



**HAL**  
open science

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

Elise Lallemand, Cédric Arvieux, Guillaume Coiffier, Jean-Louis Polard, Jean-David Albert, Pascal Guggenbuhl, Anne Jolivet-Gougeon

### ► To cite this version:

Elise Lallemand, Cédric Arvieux, Guillaume Coiffier, Jean-Louis Polard, Jean-David Albert, et al.. Use of MALDI-TOF mass spectrometry after liquid enrichment (BD Bactec™) for rapid diagnosis of bone and joint infections. *Research in Microbiology*, 2017, 168 (2), pp.122-129. 10.1016/j.resmic.2016.09.005 . hal-01446879

**HAL Id: hal-01446879**

**<https://univ-rennes.hal.science/hal-01446879>**

Submitted on 10 Apr 2017

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Accepted Manuscript



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

Elise Lallemand, Cédric Arvieux, Guillaume Coiffier, Jean-Louis Polard, Jean-David Albert, Pascal Guggenbuhl, Anne Jolivet-Gougeon

PII: S0923-2508(16)30115-2

DOI: [10.1016/j.resmic.2016.09.005](https://doi.org/10.1016/j.resmic.2016.09.005)

Reference: RESMIC 3540

To appear in: *Research in Microbiology*

Received Date: 6 February 2016

Revised Date: 19 August 2016

Accepted Date: 16 September 2016

Please cite this article as: E. Lallemand, C. Arvieux, G. Coiffier, J.-L. Polard, J.-D. Albert, P. Guggenbuhl, A. Jolivet-Gougeon, Use of MALDI-TOF mass spectrometry after liquid enrichment (BD Bactec™) for rapid diagnosis of bone and joint infections, *Research in Microbiology* (2016), doi: 10.1016/j.resmic.2016.09.005.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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

Elise Lallemand<sup>a,b</sup>, Cédric Arvieux<sup>c,d</sup>, Guillaume Coiffier<sup>d,e,f</sup>, Jean-Louis Polard<sup>d,g</sup>, Jean-David Albert<sup>d,e,f</sup>, Pascal Guggenbuhl<sup>d,e,f,h</sup>, Anne Jolivet-Gougeon<sup>a,b,d,h\*</sup>

<sup>a</sup> EA 1254 Microbiologie, Université de Rennes 1, 2, avenue du Professeur Léon Bernard, 35043 Rennes, France

<sup>b</sup> Pole Biologie Rennes University Hospital, 35043, Rennes, France

<sup>c</sup> Service des Maladies infectieuses, Rennes University Hospital, 2 rue Henri Le Guilloux, 35043, Rennes, France

<sup>d</sup> Centre de Référence en Infections Ostéo-Articulaires du Grand Ouest (CRIOGO)

<sup>e</sup> Service de Rhumatologie, Hôpital Sud, CHU F- 35000 Rennes, France

<sup>f</sup> INSERM UMR U991 F-35000 Rennes, France

<sup>g</sup> Service de Chirurgie orthopédique, Rennes University Hospital, 2 rue Henri Le Guilloux, 35043, Rennes, France

<sup>h</sup> Université de Rennes 1 F- 35000 Rennes, France

**Corresponding author\***

Anne Jolivet-Gougeon, Equipe de Microbiologie, EA 1254, Université de Rennes 1, 2, avenue du Professeur Léon Bernard, 35043 Rennes, France

Phone: (33) 2 23 23 43 05 – Fax: (33) 2 23 23 49 13

E-mail: anne.gougeon@univ-rennes1.fr

**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.

56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80

## 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

81 been extended to clinical diagnosis, either directly from positive blood culture vials [12] or  
82 from samples such as urine [13]. Research suggests that this technique is relevant for  
83 microorganism identification, with functionality comparable to routine methods used in the  
84 clinical microbiology laboratory [14]. In the case of blood culture vials, bacterial  
85 identification by MS directly on the vial pellets optimizes the rendering time result with a  
86 time-saving of 1 to 24 h over conventional methods depending on the extraction technique  
87 [15, 16]. Results quickly available contribute to reducing morbidity [17] and mortality in  
88 addition to lower cost of treatment and length of hospital stay.

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

## 98 **2. Material and methods**

### 99 *2.1. Samples - Scheme of the study*

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

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

## 108 2.2. *Bacteriological studies*

109 SF and CSs were treated according to microbiological routine techniques. Bone samples  
110 were crushed using a bead mill (Retsch® MM400 crusher: frequency 30.0 / s, for two min  
111 and 30 s). Tubes containing 10 sterile stainless steel beads (4 mm diameter) (AISI 304 Grade  
112 1000; AFBMA; Hammer & Lemarié, France) in 10 mL of molecular biology grade distilled  
113 water were prepared, sterilized, tested and stored at room temperature for a maximum of 3  
114 months in the laboratory. Following all safety protocols, contents of one tube was poured into  
115 each sterile container (HDPE) containing the OAS and grinded [4].

116 To ensure proper identification of cultures on solid media by MS, 50 µL of SF or CS  
117 were plated on Columbia agar supplemented with horse blood (5%) (Oxoid®), chocolate agar  
118 (Oxoid®) in atmosphere enriched with 5% CO<sub>2</sub> for 72 h and Columbia agar supplemented  
119 with horse blood (5%) in an anaerobic atmosphere for 5 days at 37°C.[18,19]

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

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

130 the polished steel target plate for rapid identification by MALDI-TOF. Once a positive vial  
131 was automatically detected, 1 ml of broth was extracted without delay (< 2 h, to preserve  
132 spectra) with formic acid overlay [18] and analyzed via the same method as for colonies.  
133 Criteria for interpretation of results were based on the manufacturer's recommendations  
134 (Bruker®). Identification was established through biostatistics reliability levels on the basis  
135 of a correlation between the acquired spectrum and the reference spectra. The spectrum of the  
136 unknown test organism, acquired through MALDI Biotyper CA System Software®, was  
137 electronically transformed into the peak list. Using a biostatistical algorithm, this peak list was  
138 compared to reference peak lists of organisms in the reference database, and a log(score)  
139 value between 0.00 and 3.00 was calculated. The higher the log(score) value, the more  
140 reliable the degree of similarity (to a given organism in the reference FDA-cleared database).  
141 A log(score) value of  $\geq 2.00$  indicated an excellent probability for test organism identification  
142 at the species level. The interpretation considered two independent parameters: the value of  
143 the homology score and the reproducibility of identification obtained (on 10 measurements  
144 carried out after laser impacts, the same bacterial species must be found at least three times  
145 with the highest scores, particularly in cases with a score <1.7). Identification with a score  $\geq 2$   
146 was considered reliable to the species; identification with a score  $\geq 1.7$  was considered  
147 reliable to the genus. An identification score of 1.5 was also examined in light of several  
148 prior studies suggesting that it adequately identified the bacterial genus [22-24]. Identification  
149 was considered unacceptable when the score (< 1.7) and reproducibility were insufficient, and  
150 incorrect when the score or reproducibility was acceptable, with poor identification to the  
151 species level. If necessary, 16SrDNA PCR was performed to confirm bacterially uncertain  
152 identifications, as previously described [4]. To assess the performance of MS in diagnosing  
153 bone and joint infections, we compared score and reproducibility results on bacterial  
154 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

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

180 samples or transplanting from enrichment vials) were consistent with species identification in  
181 79.8% of cases, with the genus in 90.4% of cases, and unacceptable identification in 1.9% of  
182 cases (score and insufficient reproducibility), or incorrect identification in 4.8% (score or  
183 acceptable reproducibility, but poor identification at the species level).

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

191 MS performance was better for staphylococci than for streptococci for all parameters: a  
192 high degree of identification (38.5% vs.17.6%,  $p=0.03$ ), species identification (89.7% vs.  
193 58.8%,  $p=0.001$ ), genus identification (100% vs.70.6%,  $p<0.001$ ), incorrect identification  
194 (0% vs.29.4%,  $p=0.03$ ) and acceptable reproducibility (100% vs.58.8%,  $p<0.001$ ).

195  
196 3.3. Comparison of MS score results from pellets after enrichment in blood vials from blood  
197 agar (Table 2)  
198

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

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

212

### 213 3.4. Polymicrobial samples (Table 3)

214 In polymicrobial cultures, identification of the two species by MS was acceptable in  
215 92.8% of cases [26/28 identifications (92.9%) with a score >1.5; 2/28 identifications (7.1%)  
216 with 1.5<score<1.7]. Correct identification was obtained in all cases (14/14) of a single  
217 bacterial species and in 12/14 (85.7%) for 2 bacterial species; no peak could be detected for  
218 2/14 (14.3%) vials (second identification) (Table 3).

219

## 220 4. Discussion

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

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

235 To our knowledge, few studies have evaluated the performance of MS in identifying  
236 bacteria directly on vial pellets after enrichment of bone samples. Using the Sepsityper kit on  
237 blood pellets, the percentage of correct identification was 92% at the species level; this  
238 number increased when decreasing to the threshold of 1.5, retained by some studies [24]. In  
239 our work, *P. acnes* were all correctly identified (score > 2). This finding is in contrast to a  
240 study conducted by Stevenson et al. [28] that reported 27.3% of unacceptable identifications  
241 (score < 1.7) for *P. acnes*, a result that was previously found by MS directly performed on  
242 colonies [29]. However, their study carried out only a series of five 1-to-2 min  
243 washing/centrifugation steps (without the lysis step of the Sepsityper kit) to remove red blood  
244 cells and proteins from the blood culture broths. In our study, all unidentified bacteria and the  
245 majority of incorrect identifications concerned the genus *Streptococcus* (13.6%), especially  
246 the alpha-hemolytic group. This was already demonstrated in many prior studies on blood  
247 culture vials [22, 26, 27]. Using the Sepsityper kit, the percentage of high degree of  
248 identification (score > 2.3) on enriched bone samples was higher in our study (54.9%) than  
249 what was found in blood culture vials by Kok et al. [27], who reported 47.1% for Gram-  
250 negative bacteria, 9.8% for staphylococci and 22.6% for streptococci (in our study 68.2%,  
251 60% and 34.6%, respectively). However, Kok et al. [27] detected more coagulase-negative  
252 staphylococci and non-fermenting Gram-negative bacilli, both of which are commonly less  
253 well identified, potentially explaining the differences from our work. The percentage of high  
254 degree of identification (score > 2.3) was significantly higher on vial pellets than on blood

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

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

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

280 possible with an inoculum of at least  $10^3$  UFC/mL [32]. Works carried out on blood culture  
281 vials showed acceptable identification from  $10^6$  CFU/mL. For comparison, the average  
282 inoculum was  $5 \times 10^8$  CFU/mL for detection of bacterial growth by the blood culture system  
283 [15]. Several studies also reported detection of lower inocula with Gram-positive bacteria by  
284 an automatic chamber, but when the inoculum was  $< 10^6$  CFU/mL, the analyzed spectra were  
285 close to those obtained from sterile vials [15].

286 In previous works using the Sepsityper kit on blood pellets, identifications at the  
287 species level were obtained in less than 2 h [27]. Buchan et al. [33] reported that median times  
288 to identification using the MALDI Biotyper/Sepsityper were 23 to 83 h faster than routine  
289 methods for Gram-positive isolates, and 34 to 51 h faster for Gram-negative isolates in blood  
290 samples. This extraction technique has been standardized and validated in the literature,  
291 further reducing completion time [27, 34, 35]. Many other simplified efficient extraction  
292 methods have also already been tested on blood culture vials. Several techniques reduced the  
293 extraction time by half, for example those methods using saponin,[16] ammonium chloride  
294 [26], trifluoroacetic acid or formic acid [22], or even methods composed only of a series of  
295 centrifugations.[34] It is also possible to reduce the final cost of testing [36]. However,  
296 homemade techniques easily fail to completely respond to standardized criteria required in  
297 medical biology, and results are difficult to compare between different studies.

298  
299 In conclusion, the use of MALDI-TOF MS on bone and synovial samples in culture  
300 vials can be performed for diagnosis and management of oste-articular infections. This  
301 technique reduces the time to report results to the clinician, with a reduced cost [31]. It may  
302 also allow identification of a second bacterial species in case of polymicrobial samples, but  
303 identification of streptococci to the species level remains difficult. Further improvements in

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

### 307 **Acknowledgments**

308 We thank Philippe Gautier for technical assistance for PCR 16SrDNA and Adina Pascu  
309 for his help in formatting of the manuscript. This work was supported by the Centre de  
310 Reference des Infections Ostéoarticulaires complexes du Grand Ouest (CRIOGO, France).

### 311 **Authors' contributions**

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

### 315 **Competing interests**

316 The authors declare that they have no competing interests.

### 318 **References**

- 319
- 320 [1] Societe de Pathologie Infectieuse de Langue F, College des Universitaires de Maladies  
321 Infectieuses et T, Groupe de Pathologie Infectieuse P, Societe Francaise d'Anesthesie et de R,  
322 Societe Francaise de Chirurgie Orthopedique et T, Societe Francaise d'Hygiene H, et al.  
323 [Recommendations for clinical practice. Osteo-articular infection therapy according to  
324 materials used (prosthesis, implants, osteosynthesis)]. *Med Mal Infect* 2009;39:745-74.
- 325 [2] Schafer P, Fink B, Sandow D, Margull A, Berger I, Frommelt L. Prolonged bacterial  
326 culture to identify late periprosthetic joint infection: a promising strategy. *Clin Infect Dis*  
327 2008;47:1403-9.
- 328 [3] Roux AL, Sivadon-Tardy V, Bauer T, Lortat-Jacob A, Herrmann JL, Gaillard JL, et al.  
329 Diagnosis of prosthetic joint infection by beadmill processing of a periprosthetic specimen.  
330 *Clin Microbiol Infect* 2011;17:447-50.
- 331 [4] Bemmer P, Plouzeau C, Tande D, Leger J, Giraudeau B, Valentin AS, et al. Evaluation  
332 of 16S rRNA gene PCR sensitivity and specificity for diagnosis of prosthetic joint infection: a  
333 prospective multicenter cross-sectional study. *J Clin Microbiol* 2014;52:3583-9.



- 334 [5] Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, et al.  
335 Sonication of removed hip and knee prostheses for diagnosis of infection. *N Engl J Med*  
336 2007;357:654-63.
- 337 [6] Gomez E, Cazanave C, Cunningham SA, Greenwood-Quaintance KE, Steckelberg  
338 JM, Uhl JR, et al. Prosthetic joint infection diagnosis using broad-range PCR of biofilms  
339 dislodged from knee and hip arthroplasty surfaces using sonication. *J Clin Microbiol*  
340 2012;50:3501-8.
- 341 [7] Microbiologie SFd. REMIC: référentiel en microbiologie médicale (bactériologie et  
342 mycologie). SFM, Paris 2010;4ème édition.
- 343 [8] Rogers GB, Carroll MP, Bruce KD. Studying bacterial infections through culture-  
344 independent approaches. *J Med Microbiol* 2009;58:1401-18.
- 345 [9] Bizzini A, Jatton K, Romo D, Bille J, Prod'homme G, Greub G. Matrix-assisted laser  
346 desorption ionization-time of flight mass spectrometry as an alternative to 16S rRNA gene  
347 sequencing for identification of difficult-to-identify bacterial strains. *J Clin Microbiol*  
348 2011;49:693-6.
- 349 [10] Croxatto A, Prod'homme G, Greub G. Applications of MALDI-TOF mass spectrometry  
350 in clinical diagnostic microbiology. *FEMS Microbiol Rev* 2012;36:380-407.
- 351 [11] Eigner U, Holfelder M, Oberdorfer K, Betz-Wild U, Bertsch D, Fahr AM.  
352 Performance of a matrix-assisted laser desorption ionization-time-of-flight mass spectrometry  
353 system for the identification of bacterial isolates in the clinical routine laboratory. *Clin Lab*  
354 2009;55:289-96.
- 355 [12] Ferreira L, Sanchez-Juanes F, Porrás-Guerra I, Garcia-Garcia MI, Garcia-Sanchez JE,  
356 Gonzalez-Buitrago JM, et al. Microorganisms direct identification from blood culture by  
357 matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Clin Microbiol*  
358 *Infect* 2011;17:546-51.
- 359 [13] Ferreira L, Sanchez-Juanes F, Gonzalez-Avila M, Cembrero-Fucinos D, Herrero-  
360 Hernandez A, Gonzalez-Buitrago JM, et al. Direct identification of urinary tract pathogens  
361 from urine samples by matrix-assisted laser desorption ionization-time of flight mass  
362 spectrometry. *J Clin Microbiol* 2010;48:2110-5.
- 363 [14] Garcia P, Allende F, Legarraga P, Huilcaman M, Solari S. [Bacterial identification  
364 based on protein mass spectrometry: A new insight at the microbiology of the 21st century].  
365 *Rev Chilena Infectol* 2012;29:263-72.
- 366 [15] Christner M, Rohde H, Wolters M, Sobottka I, Wegscheider K, Aepfelbacher M.  
367 Rapid identification of bacteria from positive blood culture bottles by use of matrix-assisted  
368 laser desorption-ionization time of flight mass spectrometry fingerprinting. *J Clin Microbiol*  
369 2010;48:1584-91.
- 370 [16] Meex C, Neuville F, Descy J, Huynen P, Hayette MP, De Mol P, et al. Direct  
371 identification of bacteria from BacT/ALERT anaerobic positive blood cultures by MALDI-  
372 TOF MS: MALDI Sepsityper kit versus an in-house saponin method for bacterial extraction. *J*  
373 *Med Microbiol* 2012;61:1511-6.
- 374 [17] Galar A, Leiva J, Espinosa M, Guillen-Grima F, Hernaez S, Yuste JR. Clinical and  
375 economic evaluation of the impact of rapid microbiological diagnostic testing. *J Infect*  
376 2012;65:302-9.
- 377 [18] McElvania Tekippe E, Shuey S, Winkler DW, Butler MA, Burnham CA. Optimizing  
378 identification of clinically relevant Gram-positive organisms by use of the Bruker Biotyper  
379 matrix-assisted laser desorption ionization-time of flight mass spectrometry system. *J Clin*  
380 *Microbiol* 2013;51:1421-7.
- 381 [19] Bémer P, Léger J, Tandé D, Plouzeau C, Valentin AS, Jolivet-Gougeon A, et al. Centre  
382 de Référence des Infections Ostéo-articulaires du Grand Ouest (CRIOGO) Study Team. How  
383 Many Samples and How Many Culture Media To Diagnose a Prosthetic Joint Infection: a



- 384 Clinical and Microbiological Prospective Multicenter Study. *J Clin Microbiol.* 2016 54:385-  
385 91.
- 386 [20] Rohner P, Pepey B, Auckenthaler R. Advantage of combining resin with lytic  
387 BACTEC blood culture media. *J Clin Microbiol* 1997;35:2634-8.
- 388 [21] Schulthess B, Brodner K, Bloemberg GV, Zbinden R, Bottger EC, Hombach M.  
389 Identification of Gram-positive cocci by use of matrix-assisted laser desorption ionization-  
390 time of flight mass spectrometry: comparison of different preparation methods and  
391 implementation of a practical algorithm for routine diagnostics. *J Clin Microbiol*  
392 2013;51:1834-40.
- 393 [22] La Scola B, Raoult D. Direct identification of bacteria in positive blood culture bottles  
394 by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. *PLoS One*  
395 2009;4:e8041.
- 396 [23] Moussaoui W, Jaulhac B, Hoffmann AM, Ludes B, Kostrzewa M, Riegel P, et al.  
397 Matrix-assisted laser desorption ionization time-of-flight mass spectrometry identifies 90% of  
398 bacteria directly from blood culture vials. *Clin Microbiol Infect* 2010;16:1631-8.
- 399 [24] Nonnemann B, Tvede M, Bjarnsholt T. Identification of pathogenic microorganisms  
400 directly from positive blood vials by matrix-assisted laser desorption/ionization time of flight  
401 mass spectrometry. *APMIS* 2013;121:871-7.
- 402 [25] Biswas S, Rolain JM. Use of MALDI-TOF mass spectrometry for identification of  
403 bacteria that are difficult to culture. *J Microbiol Methods* 2013;92:14-24.
- 404 [26] Prod'homme G, Bizzini A, Durussel C, Bille J, Greub G. Matrix-assisted laser desorption  
405 ionization-time of flight mass spectrometry for direct bacterial identification from positive  
406 blood culture pellets. *J Clin Microbiol* 2010;48:1481-3.
- 407 [27] Kok J, Thomas LC, Olma T, Chen SC, Iredell JR. Identification of bacteria in blood  
408 culture broths using matrix-assisted laser desorption-ionization Sepsityper and time of flight  
409 mass spectrometry. *PLoS One* 2011;6:e23285.
- 410 [28] Stevenson LG, Drake SK, Murray PR. Rapid identification of bacteria in positive  
411 blood culture broths by matrix-assisted laser desorption ionization-time of flight mass  
412 spectrometry. *J Clin Microbiol* 2010;48:444-7.
- 413 [29] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, et al. Ongoing  
414 revolution in bacteriology: routine identification of bacteria by matrix-assisted laser  
415 desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis* 2009;49:543-51.
- 416 [30] Martinez RM, Bauerle ER, Fang FC, Butler-Wu SM. Evaluation of three rapid  
417 diagnostic methods for direct identification of microorganisms in positive blood cultures. *J*  
418 *Clin Microbiol* 2014;52:2521-9.
- 419 [31] Chen JH, Ho PL, Kwan GS, She KK, Siu GK, Cheng VC, et al. Direct bacterial  
420 identification in positive blood cultures by use of two commercial matrix-assisted laser  
421 desorption ionization-time of flight mass spectrometry systems. *J Clin Microbiol*  
422 2013;51:1733-9.
- 423 [32] Kohling HL, Bittner A, Muller KD, Buer J, Becker M, Rubben H, et al. Direct  
424 identification of bacteria in urine samples by matrix-assisted laser desorption/ionization time-  
425 of-flight mass spectrometry and relevance of defensins as interfering factors. *J Med Microbiol*  
426 2012;61:339-44.
- 427 [33] Buchan BW, Riebe KM, Ledebner NA. Comparison of the MALDI Biotyper system  
428 using Sepsityper specimen processing to routine microbiological methods for identification of  
429 bacteria from positive blood culture bottles. *J Clin Microbiol* 2012;50:346-52.
- 430 [34] Juiz PM, Almela M, Melcion C, Campo I, Esteban C, Pitart C, et al. A comparative  
431 study of two different methods of sample preparation for positive blood cultures for the rapid  
432 identification of bacteria using MALDI-TOF MS. *Eur J Clin Microbiol Infect Dis*  
433 2012;31:1353-8.

434 [35] Morgenthaler NG, Kostrzewa M. Rapid identification of pathogens in positive blood  
435 culture of patients with sepsis: review and meta-analysis of the performance of the sepsityper  
436 kit. *Int J Microbiol* 2015;2015:827416.

437 [36] Martiny D, Dediste A, Vandenberg O. Comparison of an in-house method and the  
438 commercial Sepsityper kit for bacterial identification directly from positive blood culture  
439 broths by matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry. *Eur J*  
440 *Clin Microbiol Infect Dis* 2012;31:2269-81.

441

442

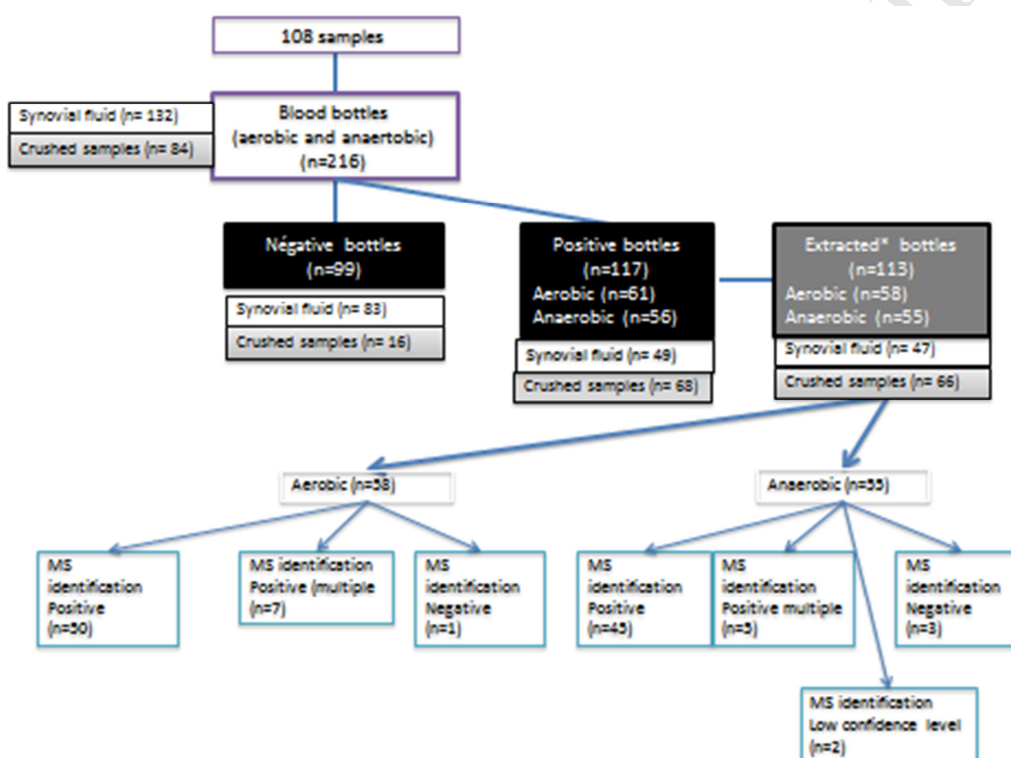
ACCEPTED MANUSCRIPT

443

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

447

448



449

450

451

452

453

454

455

456

457

458

459

460

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

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

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

466

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

472

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

476

477

ACCEPTED MANUSCRIPT

Table 2.

Results of MALDI(TOF MS identification)	Blood vials (both) (n=113)	Blood agar (n=104)	<i>Staphylococcus</i> (n=39)	<i>Streptococcus</i> <i>Enterococcus</i> (n=17)	Gram negative bacilli (n=29)	Gram positive bacilli (n=4)	Anaerobes (n=12)
	No of isolates (%)						
High degree of identification to species <i>Score</i> > 2.3	62 (54.9)	42 (40.4)	15 (38.5)	3 (17.6)	20 (69)	1 (25)	3 (25)
Identification to species <i>Score</i> > 2	91 (80.5)	83 (79.8)	35 (89.7)	10 (58.8)	29 (100)	2 (50)	7 (58.3)
Identification to genus <i>Score</i> > 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 <i>Score</i> > 1.5	107 (94.7)	94 (90.4)	39 (100)	12 (70.6)	29 (100)	4 (100)	10 (83.3)
Unacceptable identification <i>Score</i> < 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	<i>Staphylococcus aureus</i> + <i>Enterobacter cloacae</i>	<i>Staphylococcus aureus</i>	2,036	1	<i>Enterobacter cloacae</i>	2,04	8	2	SF**
1	Anaerobic	<i>Staphylococcus aureus</i> + <i>Enterobacter cloacae</i>	<i>Enterobacter cloacae</i>	2,241	9	<i>Staphylococcus aureus</i>	1,84	1	2	SF
2	Aerobic	<i>Arthrobacter cummingsii</i> + <i>Weeksella virosa</i> + <i>Oligella urethralis</i>	<i>Weeksella virosa</i>	2,041	2	<i>Arthrobacter cummingsii</i>	1,701	5	3	CS***
3	Anaerobic	<i>Peptoniphilus harei</i> + <i>Propionibacterium avidum</i>	<i>Propionibacterium avidum</i>	2,127	4	<i>Peptoniphilus harei</i>	1,849	0	0	CS
4	Aerobic	<i>Staphylococcus aureus</i> + <i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	2,235	9	<i>Escherichia coli</i>	1,96	2	2	CS
4	Anaerobic	<i>Staphylococcus aureus</i> + <i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	2,312	10	none			2	CS
5	Aerobic	<i>Enterococcus faecalis</i> + <i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	2,147	5	<i>Staphylococcus aureus</i>	1,92	5	2	CS
5	Anaerobic	<i>Enterococcus faecalis</i> + <i>Staphylococcus aureus</i> + <i>Streptococcus oralis</i>	<i>Staphylococcus aureus</i>	2,099	6	<i>Enterococcus faecalis</i>	1,964	4	3	CS
6	Aerobic	<i>Enterococcus faecalis</i> + <i>Staphylococcus aureus</i> + <i>S oralis</i>	<i>Enterococcus faecalis</i>	2,224	6	<i>Staphylococcus aureus</i>	1,886	3	3	CS
6	Anaerobic	<i>Enterococcus faecalis</i> + <i>Staphylococcus aureus</i> + <i>S oralis</i>	<i>Enterococcus faecalis</i>	2,41	8	none				CS
7	Aerobic	<i>Klebsiella pneumoniae</i> + <i>Enterobacter aerogenes</i> + <i>Eikeinella corrodens</i>	<i>Klebsiella pneumoniae</i>	2,422	8	<i>Enterobacter aerogenes</i>	1,878	2	3	CS

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

\*on pellets extracted from vial

\*\*synovial fluid

\*\*\* crushed sample