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1 **Relevance of using both aerobic and anaerobic enrichment vials for**
2 **optimizing rapid diagnosis of osteoarticular infections**

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4 Guillaume Coiffier^{1,2,3}, Emmanuelle Ducasse⁴, Arthur Keraudren⁴, Emilie Prat⁴, Hortense
5 Modeste⁴, Jean-David Albert^{1,2,3}, Olivier Loreal¹, Pascal Guggenbuhl^{1,2,3}, Anne Jolivet-
6 Gougeon^{1,3,4,5*}

7
8 ¹ *Univ Rennes, INSERM, INRAE, CHU Rennes, Institut NUMECAN (Nutrition Metabolisms and Cancer),*
9 *F-35000 Rennes, France*

10 ² *Service de Rhumatologie, CHU Rennes, F- 35000 Rennes, France*

11 ³ *Centre de Référence en Infections Ostéo-articulaires du Grand Ouest (CRIOGO)*

12 ⁴ *Univ Rennes, F- 35000 Rennes, France*

13 ⁵ *Pole Biologie, CHU Rennes, 35043, Rennes, France*

14
15 **Running head:** Aerobic and anaerobic vials for diagnosis of bone/joint infections

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18 ***Corresponding author**

19 Anne Jolivet-Gougeon, INSERM NuMeCan/CIMIAD, Université de Rennes 1, 2, avenue du
20 Professeur Léon Bernard, 35043 Rennes, France

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

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

23

24 **Abstract**

25 The performance of a pair of blood culture vials (BACTEC® Plus Aerobic/F, and Anaerobic
26 Lytic/F) were analyzed in 496 osteoarticular specimens (246 synovial fluids and 250 crushed
27 bone samples), obtained in patients during routine diagnostic procedure at the Teaching
28 Hospital of Rennes (France). The positive detection times were recorded for a 14 day-
29 incubation period, and compared between both vials and with agar cultures. For samples
30 from infected patients, the positive detection time was significantly shortened when vials
31 were used compared to agar plates ($p < 0.001$). Median positive detection time was later
32 with the Anaerobic Lytic/F vials (15.0 h) compared to the Plus Aerobic/F (13.0 h). Positivity
33 rate was similar for Anaerobic Lytic/F vials (80.4%) and Plus Aerobic/F vials (83.2%) ($p=0.25$).
34 Some microorganisms were only identified from aerobic vials (15.5%) or from anaerobic vials
35 (12.7%). The use of both atmosphere conditions for optimal positive detection time is
36 therefore critical.

37

38

39 **Key words:** bone infection; blood vials; positive detection time; synovial fluid

40

41 1. Introduction

42 Bone and joint infections constitute a heterogeneous group of infectious pathologies
43 including, among others, spondylodiscitis, prosthetic infections or septic arthritis, whose
44 bacteriological evidence is essential for the proper management of the infection. Diagnosis is
45 based on clinical, bacteriological, radiological and histological arguments, according to
46 guidelines, such as the recommendations of the "Société de Pathologie Infectieuse de
47 Langue Française" (SPILF) [1] or the "Infectious Diseases Society of America" (IDSA) [2].

48 Bacterial identification is essential and prolonged cultures beyond 7 days are needed [3],
49 especially for obligate anaerobic bacterial species [4]. An enrichment in liquid culture media
50 [5] remains essential to allow growth of a low bacterial inoculum, particularly to facilitate
51 the growth of obligate anaerobic bacteria, fastidious bacteria or "small colony variants".
52 Before inoculation into enrichment vials, a sonication step [6, 7] or grinding [8] has been
53 recommended to improve sensitivity of bacterial detection.

54 In blood, after enrichment, the minimal microorganism concentrations required for blood
55 culture vials flagged as positive, using BACTEC FX, were demonstrated $>10^7$ - 10^8 colony
56 forming units (CFU)/mL for most of the blood stream infection pathogens [9]. For blood
57 samples, the BACTEC FX systems yielded 100% positive signals with a minimal starting
58 inoculum of 30 CFU/mL, whereas there were 8.1% (11/135) false-negative results at 5
59 CFU/mL [10]. It is widely recognized that strictly aerobic bacteria are not – or poorly-
60 detected in anaerobic vials, whereas obligate anaerobic bacteria are not –or poorly-
61 detected in aerobic vials [11]. These vials content additives to improve the level of detection,
62 as lysing agents (e.g. saponin) [12] or resins (to trap some antibiotics) [13, 14]. In bone or

63 synovial samples (prosthetic or not), there are many studies demonstrating that the use of
64 an enrichment step of the sample, by using blood vials, is necessary for optimizing diagnosis
65 [15, 16]. Despite some reports, showing the importance of enclosing the two types of vials
66 (aerobic and anaerobic) for optimum positive detection time (PDT) in vials for blood cultures
67 [17], no study had proved the advantage to use one vial rather than the other, or both vials,
68 for diagnosis of osteoarticular and prosthetic joint infections.

69 The aim of this study was to evaluate the advantage of using an aerobic or anaerobic
70 enrichment vial, or both, to improve diagnostic performance of bone and joint infections,
71 using the BD BACTEC FX instrumented blood system. This study was carried out with the aim
72 of saving vials and technical staff time, while maintaining satisfactory quality and sensitivity
73 criteria. The incubation duration (PDT) was recorded, for both aerobic and anaerobic vials
74 inoculated with crushed bone or synovial fluid samples, and compared to standard culture
75 method on agar media. The type of infection was not taken into account in this study, which
76 is intended to be only a technical comparative study, aimed at defining the best performing
77 media to detect bacterial growth as quickly as possible and thus ensure optimal
78 management of the patient.

79

80 **2. Material and Methods**

81 **2.1 Design of the study**

82 A monocentric study was conducted at the University Hospital of Rennes (France).
83 Osteoarticular samples (synovial fluids and surgery bone samples) were prospectively
84 collected by surgeons, rheumatologists or radiologists, during the diagnostic procedure.

85 Synovial fluid as well as bone surgical specimens (crushed samples) were collected in sterile
86 tubes (Nalgen®), compatible with the grinding step, and analyzed at the laboratory of
87 Bacteriology. Before analysis, they were stored at room temperature for a maximum of two
88 hours after collection. Practitioners have been, prior to the study, informed of the methods
89 of analysis that would be implemented and the conditions necessary for collecting samples.
90 Synovial fluids and bone samples were then routinely analyzed at the laboratory of
91 Bacteriology (University Hospital of Rennes, France).

92

93 **2.2 Direct examination**

94 Direct examination was performed, before crushing, directly on a smear of the specimen
95 stained by Gram staining. An observation of the entire slide will be performed by an
96 experienced technician for at least 10 minutes. A positive direct examination was defined as
97 the detection of bacteria in at least two microscope fields.

98

99 **2.3 Cultures**

100 All samples were analyzed according to the microbiology laboratory's routine techniques,
101 after grinding them, using a bead mill (Retsch® MM400 crusher: frequency 30.0/s, for two
102 minutes and 30 seconds), as already described [8].

103 In accordance with usual procedure, the time of positive growth on solid agar media [8] was
104 recorded: two 50- μ l volumes were each spread onto a PolyViteX chocolate agar plate
105 (BioMerieux) and incubated under a 5% CO₂-enriched atmosphere for 3-5 days (to recover

106 strictly aerobic and facultatively anaerobic bacterial species) and onto a blood agar plate
107 incubated in an anaerobic atmosphere (Genbox anaer; BioMerieux) for 5 days (to recover
108 bacterial species, obligate anaerobic and facultatively aerobic). All plates were daily
109 inspected. All samples were also inoculated into a Schaedler broth (BBL™ Schaedler Broth,
110 BD), which was inspected for 14 days. As soon as it became cloudy (or systematically on the
111 14th day), this medium was subcultured on a blood agar plate incubated (on an anaerobic
112 atmosphere) and on a chocolate agar plate (on an aerobic atmosphere) for 48 h.

113 For each sample, 3mL of crushed sample were inoculated in each aerobic (BD BACTEC® Plus
114 Aerobic/F)(BD-AE) and anaerobic (BD BACTEC® Lytic/10 Anaerobic/F)(BD-ANA) enrichment
115 vials. As it has been widely demonstrated for blood cultures, and in order to improve the
116 positive culture rate (and therefore the performance of the technique), the two blood
117 culture bottles (aerobic and anaerobic) were systematically inoculated both (never alone).
118 Incubation was performed in automatic chambers for 10 days (BACTEC® FX, Becton
119 Dickinson). For all positive vial cultures, the PDT (hours) was also recorded for vials who
120 flagged positive by the automatic chamber of the BACTEC FX instrument.

121

122 **2.4 Bacterial identification**

123 Each bacterial species was identified from positive vial(s) or from a single colony by the
124 MALDI-TOF mass spectrometry technique (Biotyper, Bruker®) or from pure cultures by
125 16SrDNA PCR if necessary [18, 19]. Polymicrobial samples were excluded of the analyzed
126 data, because they were too few to allow a satisfactory statistical analysis, and the
127 interpretation of the results seemed more difficult with the data collected in this study.

128

129 **2.5 Statistical analysis**

130 Statistical analysis was performed with SPSS 20.0. $P < 0.05$ was considered significant.

131 Gaussian distribution for continuous quantitative were checked by the Kolmogorov-Smirnov
132 test. No continuous quantitative variables (age and time detection on Bactec or agar
133 support) were showed. So, continuous quantitative were expressed as median \pm
134 interquartile deviation (IQR). The comparison of these variables was performed by a Mann-
135 Whitney nonparametric U test ($k = 2$) or by a Fiedman nonparametric ANOVA ($k > 2$).

136 The qualitative variables are expressed in absolute number and percentage and their
137 comparison was made by a chi-2 test (χ) or the exact Fisher test if one of the theoretical
138 numbers in the contingency table was less than 5.

139

140 **3. Results**

141 **3.1 Population studied**

142 In this study, 46 bacterial species were identified from 496 samples (collected in 305
143 patients). Among the 496 samples, 246 were synovial fluids and 250 were crushed samples.

144 The different bacterial and fungal species identified, taking into account all cultural media,
145 were *Staphylococcus (aureus* and Coagulase negative *Staphylococci*, CNS), *Streptococcus*
146 (*S.pyogenes*, *S.agalactiae*, *S.dysgalactiae*, *S.anginosus*, *S bovis complex*, *S.pneumoniae*, *S.*
147 *gordonii*, *S. oralis*, *S. mitis*, *S. salivarius*), *Enterococcus sp.*, *Cutibacterium acnes*,
148 *Enterobacteriaceae (E.coli*, *K. oxytoca*, *Enterobacter sp.*, *Pantoea agglomerans*, *Citrobacter*

149 *freundii*, *Serratia marcescens*, *Morganella morganii*, *Campylobacter fetus*, *Pseudomonas*
150 *aruginosa*, *Neisseria gonorrhoeae*, and *C. tropicalis* or *M. abscessus*. *Mycobacterium*
151 *abscessus* (n=1) and *Candida tropicalis* (n=1), were detected once (Figure 1). Identification
152 was made for all microorganisms, without distinction of patient diseases, from all synovial or
153 crushed samples. Microorganisms were collected and identified as soon as a bacterial
154 culture was detected in at least one culture medium (solid or liquid).

155

156 **3.2 Direct examination**

157 Direct examination by Gram stain was positive and always concordant with results of
158 bacterial cultures for 99/496 isolates (20.0%) (51/305 patients; 16.7%): 56/246 (22.8%)(for
159 synovial fluid) and 43/250 (17.2%) (for surgery samples).

160

161 **3.3 Recovery rate and percentage of microorganisms according to culture media (Table 1)**

162 Recovery rate and percentage of microorganisms identified was tested in BD-AE and BD-ANA
163 as enrichment vials, and in agar media.

164 *C. acnes* was the most frequently anaerobic commensal bacteria isolated in osteoarticular
165 infections [4], and a significant difference was observed between the two vials for the
166 anaerobic species *C. acnes*, better recovered in anaerobic vial (93% of cases *versus* 11.9% in
167 aerobic vials) ($p < 0.001$). A better recovery was also observed in aerobic vial compared to
168 anaerobic vials for *Staphylococcus* sp. ($p = 0.03$), *Enterobacteria* ($p = 0.03$) and other Gram
169 negative bacteria ($p = 0.01$). Some strains were only identified from aerobic vials (15.5%)(*S.*
170 *aureus*, CNS, *Bacillus licheniformis*, *Corynebacterium striatum*, *Enterobacter cloacae*,

171 *Escherichia coli*, *Klebsiella oxytoca*, *Micrococcus luteus*, *Moraxella catarrhalis*,
172 *Mycobacterium abscessus*, *Pantoea agglomerans*, *Pseudomonas aeruginosa*, *Neisseria*
173 *gonorrhoeae*), or from anaerobic vials (12.7%) (*C. acnes*, *S. epidermidis*, *S. aureus*, *S. hominis*,
174 *S. capitis*, *C. fetus*, *Lactobacillus sp*, *Fingoldia magna*, *Clostridium paraputrificum*, *S.*
175 *sanguis*), indicating the importance of using both atmosphere conditions for optimal PDT . As
176 expected, the obligate anaerobic species were found preferentially in the anaerobic vials and
177 the strictly aerobic species were found preferentially in the aerobic vials.

178 A better recovery was observed for all tested bacteria in Lytic/10 Anaerobic/F vials,
179 compared to agar media. By comparing the growth time in Plus Aerobic/F vials compared to agar
180 media, only *Staphylococcus* spp. grew significantly faster in Plus Aerobic/F medium.

181 Only 2.6% of bacteria have been identified only on agar media, with a small number of
182 colonies (< 10 UFC/plate): *C. acnes* (n=1), *S. aureus* (n=4), *S. hominis* (n=1), *S. warneri* (n=3),
183 *S. epidermidis* (n=2), *S. dysgalactiae* (n=1), and *S. pyogenes* (n=1). Among those, only two
184 species were both positive by direct examination and/or combined to positive blood cultures
185 (*S. dysgalactiae* and *S. pyogenes*.), but in all the other cases, a contamination was widely
186 suspected and compatible with clinical data.

187

188 **3.4 Positive detection time (PDT)**

189 *3.4.1 Detection time according to the type of sample (synovial fluid and crushed samples)*

190 All bacterial species combined, the PDT was 13h (8-23) in BD-AE , 15h [9-30] BD-ANA,
191 and 24.0h [24-48] on agar media, attesting a faster detection in BACTEC® vials (p<0.001)
192 (Table 2). No difference was observed between the two types of samples, synovial fluid and

193 crushed samples ($p>0.05$). In BD-AE, the PDT was 12.0h [7.3-18.1] from synovial fluid and
194 13.5h [9.1-24.1] from crushed samples. In BD-ANA, the PDT was 13.2h [7.2-28.0] from
195 synovial fluids and 16.0h [9.9-31.0] from crushed samples. On agar media, the detection was
196 possible from 24.0h [24-48] of incubation from synovial fluid or crushed samples.

197

198 3.4.2 Detection time according to direct examination

199 The PDT was always significantly lower when the direct examination of the sample
200 was positive: the PDT was 6.6h [5.2-10.5] in BD-AE, 8.4h [5.2-14.2] in BD-ANA and 24.0h [24-
201 24] on agar media. When direct examination was negative, the time of detection was 15.4h
202 [10.4-27.2] in BD-AE, 17.6h [11.1-37.1] in BD-ANA and 24h [24-48] on agar media.

203

204 3.4.3 Detection time according to species and culture atmosphere (Table 2)

205 All strictly aerobic or facultative aerobic organisms were detected within 43.8h and
206 30h in BD-AE vials and BD-ANA vials, respectively. For an incubation period \leq to 24h, 77.6%
207 of the BD-AE vials *versus* 75.2% of the BD-ANA vials were detected positive by the
208 automated system. Following a 48h-period of incubation, 93.1% of BD-AE vials and 86.4% of
209 BD-ANA vials were positively detected. After 144 hours of incubation, only 2.3% of the BD-
210 ANA vials were still detected positive by the automatic chamber. Number of isolated species
211 identified from BD-AE and BD-ANA vials, with their PDT was described in Figure 1. No
212 *Enterobacteriaceae*, *Campylobacter*, *Pseudomonas*, *Neisseria* or *Moraxella* was detected
213 after 48h and 72h incubation from BD-AE and BD-ANA vials, respectively. Between 96 and
214 144 hours of incubation, only 4 BD-AE vials were detected positive (*Staphylococcus* sp., $n = 3$;
215 *Actinomyces*, $n = 1$), while 20 BD-ANA vials were detected positive (*C. acnes*, $n = 12$;

216 *Staphylococcus* sp. n = 8). Beyond 144h of incubation, only 3 BD-AE vials came out positive
217 from the automatic chamber (*C. acnes* n = 3), against 27 BD-ANA vials (*C. acnes*, n = 19;
218 *Staphylococcus* sp., n = 6; *Streptococcus*, n = 1; *Bacillus*, n = 1). Beyond 240h of incubation,
219 no BD-AE vials came out positive from the automatic chamber, against 4 BD-ANA vials (*C.*
220 *acnes*, n = 2; *Staphylococcus* sp., n = 2). In conclusion, we can therefore notice that obligate
221 anaerobic species grow later, as expected, and preferentially in anaerobic vials, but after 10
222 days of incubation, cases of contamination are most often observed.

223

224 *3.4.4 Detection time according to the type of medium used (aerobic and anaerobic vials and*
225 *agar media)*

226 The comparison of PDT of bacteria (all species combined) from BD-AE and BD-ANA
227 enrichment vials, *versus* agar media were shown in Table 3. The PDT was always significantly
228 shorter in vials than on agar media ($p < 0.001$) for almost all species studied. *C. acnes* were
229 detected, in 240h [67.5-246] (n = 5) in aerobic vials, 126h [99-183.75] (n = 40) in anaerobic
230 vials and in 84h [24-102] (n = 6) on agar media (that was considered not significantly
231 different; $p = 0.345$). However, this surprising result must be considered with
232 circumspection. It should be noted that out of the 6 cases where *C. acnes* was only detected
233 on agar, only 2 were confirmed infections according to clinical and radiological criteria, and
234 in other cases agar contamination could be suspected. *M. abscessus* was only detected in
235 aerobic vials before a 92h-incubation time, and *C. tropicalis* was only detected in vials
236 (aerobic PDT=11h and anaerobic PDT=23.5h).

237

238 4. Discussion

239 The purpose of this study was to evaluate the benefit of enrichment using a single aerobic
240 or anaerobic vial, while maintaining the diagnostic performance of bone and synovial
241 infections, and in the hope of saving technical time and money. For both aerobic and
242 anaerobic vials, the PDT required for diagnosis of bone and joint infections, was significantly
243 shorter than on agar media, and most of the time was less than 144h. The optimal PDT in the
244 two vials was variable according to the microorganisms, showing the interest in using both
245 aerobic and anaerobic vials to optimize the speed of diagnosis.

246 Direct examination has been recognized as insensitive, with positivity rates between 10
247 and 30% [19], that was confirmed by this work. The use of sterile beads to crush the samples
248 is known to improve the recovery rate of bacteria from prosthetic joint samples up to 83.7%
249 (instead of 60.8% in case of culture without grinding), with a contamination rate of 8.7%
250 [20]. Some authors used sonication method and found a sensitivity of 100% by inoculation of
251 sonication fluid in blood culture bottles, significantly higher than conventional sonication
252 fluid (87%) or intraoperative tissue cultures (59%) [7]. But although cases of contaminations
253 have been reported using this method, no study has been done to compare the performance
254 of sonication *versus* grinding.

255 Bemer et al. and the CRIOGO group [8] demonstrated the benefit of using vials rather
256 than agar plates for diagnosis of OAIs. They showed a positivity rate of 69% on chocolate
257 agar plate, 70.1% on blood agar plate, 68.8% in Schaedler anaerobic broth medium and 83%
258 in BACTEC® blood culture vials, which is in accordance with our results. Today, culture media
259 and preprocessing of samples are therefore powerful enough to detect the majority of
260 bacterial species responsible for bone and joint infections. However, the PDT must also to be

261 taken into account, especially during septic arthritis where the patient care needs to be as
262 fast as possible, and in case of surgical revision requiring a rapid therapeutic adjustment.
263 However, there is a limit to the value of this early detection: rapid clinical management must
264 therefore be in line with this improved bacteriological detection. The responsiveness of
265 clinicians must be optimal, in order to guarantee the initiation of antibiotic treatment as
266 soon as possible, treatment which can then be guided by a reliable identification, more
267 sensitive than a simple direct examination, and faster than cultures on agar media. Although
268 the growth time in a bottle can often require more than ten hours, this method is still widely
269 used, because it allows to then study the susceptibility of isolated bacteria to many various
270 antibiotics, which is not yet the case in routine today with rapid detection tests (e.g. by PCR
271 or NGS), carried out on sample vials. Moreover, in some cases during collection, PCR
272 inhibitors may also be contained in the blood or synovial fluid added. Enrichment of the
273 culture of osteoarticular specimens and the need for prolonged incubation has been widely
274 demonstrated for optimum detection. Some published works showed that Anaerobic Lytic/F
275 vials were used preferentially to Plus Anaerobic/F vials, because the recovery rate of
276 bacteria and yeast was demonstrated significantly better than in the Plus Anaerobic/F vials,
277 while performance of Plus Aerobic/F vials and Anaerobic Lytic/F vials was equivalent [16].
278 Elliott et al. [12] showed that significantly more Gram-positive organisms ($P < 0.05$) and Gram-
279 negative organisms ($P < 0.05$), including *Enterobacteriaceae* ($P < 0.05$) were recovered from
280 the lytic medium. An incubation of 14 days has been recommended [3], but without the use
281 of blood culture vials as enrichment media. The use of blood culture vials has been shown to
282 reduce incubation time to less than 14 days, with a good recovery of most of pathogenic
283 bacteria as attested by sub-culturing of negative samples [3, 5]. BACTEC® vial enrichment has

284 already been tested for different samples, such as cerebrospinal fluid samples [21] with a
285 better recovery of 95.8% *versus* 53.3% on agar media). Hugues et al. [22] analyzed samples
286 from prosthetic joint revision surgery, and showed that automated BACTEC® culture was the
287 most sensitive method (87%), as compared with cooked meat enrichment broth (83%),
288 fastidious anaerobic broth (57%) and direct plates (39%); all were highly specific (97-100%).
289 Another problem concerning the bacteriological diagnosis is the late growth that must be
290 attributed either to a contamination (at the time of the sampling or at the laboratory) or
291 either to a weak initial inoculum (chronic infection, antibiotherapy ...). The contamination
292 rate was estimated at 2.2% with agar media. With vials this was more difficult to assess,
293 because only *C. acnes* strains were identified after 144h of incubation, and it remained
294 unclear whether this was a case of contamination or whether it was a chronic infection,
295 undiagnosed by the other available criteria of infection. In our study, 2.6% of bacteria have
296 been identified only on agar media, with a small number of colonies (< 10 UFC/plate), and
297 among those, only two species were both positive by direct examination and/or combined to
298 positive blood cultures (*S. dysgalactiae* and *S. pyogenes*.). In all the other cases, a
299 contamination was widely suspected and compatible with clinical data. To confirm or
300 invalidate this hypothesis of contamination, a 16S rRNA gene amplicon sequencing could be
301 carried out on the crushed material. This would make it possible, in the event of a
302 concordant result, to exclude an agar plate contamination and provide an additional
303 argument for a torpid infection with a weak bacterial inoculum. Multiplying the number of
304 culture media and collecting at least 4 specimens for surgical sampling facilitates
305 microbiological interpretation and make it easier to suspect cases of contamination. The use

306 of 16S rRNA gene amplicon sequencing should only be used when cultures are not sufficient
307 to establish the diagnosis, due to a low sensitivity [18].

308 Regarding the PDT of blood culture vials, for sterility testing of corneal organ culture
309 media, Gain et al. [23] estimated that 78% of blood culture vials were detected within 24
310 hours and 94% within 48 hours, which joins our results. Average detection time in the lytic
311 medium was 15.8 h, compared to 22.7 h in the other medium ($P < 0.001$) [12]. Minassian et
312 al. [5] showed that the respiratory atmosphere of the inoculated blood culture vial
313 influenced the detection of microorganisms: *Staphylococci*, *Streptococci*, *Enterococci* and
314 *Enterobacteriaceae* were found in both aerobic and anaerobic vials, whereas obligate
315 anaerobic bacteria (including *C. acnes*) only grew in anaerobiosis, and *Pseudomonas* grew
316 almost exclusively in aerobic vials. Previous work showed that enrichment in vials can be
317 more effective in detecting *C. acnes* (Bemer et al. 2016), but these authors used in their
318 study pediatric vials, whose composition is different from the vials used in our study. This
319 could explain, at least in part, this difference in results. Our study showed the advantage of
320 using a set of two vials (aerobic and anaerobic) to improve the rapidity of diagnosis.

321

322 5. Conclusions

323 Using both aerobic and anaerobic vials, a large majority of bacterial species were
324 identified after only 24 hours of incubation and often less. In addition, the use of MALDI-TOF
325 to directly identify bacteria/yeasts on enrichment media has also saved valuable time for
326 diagnosis. In 15.5% of cases, some microorganisms were only identified from aerobic vials
327 and in 12.7% from anaerobic vials, indicating the importance of using both atmosphere
328 conditions for optimal PDT. The enrichment media can improve the PDT by reducing it to a

329 few hours, but the benefit of this rapid detection will be real only if the results are available
330 continuously for the clinician and if a therapeutic management is immediately conducted.

331

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337 **Conflict of interest**

338 The authors declare that they have no conflict of interests regarding this study.

339

340

341

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413 **List of Tables and Figures**

414

415 **Table 1.**

416 Recovery rate and percentage of main microorganisms identified in (BD BACTEC® Plus
417 Aerobic/F) and anaerobic (BD BACTEC® Lytic/10 Anaerobic/F) enrichment vials, and agar
418 media, attested by a positive detection time (vials) or growth on agar plate, during an
419 incubation period of 10 days for vials and 5 days for plates (for the remaining day-incubation
420 period the Schaedler agar broth was monitored and subcultured, if cloudy or systematically
421 on the 14th day, on an anaerobic blood agar plate and aerobic chocolate agar plate). Other
422 Gram-negative bacteria: *Campylobacter fetus*, *Pseudomonas aeruginosa*, *Neisseria*
423 *gonorrhoeae*, *Moraxella catarrhalis*.

424

425 **Table 2.**

426 Positive detection time of the main micro-organisms in (BD BACTEC® Plus Aerobic/F)(AE) and
427 anaerobic (BD BACTEC® Lytic/10 Anaerobic/F)(ANA) enrichment vials, compared to agar
428 media. Other Gram negative bacteria: *Campylobacter fetus*, *Pseudomonas aeruginosa*,
429 *Neisseria gonorrhoeae*, *Moraxella catarrhalis*. CNS: coagulase negative staphylococci.

430

431 **Table 3**

432 Comparison between positive detection time in BD BACTEC® Plus Aerobic/F)(A) and
433 anaerobic (BD BACTEC® Lytic/10 Anaerobic/F)(B) enrichment vials, with agar media.
434 Only positive samples, both on enrichment vials and agar plates, were counted (Aerobic n =
435 394; Anaerobic n= 347).

436

437 **Figure 1.**

438 Positivity rate and positive detection time (in hours) by each main class of microorganisms:
439 data for BD BACTEC® Plus Aerobic/F)(A) and anaerobic (BD BACTEC® Lytic/10
440 Anaerobic/F)(B) enrichment vials, for all isolates grown from bone and synovial samples
441 (n=496). Bacterial species identified by MALDI-TOF were: *Streptococcus* (*S. pyogenes*, *S.*
442 *agalactiae*, *S. dysgalactiae*, *S. anginosus*, *S. pneumoniae*, *S. gordonii*, *S. salivarius*, *S. oralis*, *S.*
443 *mitis*, *S. bovis*), *Staphylococcus* (*S. aureus*, *S. epidermidis*, *S. capitis*, *S. caprae*, *S. cohnii*, *S.*
444 *haemolyticus*, *S. hominis*, *S. lugdu*, *S. warneri*), other facultative anaerobes Gram + (9
445 species : *Enterococcus*, *Aerococcus*, *Bacillus*, *Clostridium*, *Granulicatella*, *Lactobacillus*,
446 *Micrococcus*), *Cutibacterium acnes* and other Gram positive anaerobes (2 species :
447 *Actinomyces*, *Fingoldia*), one Gram positive aerobe (*Corynebacterium*), one microaerophilic
448 bacteria (*Campylobacter fetus*), facultatively anaerobic Gram negative *Enterobacteriaceae*
449 (*Escherichia*, *Klebsiella*, *Enterobacter*, *Pantoea*, *Citrobacter*, *Serratia*, *Morganella*, *Proteus*)
450 and other strictly aerobic bacteria (*Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*,
451 *Moraxella catarrhalis*, *Mycobacterium abscessus*) or *Candida tropicalis*.

452

453

454 **Table 1**

455

	Number of isolates	Gold-standard	Recovery rate (%)				
			Agar media	Plus Aerobic/F vials	P^a	Lytic/10 Anaerobic/F vials	P^b
<i>Staphylococcus sp.</i>	311	303 (97.4)	278 (89.4)	0.006	259 (83.3)	< 0.001	0.03
<i>S. aureus</i>	178	177 (99.4)	166 (93.3)	0.003	155 (87.1)	< 0.001	0.05
CNS	133	126 (94.7)	112 (84.2)	0.006	104 (78.2)	< 0.001	0.21
<i>Streptococcus sp.</i>	58	57 (98.3)	55 (94.8)	0.62	48 (82.8)	0.008	0.07
<i>Cutibacterium acnes</i>	43	6 (14)	5 (11.9)	0.99	40 (93)	< 0.001	< 0.001
<i>Enterobacteriaceae</i>	43	42 (97.7)	41 (95.3)	0.99	34 (79.1)	0.02	< 0.05
Other Gram negative bacteria	21	19 (90.5)	18 (85.7)	0.99	9 (42.9)	0.003	0.01
All species	496	434 (87.5)	413 (83.2)	0.06	399 (80.4)	0.002	0.25

456

457 CNS: Coagulase Negative Staphylococci

458 ^a P-value for comparison between Plus Aerobic/F vials and Agar media459 ^b P-value for comparison between Lytic/10 Anaerobic/F vials and Agar media460 ^c P-value for comparison between Plus Aerobic/F vials and Lytic/10 Anaerobic/F vials

461

462 **Table 2**

	Positive detection time (h)			<i>p</i>
	Plus Aerobic/F vials	Lytic/10 Anaerobic/F vials	Agar media	
<i>Staphylococcus sp.</i>	15.0 [9.0-25.0]	16.0 [11.0-27.0]	24.0 [24-48]	<0.001
<i>S. aureus</i>	10.0 [7.0-14.0]	12.0 [9.0-19.0]	24.0 [24-24]	<0.001
CNS	28.5 [21.0-43.8]	22.0 [18.0-30.0]	48.0 [24-72]	<0.001
<i>Streptococcus sp.</i>	9.0 [6.0-12.0]	8.0 [5.25-11.0]	24.0 [24-24]	<0.001
<i>Cutibacterium acnes</i>	240.0 [67.5-246]	126 [99-183.75]	84.0 [24-102]	0.345
<i>Enterobacteria</i>	9.0 [6.0-12.0]	7.5 [5.0-11.0]	24.0 [24-24]	<0.001
<i>Other Gram negative bacteria</i>	11.0 [4.75-14.0]	7.0 [5.0-11.5]	24.0 [24-48]	0.001
All	13.0 [8.0-23.0]	15.0 [9.0-30.0]	24.0 [24-48]	<0.001

463

464 **Table 3**

A		Positive detection time on Agar media					
		24h	48h	72h	96h	120h	> 144h
	≤ 24h	244 (61,9%)	39 (9,9%)	14 (3,6%)	3 (0,75%)	4 (1%)	2 (0,5%)
Positive	>24 - ≤ 48h	10 (2,5%)	43 (10,9%)	6 (1,5%)	2 (0,5%)	/	/
detection	>48 - ≤ 72h	4 (1%)	2 (0,5%)	16 (4,1%)	/	/	/
time in	>72 - ≤ 96h	1 (0,25%)	/	/	1 (0,25%)	/	/
<u>Aerobic</u>	>96 - ≤ 120h	2 (0,5%)	1 (0,25%)	/	/	/	/
vials	>120 - ≤ 144	/	/	/	/	/	/
	>144h	/	/	/	/	/	/

465

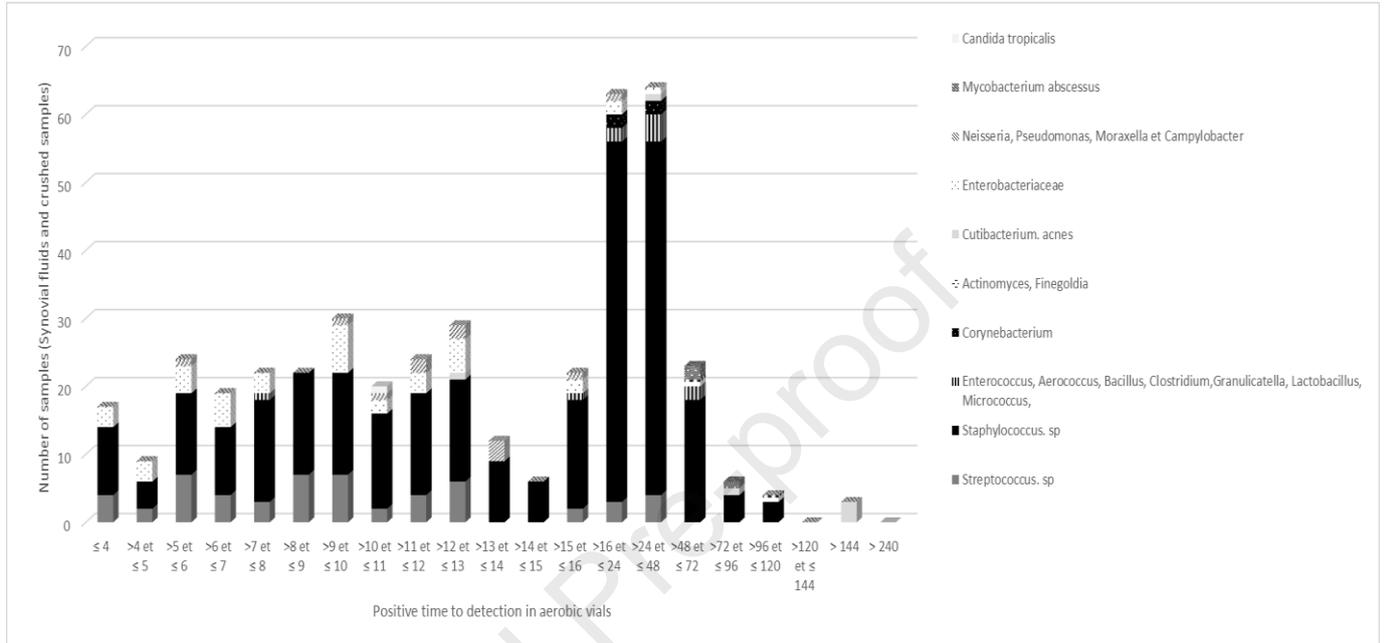
466

B		Positive detection time on Agar media					
		24h	48h	72h	96h	120h	> 144h
	≤ 24h	198 (57,1%)	45 (13%)	12 (3,4%)	3 (0,8%)	1 (0,3%)	2 (0,6%)
Positive	>24 - ≤ 48h	15 (4,3%)	14 (4%)	8 (2,3%)	2 (0,6%)	/	/
detection	>48 - ≤ 72h	7 (2%)	6 (1,7%)	4 (1,15%)	/	/	/
time in	>72 - ≤ 96h	9 (2,6%)	/	2 (0,6%)	/	/	/
<u>Anaerobic</u>	>96 - ≤ 120h	5 (1,4%)	1 (0,3%)	/	/	/	/
vials	>120 - ≤ 144	3 (0,9%)	1 (0,3%)	/	1 (0,3%)	/	/
	>144h	4 (1,2%)	3 (0,9%)	/	1 (0,3%)	/	/

467

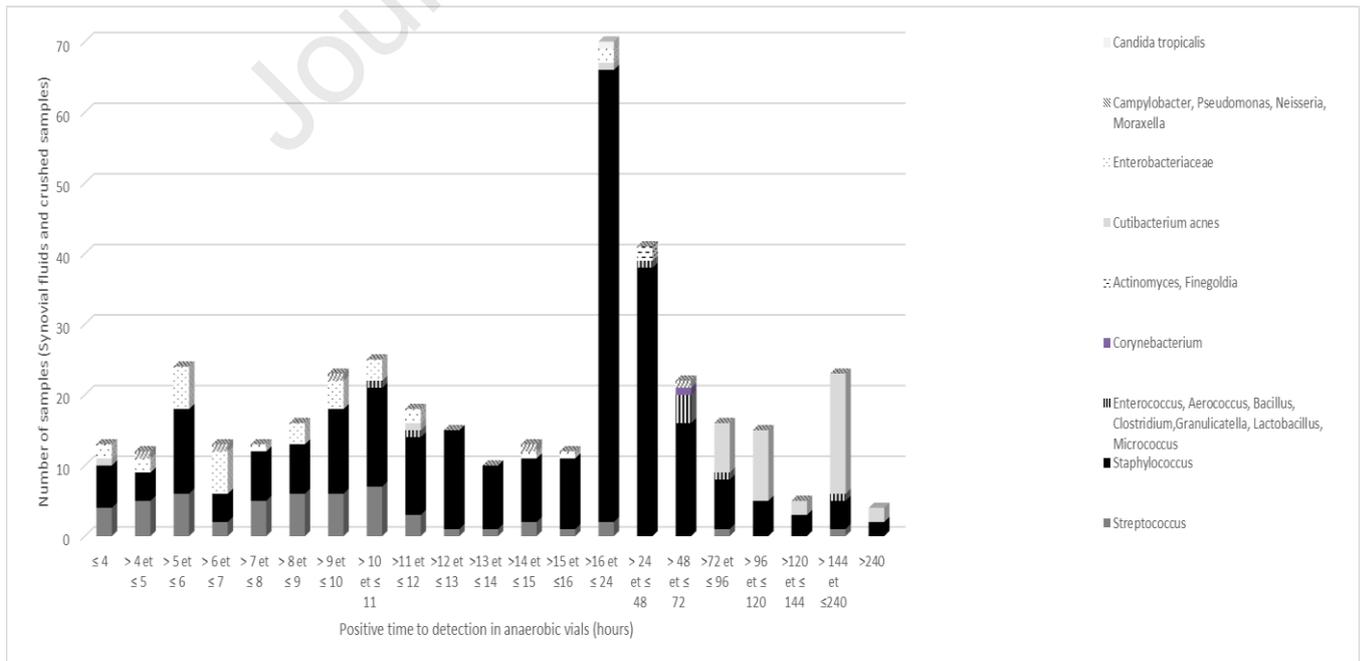
468 **Figure 1.**

469 **A**



470

471 **B**



472

473

Highlights

- After 10 days of incubation, cases of contamination are most often observed
- Use of a set of two vials is recommended to improve the rapidity of diagnosis
- Positivity rate for anaerobic and aerobic vials was > 80%

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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