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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Keywords: Prosthetic-Joint-Infection, prospective multicenter study, microbiological diagnosis

Running title: New Microbiological PJI diagnosis

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Abstract

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

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

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

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

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

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

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

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

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

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

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

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

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

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]:

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

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

3/ Bacteriological infection criterion (as specified below).

Microbiological methods

Periprosthetic tissue cultures were produced in each center following a standard protocol. For each patient, 5 perioperative specimens (tissue, bone or joint fluid specimens) were collected in sterile vials with different surgical instruments. After adding 10 ml of sterile water and stainless steel beads, the vials were shaken on a Retsch MM401 beadmill. 1 mL was inoculated into a pediatric blood culture bottle and into a Schaedler broth, as recommended, and both were incubated for 14 days. Three further 50 µL volumes were each spread onto a blood agar plate and a Polyvitex chocolate agar plate, incubated under a CO_2-enriched atmosphere for 7 days, and a blood agar plate incubated in an anaerobic atmosphere for 7 days. Synovial fluids were seeded directly onto the 3 solid and the 2 liquid culture media without beadmill processing. The Schaedler broth was inspected weekly for cloudiness, and subcultured on a Schaedler blood agar plate or a blood agar plate incubated in an anaerobic atmosphere for 48 hours as soon as it became cloudy, or systematically on the 14^{th} day.

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

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

Histological analysis

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

Statistical analysis strategy

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

Main issues

1/ Number of samples required for PJI diagnosis.

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

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

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

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

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

Secondary issues

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

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

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

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

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

Results

Diagnosis of infection

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

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

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

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

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

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

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

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

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

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

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

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

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

Times-to-positivity according to culture media.

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

93% of aerobes were isolated during the two first days of observation, and 7% between the third and the 7th day, rendering still needed the incubation of 7 days for solid media.

Most of the *P. acnes* strains were isolated from Schaedler broth (14.0% (N=7) from 2 to 4 days, and 86% (N=36) between 5 and 14 days), against 34 (68%) in 7 days from chocolate agar plates in a CO_2 atmosphere or blood agar plates in an anaerobic atmosphere, and 26 (52%) in 6 days from pediatric blood cultures. 28% (12/43) of the Schaedler broths, not cloudy after 14 days of incubation, were systematically subcultured on the 14th day.

Cost impact of sample management by the microbiology laboratory.

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

Discussion.

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

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

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

The second objective of our study was to reduce the number and incubation time of culture media while maintaining diagnosis effectiveness. We showed that seeding 3 culture media instead of 5 had no impact on the microbiological diagnosis for PJI, when using a chocolate agar plate incubated in a CO_2 atmosphere for 7 days, Schaedler broth and a blood culture bottle. Using Schaedler broth means that we can isolate more anaerobic bacteria than with an anaerobic blood agar. Besides that, we found that the chocolate agar plate was more sensitive than the anaerobic agar plate, particularly for the anaerobe *P. acnes,* which can grow in a CO_2 enriched atmosphere.

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

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

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

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

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

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

Contributors

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

Conflicts of interest

All authors declare that they have no conflicts of interest.

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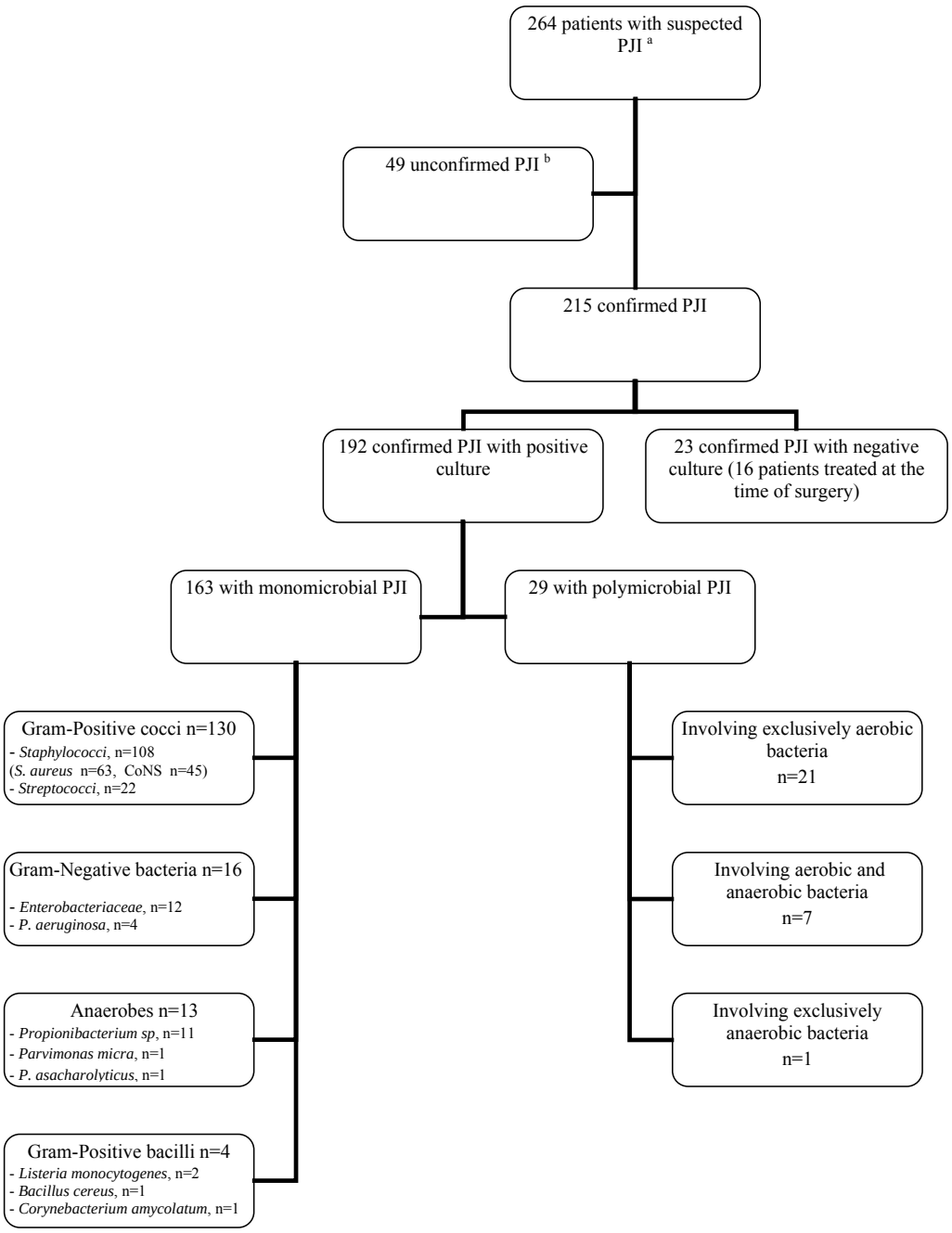
Figure 1 – Microbiological results



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492 [a]PJI: Prosthetic-Joint Infection. [b]see ref 18.

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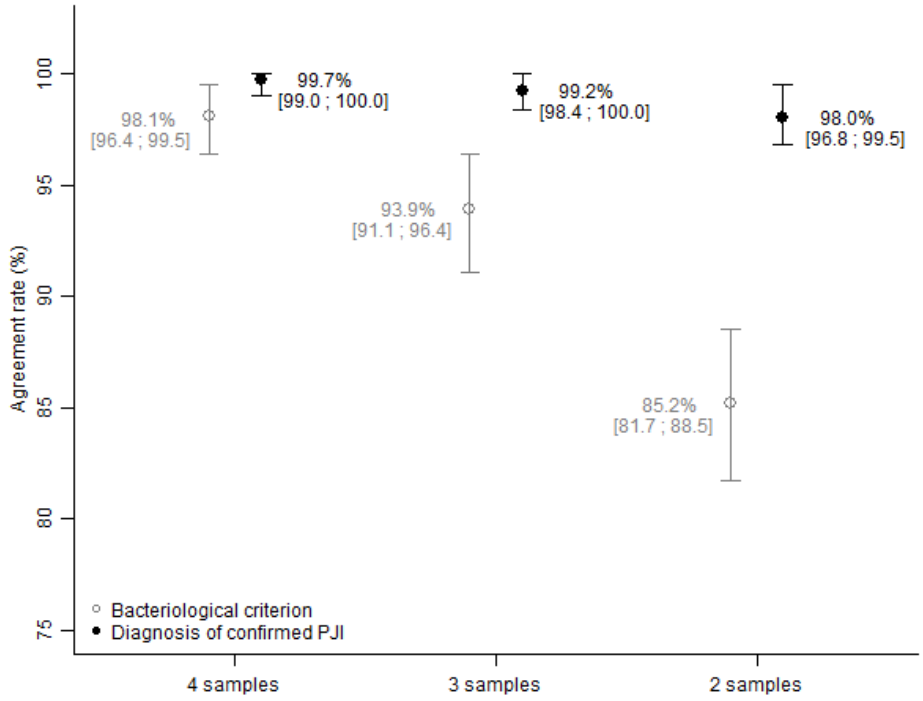

Figure 2 - Mean percentage of agreement and 95% confidence interval (97.5th; 2.5th percentiles) obtained from 1000 data sets generated according to the number of samples obtained from the simulation analysis.

Table 1 – Paired comparison of positivity rates between culture media from the 171 cases of PJI with aerobic bacteria.

	Blood agar plate P+=599 (70.1%)	Chocolate agar plate P+=590 (69.0%)	Anaerobic broth medium P+=588 (68.8%)	Blood culture bottle 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	

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

Table 2 – Paired comparison of positivity rates between culture media from the 21 cases of PJI with anaerobic bacteria*.

	Blood agar plate P+=33 (31.4%)	Chocolate agar plate P+=56 (53.3%)	Anaerobic broth medium P+=79 (75.2%)	Blood culture bottle 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	

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

*: 13 cases of monomicrobial anaerobic PJI and 8 of polymicrobial PJI with at least one anaerobe.

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

PJI : Prosthetic-Joint Infection