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Marina Oviaño, Belén Rodríguez-Sánchez, Marta Gómara, Luis Alcalá, Estrella Zvezdanova, Adrián Ruíz, David Velasco, María José Gude, Emilio Bouza, Germán Bou

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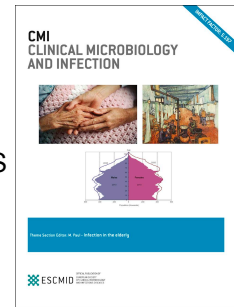
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1 **Direct identification of clinical pathogens from liquid culture media by MALDI-TOF MS**  
2 **analysis**

3 Marina OVIAÑO<sup>1</sup>, Belén RODRÍGUEZ-SÁNCHEZ<sup>2</sup>, Marta GÓMARA<sup>3</sup> Luis ALCALÁ<sup>2</sup>, Estrella  
4 ZVEZDANOVA<sup>2</sup>, Adrián RUÍZ<sup>2</sup>, David VELASCO<sup>1</sup>, María José GUDE<sup>1</sup>, Emilio BOUZA<sup>2</sup> and Germán  
5 BOU<sup>1, #</sup>  
6

7 <sup>1</sup> Servicio de Microbiología. Complejo Hospitalario Universitario A Coruña. As Xubias s/n.  
8 15006, La Coruña. Spain

9 <sup>2</sup> Servicio de Microbiología. Hospital General Universitario Gregorio Marañón. Instituto de  
10 Investigación Sanitaria Gregorio Marañón. Madrid. Spain

11 <sup>3</sup> Servicio de Microbiología. Hospital Universitario Miguel Servet. Zaragoza. Spain

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13 **Running title:** Direct MALDI-TOF-based identification of pathogen from liquid cultures

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15 #Address for correspondence:

16 Germán Bou

17 Servicio de Microbiología

18 Complejo Hospitalario Universitario A Coruña

19 Xubias de Arriba s/n, 3ª Planta Ed. Sur

20 15006 La Coruña, Spain

21 Phone: +34 981-176087

Fax: +34 981-176097

22 E-mail: German.bou.arevalo@sergas.es; german.bou@usc.es

23 **Abstract**

24 **Objectives:** We propose using MALDI-TOF MS as a tool for identifying microorganisms directly  
25 from liquid cultures after enrichment of the clinical sample in the media, in order to obtain a  
26 rapid microbiological diagnosis and an adequate administration of the antibiotic therapy in a  
27 clinical setting.

28 **Methods:** To evaluate this approach, a series of quality control isolates, were grown in  
29 thioglycollate (TG) broth and brain heart infusion (BHI) broth and extracted under 4 different  
30 protocols before finally being identified by MALDI-TOF MS. After establishing the best  
31 extraction protocol, we validated the method in a total of 300 liquid cultures (150 in TG broth  
32 and 150 in BHI broth) of different types of clinical samples obtained from two tertiary Spanish  
33 hospitals.

34 **Results:** The initial evaluation showed that the extraction protocol including a 5 min sonication  
35 step yielded 100% valid identifications, with an average score value of 2.305. In the clinical  
36 validation of the procedure, 98 % of the microorganisms identified from the TG broth were  
37 correctly identified relative to 97 % of those identified from the BHI broth. In 24 % of the  
38 samples analysed, growth by direct sowing was only successful in the liquid medium, and no  
39 growth was observed in the direct solid agar cultures.

40 **Conclusions:** Use of MALDI-TOF-MS plus the sonication-based extraction method enabled  
41 direct and accurate identification of microorganisms in liquid culture media in 15 min, in  
42 contrast to the 24 hours of subculture required for conventional identification, allowing the  
43 administration of a targeted antimicrobial therapy.

44 **Introduction**

45 Rapid and reliable identification of bacteria is essential for the diagnosis and treatment of  
46 patients with infectious diseases. Until recently, biochemical, colorimetric and even antibiotic  
47 sensitivity tests were used to identify genera and species. The main limitations of these  
48 methods include the time required and the difficulty in distinguishing between poorly reactive,  
49 very similar, or difficult-to-culture microorganisms. Many of these problems have been solved  
50 by the Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-  
51 TOF MS) [1-6]. Cost effectiveness studies have demonstrated that the early diagnosis of  
52 bacteraemia and other infectious diseases by MALDI-TOF MS has improved antimicrobial use,  
53 allowing a rapid administration of a targeted antimicrobial therapy [7-11]. However, one of  
54 the main limitations of MALDI-TOF MS is that more than  $10^5$  colony forming units (CFU)/ml are  
55 required for accurate identification of bacteria [12-13]. Direct identification of bacteria in  
56 clinical samples has therefore so far only been possible with urine samples [5-6].

57 Use of liquid cultures has increased the sensitivity and turnaround time of bacterial culture,  
58 especially for samples with low bacterial loads that do not grow in solid culture, e.g.  
59 cerebrospinal fluid (CSF), pericardial fluid and joint fluid [14]. However, a period of 24 hours is  
60 required to identify the grown up microorganism by subsequent growth on solid culture and  
61 final identification. We propose using MALDI-TOF MS as a tool for identifying microorganisms  
62 directly from liquid cultures (thioglycollate broth and brain heart infusion broth) after  
63 extracting the bacterial protein in a sonication-based procedure.

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68 **Material and methods**69 **Clinical setting and sample collection**

70 The study was performed between November 2016 and February 2017 in two tertiary teaching  
71 hospitals in Spain, the *Complejo Hospitalario Universitario A Coruña* (CHUAC) and the *Hospital*  
72 *General Universitario Gregorio Marañón* (HGUGM). For the study, each laboratory cultured  
73 150 clinical samples in enriched liquid medium. In the CHUAC, the microorganisms were  
74 cultured in thioglycollate (TG) broth supplemented with vitamin K1 and hemin (Becton  
75 Dickinson, United States), while in the HGUGM the microorganisms were cultured in brain  
76 heart infusion (BHI) broth (Becton Dickinson).

77 The following different types of samples were cultured in liquid medium: biopsy (n=50),  
78 exudate from surgical wounds (n=34), prosthetic material (n=25), cardiac valve (n=10),  
79 catheter tip (n=5), pericardial fluid (n=1), pleural fluid (n=10), synovial fluid (n=20), bile fluid  
80 (n= 20), peritoneal fluid (n=25) and CSF (n=100).

81 **Optimization of the extraction protocol for direct bacterial identification from liquid media**

82 To evaluate the optimal extraction protocol, various different quality control strains  
83 (*Escherichia coli* ATCC 25922, *Haemophilus influenzae* ATCC 49247, *Pseudomonas aeruginosa*  
84 ATCC 27083, *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 49619,  
85 *Listeria monocytogenes* ATCC 15313 and *Neisseria meningitidis*) were inoculated in parallel in  
86 both TG broth and BHI broth with the amount of bacteria filling a 1- $\mu$ l inoculation loop and  
87 incubated for 16-24 hours at 37°C. After growth was indicated by the turbidity of the media,  
88 an aliquot of 1.5 ml of each medium was transferred to an Eppendorf tube. The sample was  
89 centrifuged at 14.000 rpm for 2 min and the supernatant was discarded. Five hundred  $\mu$ l of  
90 water was added to the sample, and the agar and other debris were removed by pipetting  
91 before another 500  $\mu$ l of water was added. The subsequent steps varied depending on the

92 protocol (Supplementary material). Each sample was extracted in triplicate and each extract  
93 was analysed in duplicate. The following protocol (nº 4) was finally established:

94 Protocol 4. The sample was sonicated at 200 W (Ultrasons, JP Selecta S. A. Barcelona) for 5 min  
95 before being centrifuged at 14.000 rpm for 2 min. The supernatant was discarded and the  
96 pellet was washed with 500 µl of water. The sample was vortexed again and centrifuged at  
97 14.000 rpm for 2 min. Finally, the supernatant was discarded to yield the bacterial pellet for  
98 MALDI-TOF MS analysis.

### 99 **Clinical validation**

100 We performed a prospective clinical validation of the assay in 300 liquid cultures of clinical  
101 samples. The procedure was applied by researchers who were blinded to the type of samples.  
102 First, 139 liquid cultures incubated for 16-24 hours at 37°C and with no visually detectable  
103 turbidity were processed using the extraction protocol selected. Secondly, 161 liquid cultures  
104 with visually observed turbidity were processed using the same protocol. All liquid cultures  
105 were subcultured, in parallel with the MALDI-TOF MS direct identification, in Trypticase Soy  
106 Agar (TSA, Becton Dickinson, EEUU), Chocolate Agar (Becton Dickinson) and Schaedler Agar  
107 (Becton Dickinson). TSA and Chocolate agar plates were incubated in 5-10 % CO<sub>2</sub> atmosphere  
108 and Schaedler Agar in an anaerobic atmosphere at 37°C. Colonies grown in the subcultures  
109 were identified by MALDI-TOF MS. Cultures were considered negative after 6 days of  
110 incubation without growth of microorganisms.

### 111 **MALDI-TOF MS processing and analysis**

112 The pellet obtained at the end of the extraction procedure was spread with a pipette tip on  
113 the MALDI-TOF MS steel plate spots and allowed to dry. One µl of 70% formic acid (Sigma-  
114 Aldrich, United States) was added to the sample and allowed to air-dry. The spots were then  
115 covered with the MALDI matrix (10mg/mL α-cyano-4-hydroxy-cinnamic acid in 50% acetonitrile  
116 / 0.1% trifluoroacetic acid; Bruker Daltonik GmbH). Samples were analyzed in duplicates.

117 Spectra were acquired in a MALDI Microflex LT/SH bench-top mass spectrometer (Bruker  
118 Daltonik GmbH) equipped with a 60 Hz nitrogen laser. FlexControl v.3.0 software (Bruker  
119 Daltonik GmbH) was used to acquire the spectra and the MALDI Biotyper 3.1 (Bruker Daltonik  
120 GmbH) for real time interpretation and identification of the microorganisms. According to the  
121 manufacturer, a score > 2.0 indicates species identification, a score between 1.7 and 2.0  
122 indicates genus identification and a score < 1.7 indicates unreliable identification.

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## 124 **Results**

### 125 **Use of a sonication step is key to optimal bacterial extraction**

126 Protocol 1, based on the cell lysis using the lysis buffer from the Sepsityper Kit (Bruker Daltonik  
127 GmbH), provided 52.4% (44/84) valid identifications, and *N. meningitidis* and *H. influenzae*  
128 isolates were misidentified providing MALDI-TOF MS no peaks in the spectra acquisition (Table  
129 1, supplementary material). The average score value was 1.947 [1.444- 2.397]. Protocol 2,  
130 based on cell lysis with lysozyme, yielded 69.0 % (58/84) valid identifications, and *H. influenzae*  
131 isolates were misidentified, providing MALDI-TOF MS an unreliable identification. The average  
132 score value was 1.905 [1.163- 2.373]. Protocol 3, based on the use of SDS detergent, yielded  
133 63.1% (53/84) valid identifications, and *N. meningitidis* and *H. influenzae* isolates were  
134 misidentified. The average score value was 2.048 [1.714-2.383]. Protocol 4, based on a 5 min  
135 sonication step, yielded 100% (84/84) valid identifications with an average score value of 2.305  
136 [1.950- 2.525], and no microorganisms were misidentified. The average score value for  
137 microorganisms cultured in the BHI broth was 0.084 higher than the score for the  
138 microorganisms cultured in the TG broth.

### 139 **MALDI-TOF-MS direct identification from liquid cultures**

140 In the first part of the study, 139 liquid cultures (61 TG and 78 BHI) with no visually observable  
141 turbidity were processed using the previously optimized sonication-based extraction protocol.  
142 MALDI-TOF MS did not detect any bacteria and no growth occurred on solid agar plates, so the  
143 specificity of the direct identification by MALDI-TOF MS is 100%, for all samples tested.

144 In the second part of the study, 161 liquid cultures with visually observable turbidity were  
145 processed using the previously optimized sonication-based extraction protocol. Of the 89  
146 liquid cultures analyzed in TG broth, 84 were monomicrobial cultures (Table 1). The sensitivity  
147 of MALDI-TOF MS for detecting the pathogen in the monomicrobial cultures from the TG broth  
148 was 98 % (82/84), with a reliable identification to the species level in 74% (61/82) and an  
149 average score of 2.088. The undetected isolates were *Staphylococcus caprae*, isolated from  
150 prosthetic material, and *Streptococcus anginosus*, isolated from bile. MALDI-TOF MS  
151 successfully detected at least one microorganism in 100% (5/5) of the polymicrobial cultures  
152 from TG broth, with an average score of 1.982.

153 Of the 72 liquid cultures grown in BHI broth (Table 2), 68 were monomicrobial cultures. The  
154 sensitivity of MALDI-TOF MS for detecting the pathogen in the monomicrobial cultures grown  
155 in BHI broth was 97% (66/68), with a reliable identification to the species level in 77% (51/ 66)  
156 with an average score of 2.090. The 2 undetected isolates were *Candida albicans* and *Candida*  
157 *tropicalis*. MALDI-TOF MS enabled identification of at least one microorganism in 100% (4/4) of  
158 the polymicrobial cultures grown in BHI broth, with average score of 2.158.

159 For monomicrobial cultures carrying Gram-negative bacteria, MALDI-TOF MS yielded an  
160 average score of 2.172 with a reliable identification to the species level in 85% (33/39) for  
161 direct identification from TG broth (n=39) and 2.092 with a reliable identification to the species  
162 level in 72% (21/22) from BHI broth (n=22). For Gram-positive bacteria, MALDI-TOF MS yielded  
163 an average score of 2.017 with a reliable identification to the species level in 69% (29/42) for  
164 direct identification from TG broth (n=42) and 2.071 with a reliable identification to the species



165 level in 68% (30/44) from BHI broth (n=44). For identification of monomicrobial cultures  
166 carrying anaerobic bacteria, MALDI-TOF MS yielded an average score of 2.110 with a reliable  
167 identification to the species level in 25% (1/4) for direct identification from TG (n=4),  
168 identifying *Bacteroides fragilis* from CSF and peritoneal fluid samples and *Propionibacterium*  
169 *acnes* from two biopsy samples. For monomicrobial cultures carrying anaerobic bacteria,  
170 MALDI-TOF MS yielded an average score of 1.809, with no reliable identification to the species  
171 level in any of the samples in the direct identification from BHI (n=3), identifying *Clostridium*  
172 *perfringens* in a bile, *Propionibacterium acnes* in a joint prosthesis sample and *C. innocuum* in a  
173 surgical wound. For monomicrobial cultures carrying fungus, MALDI-TOF MS yielded an  
174 average score of 2.107 for direct identification from TG broth (n=3), identifying *Candida*  
175 *glabrata* in a derivation cardiac valve sample and in a catheter tip sample and *Cryptococcus*  
176 *neoformans* var. *grubii* in a CSF sample. MALDI-TOF MS did not reliably identify any BHI  
177 cultures carrying fungus, and subculture revealed *Candida albicans* in a bile and *Candida*  
178 *tropicalis* in a cardiac valve sample.

179 Unreliable identification by MALDI-TOF MS was not associated with any particular type of  
180 clinical sample. The MALDI-TOF MS method for direct identification from liquid cultures did  
181 not yield any false positive results. In addition, the overall positive predictive value was 100 %  
182 and the negative predictive value, 97 %.

183 In 24 % (39/ 160) of the samples analysed, growth by direct sowing was only successful in the  
184 liquid medium, and no growth was observed in the direct solid agar cultures. These samples  
185 comprised CSF (n=13), prosthetic material (n=4), biopsy (n=8), cardiac valve (n=5), catheter tip  
186 (n=2), peritoneal fluid (n=4), synovial fluid (n=1) and bile fluid (n=2). Agreement of 100% (39/  
187 39) was found between the results obtained by direct identification from the liquid medium by  
188 MALDI-TOF MS and the results obtained by identification of the corresponding subculture. The  
189 average MALDI-TOF MS score was 2.150.

190 **Discussion**

191 In the present study, we demonstrated that MALDI-TOF MS can also provide accurate, reliable  
192 and rapid identification of pathogens directly from liquid cultures after enrichment of clinical  
193 samples with the sonication-based extraction procedure. This method was able to correctly  
194 identify the main species causing meningitis and the most common bacterial species found in  
195 clinical microbiology. In the present study, 50 % (13/26) of the positive CSF samples only grew  
196 successfully in the liquid culture media, with MALDI-TOF MS providing 100% accurate  
197 identification within 16-24 hours of the sample arriving in the laboratory. The method is  
198 particularly accurate for pathogens that are scarce and difficult to detect, as was the case for  
199 *P. aeruginosa* isolated from a neurosurgery patient and others that were not detected by  
200 Gram staining, as was the case for the *Listeria monocytogenes* in an old man and even not  
201 suspected (e.g. *C. neoformans\_var\_grubii* isolated from a pulmonary transplant patient).  
202 Although the clinical impact is obvious for meningitis, other applications of this novel MALDI-  
203 TOF MS identification procedure may be of great value. We have observed that 55 % (5/9)  
204 pathogens isolated from cardiac valves were recovered exclusively in the liquid media, having  
205 a great impact in the diagnosis of endocarditis and in the management of prosthetic heart  
206 valves [15-16]. Furthermore, 33 % (4/12) of the pathogens isolated from prosthetic material  
207 were recovered exclusively in the liquid media. This is of great importance for prosthetic joint  
208 samples, in which differentiation between infection and aseptic loosening of the replacement  
209 joint is difficult to achieve clinically [17-18].

210 Regarding the extraction procedure from the liquid media, the sonication protocol yielded  
211 sensitivities close to 100 % without using as a final step the gold-standard ethanol/formic acid  
212 extraction procedure recommended by Bruker Daltonik GmbH. We recommend applying the  
213 hole procedure on a second time, only if the identification is not reliable in the first place.  
214 Regarding the culture media, we did not observe substantial differences between the TG and

215 BHI broth. The unidentified microorganisms isolated from the TG media were mainly  
216 *Streptococcus anginosus* group. (i.e. *S. anginosus* in a bile and *S. constellatus* and *S. anginosus*  
217 in two mixed cultures). The heterogeneity of members of the *Streptococcus anginosus* group  
218 has traditionally hampered their correct identification, and although MALDI-TOF MS has  
219 helped, identification to the subspecies level has not yet been clearly established [19]. The  
220 unidentified microorganisms from the BHI broth were yeasts (i.e. *C. tropicalis* and *C. albicans*),  
221 probably because these microorganisms grow less well in the media used [14]. Regarding  
222 polymicrobial cultures, at least one microorganism was correctly identified in 100 % of the  
223 samples. Use of the MALDI Biotyper MSP identification Mixture Method, relative to the  
224 Standard Method used in this study and recommended by the manufacturer for identifying  
225 mixed cultures, did not prove useful for the possible identification of mixed cultures in the  
226 liquid media (data not shown). Thus, this method must be used with caution in clinical settings.  
227 Further improvements in the software should be carried out to validate the possible use of this  
228 method in samples of polymicrobial predictable nature. Direct examination on the positive  
229 liquid culture could be performed prior extraction to confirm the presence of polymicrobial  
230 cultures and reject the direct MALDI-TOF MS identification.

231 Strengths of our study include the double-center, prospective and blinded sample adjudication  
232 of the study. Besides, once the identification is well established, this study opens a way to  
233 detect antimicrobial resistance directly from the liquid culture media as previously performed  
234 in positive blood cultures and in urine samples [6, 20], being one more step towards the early  
235 administration of adequate antimicrobial therapy.

236 Limitations of the study include the application of the technique exclusively in monomicrobial  
237 cultures, the slowness and less sensitivity compared with molecular methods [21-22] and the  
238 possibility to bring out contaminants that are further recovered in the liquid cultures, as  
239 negative-coagulase Staphylococci. We have informed all isolates recovered exclusively in the  
240 liquid media to clinicians responsible of the respective patients, although in case of negative-

241 coagulase Staphylococci we have warned to evaluate with caution the significance of the  
242 microorganism in the clinical setting.

243 The proposed MALDI-TOF MS method for direct identification from liquid media is able to  
244 provide an etiologic diagnosis of the infection only 15 min after observation of the turbidity of  
245 the medium, thus saving the 24 hours required for subculture in conventional analysis. Further  
246 studies should address the clinical impact of the proposed method by examining its capacity to  
247 adapt to different clinical situations and evaluating the yield for the different types of samples  
248 and liquid media.

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268 **Table 1.** Direct identification of the 89 positive TG broth cultures by MALDI-TOF MS.

| Clinical sample (n) <sup>1</sup> | ID solid culture (n) <sup>2</sup>               | ID liquid culture (n) <sup>3</sup> | Reliable species identification by MALDI-TOF MS (y) <sup>4</sup> |
|----------------------------------|---|------------------------------------|--|
| Biopsies (22)                    | <i>E. coli</i> (2)                              | <i>E. coli</i> (2)                 | 100% (2.109)   |
|                                  | <i>E. cloacae</i> (1)                           | <i>E. cloacae</i> (1) / (1)        | 100% (2.268)   |
|                                  | <i>M. morgani</i> (1)                           | <i>M. morgani</i> (1)              | 100% (2.267)   |
|                                  | <i>S. aureus</i> (10)                           | <i>S. aureus</i> (10) / (2)        | 70% (2.058)  |
|                                  | <i>S. epidermidis</i> (1)                       | <i>S. epidermidis</i> (1)          | 0% (1.799)   |
|                                  | <i>S. hominis</i> (1)                           | <i>S. hominis</i> (1) / (1)        | 100% (2.049)   |
|                                  | <i>S. capitis</i> (1)                           | <i>S. capitis</i> (1)              | 100% (2.070)   |
|                                  | <i>S. oralis</i> (1)                            | <i>S. oralis</i> (1) / (1)         | 0% (1.538)   |
|                                  | <i>S. pyogenes</i> (1)                          | <i>S. pyogenes</i> (1)             | 100% (2.303)   |
|                                  | <i>E. faecalis</i> (1)                          | <i>E. faecalis</i> (1) / (1)       | 100% (2.148)   |
| Surgical wound exudates (14)     | <i>P. acnes</i> (2)                             | <i>P. acnes</i> (2) / (1)          | 0% (1.835)   |
|                                  | <i>E. coli</i> (6)                              | <i>E. coli</i> (6)                 | 83% (2.211)  |
|                                  | <i>K. oxytoca</i> (1)                           | <i>K. oxytoca</i> (1)              | 100% (2.188)   |
|                                  | <i>M. morgani</i> (1)                           | <i>M. morgani</i> (1)              | 100% (2.369)   |
|                                  | <i>P. mirabilis</i> (1)                         | <i>P. mirabilis</i> (1)            | 100% (2.111)   |
|                                  | <i>P. aeruginosa</i> (2)                        | <i>P. aeruginosa</i> (2)           | 50% (2.086)  |
| Prosthetic material (4)          | <i>S. aureus</i> (3)                            | <i>S. aureus</i> (3)               | 100% (2.143)   |
|                                  | <i>P. agglomerans</i> (1)                       | <i>P. agglomerans</i> (1) / (1)    | 0% (1.946)   |
|                                  | <i>K. pneumoniae</i> (1)                        | <i>K. pneumoniae</i> (1)           | 100% (2.305)   |
|                                  | <i>S. caprae</i> (1)                            | NRI <sup>5</sup>                   | 0% (<1.6)  |
| Cardiac valves (5)               | <i>S. agalactiae</i> (1)                        | <i>S. agalactiae</i> (1)           | 100% (2.013)   |
|                                  | <i>K. pneumoniae</i> (1)                        | <i>K. pneumoniae</i> (1) / (1)     | 100% (2.305)   |
|                                  | <i>S. hominis</i> (2)                           | <i>S. hominis</i> (2) / (2)        | 50% (2.049)  |
|                                  | <i>E. faecium</i> (1)                           | <i>E. faecium</i> (1) / (1)        | 0% (1.771)   |
| Catheter tips (5)                | <i>C. glabrata</i> (1)                          | <i>C. glabrata</i> (1)             | 100% (2.203)   |
|                                  | <i>S. aureus</i> (3)                            | <i>S. aureus</i> (3) / (1)         | 66% (1.990)  |
|                                  | <i>S. epidermidis</i> (1)                       | <i>S. epidermidis</i> (1) / (1)    | 100% (2.094)   |
| Pleural fluids (3)               | <i>C. glabrata</i> (1)                          | <i>C. glabrata</i> (1)             | 100% (2.159)   |
|                                  | <i>P. aeruginosa</i> (1)                        | <i>P. aeruginosa</i> (1)           | 0% (1.978)   |
|                                  | <i>S. oralis</i> + <i>S. constellatus</i> (1)   | <i>S. oralis</i> (1)               | 100% (2.064)   |
| Synovial fluid (2)               | <i>C. albicans</i> + <i>G. adiacens</i> (1)     | <i>C. albicans</i> (1)             | 0% (1.765)   |
|                                  | <i>S. aureus</i> (2)                            | <i>S. aureus</i> (2) / (1)         | 100% (2.324)   |
| Bile fluids (7)                  | <i>E. coli</i> (3)                              | <i>E. coli</i> (3)                 | 100% (2.315)   |
|                                  | <i>E. cloacae</i> (1)                           | <i>E. cloacae</i> (1) / (1)        | 100% (2.210)   |
|                                  | <i>K. oxytoca</i> (1)                           | <i>K. oxytoca</i> (1)              | 100% (2.160)   |
|                                  | <i>S. anginosus</i> (1)                         | NP <sup>6</sup>                    | 0% (<0)  |
|                                  | <i>C. perfringens</i> + <i>S. anginosus</i> (1) | <i>C. perfringens</i> (1)          | 0% (1.750)   |
| Peritoneal fluids (12)           | <i>E. coli</i> (6)                              | <i>E. coli</i> (6)                 | 100% (2.184)   |
|                                  | <i>B. fragilis</i> (1)                          | <i>B. fragilis</i> (1) / (1)       | 100% (2.328)   |
|                                  | <i>P. aeruginosa</i> (1)                        | <i>P. aeruginosa</i> (1)           | 100% (2.001)   |

|          |  |  |              |
|----------|--|--|--------------|
|          | <i>S. epidermidis</i> (2)                    | <i>S. epidermidis</i> (2) / (2)                  | 100% (2.038) |
|          | <i>E. faecium</i> + <i>C. glabrata</i> (1)   | <i>C. glabrata</i> (1)                           | 100% (2.074) |
|          | <i>K. pneumoniae</i> + <i>E. faecium</i> (1) | <i>K. pneumoniae</i> (1)                         | 100% (2.255) |
| CFS (14) | <i>E. coli</i> (2)                           | <i>E. coli</i> (2)                               | 0% (1.759)   |
|          | <i>K. pneumoniae</i> (1)                     | <i>K. pneumoniae</i> (1)                         | 100% (2.025) |
|          | <i>P. aeruginosa</i> (2)                     | <i>P. aeruginosa</i> (2) / (1)                   | 100% (2.296) |
|          | <i>N. meningitidis</i> (1)                   | <i>N. meningitidis</i> (1) / (1)                 | 100% (2.304) |
|          | <i>B. fragilis</i> (1)                       | <i>B. fragilis</i> (1)                           | 100% (2.300) |
|          | <i>E. faecium</i> (1)                        | <i>E. faecium</i> (1)                            | 100% (2.337) |
|          | <i>S. epidermidis</i> (1)                    | <i>S. epidermidis</i> (1) / (1)                  | 0% (1.797)   |
|          | <i>S. hominis</i> (1)                        | <i>S. hominis</i> (1) / (1)                      | 100% (2.090) |
|          | <i>S. haemolyticus</i> (1)                   | <i>S. haemolyticus</i> (1)                       | 0% (1.870)   |
|          | <i>S. capitis</i> (1)                        | <i>S. capitis</i> (1) / (1)                      | 100% (2.165) |
|          | <i>S. pettenkoferi</i> (1)                   | <i>S. pettenkoferi</i> (1) / (1)                 | 100% (2.110) |
|          | <i>L. monocytogenes</i> (1)                  | <i>L. monocytogenes</i> (1) / (1)                | 100% (2.239) |
|          | <i>C. neoformans</i> var <i>grubii</i> (1)   | <i>C. neoformans</i> var <i>grubii</i> (1) / (1) | 0% (1.763)   |

269

270 <sup>1</sup> Description of the clinical samples analysed after culture in TG, classified depending on their  
 271 origin and the number of samples (n).

272 <sup>2</sup> Identification by MALDI-TOF MS after subculture in solid media with the number of isolates by  
 273 species (n).

274 <sup>3</sup> Direct identification by MALDI-TOF MS after sonication-based extraction from the TG broth,  
 275 with the number of isolates per species (n) and the number of isolates that were exclusively  
 276 isolated in the TG broth (x) and not recovered in the solid culture by direct seeding.

277 <sup>4</sup> Percentage of direct reliable identifications (score value >2.0) obtained by MALDI-TOF MS  
 278 and average scores (y) obtained with the Biotyper MSP identification Standard Method (Bruker  
 279 Daltonik GmbH). Not reliable identifications to the species level obtained scores among [1.7-  
 280 2.0], thus accurate to the genus level, excepting the cases further detailed in the table  
 281 (NRI/NP).

282 <sup>5</sup> NRI: Not reliable identification (score < 1.6)

283 <sup>6</sup> NP: No peaks

284 **Table 2.** Direct identification of the 72 positive BHI broth cultures by MALDI-TOF MS.

| Clinical sample (n) <sup>1</sup> | ID solid culture (n) <sup>2</sup>         | ID liquid culture (n) / (x) <sup>3</sup> | Reliable species identification by MALDI-TOF MS (y) <sup>4</sup> |
|----------------------------------|---|--|--|
| Biopsies (23)                    | <i>E. coli</i> (5)                        | <i>E. coli</i> (5)                       | 100% (2.481)   |
|                                  | <i>M. morgani</i> (1)                     | <i>M. morgani</i> (1)                    | 100% (2.123)   |
|                                  | <i>E. cloacae</i> (2)                     | <i>E. cloacae</i> (2)                    | 100% (2.209)   |
|                                  | <i>S. aureus</i> (4)                      | <i>S. aureus</i> (4)                     | 100% (2.234)   |
|                                  | <i>S. pyogenes</i> (2)                    | <i>S. pyogenes</i> (2)                   | 0% (1.843)   |
|                                  | <i>E. faecalis</i> (1)                    | <i>E. faecalis</i> (1)                   | 100% (2.330)   |
|                                  | <i>E. faecalis</i> (1)                    | <i>E. faecalis</i> (1)                   | 100% (2.233)   |
|                                  | <i>S. epidermidis</i> (2)                 | <i>S. epidermidis</i> (2)                | 50% (2.014)  |
|                                  | <i>S. capitis</i> (1)                     | <i>S. capitis</i> (1) / (1)              | 100% (2.146)   |
|                                  | <i>E. coli</i> + <i>S. agalactiae</i> (1) | <i>E. coli</i> (1)                       | 100% (2.071)   |
|                                  | <i>E. coli</i> + <i>C. striatum</i> (1)   | <i>E. coli</i> (1)                       | 100% (2.237)   |
| Surgical wound exudates (14)     | <i>P. aeruginosa</i> (1)                  | <i>P. aeruginosa</i> (1)                 | 0% (1.710)   |
|                                  | <i>S. marcescens</i> (1)                  | <i>S. marcescens</i> (1)                 | 100% (2.243)   |
|                                  | <i>K. pneumoniae</i> (3)                  | <i>K. pneumoniae</i> (3)                 | 100% (2.316)   |
|                                  | <i>C. innocuum</i> (1)                    | <i>C. innocuum</i> (1)                   | 0% (1.705)   |
|                                  | <i>S. anginosus</i> (2)                   | <i>S. anginosus</i> (2)                  | 50% (2.094)  |
|                                  | <i>S. aureus</i> (3)                      | <i>S. aureus</i> (3)                     | 100% (2.248)   |
|                                  | <i>S. agalactiae</i> (1)                  | <i>S. agalactiae</i> (1)                 | 0% (1.946)   |
|                                  | <i>S. epidermidis</i> (2)                 | <i>S. epidermidis</i> (2)                | 100% (2.238)   |
| Prosthetic material (7)          | <i>S. aureus</i> (2)                      | <i>S. aureus</i> (2) / (2)               | 100% (2.285)   |
|                                  | <i>S. pyogenes</i> (2)                    | <i>S. pyogenes</i> (2)                   | 0% (1.748)   |
|                                  | <i>E. faecalis</i> (1)                    | <i>E. faecalis</i> (1)                   | 100% (2.491)   |
|                                  | <i>P. acnes</i> (1)                       | <i>P. acnes</i> (1) / (1)                | 0% (1.823)   |
|                                  | <i>S. aureus</i> + <i>F. magna</i> (1)    | <i>S. aureus</i> (1)                     | 100% (2.352)   |
| Cardiac valves (4)               | <i>S. mitis</i> (1)                       | <i>S. mitis</i> (1) / (1)                | 0% (1.981)   |
|                                  | <i>S. aureus</i> (1)                      | <i>S. aureus</i> (1)                     | 100% (2.822)   |
|                                  | <i>S. epidermidis</i> (1)                 | <i>S. epidermidis</i> (1)                | 100% (2.305)   |
|                                  | <i>C. tropicalis</i> (1)                  | NRI <sup>5</sup>                         | 0% (< 1.6)   |
| Bile fluids (6)                  | <i>E. coli</i> + <i>E. faecium</i> (1)    | <i>E. coli</i> (1)                       | 100% (2.624)   |
|                                  | <i>P. mirabilis</i> (1)                   | <i>P. mirabilis</i> (1)                  | 100% (2.370)   |
|                                  | <i>S. odorifera</i> (1)                   | <i>S. odorifera</i> (1)                  | 100% (2.326)   |
|                                  | <i>C. perfringens</i> (1)                 | <i>C. perfringens</i> (1)                | 0% (1.898)   |
|                                  | <i>S. constellatus</i> (1)                | <i>S. constellatus</i> (1) / (1)         | 100% (2.124)   |
|                                  | <i>C. albicans</i> (1)                    | NP <sup>6</sup>                          | 0% (<0)  |
| Peritoneal fluids (6)            | <i>K. pneumoniae</i> (1)                  | <i>K. pneumoniae</i> (1)                 | 100% (2.593)   |
|                                  | <i>P. aeruginosa</i> (1)                  | <i>P. aeruginosa</i> (1)                 | 100% (2.472)   |
|                                  | <i>E. faecalis</i> (2)                    | <i>E. faecalis</i> (2) / (1)             | 100% (2.231)   |
|                                  | <i>E. faecium</i> (1)                     | <i>E. faecium</i> (1)                    | 100% (2.222)   |
|                                  | <i>E. coli</i> + <i>K. pneumoniae</i> (1) | <i>E. coli</i> (1)                       | 100% (2.071)   |
|                                  | <i>E. coli</i> (2)                        | <i>E. coli</i> (1)                       | 100% (2.481)   |
|                                  | <i>S. marcescens</i> (2)                  | <i>S. marcescens</i> (2)                 | 100% (2.254)   |

|          |                           |                                 |              |
|----------|---------------------------|---------------------------------|--------------|
| CFS (12) | <i>S. pneumoniae</i> (2)  | <i>S. pneumoniae</i> (2) / (1)  | 100% (2.357) |
|          | <i>S. epidermidis</i> (5) | <i>S. epidermidis</i> (5) / (4) | 40% (2.001)  |
|          | <i>S. simulans</i> (1)    | <i>S. simulans</i> (1)          | 100% (2.130) |

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286

287 <sup>1</sup> Description of the clinical samples analysed after being cultured in BHI broth, classified  
 288 depending on their origin and the number of samples (n).

289 <sup>2</sup> Identification obtained by MALDI-TOF MS after subculture on solid media with the number of  
 290 isolates per species (n).

291 <sup>3</sup> Direct identification by MALDI-TOF MS after the sonication-based extraction from BHI broth,  
 292 showing the number of isolates per species (n) and the number of isolates that were  
 293 exclusively isolated in the BHI broth (x) and not recovered in the solid culture by direct  
 294 seeding.

295 <sup>4</sup> Percentage of direct reliable identifications (score value >2.0) obtained by MALDI-TOF MS  
 296 and average scores (y) obtained with the Biotyper MSP identification Standard Method (Bruker  
 297 Daltonik GmbH). Not reliable identifications to the species level obtained scores among [1.7-  
 298 2.0], thus accurate to the genus level, excepting the cases further detailed in the table  
 299 (NRI/NP).

300 <sup>5</sup> NRI: Not reliable identification (score < 1.6)

301 <sup>6</sup> NP: No peaks



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312 **Transparency declarations**

313 None to declare.

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324 **REFERENCES**

- 325 1. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM *et al.* Ongoing  
326 revolution in bacteriology: routine identification of bacteria by matrix-assisted laser  
327 desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis* 2009; **49**: 543-  
328 51.
- 329 2. Bizzini A, Durussel C, Bille J, Greub G, Prod'hom G. Performance of matrix-assisted laser  
330 desorption ionization-time of flight mass spectrometry for identification of bacterial  
331 strains routinely isolated in a clinical microbiology laboratory. *J Clin Microbiol* 2010;  
332 **48**: 1549-54.
- 333 3. Prod'hom G, Bizzini A, Durussel C, Bille J, Greub G. Matrix-assisted laser desorption  
334 ionization-time of flight mass spectrometry for direct bacterial identification from  
335 positive blood culture pellets. *J Clin Microbiol* 2010; **8**: 1481-3.
- 336 4. Rodríguez-Sánchez B, Sánchez-Carrillo C, Ruiz A, Marín M, Cercenado E, Rodríguez-  
337 Créixems M *et al.* Direct identification of pathogens from positive blood cultures using  
338 matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. *Clin*  
339 *Microbiol Infect* 2014; **20**: 421-7.
- 340 5. Zboromyrska Y, Rubio E, Alejo I, Vergara A, Mons A, Campo I *et al.* Development of a  
341 new protocol for rapid bacterial identification and susceptibility testing directly from  
342 urine samples. *Clin Microbiol Infect* 2016; **22**: 561.e1-6.
- 343 6. Oviaño M, Ramírez CL, Barbeyto LP, Bou G. Rapid direct detection of carbapenemase-  
344 producing Enterobacteriaceae in clinical urine samples by MALDI-TOF MS analysis. *J*  
345 *Antimicrob Chemother* 2017; **72**: 1350-1354.

- 346 7. Osthoff M, Gürtler N, Bassetti S, Balestra G, Marsch S, Pargger H *et al.* Impact of  
347 MALDI-TOF-MS-based identification directly from positive blood cultures on patient  
348 management: a controlled clinical trial. *Clin Microbiol Infect* 2017; 23:78-85.
- 349 8. Verroken A, Defourny L, le Polain de Waroux O, Belkhir L, Laterre PF, Delmée M *et al.*  
350 Clinical impact of MALDI-TOF MS identification and rapid susceptibility testing on  
351 adequate antimicrobial treatment in sepsis with positive blood cultures. *PLoS One*  
352 2016; 11: e0156299.
- 353 9. Clerc O, Prod'hom G, Vogne C, Bizzini A, Calandra T, Greub G. Impact of matrix-  
354 assisted laser desorption ionization time-of-flight mass spectrometry on the clinical  
355 management of patients with Gram-negative bacteremia: a prospective observational  
356 study. *Clin Infect Dis* 2013; 56: 1101-7.
- 357 10. Huang AM, Newton D, Kunapuli A, Gandhi TN, Washer LL, Isip J *et al.* Impact of rapid  
358 organism identification via matrix-assisted laser desorption/ionization time-of-flight  
359 combined with antimicrobial stewardship team intervention in adult patients with  
360 bacteremia and candidemia. *Clin Infect Dis* 2013; 57:1237-45.
- 361 11. Maurer FP, Christner M, Hentschke M, Rohde H. Advances in rapid identification and  
362 susceptibility testing of bacteria in the clinical microbiology laboratory: implications  
363 for patient care and antimicrobial stewardship programs. *Infect Dis Rep* 2017; 9: 6839.
- 364 12. Bizzini A, Greub G. Matrix-assisted laser desorption ionization time-of-flight mass  
365 spectrometry, a revolution in clinical microbial identification. *Clin Microbiol Infect*  
366 2010; 16: 1614-9.
- 367 13. Yan Y, He Y, Maier T, Quinn C, Shi G, Li H *et al.* Improved identification of yeast species  
368 directly from positive blood culture media by combining Sepsityper specimen

- 369 processing and Microflex analysis with the matrix-assisted laser desorption ionization  
370 Biotyper system. *J Clin Microbiol.* 2011; 49: 2528-32.
- 371 14. Baron EJ. Specimen collection, transport, and processing: bacteriology. In: Jorgensen  
372 JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW, editors.  
373 Manual of clinical microbiology, 11th ed. American Society for Microbiology,  
374 Washington, D.C., 2015; pp. 270-315.
- 375 15. Liesman RM, Pritt BS, Maleszewski JJ, Patel R. Laboratory Diagnosis of Infective  
376 Endocarditis. *J Clin Microbiol* 2017; doi: 10.1128/JCM.00635-17. [Epub ahead of print]
- 377 16. Peeters B, Herijgers P, Beuselink K, Verhaegen J, Peetermans WE, Herregods MC *et al.*  
378 Added diagnostic value and impact on antimicrobial therapy of 16S rRNA PCR and  
379 Amplicon Sequencing on resected heart valves in infective endocarditis: a prospective  
380 cohort study. *Clin Microbiol Infec* 2017; doi: 10.1016/j.cmi.2017.06.008. [Epub ahead  
381 of print]
- 382 17. Friedrich MJ, Wimmer MD, Schmolders J, Strauss AC, Ploeger MM, Kohlhof H *et al.*  
383 RANK-ligand and osteoprotegerin as biomarkers in the differentiation between  
384 periprosthetic joint infection and aseptic prosthesis loosening. *World J Orthop* 2017;  
385 8: 342-349.
- 386 18. Sousa R, Serrano P, Gomes Dias J, Oliveira JC, Oliveira A. Improving the accuracy of  
387 synovial fluid analysis in the diagnosis of prosthetic joint infection with simple and  
388 inexpensive biomarkers: C-reactive protein and adenosine deaminase. *Bone Joint J*  
389 2017; 99-B: 351-357.
- 390 19. Arinto-Garcia R, Pinho MD, Carriço JA, Melo-Cristino J, Ramirez M. Comparing matrix-  
391 assisted laser desorption ionization-time of flight mass spectrometry and phenotypic

392 and molecular methods for identification of species within the *Streptococcus*  
393 *anginosus* group. J Clin Microbiol 2015; 53: 3580-8.

394 20.Oviaño M, Sparbier K, Barba MJ, Kostrzewa M, Bou G. Universal protocol for the rapid  
395 automated detection of carbapenem-resistant Gram-negative bacilli directly from  
396 blood cultures by matrix-assisted laser desorption/ionisation time-of-flight mass  
397 spectrometry (MALDI-TOF/MS). Int J Antimicrob Agents 2016; 48: 655-660.

398 21.Akkaya O, Guvenc HI, Yuksekkaya S, Opus A, Guzelant A, Kaya M *et al.* Real-time PCR  
399 detection of the most common bacteria and viruses causing meningitis. Clin Lab.  
400 2017; 63: 827-832.

401 22.Leber AL, Everhart K, Balada-Llasat JM, Cullison J, Daly J, Holt S *et al.* Multicenter  
402 evaluation of biofire FilmArray Meningitis/Encephalitis panel for detection of bacteria,  
403 viruses, and yeast in cerebrospinal fluid specimens. J Clin Microbiol. 2016; 54: 2251-  
404 61.

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