assessment of the extraction method and of the proposed criteria.

The analysis of the time-to-result clearly demonstrated a reduction of the turnaround time for identification when performing blood culture direct identification in comparison with the conventional method, whatever the extraction method. Rapidly reporting the identification of the bacteria detected in blood culture, even before the availability of antibiotic susceptibility testing results, should help clinicians to focus their empiric antibiotherapy to the identified aetiological agent, according to antibiotic guidelines based on local epidemiology.

## REFERENCES

- Barbuddhe, S. B., Maier, T., Schwarz, G., Kostrzewa, M., Hof, H., Domann, E., Chakraborty, T. & Hain, T. (2008). Rapid identification and typing of *Listeria* species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Appl Environ Microbiol* 74, 5402–5407.

- Bizzini, A. & Greub, G. (2010).** Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. *Clin Microbiol Infect* **16**, 1614–1619.
- Buchan, B. W., Riebe, K. M. & Ledebøer, N. A. (2012).** Comparison of the MALDI Biotyper system using Sepsityper specimen processing to routine microbiological methods for identification of bacteria from positive blood culture bottles. *J Clin Microbiol* **50**, 346–352.
- Descy, J., Meex, C., Melin, P., Hayette, M. P., Huynen, P. & De Mol, P. (2010).** [MALDI-TOF mass spectrometry in clinical bacteriology or how to identify a bacteria within one minute]. *Rev Med Liege* **65** (Spec no), 29–34 (in French).
- Ferroni, A., Suarez, S., Beretti, J.-L., Dauphin, B., Bille, E., Meyer, J., Bougnoux, M.-E., Alanio, A., Berche, P. & Nassif, X. (2010).** Real-time identification of bacteria and *Candida* species in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* **48**, 1542–1548.
- Gaillot, O., Blondiaux, N., Loïez, C., Wallet, F., Lemaitre, N., Herwegh, S. & Courcol, R. J. (2011).** Cost-effectiveness of switch to matrix-assisted laser desorption ionization-time of flight mass spectrometry for routine bacterial identification. *J Clin Microbiol* **49**, 4412.
- Juiz, P. M., Almela, M., Melción, C., Campo, I., Esteban, C., Pitart, C., Marco, F. & Vila, J. (2012).** A comparative study of two different methods of sample preparation for positive blood cultures for the rapid identification of bacteria using MALDI-TOF MS. *Eur J Clin Microbiol Infect Dis* **31**, 1353–1358.
- Kumar, A., Ellis, P., Arabi, Y., Roberts, D., Light, B., Parrillo, J. E., Dodek, P., Wood, G., Kumar, A. & other authors (2009).** Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. *Chest* **136**, 1237–1248.
- Loonen, A. J., Jansz, A. R., Stalpers, J., Wolffs, P. F. & van den Brule, A. J. (2012).** 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. *Eur J Clin Microbiol Infect Dis* **31**, 1575–1583.
- Martiny, D., Dediste, A. & Vandenberg, O. (2012).** Comparison of an in-house method and the commercial Sepsityper<sup>®</sup> kit for bacterial identification directly from positive blood culture broths by matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry. *Eur J Clin Microbiol Infect Dis* **31**, 2269–2281.
- Neville, S. A., Lecordier, A., Ziochos, H., Chater, M. J., Gosbell, I. B., Maley, M. W. & van Hal, S. J. (2011).** Utility of matrix-assisted laser desorption ionization-time of flight mass spectrometry following introduction for routine laboratory bacterial identification. *J Clin Microbiol* **49**, 2980–2984.
- Saffert, R. T., Cunningham, S. A., Mandrekar, J. & Patel, R. (2012).** Comparison of three preparatory methods for detection of bacteremia by MALDI-TOF mass spectrometry. *Diagn Microbiol Infect Dis* **73**, 21–26.
- Schubert, S., Weinert, K., Wagner, C., Gunzl, B., Wieser, A., Maier, T. & Kostrzewa, M. (2011).** Novel, improved sample preparation for rapid, direct identification from positive blood cultures using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. *J Mol Diagn* **13**, 701–706.
- Szabados, F., Michels, M., Kaase, M. & Gatermann, S. (2011).** The sensitivity of direct identification from positive BacT/ALERT (bioMérieux) blood culture bottles by matrix-assisted laser desorption ionization time-of-flight mass spectrometry is low. *Clin Microbiol Infect* **17**, 192–195.