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For publication

Use of MALDI-TOF mass spectrometry after liquid enrichment (BD Bactec™) for rapid diagnosis of bone and joint infections

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Abstract

Advantages of MALDI-TOF MS (MS) were evaluated for diagnosis of bone and joint infections after enrichment of synovial fluid (SF) or crushed osteoarticular samples (CSs). MS was performed after enrichment of SF or crushed osteoarticular samples CS (n=108) in both aerobic and anaerobic vials. Extraction was performed on 113 vials (SF: n=47; CS: n=66), using the Sepsityper® kit prior identification by MS. The performances of MS, score and reproducibility results on bacterial colonies from blood agar and on pellets after enrichment in vials, were compared. MS analysis of the vial resulted in correct identification of bacteria at a species and genus level (80.5% and 92% of cases, respectively). The reproducibility was superior for aerobic Gram-positive bacteria (*Staphylococci* and Gram-positive bacilli: 100% colonies), as compared to aerobic Gram-negative bacilli (89.7%), anaerobes (83.3%) and *Streptococcus/Enterococcus* (58.8%). MS performance was significantly better for staphylococci than for streptococci on all identification parameters. For polymicrobial cultures, identification (score>1.5) of two species by MS was acceptable in 92.8% of cases. Use of MS on enrichment pellets of bone samples is an accurate, rapid and robust method for bacterial identification of clinical isolates from osteoarticular infections, except for streptococci, whose identification to species level remains difficult.

Keywords: MALDI-TOF mass spectrometry; Osteoarticular infection; Sepsityper® kit; Time of detection; Beadmill processing; Polymicrobial samples.

1. Introduction

Direct examination is an unreliable method for the diagnosis of bone infections [1], with a sensitivity threshold assessed at an inoculum of approximately 10^4 UFC/mL. Achieving an enrichment step in a liquid medium with prolonged incubation of at least 14 days is essential [2] for correct diagnosis. This time is required to observe the growth of "small colony variants" or fastidious bacteria and to dilute any antibiotic potentially present in the synovial fluid (SF) or crushed bone samples (CSs). A biopsy beadmill processing step [3, 4] or a step of sonication [5] on prosthetic samples provides improvement of culture performances. This is particularly true in the case of bacterial biofilms [6], chronic or complicated infections associated with prosthetic material. Infections on osteosynthesis material may be polymicrobial (10 to 15%) [7], and diagnosis of these infections remains difficult and often fails to identify all these bacterial species.

Universal gene amplification techniques (eg. 16SrDNA, *sodA*) are a diagnostic option, particularly in case of prior antibiotic treatment, but the time consumed (due to the necessary secondary sequencing of the amplified product), the cost of this test and its low sensitivity are major disadvantages to its use [4,8]. Specific polymerase chain reactions (PCRs) (*Borrelia, K. kingae, Tropheryma whipplei,* etc.) are more sensitive and specific tests, but the procedure requires targeting a single gene with a known sequence. This is a limit to its use in the context of bone and joint infections, where the pathogen is often unknown; accurate diagnosis may require laboratories to perform several specific PCRs.

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry, or MALDI-TOF MS (MS), is frequently used for identification of a single colony (isolated on agar media) from clinical and environmental samples [9-11]. The MS system provides rapid and high-confidence identification of bacteria, yeasts and fungi, based on proteomic fingerprinting using high-throughput MALDI-TOF mass spectrometry. Its use has recently

been extended to clinical diagnosis, either directly from positive blood culture vials [12] or from samples such as urine [13]. Research suggests that this technique is relevant for microorganism identification, with functionality comparable to routine methods used in the clinical microbiology laboratory [14]. In the case of blood culture vials, bacterial identification by MS directly on the vial pellets optimizes the rendering time result with a time-saving of 1 to 24 h over conventional methods depending on the extraction technique [15, 16]. Results quickly available contribute to reducing morbidity [17] and mortality in addition to lower cost of treatment and length of hospital stay.

This study evaluated the usefulness of MS for rapid diagnosis of bone and joint infections. Synovial fluid (SF) or crushed osteoarticular samples (CSs) were enriched in aerobic and anaerobic blood vials before harvesting bacteria (from positive vial cultures), which were then rapidly identified by MALDI-TOF. To assess the performance of MS, score and reproducibility results on bacterial colonies, directly seeded on blood agar from the sample and on pellets after enrichment in blood vials, were compared. Additionally, we defined the detection rate of culture for SF and CS by bacterial species after enrichment in aerobic and anaerobic blood vials.

2. Material and methods

2.1. Samples - Scheme of the study

This was a prospective single-center study conducted at the University Hospital of Rennes (Reference Centre for Complex Osteoarticular Infections for the West of France) from January to October 2013. Osteoarticular samples (OASs) were collected and analyzed at the Laboratory of Bacteriology within 2 h of receipt after possible storage at room temperature. Synovial fluids (SFs) were collected in a sterile tube (Falcon ®) and bone samples in a sterile

jar (30 mL, HDPE Nalgen®). The articular and bone samples were included prospectively, except for laboratory closing hours (21:00-7:30).

2.2. Bacteriological studies

SF and CSs were treated according to microbiological routine techniques. Bone samples were crushed using a bead mill (Retsch® MM400 crusher: frequency 30.0 / s, for two min and 30 s). Tubes containing 10 sterile stainless steel beads (4 mm diameter) (AISI 304 Grade 1000; AFBMA; Hammer & Lemarié, France) in 10 mL of molecular biology grade distilled water were prepared, sterilized, tested and stored at room temperature for a maximum of 3 months in the laboratory. Following all safety protocols, contents of one tube was poured into each sterile container (HDPE) containing the OAS and grinded [4].

To ensure proper identification of cultures on solid media by MS, 50 µL of SF or CS were plated on Columbia agar supplemented with horse blood (5%) (Oxoid®), chocolate agar (Oxoid®) in atmosphere enriched with 5% CO_2 for 72 h and Columbia agar supplemented with horse blood (5%) in an anaerobic atmosphere for 5 days at 37°C.[18,19]

Each sample (n=108) was enriched by inoculating 1 mL (minimum volume obtained for some joints) in an aerobic blood culture vial (BD BACTEC™ Plus Aerobic/F) and in an anaerobic blood culture vial (BD BACTEC™ Lytic/10 Anaerobic/F), incubated in automatic chambers for 14 days. Aerobic blood culture vial (BD BACTEC™ Plus Aerobic/F) and anaerobic blood culture vial (BD BACTEC™ Lytic/10 Anaerobic/F) were used because they proved to be the most efficient pair of blood aerobic/anaerobic culture media [20].

After extraction performed according to manufacturer's recommendations (Sepsityper® kit; Bruker), identification of bacterial species was performed using the MS technique (Microflex LT/SH mass spectrometer Biotyper, Bruker®) either on a single colony from agar media (routine use) [21] or on extracted enriched vial pellets (Sepsityper® kit), placed onto

the polished steel target plate for rapid identification by MALDI-TOF. Once a positive vial was automatically detected, 1 ml of broth was extracted without delay (< 2 h, to preserve spectra) with formic acid overlay [18] and analyzed via the same method as for colonies. Criteria for interpretation of results were based on the manufacturer's recommendations (Bruker®). Identification was established through biostatistics reliability levels on the basis of a correlation between the acquired spectrum and the reference spectra. The spectrum of the unknown test organism, acquired through MALDI Biotyper CA System Software®, was electronically transformed into the peak list. Using a biostatistical algorithm, this peak list was compared to reference peak lists of organisms in the reference database, and a log(score) value between 0.00 and 3.00 was calculated. The higher the log(score) value, the more reliable the degree of similarity (to a given organism in the reference FDA-cleared database). A log(score) value of ≥ 2.00 indicated an excellent probability for test organism identification at the species level. The interpretation considered two independent parameters: the value of the homology score and the reproducibility of identification obtained (on 10 measurements carried out after laser impacts, the same bacterial species must be found at least three times with the highest scores, particularly in cases with a score <1.7). Identification with a score ≥ 2 was considered reliable to the species; identification with a score ≥ 1.7 was considered reliable to the genus. An identification score of 1.5 was also examined in light of several prior studies suggesting that it adequately identified the bacterial genus [22-24]. Identification was considered unacceptable when the score (< 1.7) and reproducibility were insufficient, and incorrect when the score or reproducibility was acceptable, with poor identification to the species level. If necessary, 16SrDNA PCR was performed to confirm bacterially uncertain identifications, as previously described [4]. To assess the performance of MS in diagnosing bone and joint infections, we compared score and reproducibility results on bacterial colonies from blood agar and on pellets after enrichment in blood vials.

155

156 *2.3. Statistical analysis*

157 Means were compared using the Student test and percentages using the chi-square test
158 (or Fisher's exact test when sample size was less than 5). P values less than 0.05 were
159 considered significant.

160

161 **3. Results**

162 *3.1. Scheme of the study and description of samples*

163 A total of 108 osteoarticular samples (OASs) were collected and 216 enrichment vials
164 (BD BACTEC™) were inoculated; 117 were detected positive in automatic chambers
165 (Bactec® 9240, Becton Dickinson) and 113 were analyzed within 2 h following a positive
166 detection rate of culture (for extraction consistent with the Sepsityper® kit manufacturer's
167 recommendations) (Fig. 1). During the incubation period of 336 h, all positive vials were
168 detected before 227 h.

169 After extraction (1 mL sample with the Sepsityper kit), MS identifications were
170 performed on final extracted pellets. Among the aerobic-positive vials (n=58), 50 (86.2%)
171 were considered to be monomicrobial samples, 7 (12%) polymicrobial and 1 (1.8%) negative.
172 In anaerobic vials (n =55), 45 (81.8%) were monomicrobial, 5 (9.1%) were polymicrobial and
173 3 (5.5%) were negative. The list of bacterial isolates obtained from enriched media (aerobic
174 and anaerobic vials, incubated in automatic chambers for 14 days) and from agar media is
175 shown in Table 1.

176

177 *3.2. Results of bacterial identification by MS on blood agar (Table 2)*

178 According to defined criteria, results of the identification by MS from colonies
179 picked on blood agar (n = 104) (colonies on agar plates obtained from direct spreading of

samples or transplanting from enrichment vials) were consistent with species identification in 79.8% of cases, with the genus in 90.4% of cases, and unacceptable identification in 1.9% of cases (score and insufficient reproducibility), or incorrect identification in 4.8% (score or acceptable reproducibility, but poor identification at the species level).

Aero-anaerobic bacteria species analyzed on blood agar showed highly acceptable identification rates (score>1.7) (100%), with the exception of anaerobes (83.3%) and *Streptococcus* (70.6%). No relevant misidentifications at the genus level were reported at the log(score) cut-off of 1.6. For *Streptococcus,* five incorrect identifications were detected. Reproducibility was superior for aerobic Gram-positive bacteria (*Staphylococci* and Gram positive bacilli: 100% colonies) compared to aerobic Gram-negative bacilli (89.7%), anaerobes (83.3%) and *Streptococcus/Enterococcus* (58.8%).

MS performance was better for staphylococci than for streptococci for all parameters: a high degree of identification (38.5% vs.17.6%, $p=0.03$), species identification (89.7% vs. 58.8%, $p=0.001$), genus identification (100% vs.70.6%, $p<0.001$), incorrect identification (0% vs.29.4%, $p=0.03$) and acceptable reproducibility (100% vs.58.8%, $p<0.001$).

3.3. *Comparison of MS score results from pellets after enrichment in blood vials from blood agar (Table 2)*

MS analysis on vial pellets resulted in correct identification of bacterial species at a species and genus level (80.5% and 92% of cases, respectively). There was no significant difference between MS identification on vials containing Gram-negative bacilli and staphylococci regarding the high degree of identification, identification to genus and species, unacceptable identifications, incorrect identifications, absence of identification and reproducibility. Incorrect identifications from vial pellets, as compared to the expected

identification (MS from colonies and / or PCR 16SrDNA), were observed in streptococci and related species (*S. minor/sinensis; S.oralis/pneumoniae; S.parasanguis/Gemellans haemolysans*) and *Arthrobacter cumminsii*/lipophilic *Corynebacterium* F1 group). The absence of a peak was observed in four cases: *S. oralis* (no growth on solid media), *S.sanguinis*, *S. minor* and *S.haemolyticus* (<10 colonies on agar corresponding media) and identification was un-interpretable in two cases (*S.parasanguis/Gemella haemolysans* and *Arthrobacter cumminsii*/lipophilic *Corynebacterium* gp F1).

3.4. Polymicrobial samples (Table 3)

In polymicrobial cultures, identification of the two species by MS was acceptable in 92.8% of cases [26/28 identifications (92.9%) with a score >1.5; 2/28 identifications (7.1%) with 1.5<score<1.7)]. Correct identification was obtained in all cases (14/14) of a single bacterial species and in 12/14 (85.7%) for 2 bacterial species; no peak could be detected for 2/14 (14.3%) vials (second identification) (Table 3).

4. Discussion

MALDI-TOF MS technology showed superiority in identification of most clinical isolates at the genus and species level [9, 11, 25] compared to conventional phenotypic bacterial identification systems. Moussaoui et al. [23] tested a new protocol for bacterial identification from blood culture broths in hospital routine using collection tubes with separator gels. In 503 samples tested over three months, they found that a score> 1.4 was relevant if the score (at the species level) was reproducible at least four times, providing successive proposals. Some differences in scores were observed in the literature between results found on aerobic and anaerobic vials. Christner et al. [15] described a lower estimated mean identification score in the linear mixed-effect model analysis of study data for *S. aureus*

species from aerobic (1,786) compared to anaerobic vials (2,101). In contrast, no such difference was observed in our study (2.31 and 2.30, respectively). Focusing on differences in performance according to bacterial species, our results are consistent with those found in prior literature on blood vials extracted via different methods: Gram-negative bacilli and *S. aureus* were better identified than other Gram-positive bacteria [12, 22, 24, 26, 27].

To our knowledge, few studies have evaluated the performance of MS in identifying bacteria directly on vial pellets after enrichment of bone samples. Using the Sepsityper kit on blood pellets, the percentage of correct identification was 92% at the species level; this number increased when decreasing to the threshold of 1.5, retained by some studies [24]. In our work, *P. acnes* were all correctly identified (score> 2). This finding is in contrast to a study conducted by Stevenson et al. [28] that reported 27.3% of unacceptable identifications (score <1.7) for *P. acnes*, a result that was previously found by MS directly performed on colonies [29]. However, their study carried out only a series of five 1-to-2 min washing/centrifugation steps (without the lysis step of the Sepsityper kit) to remove red blood cells and proteins from the blood culture broths. In our study, all unidentified bacteria and the majority of incorrect identifications concerned the genus *Streptococcus* (13.6%), especially the alpha-hemolytic group. This was already demonstrated in many prior studies on blood culture vials [22, 26, 27]. Using the Sepsityper kit, the percentage of high degree of identification (score> 2.3) on enriched bone samples was higher in our study (54.9%) than what was found in blood culture vials by Kok et al. [27], who reported 47.1% for Gram-negative bacteria, 9.8% for staphylococci and 22.6% for streptococci (in our study 68.2%, 60% and 34.6%, respectively). However, Kok et al. [27] detected more coagulase-negative staphylococci and non-fermenting Gram-negative bacilli, both of which are commonly less well identified, potentially explaining the differences from our work. The percentage of high degree of identification (score> 2.3) was significantly higher on vial pellets than on blood

agar in our study. This may be related to the fact that, for identification from vial pellets, Sepsityper extraction was followed by extraction with ethanol/formic acid, increasing efficiency.

Using the Bactec FX automated blood culture system, Kok et al. [27] reported 6.1% polymicrobial blood vials, with unidentified (32.3%) or misidentified vials (3.2%) at the species level. In case of multiple identifications, it was possible to take into account the presence of any species with a score and/or acceptable reproducibility. Conversely, the presence of a single bacterial species by MS, after extraction, did not exclude the presence of other species in the sample. A study by Martinez et al. [30] found that none of the tested methods were capable of consistently identifying polymicrobial cultures in their entirety. In most studies, only the predominant species was identified from cultures of polymicrobial clinical specimens, which might be explained by bacterial growth competition, with the elimination of one (or more) species in the liquid medium. Chen et al. [31] demonstrated that, for 21 blood cultures composed of two bacterial species, the Bruker Biotyper® was the only system that generated polymicrobial identification: in five out of the 21 mixed-culture specimens (23.8%), the two species present were identified (with >1.6 confidence scores); in the remaining 16 mixed-culture specimens (76.2%), MS identified only the major species of the mixed cultures. A better result was obtained in our study, with an acceptable score of reproducibility, identifying two species in bone samples in 92.8% of cases.

The bacterial inoculum of bone sample introduced into blood vials is another important element to take into account, based on the fact that the threshold proposed by the manufacturer underestimates the proportion of correct identifications, resulting in a lower score (that is an artifact of the sample quality: low inoculum and the presence of background noise), rather than a low degree of correlation between the mass spectrum of the sample and the best profile in the database [15]. Direct detection of bacteria in urine by MS was only

possible with an inoculum of at least 10^3 UFC/mL [32]. Works carried out on blood culture vials showed acceptable identification from 10^6 CFU/mL. For comparison, the average inoculum was 5×10^8 CFU/mL for detection of bacterial growth by the blood culture system [15]. Several studies also reported detection of lower inocula with Gram-positive bacteria by an automatic chamber, but when the inoculum was $< 10^6$ CFU/mL, the analyzed spectra were close to those obtained from sterile vials [15].

In previous works using the Sepsityper kit on blood pellets, identifications at the species level were obtained in less than 2 h [27]. Buchan et al. [33] reported that median times to identification using the MALDI Biotyper/Sepsityper were 23 to 83 h faster than routine methods for Gram-positive isolates, and 34 to 51 h faster for Gram-negative isolates in blood samples. This extraction technique has been standardized and validated in the literature, further reducing completion time [27, 34, 35]. Many other simplified efficient extraction methods have also already been tested on blood culture vials. Several techniques reduced the extraction time by half, for example those methods using saponin,[16] ammonium chloride [26], trifluoroacetic acid or formic acid [22], or even methods composed only of a series of centrifugations.[34] It is also possible to reduce the final cost of testing [36]. However, homemade techniques easily fail to completely respond to standardized criteria required in medical biology, and results are difficult to compare between different studies.

In conclusion, the use of MALDI-TOF MS on bone and synovial samples in culture vials can be performed for diagnosis and management of oste-oarticular infections. This technique reduces the time to report results to the clinician, with a reduced cost [31]. It may also allow identification of a second bacterial species in case of polymicrobial samples, but identification of streptococci to the species level remains difficult. Further improvements in

the technique are possible, including optimization of extraction methods for CS and SF before switching on the MS, and continued enrichment of the MS database.

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Authors' contributions

EL and AJG designed the research, designed experiments, assessed and interpreted the results and prepared the manuscript. CA, GC, JLP, JDA and PG took clinical samples and carried out data analysis. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Fig. 1. Scheme of the study for 216 vials and results of MALDI-TOF MS identification after extraction on 113 positive vials. *Vials were extracted with the Sepsityper kit before MS identification.

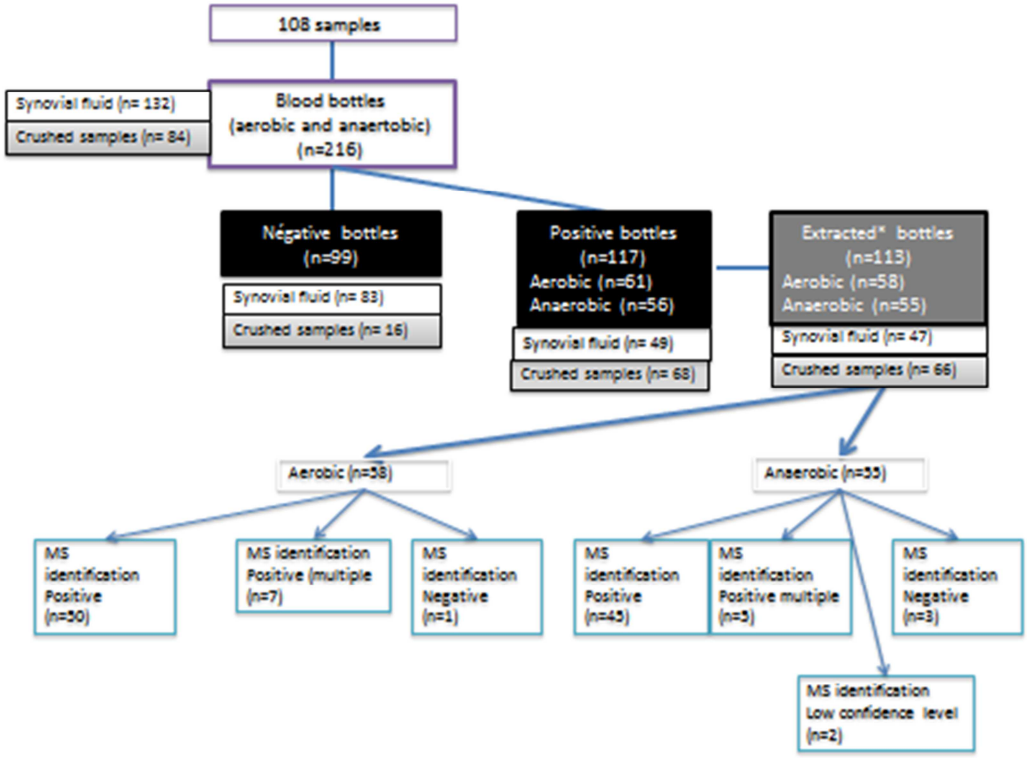

Table 1. List of bacterial isolates obtained from (1) enriched media (aerobic vial (BD BACTEC Plus Aerobic/F and anaerobic vial (BD BACTEC Lytic/10 Anaerobic/F), incubated in automatic chambers for 14 days (Bactec 9240, Becton Dickinson) and (2) agar media (blood agar).

Bacterial species	**No. of isolates**			**16SrDNA PCR identification**
	Enrichment broth		**Standard cultures (blood agar)**	
	Aerobic incubation	**Anaerobic incubation**		
	(n=58)	**(n=55)**	**(n=42)**	
Arthrobacter cumminsii	1	1	0	+
Clostridium subterminale	0	2	2	
Enterobacter cloacae	3	3	5	
Enterococcus faecalis	4	4	3	
Escherichia coli	5	5	4	
Kingella kingae	1	0	0	+
Klebsiella pneumoniae	1	0	0	
Listeria monocytogenes	1	1	0	
Propionibacterium sp.	0	4	4	
Pseudomonas aeruginosa	3	1	3	
Staphylococcus aureus	20	19	20	
Staphylococcus (negative coagulase)	5	4		
Staphylococcus epidermidis	5	3	0	
Streptococcus agalactiae	2	2	0	
Streptococcus pneumoniae	1	1	1	+
Streptococcus	6	5	5	+

Species identified by 16SrDNA PCR are indicated (+).

Table 2. Results of identification scores obtained with the MALDI-TOF MS technique on each bacterial group, i.e. from bacterial colonies (on agar plates obtained from direct spreading of samples or transplanting from enrichment vials) and from pellets after enrichment in blood vials (aerobic and anaerobic). *Vials were extracted with the Sepsityper® Kit before MS identification.

Table 3. Results of score and reproducibility of extracted bone and articular samples with multiple identifications with MALDI-TOF mass spectrometry (MS) technique. * Vials were extracted with the Sepsityper kit before MS identification.

Table 2.

Results of MALDI(TOF MS identification	Blood vials (both) (n=113)	Blood agar (n=104)	*Staphylococcus* (n=39)	*Streptococcus Enterococcus* (n=17)	Gram negative bacilli (n=29)	Gram positive bacilli (n=4)	Anaerobes (n=12)
				No of isolates (%)			
High degree of identification to species *Score > 2.3*	62 (54.9)	42 (40.4)	15 (38.5)	3 (17.6)	20 (69)	1 (25)	3 (25)
Identification to species *Score > 2*	91 (80.5)	83 (79.8)	35 (89.7)	10 (58.8)	29 (100)	2 (50)	7 (58.3)
Identification to genus *Score > 1.7*	104 (92)	94 (90.4)	39 (100)	12 (70.6)	29 (100)	4 (100)	10 (83.3)
Identification to genus with modified threshold *Score > 1.5*	107 (94.7)	94 (90.4)	39 (100)	12 (70.6)	29 (100)	4 (100)	10 (83.3)
Unacceptable identification *Score < 1.7*	2 (1.8)	2 (1.9)	0	0	0	0	2 (16.7)
Incorrect identification	2 (1.8)	5 (4.8)	0	5 (29.4)	0	0	0
No identification	4 (3.5)	0	0	0	0	0	0
Acceptable reproducibility	99 (87.6)	89 (85.6)	39 (100)	10 (58.8)	26 (89.7)	4 (100)	10 (83.3)

Sample identification	Type of blood vial	Bacterial species identified from solid media	1st bacterial species identified from vials after extraction by Sepsis typer® kit	MALDI-TOF score*	Reproducibility MALDI-TOF*	2nd bacterial species identified from vials after extraction by Sepsis typer ® kit (if polymicrobial)	MALDI-TOF score*	Reproducibility MALDI-TOF*	Total number of different bacterial species identified in the sample	Sample
1	Aerobic	*Staphylococcus aureus + Enterobacter cloacae*	*Staphylococcus aureus*	2,036	1	*Enterobacter cloacae*	2,04	8	2	SF**
1	Anaerobic	*Staphylococcus aureus + Enterobacter cloacae*	*Enterobacter cloacae*	2,241	9	*Staphylococcus aureus*	1,84	1	2	SF
2	Aerobic	*Arthrobacter cumminsii + Weeksella virosa + Oligella urethralis*	*Weeksella virosa*	2,041	2	*Arthrobacter cumminsii*	1,701	5	3	CS***
3	Anaerobic	*Peptoniphilus harei + Propionibacterium avidum*	*Propionibacterium avidum*	2,127	4	*Peptoniphilus harei*	1,849	0	0	CS
4	Aerobic	*Staphylococcus aureus + Escherichia coli*	*Staphylococcus aureus*	2,235	9	*Escherichia coli*	1,96	2	2	CS
4	Anaerobic	*Staphylococcus aureus + Escherichia coli*	*Staphylococcus aureus*	2,312	10	none			2	CS
5	Aerobic	*Enterococcus faecalis + Staphylococcus aureus*	*Enterococcus faecalis*	2,147	5	*Staphylococcus aureus*	1,92	5	2	CS
5	Anaerobic	*Enterococcus faecalis + Staphylococcus aureus + Streptococcus oralis*	*Staphylococcus aureus*	2,099	6	*Enterococcus faecalis*	1,964	4	3	CS
6	Aerobic	*Enterococcus faecalis + Staphylococcus aureus + S oralis*	*Enterococcus faecalis*	2,224	6	*Staphylococcus aureus*	1,886	3	3	CS
6	Anaerobic	*Enterococcus faecalis + Staphylococcus aureus + S oralis*	*Enterococcus faecalis*	2,41	8	none				CS
7	Aerobic	*Klebsiella pneumoniae + Enterobacter arerogenes + Eikeinella corrodens*	*Klebsiella pneumoniae*	2,422	8	*Enterobacter aerogenes*	1,878	2	3	CS

7	Anaerobic	*Klebsiella pneumoniae* + *Enterobacter arerogenes* + *Streptococcus anginosus* + *Actinomyces radingue* + *Parvimonas micra*	*Klebsiella pneumoniae*	2,188	8	*Streptococcus anginosus*	1,684	1	5	CS
8	Aerobic	*Pseudomonas aeruginosa* + *Eikenella corrodens* + *Actinomyces odontolyticus* + *Aggregatibacter aphrophilus*	*Pseudomonas aeruginosa*	2,234	6	*Streptococcus anginosus*	1,597	2	4	CS
8	Anaerobic	*Pseudomonas aeruginosa* + *Eikenella corrodens* + *Aggregatibacter aphrophilus* + *Staphylococcus epidermidis*	*Staphylococcus epidermidis*	2,017	6	*Pseudomonas aeruginosa*	1,804	4	4	CS

*on pellets extracted from vial

**synovial fluid

*** crushed sample