

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

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► **To cite this version:**

Pascale Bémer, Julie Leger, Didier Tandé, Chloé Plouzeau, Anne Sophie Valentin, et al.. How many samples and how many culture media to diagnose a Prosthetic Joint Infection: A clinical and microbiological prospective multicenter study. *Journal of Clinical Microbiology*, American Society for Microbiology, 2016, 54 (2), pp. 385-391 <10.1128/JCM.02497-15>. <hal-01255868>

HAL Id: hal-01255868

<https://hal-univ-rennes1.archives-ouvertes.fr/hal-01255868>

Submitted on 26 Sep 2017

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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 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 μ L volumes were each spread onto a blood agar plate and a
133 Polyvitex chocolate agar plate, incubated under a CO₂-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 14th 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 7th 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 CO₂ 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 14th 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 CO₂ 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 CO₂ 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 14th 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.

352 **Acknowledgements**

353 This study was supported by a grant from the French Ministry of Health [Programme
354 Hospitalier de Recherche Clinique Interrégional API/N/041], and a grant from the
355 CRIOGO (Centre de Référence des Infections Ostéo-articulaires du Grand Ouest).

REVISED MANUSCRIPT JCM02497-15

356 We thank Louis Bernard for his clinical and methodological assistance. We thank
357 Karine Fèvre and Line Happi for their help with the study and technical assistance.
358 We are indebted to Jane Cottin† for her enthusiasm until the end. We are also very
359 grateful to all the members of the CRIOGO for their continuing support.

360 The CRIOGO Study Team members included the following : J. Cottin†, P. Bizot, P.
361 Abgueguen (CHU Angers); R. Gérard, E. Stindel, S. Ansart (CHU Brest); S.
362 Touchais, F. Gouin, D. Boutoille, N. Asseray (CHU Nantes); A. Guigon, J. Guinard, F.
363 Razanabola, C. Mille (CHU Orléans); A.S. Cognée, L.E. Gayet, F. Roblot, G. Le Moal
364 (CHU Poitiers); J. Guinard, J.L. Polard, C. Arvieux (CHU Rennes); L. Bernard, P.
365 Rosset, G. Gras (CHU Tours).

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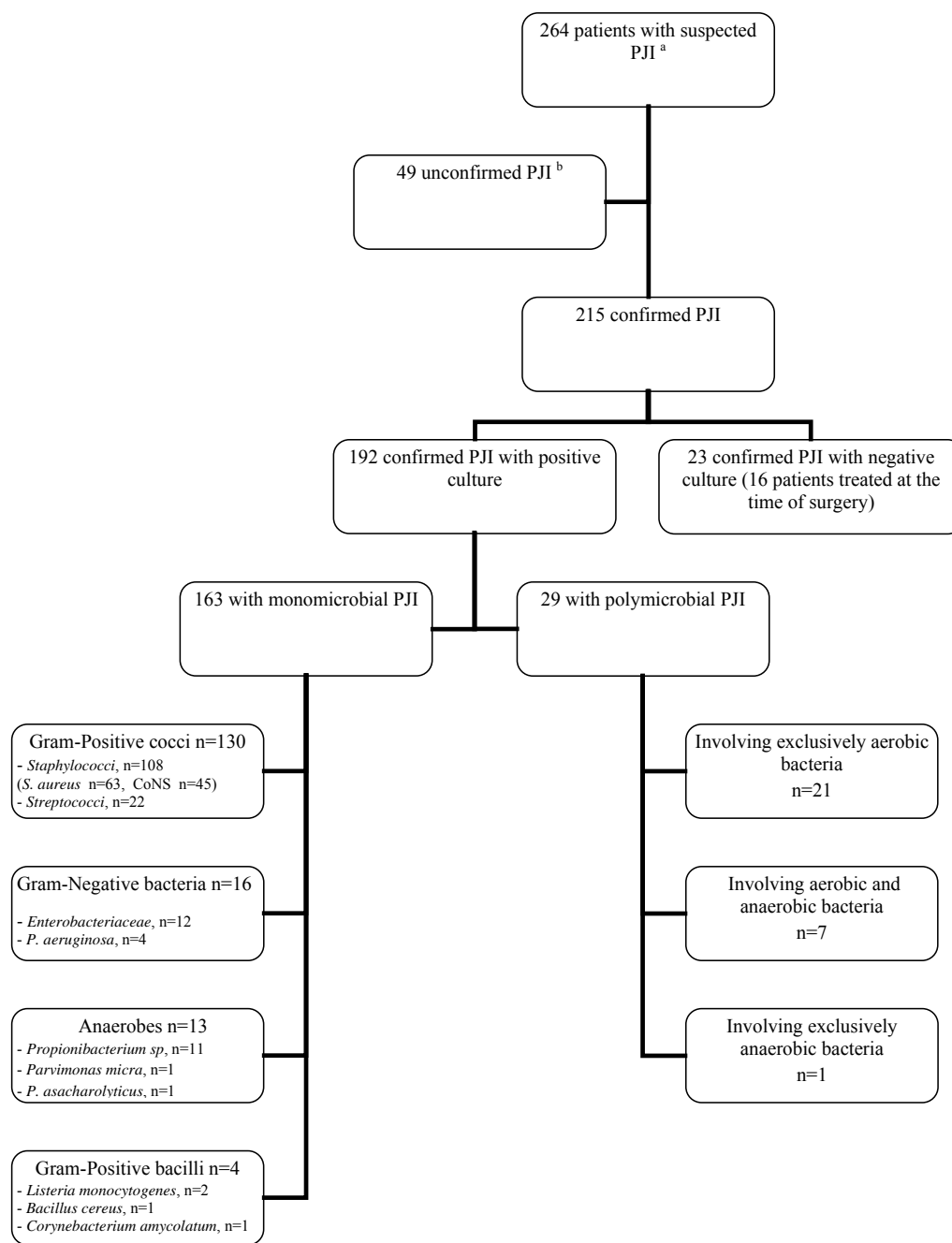
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Figure 1 – Microbiological results

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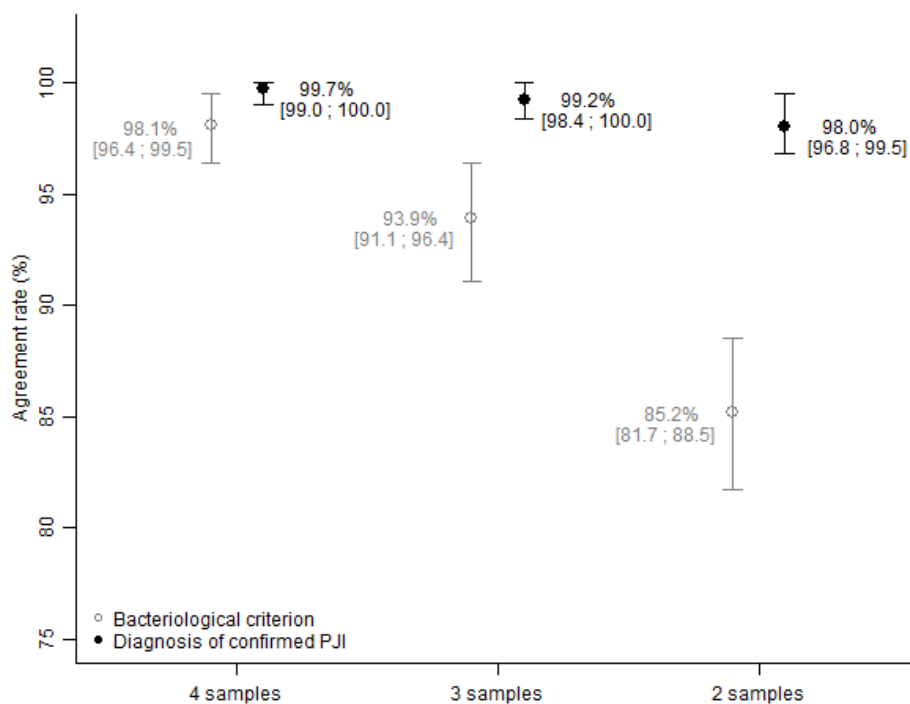
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499 Figure 2 - Mean percentage of agreement and 95% confidence interval (97.5th; 2.5th

500 percentiles) obtained from 1000 data sets generated according to the number of

501 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 plate	Chocolate agar plate	Anaerobic broth medium	Blood culture bottle
	P+=599 (70.1%)	P+=590 (69.0%)	P+=588 (68.8%)	P+=710 (83.0%)
Blood agar plate P+=599 (70.1%)				
Chocolate agar plate P+=590 (69.0%)	p=0.3507			
Anaerobic broth medium P+=588 (68.8%)	p=0.4510	p=0.8892		
Blood culture bottle 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 plate	Chocolate agar plate	Anaerobic broth medium	Blood culture bottle
	P+=33 (31.4%)	P+=56 (53.3%)	P+=79 (75.2%)	P+=57 (54.3%)
Blood agar plate P+=33 (31.4%)				
Chocolate agar plate P+=56 (53.3%)	p<0.0001			
Anaerobic broth medium P+=79 (75.2%)	p<0.0001	p<0.0001		
Blood culture bottle 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 media	Bacteriological criterion		Confirmed diagnosis of PJI	
	%		%	
	Aerobes 171 PJI*	Anaerobes 21 PJI	Aerobes 171 PJI	Anaerobes 21 PJI
Blood culture bottle & chocolate agar plate & anaerobic broth medium	98.3	85.7	100.0	100.0
Blood culture bottle & blood agar plate & anaerobic broth medium	97.7	85.7	100.0	100.0
Chocolate agar plate & anaerobic broth medium	92.4	81.0	98.3	100.0
Blood agar plate & anaerobic broth medium	92.4	76.2	99.4	100.0
Blood culture bottle & chocolate agar plate	97.7	66.7	100.0	95.2
Blood culture bottle & blood agar plate	96.5	47.6	100.0	90.5

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531 PJI : Prosthetic-Joint Infection

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