



## How many samples and how many culture media to diagnose a Prosthetic Joint Infection: A clinical and microbiological prospective multicenter study

Pascale Bémer, Julie Leger, Didier Tandé, Chloé Plouzeau, Anne-Sophie Domelier-Valentin, Anne Jolivet-Gougeon, Carole Lemarié, Marie Kempf, Geneviève Héry-Arnaud, Laurent Bret, et al.

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25 **Abstract**

26 **Background** Although numerous perioperative samples and culture media are  
27 required to diagnose prosthetic joint infection (PJI), their exact number and types  
28 have not yet been definitely determined with a high level of proof.

29 **Objectives** We conducted a prospective multicenter study to determine the minimal  
30 number of samples and culture media required for accurate diagnosis of PJI.

31 **Methods** Over a 2-year period, consecutive patients with clinical signs suggesting  
32 PJI were included, with 5 perioperative samples per patient. The bacteriological and  
33 PJI diagnosis criteria were assessed using a random selection of 2, 3 or 4 samples,  
34 and compared with those obtained using the 5 recommended samples (references  
35 guidelines. The results obtained with 2 or 3 culture media were then compared with  
36 those obtained with 5 culture media for both criteria. The times-to-positivity of the  
37 different culture media were calculated.

38 **Results** PJI was confirmed in 215/264 suspected cases, with a bacteriological  
39 criterion in 192 (89%). The PJI was monomicrobial (85%) or polymicrobial (15%).  
40 Percentages of agreement of 98.1% and 99.7% respectively for the bacteriological  
41 criterion and confirmed PJI diagnosis were obtained when 4 perioperative samples  
42 were considered. The highest percentages of agreement were obtained with the  
43 association of 3 culture media, a blood culture bottle, a chocolate agar plate and  
44 Schaedler broth, incubated for 5, 7 and 14 days respectively. This new procedure  
45 would lead to significant cost saving.

46 **Conclusion** Our prospective multicenter study showed that 4 samples seeded on 3  
47 culture media are sufficient for diagnosing PJI.

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50 **Introduction.** There is still no standard definition of prosthetic joint infection (PJI).  
51 Although numerous perioperative samples are needed to diagnose PJI, their optimal  
52 number is not yet definitively known. In 1998, Atkins et al, using a mathematical  
53 model, demonstrated that five or six perioperative samples with a cut-off of three or  
54 more culture-positive samples are needed to diagnose PJI with high sensitivity and  
55 specificity [1]. Recent guidelines still recommend that about 5 samples should be  
56 cultured for optimal diagnosis of PJI [2, 3]. Since each tissue specimen is cultured on  
57 4 to 5 culture media, a total of 20 to 25 culture media have to be analyzed per  
58 patient. The optimal duration of incubation for periprosthetic cultures was extended to  
59 13 or 14 days especially for *Propionibacterium acnes* recovery [4, 5]. The  
60 implementation of 14-day culture incubation in combination with the use of multiple  
61 solid and liquid aerobic and anaerobic culture media makes the daily monitoring of  
62 PJI by the laboratory very complex, costly and time-consuming. Moreover, the  
63 number of cases of PJI will continue to increase in the coming decades. US forecasts  
64 predict that by 2030, the demand for total hip and knee arthroplasties will grow to 4  
65 million in the United States [6]. A major economic effect is that device-associated  
66 costs are significantly higher than native bone and joint infections, as recently  
67 reported [7]. This will inevitably lead to a dramatic increase in the number of tissue  
68 specimens to be processed by the bacteriology laboratory with major economic  
69 impact on the cost of microbiological diagnosis.

70 Furthermore, the sensitivity of microbiological diagnosis was recently improved by the  
71 use of new techniques for preparing tissue specimens and new culture media,  
72 allowing us to redefine the diagnostic modalities of PJI.

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73 Sonication of explanted prosthesis has been used to dislodge the bacteria from the  
74 surface. Prosthesis sonication culture has been shown to increase culture sensitivity  
75 compared with periprosthetic tissue cultures [8].

76 Bacterial extraction using beadmill specimen processing was shown to improve  
77 bacteriological diagnosis of PJI in a single-center study [9]. Among the clinical  
78 questions recently identified by IDSA guidelines authors, the role of beadmill  
79 processing in the diagnosis of PJI was raised and should be more specifically studied  
80 [2].

81 Another method to increase the diagnostic yield of microbiological diagnosis is to  
82 inoculate samples directly into blood culture bottles. A diagnostic gain was reported  
83 not only for joint aspirate fluids, but also for periprosthetic tissues from several single-  
84 center studies without preliminary beadmill processing [10-16].

85 Finally, the wide range of culture sensitivity observed in the different single-center  
86 studies published underlines the need for multicenter studies to accurately evaluate  
87 microbiological methods [17]. Moreover, the most cost-effective algorithms for  
88 microbiological diagnosis in the light of clinical effectiveness still have to be defined.

89 Our West-French network organization for the multidisciplinary diagnosis and  
90 treatment of bone and joint infections in seven referral centers allowed us to carry out  
91 the first prospective multicenter study related to the microbiological diagnosis of PJI  
92 [18].

93 The main issues of the current study study were: i/ how many samples, and which  
94 samples are needed to obtain an accurate PJI diagnosis, and can we reduce the  
95 number of intraoperative samples? ii/ how many culture media would we need and  
96 how long should we keep them incubated to obtain an accurate PJI diagnosis? iii/

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97 can we confirm the improvement obtained by using blood culture bottles in a  
98 multicenter study? iv/ what is the cost impact of sample care by the microbiology  
99 laboratory?

100 **Patients and methods**101 **Study design**

102 This study was designed as a multicenter, prospective, observational, cross-sectional  
103 study of adult patients suspected of having PJI. The study protocol [Programme  
104 Hospitalier de Recherche Clinique Interrégional API/N/041] was approved by the  
105 institutional review board and ethics committee. Informed consent was obtained from  
106 each patient before inclusion.

107 **Study population**

108 Consecutive patients with clinical signs suggesting acute or chronic PJI were  
109 included in 7 French University hospitals from December 2010 through March 2012.  
110 Six tissue samples were collected during surgery, five samples for the culture and  
111 16S rDNA PCR, and one periprosthetic membrane sample for histological analysis.  
112 An electronic case-report form was created to collect the following data for each  
113 patient: patient demographic characteristics, arthroplasty localization, presentation of  
114 infection, and antibiotic treatment over the 15 days before surgery.

115 **Definition of a PJI**

116 Acute PJI was suspected in patients with pain, disunion, necrosis or inflammation of  
117 the scar in the 3 months following prosthesis implantation. Chronic infection was  
118 suspected in the presence of chronic pain without systemic symptoms, as well as a  
119 loosened prosthesis. According to IDSA recent proposals, PJI was diagnosed when  
120 at least one of the following criteria was positive [2]:

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121 1/ Clinical criterion with a sinus tract communicating with the prosthesis and/or  
122 purulence around the prosthesis.

123 2/ Histology positive for infection (as specified below).

124 3/ Bacteriological infection criterion (as specified below).

125 **Microbiological methods**

126 Periprosthetic tissue cultures were produced in each center following a standard  
127 protocol. For each patient, 5 perioperative specimens (tissue, bone or joint fluid  
128 specimens) were collected in sterile vials with different surgical instruments. After  
129 adding 10 ml of sterile water and stainless steel beads, the vials were shaken on a  
130 Retsch MM401 beadmill. 1 mL was inoculated into a pediatric blood culture bottle  
131 and into a Schaedler broth, as recommended, and both were incubated for 14 days.  
132 Three further 50  $\mu$ L volumes were each spread onto a blood agar plate and a  
133 Polyvitex chocolate agar plate, incubated under a CO<sub>2</sub>-enriched atmosphere for 7  
134 days, and a blood agar plate incubated in an anaerobic atmosphere for 7 days.  
135 Synovial fluids were seeded directly onto the 3 solid and the 2 liquid culture media  
136 without beadmill processing. The Schaedler broth was inspected weekly for  
137 cloudiness, and subcultured on a Schaedler blood agar plate or a blood agar plate  
138 incubated in an anaerobic atmosphere for 48 hours as soon as it became cloudy, or  
139 systematically on the 14<sup>th</sup> day.

140 Time-to-positivity of blood culture bottles was defined as a positive detection by the  
141 automatic blood culture system. Isolated bacteria were identified using standard  
142 laboratory procedures. Antibiotic susceptibility testing was determined as  
143 recommended [19]. The bacteriological criterion was considered positive when at  
144 least one culture yielded a strict pathogen (*Staphylococcus aureus*, *Pseudomonas*

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145 *aeruginosa*, *Enterobacteriaceae*, anaerobes), or two cultures if the isolated strain was  
146 a skin commensal (such as coagulase-negative staphylococci (CoNS) or *P. acnes*)  
147 [2, 3].

148 **Histological analysis**

149 The periprosthetic membrane samples were fixed in buffered formalin, and paraffin  
150 block sections were stained with haematoxylin and eosin. Following Feldman's  
151 criteria (adapted from Mirra's criteria), the histology was considered positive for  
152 infection when at least 5 neutrophils per high-power field (x400) were found after  
153 examination of at least five separate microscopic fields [20, 21]. The specimens were  
154 examined by pathologists blinded to the suspicion of PJI and to the results of the  
155 cultures.

156 **Statistical analysis strategy**

157 Statistical analyses were performed with SAS software, version 9.3 (SAS institute,  
158 Cary, NC, USA) and R 3.1.3 [22].

159 **Main issues**160 **1/ Number of samples required for PJI diagnosis.**

161 Our first study objective was to estimate the impact of processing 2, 3, or 4 samples,  
162 instead of the 5 samples usually recommended, on bacteriological and PJI diagnostic  
163 criteria. For each patient, we randomly selected 4 samples and re-assessed the  
164 bacteriological and the PJI diagnosis criteria. We then checked whether these re-  
165 assessed bacteriological and PJI diagnosis criteria agreed with those obtained using  
166 the full microbiological result dataset. This procedure was re-run 1000 times to  
167 estimate an average agreement rate (proportion of correct results, whether PJI or



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168 non-PJI correctly classified), with its associated 95% confidence interval. The same  
169 operation was also conducted using sub-samples of 3 and 2 samples.

170 **2/ Number and type of culture media required for PJI diagnosis.**

171 The Cochran Q test was used to evaluate the overall effect of the culture medium on  
172 the proportion of samples that tested positive for aerobic and anaerobic bacteria. The  
173 positivity rates of samples from paired culture media were then compared using  
174 McNemar's Test.

175 A secondary study objective was to estimate the impact of eliminating 2 or 3 culture  
176 media on the bacteriological criterion and PJI diagnosis. The results obtained with 2  
177 or 3 culture media were compared with those obtained with 5 culture media for both  
178 bacteriological and PJI diagnostic criteria. The results of the different analyses were  
179 presented with the percentage of agreement for each criterion.

180 **Secondary issues**

181 **1/ Incubation period of culture media required for PJI diagnosis.**

182 As the time-to-positivity in liquid media cannot be determined for polymicrobial  
183 infection, they were determined for monomicrobial infections only. The time-to-  
184 positivity of the different culture media were determined separately for aerobic and  
185 anaerobic bacteria.

186 **2/ Considerations on cost management of PJI samples for the bacteriology**  
187 **laboratory and its possible reduction.**

188 A cost per patient was evaluated, including 5 perioperative samples, each seeded in  
189 5 different culture media, with part of the technical time spent on the culture and  
190 processing of positive cultures. This cost was then compared to that estimated for the

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191 optimal number of samples and culture media as determined from our statistical  
192 analysis.

**193 Results****194 Diagnosis of infection**

195 After analysis of clinical, bacteriological and histological criteria, a definitive diagnosis  
196 of infection was confirmed in 215 out of 264 suspected cases of PJI (Figure 1). The  
197 PJI were chronic for 168/215 (78.1%) and acute for 47/215 (21.9%) of them. For  
198 patient characteristics, see reference [18].

199 Of the 215 patients with confirmed PJI, 192 (89.3%) had a positive bacteriological  
200 criterion, monomicrobial in 163 (84.9%) cases, and polymicrobial in 29 (15.1%). Of  
201 the monomicrobial infections, staphylococci were isolated in 66.3%, streptococci and  
202 enterococci in 13.5%, Gram-negative bacilli in 9.8%, anaerobes in 8.0%, and Gram-  
203 positive bacilli in 2.5% (Figure 1).

204 Of the 29 polymicrobial infections, 21 involved 2 or more different aerobic bacteria, 7  
205 involved both aerobic and anaerobic bacteria, and 1 involved exclusively anaerobes  
206 (Figure 1).

207 Among 70 confirmed cases of PJI in patients treated with antibiotics at the time of  
208 surgery, 54 (77%) were positive in culture (44 monomicrobial and 10 polymicrobial  
209 PJI), and 16 (23%) remained negative in culture.

210 **Minimal number and best types of samples required for an accurate PJI**  
211 **diagnosis.**

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212 Of the 192 patients with a positive bacteriological criterion, a total of 849 (88.4%) out  
213 of 960 samples were positive in culture, i.e. an average number of 4.4 per patient.  
214 Tissues in contact with material and joint fluids accounted for 45.6% of the collected  
215 samples. The rate of positivity ranged from 76.6% to 91.7% depending on the nature  
216 of the sample: cancellous bone 76.6% (N=36/47), bone in contact with cement 78.8%  
217 (N=41/52), capsule 84.8% (N=67/79), cortical bone 87.1% (N=81/93), synovial tissue  
218 88.2% (N=90/102), subfascial tissue 89.3% (N=133/149), tissue in contact with  
219 material 91.5% (N=247/270) and joint fluid 91.7% (N=154/168).

220 From the 1000 generated data sets, the bacteriological criterion and diagnosis of  
221 confirmed PJI had a mean percentage of agreement of 85.2% and 98.0%  
222 respectively when 2 samples were considered, and 93.9% and 99.2% respectively  
223 when 3 samples were considered. Percentages of agreement of 98.1% and 99.7%  
224 respectively for both criteria were obtained when 4 perioperative samples were  
225 considered (Figure 2).

**226 Number and type of culture media required for an accurate PJI diagnosis .**

227 Preliminary comparison of anaerobic culture media showed the superiority of the  
228 broth medium, as 75.2% of anaerobic bacteria grew on Schaedler broth *versus*  
229 56.2% on anaerobic blood agar. Thus, for the simplicity of analysis, the Schaedler  
230 broth was the only anaerobic medium considered in the statistical analysis.

231 Of the 171 cases of PJI involving aerobes (150 monomicrobial and 21 polymicrobial),  
232 the rate of positive samples was 70.1% on blood agar plate, 69.0% on chocolate  
233 agar plate, 68.8% on Schaedler broth, and 83.0% in the blood culture bottle (Table  
234 1). Of the 21 cases of PJI involving anaerobes (13 monomicrobial and 8  
235 polymicrobial), the rate of positive samples was 31.4% on blood agar plate, 53.3% on

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236 chocolate agar plate, 75.2% on Schaedler broth, and 54.3% in the blood culture  
237 bottle (Table 2).

238 As the Cochran Q test showed at least one of the four culture media differed  
239 significantly from another, whether for aerobes ( $p < 0.0001$ ) or anaerobes ( $p < 0.0001$ ),  
240 so paired comparisons were performed. These comparisons using the McNemar test  
241 showed that the rate of positive samples was significantly higher in the blood culture  
242 bottle for aerobes (Table 1), and in Schaedler broth for anaerobes (Table 2)  
243 compared to other media. Compared to what was observed on the blood agar plate,  
244 the rate of positive cultures on the chocolate agar plate was similar for aerobic  
245 bacteria, and much higher for anaerobic bacteria due to the predominance of *P.*  
246 *acnes*. There were 4 chronic cases of PJI with bacteria detected using blood cultures  
247 only, 3 due to *S. aureus* (2 susceptible and 1 methicillin resistant) and 1 to methicillin-  
248 susceptible *S. epidermidis*. Two of the 4 patients were being treated by antibiotics at  
249 the time of surgery.

250 Finally, a combination of 3 culture media gave results similar to those obtained with 4  
251 or 5 culture media. The highest percentages of agreement of the bacteriological  
252 criterion (98.3% for aerobes and 85.7% for anaerobes) and the confirmed diagnosis  
253 of PJI (100% for both aerobes and anaerobes) were obtained with a combination of a  
254 blood culture bottle, a chocolate agar plate and the Schaedler broth (Table 3).

**255 Times-to-positivity according to culture media.**

256 All the aerobic bacteria were detected from positive pediatric blood culture bottles in  
257 5 days (except for 1 sample which was positive in 10 days), and grew in 7 days from  
258 chocolate or blood agar plates. Of the aerobic bacteria, 84.1% grew in less than 10  
259 hours in blood culture bottles versus 71.4% in 18 to 24 hours on agar media.

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260 93% of aerobes were isolated during the two first days of observation, and 7%  
261 between the third and the 7<sup>th</sup> day, rendering still needed the incubation of 7 days for  
262 solid media.

263 Most of the *P. acnes* strains were isolated from Schaedler broth (14.0% (N=7) from 2  
264 to 4 days, and 86% (N=36) between 5 and 14 days), against 34 (68%) in 7 days from  
265 chocolate agar plates in a CO<sub>2</sub> atmosphere or blood agar plates in an anaerobic  
266 atmosphere, and 26 (52%) in 6 days from pediatric blood cultures. 28% (12/43) of the  
267 Schaedler broths, not cloudy after 14 days of incubation, were systematically  
268 subcultured on the 14<sup>th</sup> day.

**269 Cost impact of sample management by the microbiology laboratory.**

270 Cost assessment was based on 5 samples grown on 5 culture media per patient as  
271 in our multicenter protocol. The total cost was 50.5 euros (53.5 dollars) per patient  
272 (23.0 euros for the culture media including cost of vials with beadmills, and 27.5  
273 euros for the technical manipulation time), against 37.4 euros (39.6 dollars) for 4  
274 samples grown on 3 culture media, i.e. a difference of 13.1 euros (14 dollars) per  
275 patient.

**276 Discussion.**

277 Our study is the first prospective multicenter study to have performed multicenter  
278 standardization of culture techniques as recently published [18]. A uniform number of  
279 5 samples were collected per patient, with many at the bone-prosthesis interface and  
280 the interface membrane. The beadmilled suspensions were used to seed solid and  
281 liquid culture media including a blood culture bottle [18]. This methodology enabled  
282 us to bacteriologically document PJI in 89.3% of cases, compared with 61% or 70%

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283 in other studies [8, 23]. Furthermore, we were able to document PJI for 95.1% of  
284 patients who were not receiving antibiotics at the time of surgery.

285 The first objective of the present study was to analyze the impact that  
286 reduction of 1, 2 or 3 samples would have on the microbiological criteria and PJI  
287 diagnosis using a random sampling method repeated 1000 times. We showed that  
288 bacterial analysis of 4 samples instead of 5 had no impact on the clinical  
289 effectiveness of microbiological diagnosis for PJI. Concerning the most adequate  
290 samples, our study did not confirm the superiority of interface membranes over  
291 capsule and synovial fluid specimens, as reported by Bjerkan et al [24]. In fact, the  
292 positivity rate varied little according to the various types of samples. However, it  
293 seems preferable to privilege tissue in contact with the material and the joint fluid.  
294 Bone samples may be more difficult to grind. Joint fluid should be seeded directly into  
295 a blood culture bottle by the surgeon in the operating room in a daily routine.

296 The second objective of our study was to reduce the number and incubation  
297 time of culture media while maintaining diagnosis effectiveness. We showed that  
298 seeding 3 culture media instead of 5 had no impact on the microbiological diagnosis  
299 for PJI, when using a chocolate agar plate incubated in a CO<sub>2</sub> atmosphere for 7 days,  
300 Schaedler broth and a blood culture bottle. Using Schaedler broth means that we can  
301 isolate more anaerobic bacteria than with an anaerobic blood agar. Besides that, we  
302 found that the chocolate agar plate was more sensitive than the anaerobic agar plate,  
303 particularly for the anaerobe *P. acnes*, which can grow in a CO<sub>2</sub> enriched  
304 atmosphere.

305 The decision to inoculate a blood culture bottle, and the choice of bottle, i.e.  
306 aerobic, anaerobic or pediatric, is important. Blood culture bottles have been  
307 previously shown to improve sensitivity in detecting microorganisms from synovial

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308 fluids for the diagnosis of infectious arthritis or prosthetic-joint infection [10-13]. This  
309 improvement can easily be explained by optimal culture conditions such as shaking,  
310 inactivating antimicrobial agents with resins, and inoculating larger volumes of  
311 samples. Other studies have investigated the benefit of using blood culture bottles for  
312 culturing tissue samples pre-treated by manual dissection or vigorous shaking [14-  
313 16]. A prospective single-center study comparing 8 culture media (4 liquid media and  
314 4 solid ones) showed the high sensitivity of blood culture bottles for the diagnosis of  
315 23 cases of PJI [14]. In another recent single-center prospective study with 79 cases  
316 of PJI, the sensitivity of blood cultures was 82.3% after 3 days of incubation [16]. The  
317 authors recommended seeding 2 blood culture vials, one aerobic and one anaerobic,  
318 for each sample, representing 10 vials per patient to be inserted into the automatic  
319 blood culturing system [16]. Because of the high number of cases of PJI, present  
320 and future, it seems important to propose an alternative solution.

321 In our study, pediatric blood culture bottles proved less efficient than Schaedler broth  
322 for cultivating anaerobes, as shown previously [15]. However, the pediatric bottle,  
323 seeded with a small amount of beadmilled suspension, led to the automatic detection  
324 of 83% of aerobes in less than 18 hours in our study. The bacteria could be identified  
325 within 24 hours of collection, allowing bacteriological documentation of 85% of cases  
326 of PJI. This represents a remarkable advance for the choice of antibiotics.

327 Our study showed that Schaedler broth could be used to isolate most  
328 anaerobes and was particularly useful in polymicrobial PJI. The optimal duration of  
329 culture could not be determined from our study as the majority of broths were  
330 subcultured on the 14<sup>th</sup> day. Nevertheless, one might suggest subculture once at day  
331 7 and then at day 14 in the event of negative subculture, according to a recent study  
332 [25].

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333 This new procedure would lead to significant cost saving when considering  
334 the increasing number of cases of PJI that can be expected in the near future [6].  
335 This is particularly important as the cost of microbiological diagnosis is not yet taken  
336 into account in the overall cost assessment of PJI. No impact was found on the  
337 clinical efficacy of PJI diagnosis.

338 In conclusion, our prospective multicenter study showed that the minimal  
339 number of samples required to confirm the diagnosis of PJI can be decreased to four  
340 per patient instead of five as recommended by current consensus guidelines. Three  
341 culture media, including a blood culture bottle incubated for only 5 days could be  
342 proposed for an accurate microbiological diagnosis of PJI.

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345 **Contributors**

346 PB, DT, CP, LB, and CB conceived and designed the study. PB, DT, CP, ASV, AG,  
347 CL, MK, GHA, LB, MEJ, SC, and CB were site investigators. JL and BG were study  
348 statisticians. PB, JL, CB, CP, DT, ASV, AG, SC, CL, MK wrote the paper. PB was the  
349 principal investigator.

350 **Conflicts of interest**

351 All authors declare that they have no conflicts of interest.

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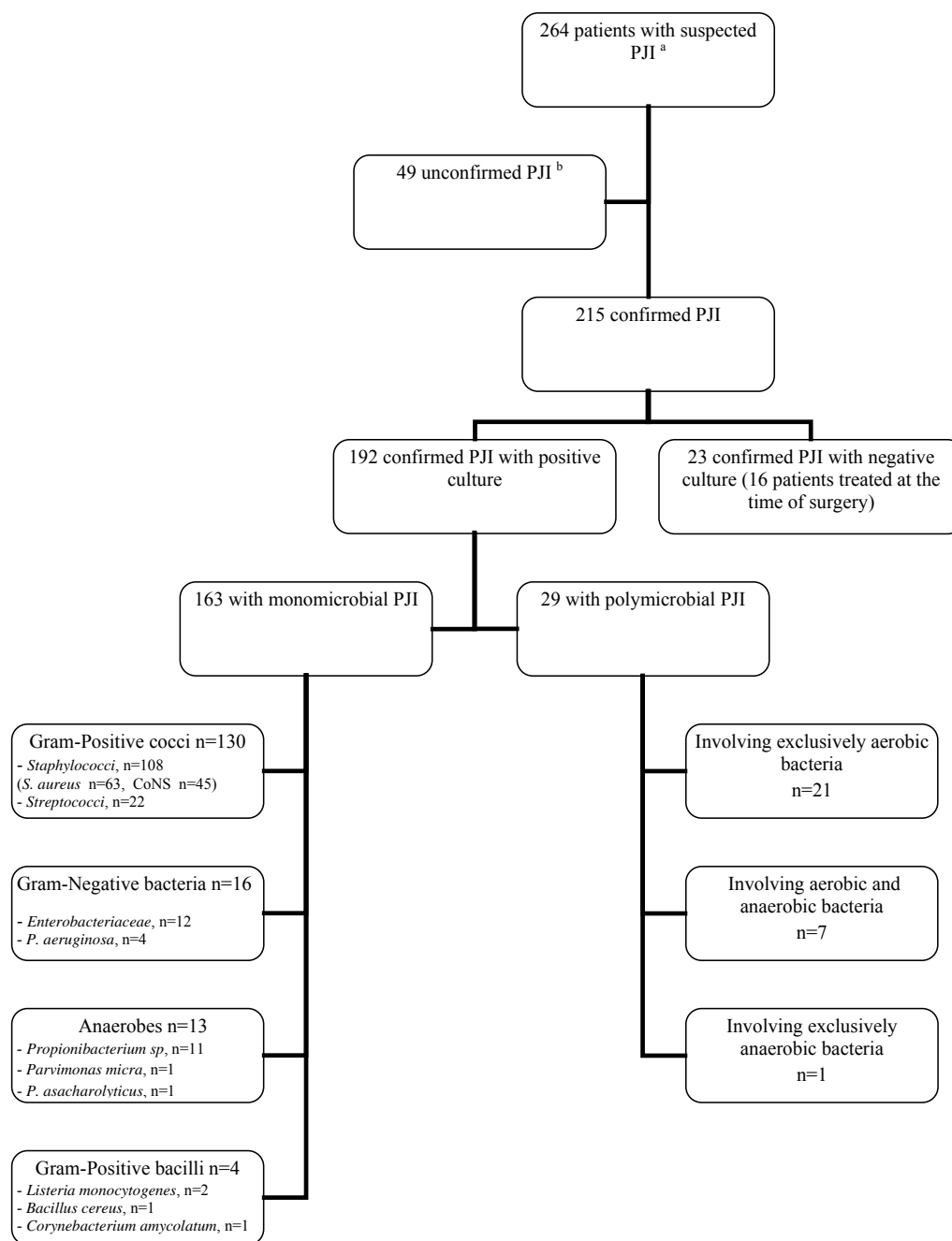
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Figure 1 – Microbiological results

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492 <sup>a</sup>PJI: Prosthetic-Joint Infection. <sup>b</sup>see ref 18.



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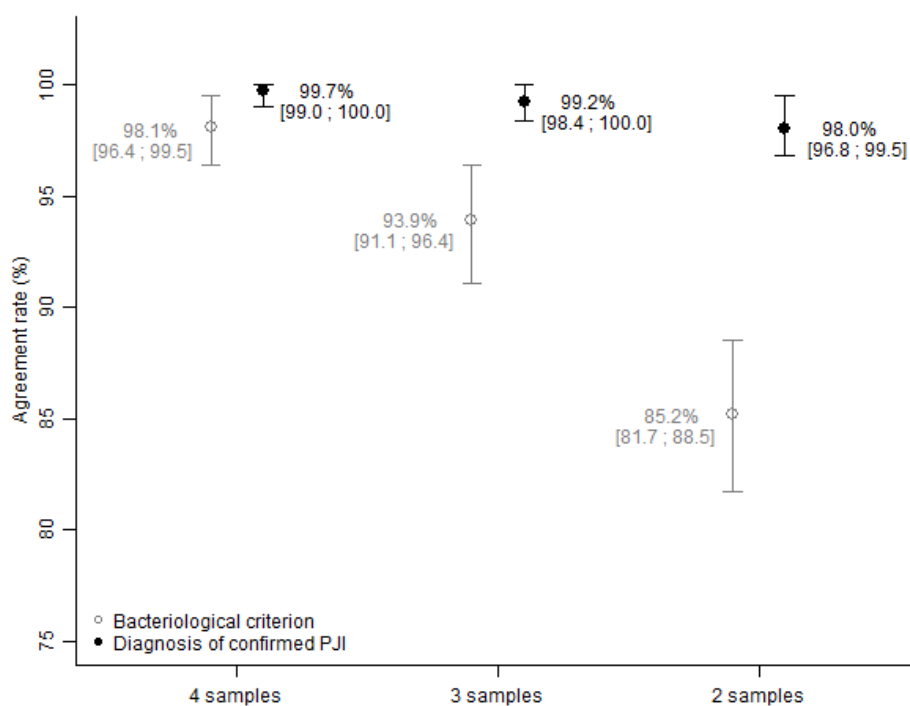


Figure 2 - Mean percentage of agreement and 95% confidence interval (97.5th; 2.5th percentiles) obtained from 1000 data sets generated according to the number of samples obtained from the simulation analysis.

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Table 1 – Paired comparison of positivity rates between culture media from the

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171 cases of PJI with aerobic bacteria.

|                                          | Blood agar<br>plate | Chocolate agar<br>plate | Anaerobic<br>broth medium | Blood<br>culture bottle |
|------------------------------------------|---------------------|-------------------------|---------------------------|-------------------------|
|                                          | P+=599<br>(70.1%)   | P+=590<br>(69.0%)       | P+=588<br>(68.8%)         | P+=710<br>(83.0%)       |
| Blood agar plate<br>P+=599 (70.1%)       |                     |                         |                           |                         |
| Chocolate agar plate<br>P+=590 (69.0%)   | p=0.3507            |                         |                           |                         |
| Anaerobic broth medium<br>P+=588 (68.8%) | p=0.4510            | p=0.8892                |                           |                         |
| Blood culture bottle<br>P+=710 (83.0%)   | p<0.0001            | p<0.0001                | p<0.0001                  |                         |

508 P+: positive samples. Total number of samples: 855.

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517 Table 2 – Paired comparison of positivity rates between culture media from the

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21 cases of PJI with anaerobic bacteria\*.

|                                         | Blood agar<br>plate | Chocolate<br>agar plate | Anaerobic<br>broth medium | Blood<br>culture bottle |
|-----------------------------------------|---------------------|-------------------------|---------------------------|-------------------------|
|                                         | P+=33<br>(31.4%)    | P+=56<br>(53.3%)        | P+=79<br>(75.2%)          | P+=57<br>(54.3%)        |
| Blood agar plate<br>P+=33 (31.4%)       |                     |                         |                           |                         |
| Chocolate agar plate<br>P+=56 (53.3%)   | p<0.0001            |                         |                           |                         |
| Anaerobic broth medium<br>P+=79 (75.2%) | p<0.0001            | p<0.0001                |                           |                         |
| Blood culture bottle<br>P+=57 (54.3%)   | p<0.0001            | p=0.8759                | p=0.0002                  |                         |

519 P+: positive samples. Total number of samples: 105.

520 \*: 13 cases of monomicrobial anaerobic PJI and 8 of polymicrobial PJI with at least

521 one anaerobe.

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528 Table 3 - Percentages of agreement according to the combination of culture

529 media for the 192 PJI.

| Combination of culture<br>media                                            | Bacteriological criterion |                     | Confirmed diagnosis of PJI |                     |
|----------------------------------------------------------------------------|---------------------------|---------------------|----------------------------|---------------------|
|                                                                            | %                         |                     | %                          |                     |
|                                                                            | Aerobes<br>171 PJI*       | Anaerobes<br>21 PJI | Aerobes<br>171 PJI         | Anaerobes<br>21 PJI |
| Blood culture bottle &<br>chocolate agar plate &<br>anaerobic broth medium | 98.3                      | 85.7                | 100.0                      | 100.0               |
| Blood culture bottle & blood<br>agar plate & anaerobic<br>broth medium     | 97.7                      | 85.7                | 100.0                      | 100.0               |
| Chocolate agar plate &<br>anaerobic broth medium                           | 92.4                      | 81.0                | 98.3                       | 100.0               |
| Blood agar plate &<br>anaerobic broth medium                               | 92.4                      | 76.2                | 99.4                       | 100.0               |
| Blood culture bottle &<br>chocolate agar plate                             | 97.7                      | 66.7                | 100.0                      | 95.2                |
| Blood culture bottle & blood<br>agar plate                                 | 96.5                      | 47.6                | 100.0                      | 90.5                |

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531 PJI : Prosthetic-Joint Infection

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