# CAT
# Critically Appraised Topic

## Diagnosis of prosthetic joint infections: towards a BILULU consensus

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### CLINICAL BOTTOM LINE

Prosthetic joint infections are feared complications of joint replacement surgery. They require both surgical intervention and prolonged courses of antibiotics. Therefore, a correct diagnosis and bacteriological documentation of the causative pathogen is important. Acute prosthetic joint infections generally don't pose a diagnostic challenge. Chronic infections, however, caused by low-virulent organisms, are much harder to diagnose. They mostly present with non-specific clinical signs, don't induce an overt inflammatory response and the involved bacteria are concentrated in a biofilm located on the surface of the implant, making them hard to culture. To establish a diagnosis, optimal laboratory testing is necessary.

Currently, no uniformly accepted procedure is available as diagnostic work-up. International definitions were already created as an aid, but no single set of criteria is accepted as the gold standard. Additionally, there are many studies available on the contributions of different sample types and culture conditions in the diagnosis of prosthetic joint infections, but due to many methodological differences, they are difficult to compare. It seems, however, that the following aspects are important in the diagnostic workup: synovial white blood cell count and differential, aerobic and anaerobic culture of multiple periprosthetic tissue samples (optimally 5, at least 3) aside from synovial fluid, use of blood culture bottles for all sample types and histological analysis of periprosthetic tissue (to detect acute inflammation). Sonication of the explanted prosthesis seems to have an added value in the difficult-to-diagnose chronic infections. A definite conclusion about this topic, however, is difficult due to the many variations between the different studies.

Following this CAT, the existing evidence will be used to create a consensus between the BILULU laboratories in order to optimize and standardize the diagnosis of prosthetic joint infections. This consensus document should be a workable tool that can be used by both orthopedic surgeons and laboratories in their daily routine.

### CLINICAL/DIAGNOSTIC SCENARIO

Joint replacement surgery is a procedure performed worldwide in many patients with chronic disabling joint pain which can successfully provide pain relief and improve quality of life. One of the feared complications of this procedure is infection of the implanted prosthesis. These infections are seen in

approximately 0.5 to 1.0 percent in hip replacements, 0.5 to 2 percent in knee replacements and less than 1 percent for shoulder replacements (1). Although risk of prosthetic joint infection (PJI) is low, the high frequency of joint replacements results in a substantial burden of these infections.

Clinical manifestations of prosthetic joint infections vary from overt inflammatory symptoms with systemic response to more chronic and indolent cases. They depend on time of onset after surgery, virulence of the infecting organisms, route of infection and host responses. Highly virulent organisms (like *Staphylococcus aureus* and Gram-negative bacilli) will more likely present with typical signs of acute inflammation like erythema, swelling, acute pain, wound drainage or fever. Low-virulent organisms on the other hand have a more indolent course and usually present with non-specific symptoms like persistent pain or implant loosening (2,3). They mostly present months after surgery. Generally, the most common presenting symptom is joint pain (4).

The most frequently involved pathogens are listed in table 1. *Staphylococcus aureus* and coagulase-negative staphylococci are the leading causes of acute and chronic prosthetic joint infections respectively. Other less frequent causes are streptococci, enterococci, Gram-negative bacilli, anaerobes (including *Cutibacterium acnes*) and rarely fungi or mycobacteria. Polymicrobial infections account for 10-20% of all infections. Culture negative infections for 10-30% (5).

**TABLE III** - FREQUENCY OF MICROORGANISMS CAUSING PROSTHETIC JOINT INFECTION

| Microorganism | Frequency (%) |
|---|---|
| Coagulase-negative staphylococci | 30-43 |
| *Staphylococcus aureus* | 12-23 |
| Streptococci | 9-10 |
| Enterococci | 3-7 |
| Gram-negative bacilli | 10-17 |
| Anaerobes | 2-4 |
| *Candida* spp. | 1-3 |
| Polymicrobial | 10-20 |
| Unknown (culture false-negative) | 10-30 |

Table 1: Frequency of microorganisms causing prosthetic joint infection (5).

Prosthetic joint infections can be classified according to their timing of onset. These different categories reflect the pathogenesis of the infection, in particular route of infection and virulence of the pathogens involved. Different classification schemes exist (6). The most frequently used classification divides prosthetic joint infections into early (less than 3 months after surgery), delayed (3 to 24 months after surgery) and late onset infections (more than 24 months after surgery). Early infections present as acute infections and are caused by highly virulent organisms, whereas delayed infections present as chronic, low-grade infections due to low-virulent organisms (2). Early and delayed infections are usually acquired during implantation of the prosthesis. Late infections, however, are generally caused by hematogenous seeding in case of bacteremia, and mostly present as acute infections, but they can also be acquired during surgery in case of extremely indolent organisms and present as chronic infections (table 2) (4, 6).

**Table 2**
**Classification and clinical presentation of prosthetic joint infections**

| Type of Infection | Time to Presentation | Mechanism of Infection | Organisms | Clinical Presentation | |
|---|---|---|---|---|---|
| Early | <3 mo | Intraoperative contamination | Virulent bacteria (ie, *Staphylococcus aureus*) | Acute | Sudden onset erythema, edema, warmth, and tenderness |
| Delayed | 3–12 mo | Intraoperative contamination | Low virulent bacteria (coagulase-negative staphylococci) | Chronic | Joint pain and stiffness |
| Late | >12 mo | Hematogenous seeding | Virulent bacteria (ie, *S.aureus*) | Acute | Sudden-onset erythema, edema, warmth, and tenderness |
| | | Intraoperative contamination | Low virulent bacteria (ie, *Propionibacterium acnes*) | Chronic | Joint pain, sinus tract |

*Adapted from* Parvizi J, Fassihi SC, Enayatollahi MA. Diagnosis of periprosthetic joint infection following hip and knee arthroplasty. Orthop Clin North Am 2016;47(3):509; with permission.

Table 2: Classification and clinical presentation of prosthetic joint infections (early, delayed, late) (4).

Aside from this classification, another frequently used classification scheme divides infections in two categories: acute and chronic infections. Acute infections are those infections presenting within 4 weeks after surgery or as a result of bacteremia (with an onset less than 3 weeks ago). Chronic infections present after 4 weeks of surgery or after an episode of bacteremia with an onset more than 3 weeks ago. This classification is based on the maturity of the biofilm and is therefore useful to guide the surgical approach. Acute infections can be managed with debridement, exchange of mobile parts and prosthesis retention. Chronic infections, with a mature biofilm, require a complete removal of the prosthesis. Similar to the first classification, acute infections are typically caused by highly virulent organisms and chronic infections by low virulent organisms (table 3)(2, 7, 8). Other classification schemes also exist (Tsukayama et al, McPherson et al.).

| Type of PJI | Acute PJI | Chronic PJI |
|---|---|---|
| Pathogenesis | | |
| - Perioperative origin | Early postoperative <4 weeks after surgery | Delayed postoperative (low-grade) ≥4 weeks after surgery |
| - Hematogenous origin | <3 weeks of symptoms | ≥3 weeks of symptoms |
| Biofilm age (maturity) | Immature | Mature |
| Clinical features | Acute joint pain, fever, red/swollen joint | Chronic pain, loosening of the prosthesis, sinus tract (fistula) |
| Causative microorganism | High-virulent: *Staphylococcus aureus*, gram-negative bacteria (e.g. *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*) | Low-virulent: Coagulase-negative staphylococci (e.g. *Staphylococcus epidermidis*), *Propionibacterium acnes* |
| Surgical treatment | Débridement & retention of prosthesis (change of mobile parts) | Complete removal of prosthesis (exchange in one-, two-, or multiple stages) |

Table 3: Classification and clinical presentation of prosthetic joint infections (acute, chronic) (8).

Management of prosthetic joint infections is complex. It requires both surgical intervention and prolonged courses of antibiotics. Since signs like pain or prosthesis loosening can also be attributable to various other conditions like aseptic loosening or crystal induced arthropathy, which require a different therapeutic approach, it's important to establish the correct diagnosis (9). Therefore, when there is a clinical suspicion for infection, additional laboratory investigations are necessary.

Laboratory testing includes blood chemistry tests (like erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)), synovial white blood cell count with differential and microbiological evaluation of synovial fluid and periprosthetic tissue biopsies. Additional culture of the explanted prosthesis material is also a possibility. Isolation and identification of the causative organism provides proof of infection and gives the opportunity for antibiotic susceptibility testing. However, differentiating between true pathogen or contaminant can be difficult and culture methods may fail to detect the causative organism, making definite bacteriological diagnosis challenging. Additionally, histopathological examination of intraoperative tissue samples is also recommended and blood cultures should be taken in patients who are acutely ill or who present with fever (7, 8, 10, 11, 12, 13)

This combination of different analyses and multiple cultures is essential to increase the likelihood of definitive bacteriological diagnosis and isolation of the causative organism, since no single sign or test is accurate enough. Multiple cultures improve sensitivity, but also help with the interpretation in case of growth of a skin flora organism (eg. coagulase-negative staphylococci) (14).

The current experience, however, shows that these laboratory investigations lack standardization and are not optimally used or processed to establish the diagnosis. First, laboratory requests are not always complete (eg. white blood cell count in synovial fluid is not requested or synovial fluid is only sent in EDTA collection tubes, etc.). Second, periprosthetic swabs are often used for culture instead of periprosthetic tissue biopsies (or the explanted prosthesis). Third, there is currently no specific procedure for microbiological culture in the case of suspicion of prosthesis infection. Samples are processed according to sample type (sterile body fluids, biopsies or swabs) and not according to pathology. Culture conditions and incubation periods might therefore not be optimal for isolation of organisms involved in prosthetic joint infections, like fastidious slow-growing organisms. Finally, histopathological examination of intraoperative biopsies is not currently done.

We can therefore conclude that both the pre-analytical and the analytical phase in diagnosis for prosthetic joint infections are suboptimal at this moment. This may potentially fail to give a diagnosis and a causative organism. Therefore, there is a need for a procedure for both orthopedic surgeons and laboratories to optimize this pre-analytical and analytical phase in order to improve and standardize this diagnostic process.

The purpose of this critically appraised topic is to review the existing recommendations in the literature about diagnosis of prosthetic joint infections, with a focus on microbiological investigations and culture conditions. Based on the available evidence, an interlaboratory consensus protocol between the BILULU laboratories will be made. This should be a workable tool that, on the one hand, should optimize the diagnostic procedures and, on the other hand, should be achievable in a routine practice. This means that both the orthopedic surgeons (pre-analytical phase) and laboratories (analytical phase) should be able to use this protocol in their daily organization and workflow.

## QUESTION(S)

1) *Diagnosis of prosthetic joint infections: what are the current challenges?*
2) *Diagnosis of prosthetic joint infections: what is the current evidence on laboratory testing?*
3) *Diagnosis of prosthetic joint infections: towards a BILULU consensus*

## APPRAISAL

## 1. Prosthetic joint infections: What are the current challenges?

While clinical findings may at times be more obvious and diagnosis may be clear (mainly in acute presentations), prosthetic joint infections present with a wide variation of symptoms, which are often non-specific. Especially in the more indolent infections, patients often only present with signs like chronic pain, making the diagnosis more challenging, since other causes should also be considered (15).

There are several reasons why these infections often present as a diagnostic challenge. First, there is no single definition that is accepted as the gold standard. Second, bacterial isolation of a causative pathogen is often difficult, especially in chronic infections. Last, the combination of different sample types and different analysis makes the diagnostic algorithm complex. Even though there are more and more recommendations available in the literature about the optimal diagnostic strategy, they still need to be translated into a procedure that guaranties the same work-up for every patient and is workable for both orthopedic surgeons and laboratories.

### 1.1 Definitions

For many years, research on prosthetic joint infections has been limited due to the lack of standardized criteria. This has improved over the last few years though, since sets of diagnostic criteria have been generated by different groups. They are now widely accepted and used as an aid in the diagnosis. Moreover, these criteria have already created a little bit more consistency in the literature. However, this is not absolute as currently no single set of criteria is yet accepted as the gold standard. Studies should therefore still be interpreted with caution, because different definitions could be used. This often creates difficulties to compare study results and draw up conclusions. The accuracy of different tests can only be measured by comparing the results to a gold standard definition, which currently does not yet exist (16). Efforts are continuously made to improve these criteria in order to enhance diagnostic sensitivity and medical research about this topic.

The most commonly used definitions at this moment are those by the Infectious Diseases Society of America (IDSA), the Musculoskeletal Infection Society (MSIS), the European Bone and Joint Infection Society (EBJIS) and the international consensus meeting (ICM) (10, 11, 12, 23). These are summarized in table 4 (IDSA, MSIS, ICM) and table 5 (EBJIS).

| Criterion | Definition of prosthetic joint infection | | | | | |
|---|---|---|---|---|---|---|
| | Musculoskeletal Infection Society | | International consensus | | Infectious Diseases Society of America | |
| | Definitive evidence | Supportive evidence | Definitive evidence | Supportive evidence | Definitive evidence | Supportive evidence |
| Sinus tract communicating with the prosthesis | x | | x | | x | |
| Identical microorganisms isolated from 2 or more cultures | x | | x | | x | |
| Purulence surrounding the prosthesis | | x | | | x | |
| Acute inflammation upon histological examination of periprosthetic tissue | | x | | x | | x |
| Single culture with any microorganism | | x | | x | | |
| Single culture with a virulent microorganism | | | | | | x |
| Elevated synovial fluid leukocyte count[b] | | x | | x | | |
| Elevated synovial fluid neutrophil percentage | | x | | x | | |
| Elevated serum ESR and CRP values | | x | | x | | |

[a] The MSIS definition requires 4 supportive criteria; the International Consensus Meeting definition requires 3 supportive criteria. Data are from references 60, 61, and 251. ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.
[b] The International Consensus Meeting definition also includes a "++" result on the leukocyte esterase strip.

Table 4: Overview of IDSA, ICM and MSIS criteria (6)

**Table 2.** Definition of Periprosthetic Joint Infection, if at least one of the following 4 criteria is fulfilled

| Diagnostic test | Criteria | Sensitivity (%) | Specificity (%) |
|---|---|---|---|
| Clinical features | Sinus tract or visible purulence* | 20-30 | 100 |
| Histology in periprosthetic tissue | Acute inflammation in periprosthetic tissue† | 95-98 | 95-98 |
| Leukocyte count in synovial fluid‡ | >2,000/μL leukocytes or >70% granulocytes | 93-96 | 93-96 |
| Microbiology (culture) | Synovial fluid or | 60-80 | 97 |
| | Tissue samples§ or | 70-85 | 92 |
| | Sonication fluid (≥50 CFU/mL)‖ | 85-95 | 95 |

* Metal-on-metal bearing components can simulate pus, but leukocyte count is usually normal, but metal debris visible.
† Acute inflammation defined as ≥2 granulocytes per high-power field.
‡ Leukocyte cutoffs are not interpretable within 6 weeks of surgery, in rheumatic joint disease, periprosthetic fracture or luxation. Leukocyte count should be determined within 24 hours; clotted specimens are treated with 10 μL hyaluronidase.
§ For highly virulent organisms (e.g., *Staphylococcus aureus*, *Escherichia coli*) already one positive sample confirms infection.
‖ Under antibiotics and for anaerobes, <50 colony-forming unit (CFU)/mL can be significant.

Table 5: EBJIS criteria (used by the Pro-Implant foundation) (7, 8, 23)

The major criteria are identical between these definitions: presence of a communicating sinus tract, which is believed to be pathognomonic for PJI, or isolation of identical microorganisms in 2 or more cultures. These are two criteria that confirm the diagnosis of a prosthetic joint infection. Differences are seen in the minor criteria, where some criteria are or aren't included depending on the definition. Serum C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) for example are included by some (MSIS, ICM), but not all definitions (IDSA, EBJIS).

In 2018, a new definition was proposed by Parvizi et al. to further optimize the diagnostic performance of the existing criteria. They created a scoring system that categories patients as 'infected', 'possibly infected', 'not infected' or 'inconclusive'. The two major criteria remain the same, namely 'communicating sinus tract' and/or 'two positive cultures of the same organism'. The minor criteria contain many of the criteria of MSIS and the International Consensus Meeting, but also include newer diagnostic tests like alpha-defensin and synovial CRP (13).

These differences in minor criteria, however, mean that patients may or may not be diagnosed with an infection depending on the definition used. Additionally, some patients may remain undiagnosed, even though they will have a prosthetic joint infection. Only 2 major criteria are considered definite proof of diagnosis, namely presence of a sinus tract and isolation of an identical microorganism in 2 separate cultures. Since a sinus tract is often not present, this means that microbiology is a very

important tool in this diagnostic process. Optimal laboratory testing, especially microbiological culturing conditions should thus be optimized to minimize the proportion of undiagnosed patients.

***Conclusion:***

- Different definitions are available (IDSA, MSIS, EBJIS, ICM).
- Major criteria for diagnosis of prosthetic joint infections are:
    - Presence of a sinus tract
    - Identical microorganisms in 2 or more cultures
- A diagnosis of prosthetic joint infection can also be made based on a combination of minor criteria. These criteria slightly differ between definitions. Examples of minor criteria:
    - Elevated synovial white blood cell count and polymorphonuclear percentage
    - Acute inflammation on histological analysis of periprosthetic tissue
    - Single positive culture
    - Elevated ESR and CRP
- There is no single set of criteria that is currently accepted as the gold standard for prosthetic joint infections.

### 1.2 Microbiological challenges

As described in the introduction, bacteriological investigations have their own challenges. First, bacterial culture may fail to grow the causative organism. This may be because of prior antibiotic use or the involvement of fastidious bacteria which require long incubation periods (like *Cutibacterium acnes*), but also because of the pathogenesis of prosthetic joint infections. Bacteria involved in prosthetic joint infections are usually only present in low numbers in the periprosthetic fluid and tissue, which can be explained by the presence of a biofilm (3, 6, 17, 18). These biofilms are complex communities that consist of microorganisms embedded in an extracellular matrix that forms on prosthetic material. It allows non-virulent commensals to become pathogens and protects them from the host immune-system and antibiotics (6). The majority of organisms involved in prosthetic joint infections are concentrated in this biofilm attached to the implant surface, making it difficult to treat, but also difficult to culture (3, 17, 18). This is particularly an issue in chronic, delayed infections. It also explains the lack of overt inflammatory response in this type of infections in contrast to acute infections. These latter are usually caused by highly virulent, rapid-growing organisms which are not yet trapped in a mature biofilm and are present in high numbers in the synovial fluid. They frequently present with more obvious inflammatory symptoms than chronic infections, where clear inflammatory signs are often missing and the clinical presentation may be indistinguishable with aseptic loosening (17, 18). To improve the sensitivity, multiple samples, enrichment media (including blood culture bottles) and prolonged incubation are recommended (6, 15). There is also increasing interest in sonication of the removed prosthesis, a promising strategy that can dislodge these bacteria from the biofilm attached to the surface (19). This will be discussed later.

As mentioned, Infections can be classified into acute and chronic infections based on this biofilm formation (this classification is used by the pro-implant foundation for example). Acute infections (<4 weeks after surgery or <3 weeks after hematogenous onset) still have an immature biofilm, which can be eradicated without complete removal of the prosthesis. Chronic infections however, have a mature biofilm and do require complete surgical removal (8).

Second, bacterial culture of joint samples also poses a challenge in discriminating between possible contamination or real pathogen since the causative organisms in prosthetic joint infections predominantly belong to the skin flora. Growth of such an organism (e.g. coagulase-negative staphylococci or *Cutibacterium acnes*) in only one sample makes it difficult to decide whether this is a contaminant or a clinically significant pathogen (6, 14, 17). Therefore, multiple samples are necessary to help in this interpretation. According to the internationally designed definitions for prosthetic joint infections, growth in 2 or more independent samples can be considered as confirmation of the diagnosis. In case of a virulent organism like *Staphyloccocus aureus*, already one positive culture is enough (6, 8, 10, 11). Growth of a non-virulent organism in only one sample should be evaluated in the context of the other available evidence (10).

***Conclusion:***

- Isolation of the causative microorganism may be difficult for a few reasons:
    - Prior antibiotic treatment
    - Involvement of fastidious bacteria
    - Involvement of a biofilm
- Involved pathogens in PJI typically belong to the skin flora. Discriminating between contamination and clinically significant can be difficult. Multiple cultures are necessary to help in this interpretation.
- Acute infections caused by virulent organisms generally pose less diagnostic problems than chronic infections.

### 1.3 Practical challenges

Aside from these theoretical challenges, the complex work-up also presents with practical challenges. Since there is no single test with absolute accuracy, a combination of clinical findings and different laboratory analysis, including histopathology, multiple samples and different culturing methods, is necessary (13, 10, 11, 6). This leads to a complex diagnostic work-up.

First of all, different sample types (peripheral blood, synovial fluid, tissue biopsies and prosthesis material) should be sent to the laboratory by the orthopedic surgeon. Second, different kinds of analysis are necessary on the same sample type, like both white blood cell count with differential and microbiological culturing on synovial fluid. Last, bacteriological culturing requires processing of multiple samples with different agar media, enrichment steps and prolonged incubation, which adds to the complexity.

Laboratory evaluation thus requires a well-structured workflow and a good collaboration between orthopedic surgeons and laboratories. Orthopedic surgeons, on the one hand, are responsible for the correct samples and test requests. Laboratories, on the other hand, are responsible for the test results. Microbiological investigations should therefore have procedures that provide optimal culturing conditions for isolation of pathogens that are involved in this type of pathology.

### 1.4 Current situation

To understand the current practice and these practical challenges, a survey was conducted in the BILULU laboratory. Both the pre-analytical and analytical phase were questioned.

Questions concerning pre-analytical phase included a general impression about the current practice and questions concerning the samples types that are currently used. Questions concerning analytical phased mainly focused on culture conditions (agar media and incubation time).

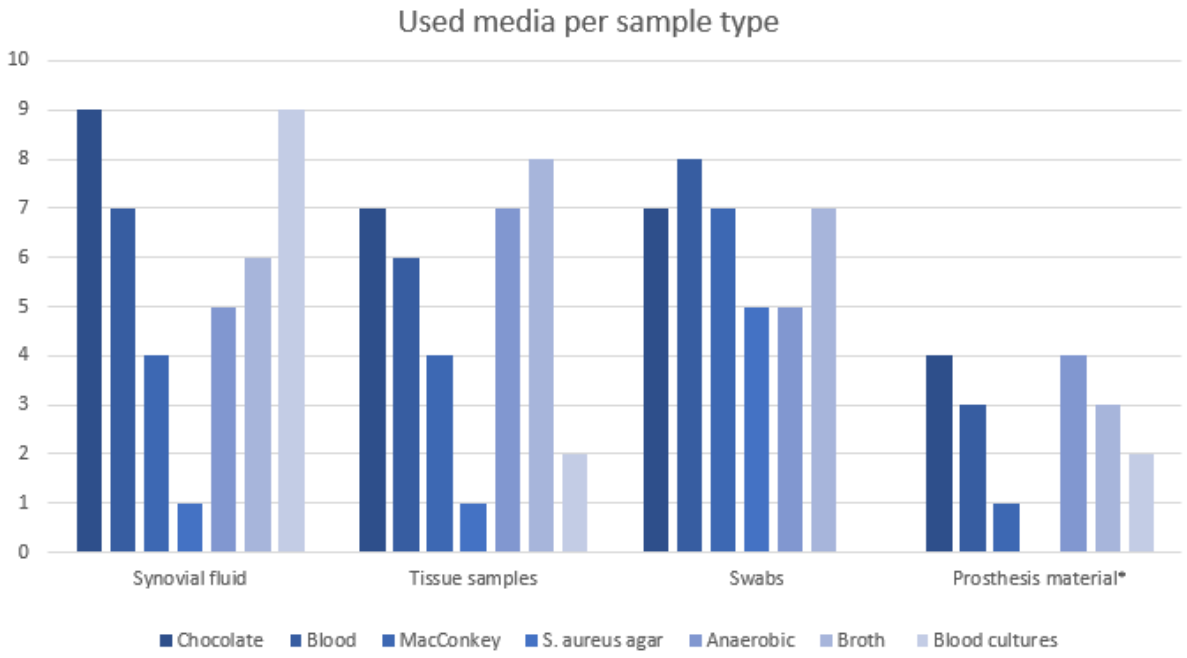
Regarding the pre-analytical phase, our own experience demonstrates some problems in the current practice. First, we often receive intraoperative swabs instead of biopsies. Second, if biopsies are taken, they are usually limited to 1 or 2 specimens. Third, synovial white blood cell count is not always requested. And lastly, it may happen that synovial fluid is only sent in an EDTA tube, which may have an inhibitory effect on bacterial growth (33).

Other laboratories experience similar problems, mainly about the synovial blood cell count, which was often not requested. Some laboratories also confirmed the use of swabs or combination of swabs with a biopsy. However, some also already implemented the use of multiple tissue biopsies in their diagnostic algorithm and therefore did only receive swabs on a rare occasion.

Aside from these general impressions, other areas of interest were the use of sonication of the explanted prosthesis and histological analysis of tissue biopsies. Sonication is done by 3 out of 9 laboratories. Two laboratories culture prosthesis material by the use of TSB. The other four laboratories don't culture prosthesis material. Histological analysis is only done by 2 laboratories.

In addition to these questions, all laboratories were questioned about their current bacteriological procedures. An overview of used media per sample type is given in figure 1. These results demonstrate that non-selective plates are used by all laboratories for all sample types (chocolate agar, blood agar or both). Selective media (like MacConkey and *S. aureus* media) are less frequently used. All laboratories use blood culture bottles for synovial fluid, in contrast to only 2 out of 9 laboratories for tissue biopsies. Five laboratories culture prosthesis material of which three use sonication. Two of these laboratories also inoculate sonication fluid in blood culture bottles. Anaerobic agar media are not routinely used in all laboratories for synovial fluid, but they do all use some alternative method for anaerobic recovery. Anaerobic agar media included brucella agar, schaedler agar or blood agar. Alternative methods used for anaerobic recovery are enrichments broths (thioglycolate, brain heart infusion) or anaerobic blood culture bottles. Broth enrichment media are used by all, except one laboratory in case of biopsies (who uses anaerobic blood culture bottles). In case of synovial fluid, broth enrichment media are only used by 6 out of 9 laboratories. For prosthesis material either enrichment broths are used (3 laboratories) or blood culture bottles (the other 2).

Swabs are included in this overview, since these are currently still part of the diagnostic process in most laboratories. They are, however, of limited value in the diagnosis of prosthetic joint infections and their use should be discouraged in the future. Because swabs are often used for wound culture, selective plates are more commonly used in this type of specimen.




*Sonication fluid: Only 5/9 laboratoria culture prosthesis material. 2/5 use TSB, 3/5 use sonication

Figure 1: Overview of used media per sample (BILULU survey)

The second important aspect is incubation time. These results are summarized in figure 2 and 3. Incubation periods for tissue biopsies and synovial fluid are presented since these are to date the 'gold standard' specimens. Incubation periods vary from 2 days to 14 days. They also vary according to specimen type. This could be potentially explained because most laboratories don't apply a procedure specifically for prosthetic joint infections, but rather have a procedure per specimen type. One laboratory, however, already applies prolonged incubation for 14 days in case of suspected prosthetic joint infections. Broth media and blood culture bottles (if used) are generally incubated longer then agar plates.

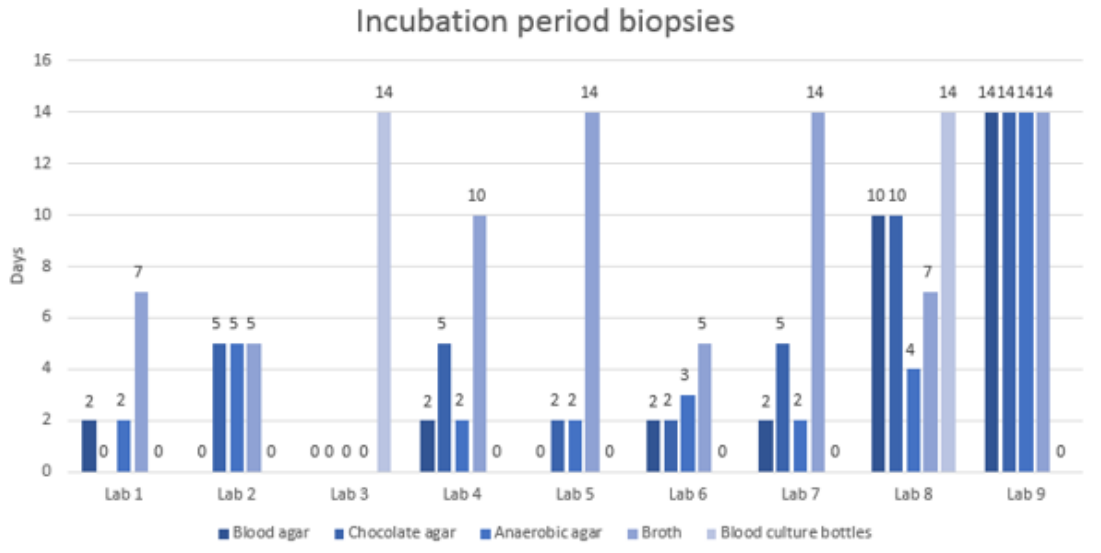



Figure 2: Overview of incubation periods for tissue samples (BILULU survey)

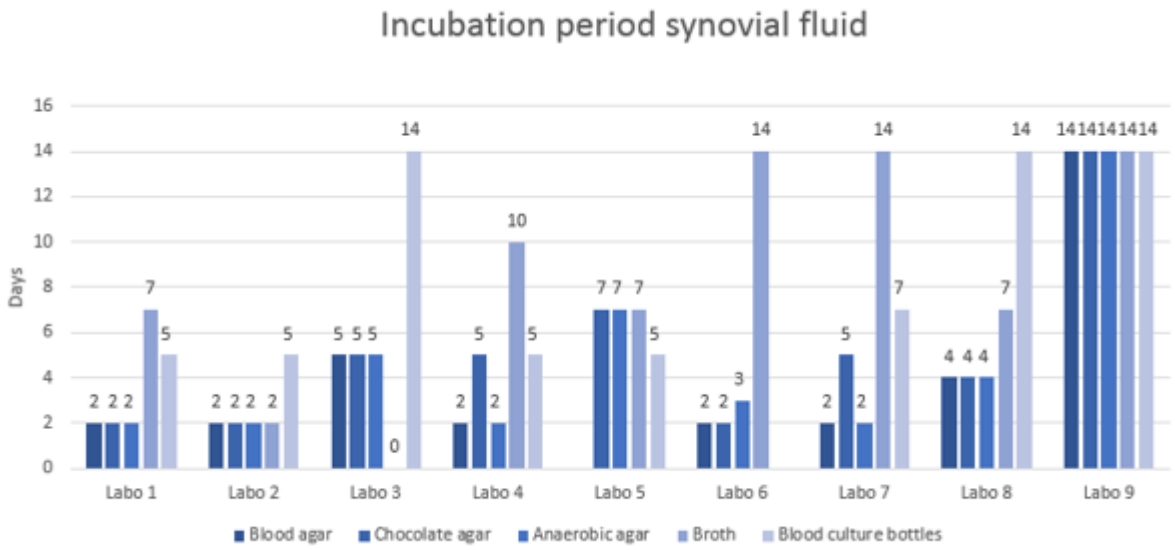



Figure 3: Overview of incubation periods for synovial fluid (BILULU survey)

Last, an overview of used cut-off levels for synovial white blood cell count and polymorphonuclear percentage is presented in figure 4. Four laboratories use the cut-off levels as proposed by the EBJIS (also used by the Pro-Implant Foundation) (7,8). They, however, don't use these cut-off levels as fixed thresholds, but rather as an aid in the interpretation in case of suspected infection. The other four laboratories currently don't use a specific cut-off.

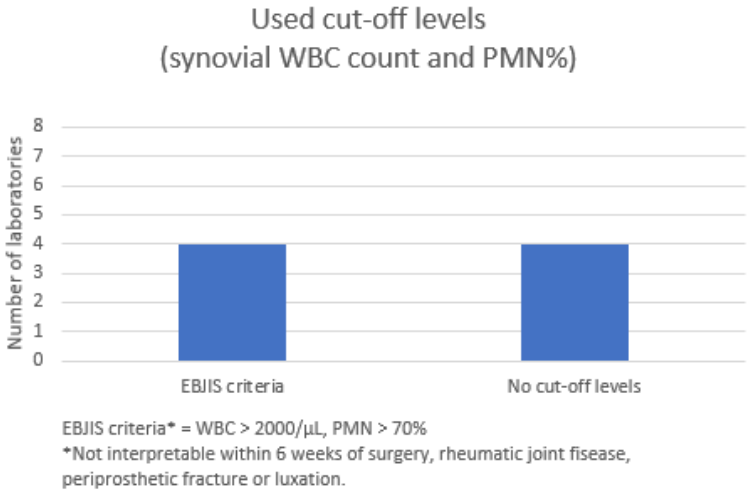



Figure 4: Overview of used cut-off levels for synovial WBC count and PMN% (BILULU survey)

***Conclusion:***

- Only a minority of the questioned laboratories currently implements histological analysis and sonication in their diagnostic algorithm.
- Most questioned laboratories do not use blood culture bottles for tissue biopsies
- There are many differences in incubation periods between laboratories, varying from 2 days to 14 days.
- Half of the questioned laboratories already use a specific cut-off level for prosthetic joint infection. They all use the thresholds as proposed by the EBJIS (7,8).

## 2. Prosthetic joint infections: what is the current evidence on laboratory testing?

Laboratory testing can be divided into preoperative and intraoperative investigations. The preoperative evaluation includes erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and synovial fluid analysis with white blood cell count, differential and microbiological culture. Intraoperative testing should consist of bacteriological culture of multiple intraoperative tissue samples. Additionally, culture of the explanted prosthesis by sonication is also an option. Aside from microbiological testing, histopathological examination of periprosthetic tissue is also recommended in diagnosing prosthetic joint infections (13, 20,).

### 2.1 Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)

Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are frequently used serum markers that indicate inflammation or infection. They are routinely used in the initial assessment of patients with suspected PJI (8). The definitions of MSIS, the International Consensus and the updated version include these parameters as minor criteria (11, 12, 13). The IDSA guidelines and the criteria proposed by the EBJIS don't use these markers in their definition, but they do however include them as part of the diagnostic work-up (7, 8, 10).

However, the diagnostic utility of these parameters for PJI is limited. They are non-specific and can be elevated in a variety of other conditions like inflammatory joint diseases and postoperatively (6). On the other hand, prosthetic joint infection may present with normal serum levels of ESR and CRP, especially in low-virulent cases. Though the combination of normal ESR and CRP levels may be useful to lower the probability of infection, it may not be accurate to definitively rule out infection in case there is a clinical suspicion. In recent publications, percentages of seronegative cases (both ESR and CRP negative) range from 4% (15) to 32% (21, 22). They were mostly associated with low-virulent infections, though some cases were also due to *S. aureus* or Gram-negative bacilli (15, 21). The great variations between these percentages are most likely attributable to the different definitions used (5, 21, 22,). Due to these limitations in sensitivity and specificity, pre-operative ESR and CRP seems to be of limited value in the definitive diagnosis of PJI.

***Conclusion:***
ESR and CRP are of limited value in the diagnosis of prosthetic joint infections. These tests do have the main advantage of being widely and easily available in a routine laboratory. They have a rapid turn turnaround time, are inexpensive and don't require invasive sampling (6). Therefore, they can be used in the initial assessment of the patient and as an additional tool in the diagnostic work-up to increase or decrease the likelihood of infection. However, though it may be helpful for lowering the probability of infection in case both parameters are negative, a small subset of patients, particularly the chronic low-virulent infections, will present with both negative ESR and CRP serum levels. Therefore, even if ESR and CRP are combined, they can't replace further investigations like synovial fluid aspiration in case of clinical suspicion (6, 9, 15, 21, 22).

### 2.2 Synovial fluid white blood cell count and differential

Synovial white blood cell count and percentage of polymorphonuclear neutrophils (PMN) are important tools in the pre-operative evaluation (10, 11 ,12). In contrast to native joint infections, there is however little consensus about the cut-off values that should be used in prosthetic joint infections.

For total knee arthroplasties (TKA), Trampuz et al. propose cut-off values of 1700 white blood cells/µL for synovial white blood cell count and 65% for PMN. This study however only included patients who undergone surgery more than 6 months ago (24). Another study by Ghanem et al. suggests cut-off values for infection of 1100 white blood cells/µL and 64% PMN. When both parameters were below these thresholds, the negative predictive value was 99,6% (25). Finally, Zmistowski et al. found higher thresholds of 3000 white blood cell/µL and 75% PMN (26).

For total hip arthroplasties (THA), reported thresholds appear to be higher than for TKA. For example, a recent multicenter study by Higuera et al. found cut-off levels of 3966 cells/µL for white blood cells and 80% for PMN (27). Another study by Schinksy et al. found optimal cut-off levels of 4200 white blood cells/µL and 80% for PMN, which is in the same range as the previous study (28). Finally, a study by Cipriano et al., which included 810 patients with knee and hip replacements, suggested a threshold of 3450 white blood cells/µL and 78% PMN. They did not analyze this values for hip and knee separately, but 55% of the arthroplasty types included hip replacements (29). In contrast, a smaller study of 75 patients, also including both knee and hip replacements, found white blood cell count values that were notably lower than those reported in other studies. They found an optimal cut-off of 1425 white blood cells/µL and 65% PMN (30).

An important caveat in these studies is that patients with inflammatory joint diseases were excluded. As for ESR and CRP, it would be expected that synovial white blood cell count is less specific in these patients and higher thresholds would be necessary. However, the aformentioned study by Cipriano et al. found no difference in test performance between patients with or without inflammatory joint diseases. These findings should still be confirmed by larger studies, since this study only had 19 patients with inflammatory joint disease who had a prosthetic joint infection (6, 29).

These suggested thresholds also do not apply in the early postoperative period, since baseline cell counts may be elevated due to surgery. It has however been shown that synovial white blood cell count and differential is still useful in this period if higher thresholds are used. Bedair et al. demonstrated that in patients presenting with a prosthetic joint infection within 6 weeks after surgery, a threshold of 27800 white blood cells/µL and 89% PMN could be predictive of infection (31).

In contrast to knee and hip arthroplasties, only a few reports with a limited number of included patients are published on shoulder arthroplasties. These results indicate that optimal thresholds for synovial white blood cell count and differential are presumably higher than the thresholds described for TKA and THA. More studies are needed however to establish these cut-offs (32).

As to the international definitions, the diagnostic criteria by MSIS do include elevated synovial white blood cell count and elevated PMN% in their criteria, but they do not include thresholds. The international consensus meeting however suggests using a threshold of 3000 white blood cells/µL and 80% PMN. They don't exclude patients with inflammatory joint diseases. The EBJIS suggests a threshold of 2000 white blood cells/µL and 70% PMN. They exclude inflammatory joint disease, periprosthetic fracture or luxation (8, 7). They both refer to a period >6 weeks after surgery and don't differentiate between type of affected joint. These criteria do offer a useful consensus which can be used in practice.

***Conclusion:***

- Cut-off values differ between studies and between affected joint.
- Cut-off values in studies appear to be higher for THA than TKA.

- The proposed thresholds mainly apply in the context of chronic prosthetic joint infections and in in patients without inflammatory diseases.
- The International Consensus Meeting and EBJIS suggest the following thresholds:
  - ICM: 3000 white blood cells/µL and 80% PMN (>6 weeks post-surgery)
  - EBJIS: 2000 white blood cells/µL and 70% PMN (exclusions: <6 weeks post-surgery, inflammatory joint disease, periprosthetic fracture or luxation)

### 2.3 Gram staining

A number of studies reported very low sensitivities (ranging from 0-27%) for tissue gram staining. This can be explained by the very low numbers of bacteria present in the sample. On the other hand, patients with a positive gram stain are frequently those who present with acute infections for whom the diagnosis does not present a challenge. Tissue gram staining has thus little value in the diagnosis of prosthetic joint infections and is not recommended (6, 17).

### 2.4 Bacteriological culture

Bacteriological culture is an important tool in the diagnosis of prosthetic joint infections. Isolation and identification of the causative organism confirms the diagnosis, directs antimicrobial therapy and optimizes patient outcome (34). As described earlier, presence of a biofilm complicates isolation of a pathogen. The majority of organisms are concentrated in this biofilm on the surface of the prosthesis and are present in a slow-growing phase. Only a low number of free-floating bacteria are present in the surrounding tissue and fluid (19). Recovery of the causative organism can therefore be challenging (35).

#### *2.4.1 Sample types*

Different sample types, both pre- and intraoperative, should be sent for culture. Currently, synovial fluid and intraoperative tissue biopsies are considered gold standard (35). Recent years, there's a growing interest in the use of sonication of the removed prosthesis. This technique applies ultrasound to dislodge bacteria from the biofilm on the surface of the prosthesis to enhance bacterial growth. Therefore, the explanted prosthesis needs to be sent to the laboratory (35). Intraoperative swabs, which are commonly used due to their ease, should be discouraged. Their sensitivity and specificity is too low compared to intraoperative tissue samples (36).

It is recommended that antibiotics be discontinued for at least 14 days prior to culture, if possible, since recent antibiotic use could be a reason for culture-negative PJI.

*2.4.1.1 Synovial fluid and periprosthetic tissue biopsies*

Culturing of multiple samples is essential to increase the chance of isolation of a pathogen and to help differentiate between contaminant and true pathogen (19, 14). Bacteria involved in prosthetic joint infections typically belong to the normal skin flora and could also represent contamination from the environment (during transport or processing) or from the patient himself (during sampling). In case of growth of such an organism in a single specimen, it could therefore be difficult to distinguish between contamination or clinically significant. Growth in multiple specimens could help in the interpretation and is strongly predictive for infection.

An old study from 1980 by Kamme and Lindberg already recognized these challenges and reported that five intraoperative biopsy samples should be taken and processed separately to distinguish clinical significant bacteria from contaminating bacteria. Growth in 3 or more specimens strongly indicated an infection, growth in only one or 2 specimens strongly indicated contamination (37). Subsequent studies repeatedly confirmed the advantage of obtaining multiple tissue samples with a cut-off of 3 or more positive culture samples for infection (14, 17, 33).

For example, the study by DeHaan et al. evaluated the impact of obtaining 5 or more tissue samples on microbiological diagnosis and antibiotic use. Tissue samples were cultured in thioglycolate and anaerobic conditions for 10 days. They defined a skin flora commensal as pathogen if 3 or more samples were positive and as contaminant if 2 or less samples were positive. Growth of a virulent organism was always considered relevant. In total, 77 cases were included. The use of this protocol identified 7 cases of definite infection by a skin flora commensal and 11 cases of definite contamination by a skin flora commensal. There were also 8 cases with virulent organisms that were only identified by the use of multiple cultures, that would have been missed if only 1 or 2 biopsies were sent for examination. The use of multiple cultures had thus an added value in 26/77 cases (34%). They also evaluated the antibiotic use in these patients and concluded that this protocol altered antibiotic therapy (targeted antibiotic therapy or no antibiotics in case of contamination) in 23 cases. This protocol presumably also correctly predicted joint sterility in 95% of the remaining cases if cultures were negative after 10 days. Therefore, this could mean that prosthetic joint infection is very unlikely when all cultures of all samples are negative. This should, however, always be combined with clinical judgement and other results (14).

A more recent study by Bemer et al. did not confirm the superiority of 5 samples and suggested that 4 intraoperative samples (which included synovial fluid) were equally effective. They used a minimum of 2 (instead of 3) positive cultures as one of the criteria for prosthetic joint infection (38).

The aforementioned definitions by IDSA, MSIS, ICM and EBJIS al recommend multiple tissue samples (at least 3 and maximum 6 intraoperative tissue biopsies). They don't specify the exact number. They include growth of the same organism in minimal two, and not three, specimens (two intraoperative specimens or combination of preoperative aspiration and intraoperative culture) as diagnostic for prosthetic joint infections. In case of isolation of a virulent organism like *S. aureus*, growth in only one specimen may already confirm the diagnosis (8, 10, 11, 14). Growth of a non-virulent organism in only one sample should be interpreted with caution in combination with other findings.

*Conclusion:*

- Synovial fluid and intraoperative periprosthetic tissue biopsies should be sent for culture.
- Multiple periprosthetic tissue samples are necessary to improve the sensitivity and to help discriminate between contaminant and clinically significant pathogen.
- The international available definitions suggest taking at least 3 and maximum 6 intraoperative biopsies. They don't specify the exact number. According to these definitions, diagnosis can be confirmed when two independent cultures grow the same microorganism.

*2.4.1.2 Prosthesis material*

To improve isolation of organism, there is a growing interest in other techniques that can help in the diagnosis of prosthetic joint infections. Sonication has been introduced many years ago for diagnosis and uses ultrasound waves to dislodge the biofilm and the associated bacteria from the implant. The implant is placed in a large sterile container and sent to the laboratory. Fluid is added and can be used, after the sonication process for culture (6, 19, 35). As this technique targets the biofilm organisms, it may therefore improve microbiological yield, especially in the chronic prosthetic joint infections.

Trampuz et al. (NEJM, 2007) was one of the first groups who evaluated the added value of sonication in the diagnosis or prosthetic joint infections. They concluded that sonication was more sensitive than conventional microbiological methods, especially in patients who had received prior antibiotic therapy (19). Many other studies have since then been performed over the last decade and often came to the same conclusion (19, 35, 39, 40). A meta-analysis published in 2017 by Liu et al. showed that sonication was of great value in the diagnosis of prosthetic joint infections with a pooled sensitivity of 79% and specificity of 95%. They concluded that sonication was more sensitive than conventional microbiological methods (35). These data were very similar to those published in an earlier meta-analysis in 2014 by Zhai et al. (41).

Despite these many promising results, the value of sonication, however, remains a point of discussion. There are many differences between the performed studies, which makes correct interpretation difficult (42). First of all, there are methodological differences, in particular, differences in the number of tissue biopsies and culture conditions. The sensitivity of tissue culture largely depends on the number of tissue samples obtained during surgery. As mentioned above, a minimum of 3 biopsy samples and ideally 4 or 5, are recommended. Comparison of sonication results with culturing results of a suboptimal number of biopsies may overestimate the value of sonication. Additionally, differences in culturing conditions between tissue samples and sonication fluid (conventional agar media versus blood culture bottles), may also result in suboptimal conditions for tissue samples (19, 39, 40, 42). Second, included cases differ due to differences in used definitions and therefore inclusion criteria. Last, there is a variation between used thresholds and culture duration between studies. A summary of all these studies with used definitions, methodology and other variables can be found in the attachments (supplementary file 1) (42).

A recent large study by Dudareva et al. (JCM, 2018) addressed these issues and compared the diagnostic accuracy between culture of tissue samples with optimal culture conditions (a median of 5 tissue specimens and blood culture bottles) and sonication. In total, 528 procedures were performed of which 23 were excluded since <2 tissue samples were taken. Antibiotics were withheld prior to surgery if possible. Each tissue sample was obtained using separate instruments and the implant was placed in a sterile container. The tissue samples were homogenized in sterile saline and sterile glass beads. Equal aliquots of the resulting suspension were inoculated in blood culture bottles (Bactec Plus Aerobic/F and Bactec Lytic/10 Anaerobic/F bottle) and incubated for 10 days. Sterile saline was added to the container containing the implant. Every implant was than vortexed for 30 seconds, sonicated for 1 min and vortexed again for 30 seconds. Aliquots of the sonication fluid (100µL) were inoculated onto blood and chocolate agar and incubated at 37°C for 5 days aerobically and 10 days anaerobically. Patients were classified as having a prosthetic joint infection based on a combination of published definitions (IDSA and MSIS) and criteria used in previous studies (presence of a sinus tract, visible purulence or positive histology). They concluded that tissue sample culture was more sensitive than

sonication, except in case of less virulent organisms where sensitivities were equal (table 6). They could also not confirm the higher sensitivity of sonication in cases with prior antibiotic exposure (42).

**TABLE 2** Sensitivity of tissue culture and sonication for diagnosis of prosthetic joint and other orthopedic device-related infections

| Reference standard (definition of infection) | Total no. of infected cases | No. of cases positive by each method | | | | Sensitivity, % (95% CI) | | | *P* value (tissue vs. sonication) |
|---|---|---|---|---|---|---|---|---|---|
| | | Sonication positive | | Sonication negative | | | | | |
| | | Tissue positive[a] | Tissue negative | Tissue positive | Tissue negative | Tissue or sonication positive | Tissue | Sonication | |
| PJI | | | | | | | | | |
| Clinical | 169 | 96 | 9 | 25 | 39 | 77 (70-83) | 72 (64-78) | 62 (54-69) | 0.006 |
| Consensus | 150 | 99 | 6 | 30 | 15 | 90 (84-94) | 86 (79-91) | 70 (62-77) | <0.001 |
| IDSA | 177 | 100 | 8 | 33 | 36 | 80 (73-85) | 75 (68-81) | 61 (53-68) | <0.001 |
| Composite | 182 | 99 | 14 | 30 | 39 | 79 (72-84) | 71 (64-77) | 62 (55-69) | 0.016 |
| Other orthopedic device-related infection | | | | | | | | | |
| Clinical | 77 | 32 | 3 | 17 | 25 | 68 (56-78) | 64 (52-74) | 45 (34-57) | 0.002 |
| Composite | 91 | 34 | 11 | 21 | 25 | 73 (62-81) | 60 (50-71) | 49 (39-60) | 0.077 |
| All device-related infections (PJI and non-PJI) | | | | | | | | | |
| Clinical | 246 | 128 | 12 | 42 | 64 | 74 (68-79) | 69 (63-75) | 57 (50-63) | <0.001 |
| Composite | 273 | 133 | 25 | 51 | 64 | 77 (71-81) | 67 (61-73) | 58 (52-64) | 0.003 |

[a]Sonication positive, sonication culture positive (≥50 CFU/ml). Tissue positive, tissue culture positive (i.e., indistinguishable organisms isolated from at least two tissue specimens).

Table 6: Dudereva et al. Sensitivity of tissue samples and sonication fluid (42)

Another recent study by Yan et al. (JCM, 2018) had a similar study design, but used different inclusion criteria. Patients were classified as having prosthetic joint infections based on non-microbiological IDSA criteria (sinus tract, positive histology or purulence surrounding the prosthesis). This may have resulted into misclassifying PJI patients who did not meet these criteria, but did have multiple positive cultures, into the aseptic failure group. Therefore, they applied Bayesian Latent Class modeling to estimate the diagnostic performance in the absence of a gold standard. They concluded that culture of tissue samples (when using blood culture bottles) had a similar sensitivity to sonication (86,3% versus 88,7%) (43). Both studies did report that culture yield was highest if both methods were combined (table 6 and 7) (42, 43).

**TABLE 3** Sensitivity and specificity of microbiological tests using IDSA PJI criteria as gold standard and Bayesian latent-class modeling[a]

| Specimen or culture | Patients with PJI | Patients with aseptic failure | IDSA PJI criteria as gold standard[b] | | | | Bayesian latent-class modeling[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Sensitivity | Specificity | PPV | NPV | Prevalence | Sensitivity | Specificity |
| All joints | 104 | 125 | | | | | 37.2 (31.1, 43.5) | | |
| Tissue culture | 69 | 5 | 66.4 (56.4, 75.3)* | 96.0 (90.9, 98.7) | 93.2 (84.9, 97.8) | 77.4 (70.0, 83.7) | | 86.3 (78.3, 92.4) | 99.6 (97.7, 100.0) |
| Sonicate fluid culture | 76 | 0 | 73.1 (63.5, 81.3)* | 100.0 (97.1, 100.0) | 100.0 (95.3, 100.0) | 81.7 (74.7, 87.5) | | 88.7 (81.0, 94.3) | 99.6 (97.7, 100.0) |
| Combination[c] | 80 | 5 | 76.9 (67.6, 84.6)* | 96.0 (90.9, 98.7) | 94.1 (86.8, 98.1) | 83.3 (76.2, 89.0) | | 99.1 (95.7, 100.0) | 99.5 (97.6, 100.0) |
| Hips and knees | 86 | 101 | | | | | 34.8 (28.4, 41.8) | | |
| Tissue culture | 56 | 2 | 65.1 (54.1, 75.1)* | 98.0 (93.0, 99.8) | 96.6 (88.1, 99.6) | 76.7 (68.5, 83.7) | | 88.4 (79.6, 94.7) | 99.5 (97.1, 100.0) |
| Sonicate fluid culture | 60 | 0 | 69.8 (58.9, 79.2) | 100.0 (96.4, 100.0) | 100.0 (95.3, 100.0) | 79.5 (71.5, 86.2) | | 91.4 (83.3, 96.5) | 99.4 (97.2, 100.0) |
| Combination | 63 | 2 | 73.3 (62.6, 82.2)* | 98.0 (93.0, 99.8) | 96.9 (89.3, 99.6) | 81.2 (73.1, 87.7) | | 98.9 (94.7, 100.0) | 99.4 (96.9, 100.0) |
| Shoulders and elbows | 18 | 24 | | | | | 50.9 (36.1, 65.5) | | |
| Tissue culture | 13 | 3 | 72.2 (46.5, 90.3)* | 87.5 (67.6, 97.3) | 81.3 (54.4, 96.0) | 80.8 (60.7, 93.5) | | 68.4 (48.2, 84.3) | 96.8 (84.3, 99.9) |
| Sonicate fluid culture | 16 | 0 | 88.9 (65.3, 98.6) | 100.0 (85.8, 100.0) | 100.0 (79.4, 100.0) | 92.3 (74.9, 99.1) | | 80.7 (62.8, 93.1) | 96.8 (84.7, 99.9) |
| Combination | 17 | 3 | 94.4 (72.7, 99.9)* | 87.5 (67.6, 97.3) | 85.0 (62.1, 96.8) | 95.5 (77.2, 99.9) | | 97.0 (84.8, 99.9) | 96.6 (82.8, 99.9) |

[a]PPV, positive predictive value; NPV, negative predictive value; *, statistically significant difference from other tests in the same comparison group, $P < 0.05$.
[b]Values are % (95% confidence interval) for IDSA PJI and % (95% credible interval) for Bayesian latent-class modeling.
[c]Combination includes tissue culture and sonicate fluid culture.

Table 7: Yan et al. Sensitivity and specificity of tissue samples and sonication fluid (43)

Increase in microbiological yield was also reported by Prieto-Borja et al. (EJCMID, 2018) when a combination of sonication fluid and conventional culture (periprosthetic tissue and/or synovial fluid) was used. They describe that culture from synovial fluid and tissue biopsies performed better in acute than chronic infections, in contrast to sonication fluid. However, since they compared sonication with periprosthetic tissue and/or synovial fluid in their study, optimal tissue sampling and processing could have been missing in some cases (44).

Another recent study from 2018 investigated the differential contributions of specimen types to diagnosis of prosthetic joint infections (synovial fluid, tissue biopsies, bone biopsies, swabs and sonication). They concluded that the combination of synovial fluid, tissue biopsies and sonication fluid was the ideal combination for diagnosing prosthetic joint infections. However, they didn't use blood

culture bottles. Therefore, it's not known if the added value of sonication fluid would have been the same in case blood culture bottles were used for synovial fluid and tissue biopsies. They did however confirm that swabs and bone biopsies had no additional value (46).

*Conclusion:*
Sonication remains a subject of debate. Sensitivities of sonication and tissue samples vary widely due to variations in methodology between studies and are therefore difficult to compare. It seems however that there are no strong arguments that one method is better than the other if rigorous tissue sampling with optimal culturing conditions can be established. They could however be complementary to each other to further optimize the diagnosis, especially for difficult to diagnose chronic/low-virulent infections (42, 43, 44). Future studies will hopefully provide definite clarity about this subject and suggest the optimal diagnostic protocol that can be used in a daily routine.

*2.4.1.3 Swabs*

Swabs have a limited role in the microbiological diagnosis of prosthetic joint infections and should therefore be discouraged.

Superficial swab cultures of the drainage from a sinus tract is neither sensitive nor specific. There is a low concordance between the culture from the superficial tract and the causative pathogen. This poses a risk of incorrectly identifying the infecting organism and misguide the diagnosis and treatment (45).

There is also no place for intraoperative swabs in the diagnosis of prosthetic joint infections. Both sensitivity and specificity is lower for intraoperative swabs than for intraoperative tissue samples (6, 36). In one study by font-Vizcarra et al. (2010), swabs were positive in only 44% of the patients with chronic infections, in contrast to 82% for synovial fluid and 74% for periprosthetic tissue (18).

A study by Larsen et al. (JCM, 2018) investigated the differential contributions of specimen types and culturing conditions in the diagnosis of prosthetic joint infections. They obtained multiple sample types from 114 patients, of which 43 patients underwent revision surgery for presumable prosthetic joint infection. A total of 42 patients was finally diagnosed with infection. The collected specimen set consisted of 3 tissue biopsies, 3 bone biopsies, 3 swabs from the prosthesis, synovial fluid and prosthetic material. Swabs and bone biopsies were only obtained from 32 PJI cases. Swabs were positive in 9/32 cases after 6 days of incubation and 16/32 cases after 14 days of incubation. Bone biopsies were also positive in 9/32 cases after 6 days and 13/32 cases after 14 days. This demonstrates the very low diagnostic yield of swab samples. They also did not contribute independently to the diagnosis, as the other specimen types were also positive in these patients (46).

*Conclusion:*
Neither superficial swabs from a draining sinus tract nor intraoperative swabs should be used in the diagnosis of prosthetic joint infections (5, 6, 18, 45).

### *2.4.2 Culture conditions*

As described earlier, culture of joint samples in the context of prosthetic joint infections is difficult. Aside from collecting the correct number of samples, culturing conditions should be optimized in order to improve the chance to isolate the causative microorganism. Different studies evaluated these optimal conditions. Their main focus however was on the use of blood culture systems, sonication and incubation period.

*2.4.2.1 Conventional agar media and enrichment broths*

Studies that formally evaluate the performance of different agar media in the diagnosis of PJI are lacking. Therefore, very few information is available on the optimal use of agar media and broths. Since bacteria are present in low numbers in the sample and could be difficult to isolate, it is generally accepted that culture regimens should include enrichment media (also see the section on 'blood cultures'). (17).

In general, the media that are most commonly used for prosthetic joint infections in different studies are nonselective agar media enriched with blood or blood products (chocolate agar or blood agar) both for aerobic as anaerobic incubation and different kinds of enrichment broths (most frequently thioglycolate broth or brain heart infusion broth) (see supplementary file 2 in attachments). These media allow for growth of the most commonly involved pathogens.

One recent study, published in 2015, did compare the use of three different culture media with the use of five different media for both tissue samples and synovial fluid: blood agar (one incubated for 7 days in CO2 enriched and one for 7 days in anaerobic atmosphere), chocolate agar (incubated 7 days in CO2 enriched atmosphere), a pediatric blood culture (incubated for 14 days) and Schaedler broth (incubated for 14 days). They reported that the use of 3 culture media (chocolate agar, pediatric blood culture bottle and Schaedler broth) could document PJI in 95,1% of patients who did not receive prior antibiotic therapy. They found that chocolate agar was more sensitive than the anaerobic blood agar for isolation of *Cutibacterium acnes* (which can also grow in CO2-enriched atmosphere). Additional blood agar (both under aerobic and anaerobic conditions) did not yield an extra advantage (38).

*Conclusion:*

- There is no formal evidence about the optimal combination of different agar media and enrichment broths.
- The most commonly used media in different studies are nonselective blood containing agar media (aerobic and anaerobic) and thioglycolate broth (see supplementary file 2 in attachments).

*2.4.2.2 Blood cultures*

In contrast to solid agar media and enrichment broths, there are many studies available on the advantages of blood culture bottles in the diagnosis of prosthetic joint infections.

These advantages are related to several factors. First, larger amounts of sample volume can be used to inoculate blood culture bottles. Second, resins, present in the BACTEC Plus bottles, have an inhibitory effect on antibiotics, enhancing recovery of microorganisms in patients who already received antimicrobial therapy (43). These advantages have been demonstrated in all specimen types (synovial fluid, tissue samples and sonication fluid).

Hughes et al. demonstrated the advantage of BACTEC Peds Plus/F bottle for detection of pathogens in synovial fluid of patients with septic arthritis. The use of these bottles detected significantly more pathogens (62 versus 51) in comparison to conventional methods (blood agar, chocolate agar and thioglycolate). Bottles were incubated for 5 days (47).

Another study by Font-Vizcarra et al. compared the use of blood culture bottles (both aerobic and anaerobic bottles for the BACTEC 9240 system) for synovial fluid to tissue and swab samples cultured with conventional methods (blood agar, Schaedler agar and thioglycolate). All cultures were incubated for 5 days. They demonstrated higher sensitivities and specificities for synovial fluid in blood culture bottles than tissue and swab samples cultured with conventional methods (table 8). The accuracy was higher for acute than chronic infections (18).



**Table 4.** Sensitivity, specificity, PPV and NPV of each sample according to the type of infection (acute or chronic)

| Type of infection | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) | Accuracy |
|---|---|---|---|---|---|
| Acute | | | | | |
| Synovial fluid | 91.39 | 100 | 100 | 93.6 | 96.19 |
| Periprosthetic tissue | 78.94 | 80.95 | 78.95 | 80.95 | 80 |
| Swab | 80.65 | 99.3 | 98.68 | 88.68 | 91.91 |
| Chronic | | | | | |
| Synovial fluid | 78.94 | 100 | 100 | 87.96 | 91.7 |
| Periprosthetic tissue | 56.98 | 80.95 | 67.12 | 73.38 | 71.23 |
| Swab | 39.53 | 99.29 | 97.14 | 73.06 | 76.75 |

Table 8: Font-Vizcarra et al. Sensitivities and specificities for synovial fluid (blood culture bottles) versus conventional media for periprosthethic tissue samples and swabs (18)

Later studies demonstrated that the diagnostic yield of periprosthetic tissue samples can also be optimized by the use of blood culture bottles (34, 47, 48). One study compared the use of four different media for tissue biopsies in the diagnosis of prosthetic joint infections, including blood culture bottles: direct plating (chocolate agar and blood agar aerobically and one blood agar anaerobically), enrichment broths (Robertson's cooked meat broth and fastidious anaerobic broth) and Bactec blood culture bottles (Bactec Pus Aerobic/F and Bactec Standard Anaerobic/F bottle). All were incubated for 5 days. Sensitivities were 87% for blood culture bottles, 39% for direct plating, 57% for fastidious anaerobic broth and 83% for Robertson's cooked meat broth. This demonstrates that enrichment (mainly Robertson's cooked meat) broths were more sensitive than direct plating and that blood culture bottles were the most sensitive. Blood culture bottles also had the shortest time to positivity (57).

These positive results were confirmed by a later study in 2014 by Minassian et al. They inoculated the same blood culture bottles as the former study (Bactec Plus Aerobic/F and Bacted Lytic/10 Anaerobic/F bottles). Tissue samples were homogenised in sterile saline and steril glass beads before inoculation of the bottles. They reported a sensitivity of 83,5% after 8 days of incubation. They did not found an increase in diagnostic yield with prolonged incubation. Their median detection time was 5 days for *Cutibacterium acnes* and 1 day for other bacterial species. They emphasize the need for both aerobic and anaerobic bottles, since 14% of organisms were only identified in the aerobic bottles and 27% in the anaerobic bottles. However, tissue samples were not inoculated onto other media. It is thus unclear if enrichment media (like thioglycolate) could have been of value in these missing cases (48).

Velay et al. evaluated the Bactec Peds Plus/F bottles for tissue samples which resulted in a bacterial detection in 69% of samples, compared to 53% for conventional media (agar plates and broth media) (60).

Another study by Peel et al. compared standard media (blood agar and chocolate agar incubated for 5 days and CDC anaerobic blood agar and thioglycolte incubated for 14 days) with Bactec Plus Aerobic/F and Bacted Lytic/10 Anaerobic/F bottles, incubated for 14 days. They calculated sensitivities for all individual media and combined use of different media. Tissue samples were first homogenised using a Seward Stomacher in 5 ml brain heart infusion broth before inoculating the different media. Sensitivity for blood culture bottles was 92,1% versus 62,6% for the standard media. The table below gives an overview of sensitivities and specificities for individual culture media and a combination of different media (table 9). The highest sensitivity was achieved when all media were combined. Blood culture bottles did not only improve sensitivity, but also gave faster results (34).

**TABLE 2** Sensitivity and specificity of periprosthetic tissue culture techniques using Bayesian latent class modeling and Infectious Diseases Society of America criteria for prosthetic joint infection diagnosis as gold standards[a]

| | No gold standard (Bayesian LCM) | | IDSA PJI criteria as gold standard | |
|---|---|---|---|---|
| Culture medium[b] | Sensitivity (95% credible interval) | Specificity (95% credible interval) | Sensitivity (95% confidence interval) | Specificity (95% confidence interval) |
| Individual culture media | | | | |
| Aerobic agar | 59.4 (45.3, 72.5) | 99.5 (98.3, 100.0) | 26.5 (18.8, 35.5) | 100.0 (98.6, 100.0) |
| Anaerobic agar | 32.2 (20.8, 45.7) | 99.5 (98.3, 100.0) | 14.5 (8.7, 22.2) | 100.0 (98.6, 100.0) |
| Thioglycolate | 74.8 (61.5, 85.8) | 99.4 (98.1, 99.9) | 33.3 (24.9, 42.6) | 100.0 (98.6, 100.0) |
| Aerobic blood culture bottle | 82.0 (69.5, 91.1) | 97.1 (94.8, 98.6) | 42.7 (33.6, 52.2) | 100.0 (98.6, 100.0) |
| Anaerobic blood culture bottle | 90.2 (79.4, 96.5) | 96.3 (93.7, 98.1) | 47.9 (38.5, 57.3) | 99.6 (97.8, 100.0) |
| Combinations of culture media | | | | |
| Aerobic and anaerobic agars | 48.9 (38.3, 59.7) | 99.7 (98.7, 100.0) | 33.3 (24.9, 42.6) | 100.0 (98.6, 100.0) |
| Aerobic and anaerobic agars and thioglycolate | 62.6 (51.7, 72.5) | 98.1 (96.1, 99.3) | 44.4 (35.3, 53.9) | 98.8 (96.6, 99.8) |
| Aerobic and anaerobic BCBs | 92.1 (84.9, 97.0) | 99.7 (98.7, 100.0) | 60.7 (51.2, 69.6) | 98.8 (96.6, 99.8) |
| Aerobic and anaerobic BCBs and thioglycolate | 92.1 (84.9, 97.0) | 98.8 (97.0, 99.6) | 63.3 (53.8, 72.0) | 98.8 (96.6, 99.8) |
| Aerobic and anaerobic BCBs and aerobic agar | 94.6 (88.1, 98.6) | 99.7 (98.7, 100.0) | 62.4 (53.0, 71.2) | 98.8 (96.6, 99.8) |
| Aerobic and anaerobic BCBs and anaerobic agar | 96.8 (91.3, 99.3) | 99.8 (98.7, 100.0) | 62.4 (53.0, 71.2) | 98.0 (95.4, 99.4) |
| Aerobic and anaerobic BCBs and aerobic and anaerobic agars | 99.1 (95.7, 100.0) | 99.7 (98.7, 100.0) | 64.1 (54.7, 72.8) | 98.0 (95.4, 99.4) |
| All media combined | 99.1 (95.7, 100.0) | 97.3 (94.8, 98.7) | 67.5 (58.2, 75.9) | 96.8 (93.8, 98.6) |

[a] Using individual culture media in periprosthetic tissue culture techniques, the prevalence of prosthetic joint infection (PJI) was 13.7% (95% credible interval of 10.4% to 17.6%) with no gold standard (Bayesian latent class modeling [LCM]), and the prevalence of PJI was 31.7% (95% confidence interval of 27.0% to 36.7%) with Infectious Diseases Society of America (IDSA) PJI criteria as the gold standard. Using combinations of culture media in periprosthetic tissue culture techniques, the prevalence of PJI was 21.7% (95% credible interval of 17.7% to 26.1%) with no gold standard (Bayesian LCM), and the prevalence of PJI was 31.7% (95% confidence interval of 27.0% to 36.7%) with IDSA PJI criteria as the gold standard.
[b] BCBs, blood culture bottles.

Table 9: Peel et al. Sensitivities and specificities of different culture media for periprosthetic tissue samples (34)

Finally, blood culture bottles also proved useful for sonication fluid. A study by Shen et al. compared synovial fluid cultures in blood culture bottles with sonication fluid cultured in blood culture bottles. They reported a higher sensitivity for sonication fluid (88%) versus synovial fluid (64%). The specificity however was lower (87% versus 98%). By inoculation of sonication fluid in blood culture bottles, a decrease in specificity may be explained by losing the ability of colony count, which is used to define contamination versus relevant. Given the size of the specimen that is collected and processed, presence of contaminants may be a potential problem. This study did not compare with solid agar media nor with tissue samples (61)

A second study by Portillo et al. also demonstrated the advantages of blood culture bottles for sonication fluid. They reported an increased sensitivity of sonication fluid in blood culture bottles compared to conventional media. They had no culture-negative cases by the use of blood culture bottles and also no false-positive results. This is in contrast with the aforementoned study, who did report a lower specificity for sonication fluid in blood culture bottles. This study, however, does not specificate the number of tissue samples used per case and also did not use blood culture bottles for this specimen type (39).

Culturing of sonication fluid in blood culture bottles thus also seems a promising strategy to improve diagnosis of prosthetic joint infections. The main disadvantage of using blood culture bottles for sonication fluid is the loss of semi-quantitative analysis, which allows to distinguish with contamination. Therefore, in case blood culture bottles are used for sonication fluid, they cannot replace additional tests like the conventional media and tissue samples.

*Aerobic vs Anaerobic vs Pediatric blood culture bottles?*

Both the study by Minassian et al. and Hughes et al. mention the importance of using both aerobic and anaerobic blood culture bottles (48, 47). Since both synovial fluid and multiple tissue samples are recommended in the diagnostic work-up, inoculating both blood culture bottles could result in a high amount of vials per patient that need to be inserted into the automatic blood culturing system. Use of a pediatric blood culture bottle may be an alternative. It should however be kept in mind that pediatric bottles are less efficiënt for cultivating anaerobes and thus additional media should certainly be used. A study by Bemer et al. demonstrated a bacteriological documentation of 89,3% of al PJI cases when using a pediatric blood culture bottle in combination with a chocolate agar and Schaedler broth (38).

However, table 9 demonstrates that conventional agar media also have an added value when both aerobic and anaerobic blood culture bottles are used. In 5 cases, the pathogen was only detected from another culture medium: two cases of *Cutibacterium acnes* (from thioglycolate), one case of *Staphylococcus hominis* (from aerobic agar media) and two cases of *Parvimonas micra* (anaerobic agar media) (34).

A very recent study by Van den Bijllaardt et al. (2019) also demonstrated the added value of combining multiple culture media (table 10). In 17 cases, pathogens were only found in blood culture bottles (Bactec Plus Aerobic/F and Bactec Plus Anaerobic/F). These also included virulent microorganisms, which are generally not considered to be difficult to culture. For six episodes, causative pathogens (mostly low-virulent organisms) were only isolated from broths. This demonstrates that blood cultures should be used in addition to conventional media. Sensitivities per culture set are displayed in the table below. Conventional agar media consisted of bloodagar, chocolate agar, MacConkey, Wilkins-Chalgren anaerobic agar (all incubated for 4 days), thioglycolate and brain heart infusion broth (incubated for 14 days). Both periprosthetic tissue samples and synovial fluid were cultured (49).



**Table 2** Sensitivity and specificity of PPT culture for 45 PJI cases and 45 non-PJI cases according to the IDSA criteria

| Culture set | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|
| Solid agars and broths | 84.44 (70.54–93.51) | 100.00 (92.13–100.00) |
| Solid agars and BCBs | 88.89 (75.95–96.29) | 100.00 (92.13–100.00) |
| Solid agars, broths and BCBs | 93.33 (81.73–98.60) | 100.00 (92.13–100.00) |

Table 10: Van den Bijlaardt et al. Sensitivities and specificities of different culture media (49)

*Conclusion*:

- Blood culture bottles can improve the detection rate for microorganisms in synovial fluid, periprosthetic tissue samples and sonication fluid.
- Aerobic, anaerobic and pediatric blood culture bottles can be used. Pediatric blood culture bottles, however, don't allow for anaerobic recovery.
- Combining blood culture bottles with conventional media (agar media and enrichment broths) seems to result in the highest detection rate.

*2.4.2.3 Incubation time*

Variations in incubation periods are seen between different studies. In general, the majority of studies applied incubation periods of 5-7 days for aerobic cultures and 7-14 days for anaerobic cultures. Extended incubation beyond 7 days may be necessary for recovery of *Cutibacterium* species.

Prolonged incubation for 14 days was recommended by Schafer et al. to optimize recovery of *Cutibacterium* species and *Peptostreptococcus* species. They had a detection rate of merely 73,6% after 7 days. *Cutibacterium* species were almost exclusively detected in the second week of incubation. Additionally, almost one-fourth of the coagulase-negative staphylococci were not detected until the second week (which may reflect low concentrations of bacteria in the sample). A total of 26,4% of patients would not have had a bacteriological diagnosis of prosthetic joint infections if culture duration had only been 1 week. They also did not detect overrepresentation of contaminants in the second week of incubation. The median time to detection of contaminants was 7 days and 52% of all contaminating strains were isolated in the first week. It should however be mentioned that no blood culture bottles were used in this study (50).

This was confirmed by Butler-Wu et al. They suggested that both aerobic and anaerobic cultures should be incubated for 13 days. If only anaerobic cultures had extended incubation, 29,4% of prosthetic joint infections by *Cutibacterium* acnes would have been missed in their study. They could not demonstrate that one culture medium was superior to another for isolation of this species. Extending incubation beyond 13 days was not associated with an increase in diagnosis of prosthetic joint infections, but was associated with an increasing recovery of nondiagnostic isolates. As for the former study, no blood culture bottles were used (51).

A smaller study by Shannon et al. evaluated the use of thioglycolate broth for isolation of *Cutibacterium acnes.* They suggested a 7-day incubation for recovery of *Cutibacterium acnes* when using anaerobic thioglycolate broth and specimen collection into anaerobic tissue and fluid vials (52).

A more recent article by Minassian et al., which evaluated the use of blood culture bottles (aerobic and anaerobic) in diagnosis of prosthetic joint infections, could not confirm the need for prolonged incubation of 14 days. No relevant *Cutibacterium acnes* were isolated after day 8. They included 322 patients with suspected prosthetic joint infection, which resulted in 1328 samples (48).

Peel et al. did report additional recovery of relevant *Cutibacterium* species due to prolonged incubation in the anaerobic blood culture bottle. However, they also recovered three additional contaminants. They did not recover any additional relevant species in the aerobic bottle after 7 days (see appendix with figures). They suggest that incubating the aerobic bottle for 7 days and anaerobic bottle for 14 days would be a reasonable approach (34).

Finally, Bemer et al. suggest a practical protocol to use in the routine for both synovial fluid and tissue samples: one pediatric bottle incubated for 5 days, one chocolate agar incubated for 7 days and one schaedler broth incubated for 14 days (and subcultured afterwards). Using this protocol, they had a bacteriological documentation in 89% of the cases (38).

*Conclusion:*

- Prolonged incubation is advised. Suggested incubation periods vary from 5-14 days.
- Generally, longer incubation periods are recommended for anaerobic than aerobic cultures.
- The main profit of prolonged incubation is in the increased detection of *Cutibacterium* species.

### 2.5 Histopathological examination

Histological evaluation demonstrating acute inflammation (neutrophilic infiltrate) is a helpful tool in the diagnosis of prosthetic joint infections. All internationally accepted definitions include histological evaluation in their supportive evidence (8, 10, 11, 12, 13).

It has a high sensitivity (>80%) and specificity (>90%) (8, 10). It can also be done intraoperatively on a frozen-section and can therefore give information to the surgeon on best surgical approach (if diagnosis and approach weren't already known through pre-operative analysis). A meta-analysis performed in 2012 found that the presence of acute inflammation provided a high positive likelihood ratio of 12. The absence of acute inflammation had a more modest negative likelihood ratio. They did not calculate pooled sensitivity and specificity, but these ratios do suggest that frozen-section analysis is helpful as an additional tool (6, 53).

However, results of pathological evaluation can vary due to sampling bias or expertise of the pathologist. Acute inflammation can also be absent in the case of low-virulent organisms, which may not elicit such a strong inflammatory response (6, 8, 10, 51).

There is no clearly accepted definition about acute inflammation. The classical definition of acute inflammation differs between authors and varies from 1 to 10 neutrophils per high-power field at a magnification of 400 (5). For example, the MSIS criteria define acute inflammation as the presence of 5 or more neutrophils per high-power field in at least five fields observed at a magnification of 400. Other definitions don't give exact specifications about acute inflammation (6, 8, 11).

A few years ago, histopathological criteria for evaluation of periprosthetic membrane tissue were defined by Krenn and Morawietz. Based on some morphological aspects, periprosthetic membrane tissue can be classified into 4 groups: type 1 to 4. Type 2 represents the infectious histology and is characterized by a neutrophilic infiltrate. Type 3 represents a combination of infectious histology and wear particle induced reaction. The Pro-Implant Foundation, for example, uses the suggested cut-off of 23 neutrophils in 10 high-power fields that is used to define inflammation in periprosthetic tissue by this classification (5, 7, 62, 64).

### 2.6 Other

In addition to the previous tests, other possible tests could be used in the diagnosis of prosthetic joint infections. Usually, these are not used in a routine practice. An extensive review of this tests is, however, beyond the scope of this critically appraised topic.

#### 2.6.1 PCR (16S rRNA PCR)

A recent critically appraised topic (D. Van den Bossche, 2015) evaluated the value of 16S rRNA PCR in the diagnosis of prosthetic joint infections. The included studies reported different sensitivities and specificities for 16S rRNA (synovial fluid, periprosthetic tissue biopsies or sonication fluid) in the diagnosis of prosthetic joint infections. The main conclusion was that 16S rRNA PCR should not be used routinely, but could be useful for culture-negative cases with a high suspicion for infection. It is, however, not clear which sample type is the most useful in these cases (54).

2.6.2 Alpha defensin

Determination of alpha defensin in synovial fluid is a promising test that can aid in the diagnosis of prosthetic joint infections. This test is now available as a lateral flow assay, which can generate results after 10 minutes. A study by Renz et al. demonstrated a sensitivity of 54-84% (depending on the used diagnostic criteria), limiting the use of this test in excluding prosthetic joint infections. They did however report high specificities (>95%), indicating that it can be used to confirm the diagnosis (55). A meta- analysis published in 2018 showed a lower diagnostic performance for the lateral flow assay compared to the laboratory ELISA test, with a pooled sensitivity and specificity for the lateral flow assay of 85% and 90% respectively. They conclude that further studies are required to evaluate the use of the lateral flow assay in a clinical environment before its routine adoption to diagnose PJI. Another limiting factor for routine use of this assay is the high cost per test (+/-170 euros per test), which is not reimbursed at this moment (56).

## 3. Prosthetic joint infections: towards a BILULU consensus

We can conclude that many studies are published and a lot of information is available on this topic. Due to a lack of standardization and many methodological differences between all these publications, it is however difficult to compare all these results. These theoretical challenges will hopefully be addressed in the future, so more standardized definitions and study protocols can be designed. This will make reported results more comparable and can possible lead to internationally accepted standardized diagnostic algorithms.

Aside from these theoretical obstacles, implementing the most optimal culture conditions can already improve diagnostic yield and reduce the percentage of culture-negative prosthetic joint infections.

Since there is no formal evidence on the optimal combination of culture media and incubation periods, a consensus will be made, which will take into account all the available information. The goal of this consensus is to optimize the current diagnostic process and to standardize this process in and between hospitals. On the one hand, the procedure should include the most optimal sampling and culture conditions that are now suggested in the literature to generate the highest microbiological yield. On the other hand, it should be a workable tool for a daily routine. It should therefore also take into account the organizational impact, additional workload and cost.

The key components for improvement that are suggested in literature at this moment and should be discussed when making the consensus are:

- Collection and culturing of multiple samples
- Use of blood culture vials for both synovial fluid and tissue samples
- Prolonged incubation
- Sonication (though this is still debatable)
- Elimination of swabs
- Histological analysis of periprosthetic tissue samples

### *3.1 Suggestions for sampling protocol*

A. Synovial fluid

Synovial fluid should be sent for analysis and can be obtained preoperatively. Analysis should include:

- Synovial white blood cell count and differential
- Aerobic and anaerobic culture
- Crystals for differential diagnosis with crystalarthropathy

To aid the orthopedic surgeon to request the correct tests, a prepacked sampling kit can be prepared by the laboratory and distributed. The following is an example of what this sample kit may contain:

- EDTA blood tube for cell count and differential
- Sterile syringe: to be sent to the laboratory for aerobic and anaerobic culture
- Blood culture bottle: to be directly inoculated bedside
- Request form: specifically made for (prosthetic) joint infections. This form contains instructions for sampling, priority order and necessary tests.

This kit can also be used in case of native joint infections.

In case too little sample volume is available for all these tests, a decision must be made about the priority of analysis. Since cell count is the most sensitive tool, testing for white blood cells and differential seems to be the most important tool (7, 63). In case sample volume is enough for both cell count and microbiology, inoculation of blood cultures bottles will depend on the remaining amount of volume. Inoculation of blood culture bottles should be done if possible (the volume depends on the blood culture bottle that is used: pediatric bottles need less volume than aerobic/anaerobic bottles). The exact method will be documented in the consensus.

Additionally, synovial fluid should also be taken during revision intra-operatively.

B. Tissue sample biopsies

Multiple tissue samples should be sent for diagnosis. At least 3 and maximum 6 tissue samples are recommended in the international definitions. Some studies advocate for 5 tissue samples, however, a more recent study (2016) did not confirm the superiority of 5 samples and suggested that 4 intraoperative samples (which included synovial fluid) were equally effective (38).

Since every biopsy needs to be processed separately and additional biopsies are needed for histopathological examination, it raises the question whether it's achievable to routinely request for 5 tissue samples for microbiology. A decision needs to be made about the number of tissue samples that will be used (with a minimum of 3).

As for synovial fluid, a prepacked sampling kit for intraoperative sampling can also be used to aid the orthopedic surgeon in obtaining the correct number and type of samples. This kit should contain the following:

- Sterile recipients for microbiology (number to be discussed)
- Recipients for histopathology (to be discussed with histopathology per center)
- Sterile syringe and blood culture bottle for intraoperative synovial fluid
- Request form: specifically made for prosthetic joint infections. This form contains instructions for sampling (both for tissue biopsies and synovial fluid) and necessary tests.

To minimize the manipulation of biopsies and therefore the risk of contamination, it is a possibility for the surgeon to deposit the obtained tissue biopsies directly into the sterile recipient with beads, used to homogenize in the laboratory.

The concept of these pre-packed boxes was evaluated in a research project by Larsen et al. During a 2-year period, these boxes were offered to the surgeons to use during revision surgery. They reported an overall completeness around 90%. Use of pre-packed boxes seems a promising tool to aid in the complexity of the work-up (58).

There is no protocol available for the optimal processing of tissue samples before inoculation of the different media. Most studies however used mechanical disruption to homogenize the samples (eg. sterile glass beads). Different liquid media were used like sterile saline or brain heart infusion broth. Roux et al. reported a high documentation rate of prosthetic joint infections by using beadmill processing of periprosthetic specimens (59). It seems best for tissue samples to be homogenized before inoculation of the different media.

C. Prosthesis material

As discussed, there are many variations between studies on this topic, making it difficult to compare diagnostic accuracies. Many studies report a better sensitivity for sonication. However, recent studies could not find a significant difference in sensitivity between sonication and tissue culture. They do suggest it may be used as complementary tool to synovial fluid and tissue biopsies, especially in the difficult-to-diagnose infections. The many methodological differences however prevent us from drawing a clear conclusion.

Sonication requires large sterile containers and an ultrasound bath and consists of multiple steps including vortexing, sonication, vortexing, centrifugation and eventually inoculation of the different media. Therefore, this technique is time consuming and requires additional material resources (if not yet present in the laboratory). Since it remains unclear to which extent the use of sonication impacts the bacteriological diagnosis, it remains to be discussed if these additional investments are justified. One can however argument that processing multiple tissue is also time consuming. This issue will be further addressed when discussing the consensus.

D. Swabs

Swabs should not be used in the diagnosis of prosthetic joint infections due to their low sensitivity. This applies to both swabs taken from sinus tracts as intraoperative swabs.

A decision should however be made about what to do if swabs do arrive in the laboratory. A good collaboration between laboratory and orthopedic surgery is therefore necessary and can help to avoid these situations. The use of a prepacked intraoperative sampling kit should also help to stimulate orthopedic surgeons to obtain the correct number of tissue biopsies instead of swabs.

### *3.2 Suggestions for culture conditions*

#### *3.2.1 Culture media*

There is no standard protocol available for optimal combination of different media and incubation periods. Many studies do advocate the use of blood culture bottles and prolonged incubation. It is not clear which type of blood culture bottle should be preferred and which combination is the best. Regarding conventional media, studies that compare different media are lacking. The majority of studies however used blood containing non-selective agar media and enrichment broths (thioglycolate being the most used broth medium).

As mentioned, the optimal combination of all these media is not known. In case a pediatric blood culture bottle or only an aerobic blood culture bottle is used, enrichment broths for anaerobic recovery are necessary. If both an aerobic as an anaerobic blood culture bottle is used, additional media can also still yield additional pathogen recovery, as is demonstrated by the study of Peel et al. and Van den Bijlaardt et al. (34, 49). Since isolation of additional causative organisms has important consequences in the treatment strategy of prosthetic joint infections, it seems acceptable that a combination of blood culture bottles and conventional media are used to create the most optimal culture conditions.

Considering all the pathogens that could be involved and based on all the available information, it seems reasonable that the following combination of media should be used:

- Nonselective blood containing media (eg. chocolate agar, blood agar, etc.), both aerobic and anaerobic
- Enrichment broths that favor growth of fastidious and anaerobic bacteria
- Blood culture bottles

*3.2.2 Incubation period*

As discussed earlier, studies recommend prolonged incubation for optimal recovery of *Cutibacterium acnes*. There is however no single accepted incubation protocol available. Incubation periods between 7 (aerobic) and 14 days (anaerobic) seem to be reasonable.

Two studies report workable suggestions:
- Peel et al. suggest, when using both aerobic and anaerobic culture bottles, an incubation period of 7 and 14 days respectively (34).
- Bemer et al. suggest an entire protocol for microbiology, namely the use of 3 different media: chocolate agar (incubated for 7 days in aerobic conditions), a pediatric blood culture bottle (incubated for 5 days) and a Schaedler broth incubated for 14 days.

### 3.3 *Introduction of histological analysis*

To date, many laboratories do not currently use histological analysis in their routine diagnostic algorithm for prosthetic joint infections. This is however recommended as a useful tool that is included in all internationally defined diagnostic criteria. It is thus highly recommended to add histological analysis in the diagnostic process for prosthetic joint infections.

The use of frozen-section analysis could give the advantage to detect the presence of acute inflammation during revision surgery. This is however something that needs to be discussed with the pathologist.

### *3.4 Inconclusive results*

In culture-negative cases, which remain suspicious for infection, additional molecular testing could be an option. This is also suggested in the new definition, published by Parivizi et al. in 2018. Based on a scoring system, they classify patients into different categories: Infected, possibly infected, inconclusive and not infected. In inconclusive cases, additional molecular testing is advised (13).

### *3.5 Conclusion*

Following this CAT, a BILULU consensus will be made which will represent a procedure for diagnosing prosthetic joint infections and will contain exact specifications about both the pre-analytical and analytical process. The aforementioned suggestions will be discussed between the different BILULU laboratories and a definite decision will be made regarding all these different aspects. As there are no formal evaluations about the best protocol for microbiological diagnosis and the many methodological differences between studies make it difficult to draw a hard conclusion, this consensus will try to create a workable instrument that includes the available evidence as well as possible. The main goal is to create more standardization between laboratories and improve the diagnostic process for prosthetic joint infections. The resulting consensus will contain exact specifications about:

- Necessary samples and tests
- Priority of testing in synovial fluid in case of low sample volume
- Number of tissue biopsies for microbiology
- Number of tissue biopsies for histopathology
- Sonication yes or no?
- Culture conditions:
    - Which agar media
    - Which enrichment broths
    - Which blood culture bottles
- Incubation time

Based on this consensus, a new standard operating procedure will be introduced in the laboratory specifically for processing samples of suspected prosthetic joint infections. Therefore, samples (eg. periprosthetic biopsies) will be processed according to pathology and no longer according to a general specimen type.

Aside from a specific SOP, histological analysis will be implemented and pre-packed sample kits will be offered to orthopedic surgeons to guide them to request the correct tests and obtain the right samples.

Finally, a good collaboration and communication between orthopedic surgeons and the laboratory is and will remain important for an optimal use of all the available tools.

## TO DO

- BILULU consensus meeting
- Draw up the BILULU consensus
- Implement the BILULU consensus in HHZHLIER

## ATTACHMENTS

Supplementary file 1: Overview of different studies regarding sonication (Dudereva et al.) (42)

**Table S - 1 Summary of published studies comparing sonication and tissue culture for diagnosis of prosthetic joint infection (PJI) and other (Trampuz et al., 2006) orthopaedic device-related infections (DRI).**

| Author & Year (reference) | Case mix[k] | Definition of infection[j] | No. infected cases | No. aseptic cases | % early infection cases[a] | Antibiotics in 14 days prior to surgery, % | Methodology | | | | Sensitivity, % | | Specificity, % | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Number of tissue specimens[b] | Automated liquid culture[p] | Sonication fluid concentrated | Sonication threshold (cfu/ml[c]) | Sonication culture | Tissue culture | Sonication culture | Tissue culture |
| Trampuz 2006[1] | PJI | Clinical[2] | 24 | 54 | | 54 | ≥2 | Synovial only | No | 2 | 75 | 54 | 87 | 98 |
| Dora 2007[3] | PJI | Other clinical | 14 | 55 | | | mean 4 | | No | 2 | 71 | 79 | 96 | 98 |
| Trampuz 2007[2] | PJI | Clinical[2] | 79 | 252 | | 56 | ≥2 | Tissue | No | 10 | 79 | 61 | 89 | 99 |
| Esteban 2008[4] | PJI, DRI | Modified clinical | 17[d] | 14[d] | | | 3 to 5 | | Yes | 10[m] | 94 | 88 | 43 | 100 |
| Piper 2009[5] | PJI | Clinical[2] | 33 | 101 | [g] | 24 | ≥2 | | No/Yes | 10 | 67 | 55 | 98 | 95 |
| Sampedro 2010[6] | DRI | IDSA[7] | 36 | 76 | 82 | >39 | 2 to 9 | | No/Yes | 10 to 1000[i] | 91 | 73 | 97 | 93 |
| Verdigis 2011[8] | PJI | IDSA[7] | 9 | 27 | [g] | 33 | ≥2 | | Yes | 0.1 to 2[i] | 89 | 55 | 100 | 93 |
| Bjerkan 2012[9] | PJI | IDSA[7] | 18 | 36 | | | 5 | | Yes | 1.6 | 82 | 100 | 100 | 100 |
| Gomez 2012[10] | PJI | Clinical[2] | 135 | 231 | 76[h] | 51[e] | ≥2 | Synovial only | Yes | 2 | 73 | 70 | 98 | 99 |
| Borens 2013[11] | PJI, DRI | Modified MSIS | 12 | 27 | 66 | | Not stated | | No | 10[m] | 83 | 100 | | |
| Evangelopoulos 2013[12] | PJI | MSIS[13] | 24 | 10 | | 0 | ≥2 | | Not stated | Not stated | 71 | 47 | | |
| Janz 2013[14] | PJI | Modified IDSA | 37 | 65 | | 0 | 2 to 6 | | No | Not stated | 89 | 52 | 72 | 100 |
| Esteban 2013[15] | PJI, DRI | Modified IDSA | 70[d] | 33[d] | | 46[f] | 3 to 5 | | Yes | 100[n] | 68 | 60 | 94 | 98 |
| Puig-Verdie 2013[16] | PJI | IDSA[7] | 109 | 208 | 85 | 6 | ≥5 | | No | 50 | 90 | 67 | 99 | 100 |
| Cazanave 2013[17] | PJI | Clinical[2] | 144 | 290 | | 43 | ≥2 | Synovial only | Yes | 2 | 73 | 70 | 98 | 98 |
| Portillo 2014[18] | PJI | Modified MSIS | 69 | 162 | 65 | 48 | 5 | | No | 2 to 50[im] | 81 | 61 | 99 | 100 |
| Yano 2014[19] | DRI | Clinical[2] | 125 | 55 | | 31 | ≥2, mean 2.9 | | Yes | 0.1 to 5[i] | 90 | 57 | 91 | 96 |
| Janz 2015[20] | PJI | Modified IDSA | 31 | 88 | | | 2 to 6 | | No | Not stated[i] | 74 | 65 | 85 | 100 |
| Portillo 2015[21] | PJI, DRI | Modified MSIS | 69 | 162 | | 56 | Not stated | Sonication only | No | 50 | 87 | 59 | 100 | 100 |
| Puchner 2016[22] | DRI | MSIS[13] | 23 | 8 | | 61 | Not stated | | No | 2[m] | 91 | 52 | 100 | 100 |
| Hischebeth 2016[23] | PJI | Histopathology | 47 | 33 | | | Not stated | Sonication only | Yes | Not stated | 72 | 61 | 76 | 77 |
| Rak 2016[24] | PJI | MSIS[13] | 58 | 29 | [g] | 28 | 5 to 10 | | No | Not stated | 93 | 76 | 77 | 69 |
| Grosso 2017[25] | PJI | Modified clinical | 25 | 28 | | 0 | 2 to 8 | | No | 2 to 20 | 56 | 96 | 93 | 75 |
| Tani 2017[26] | PJI | IDSA[7] | 61 | 53 | [g] | 0 | ≥5 | | No | 50 | 77 | 56 | 98 | 94 |
| Van Diek 2017[27] | PJI | MSIS[13] | 75 | 177 | 81 | 0 | ≥6 | | No | 50 | 47 | 68 | 99 | 80 |
| Renz 2017[28] | PJI | EBJIS[32] | 78 | 33 | | 0 | Not stated | | No | 10 to 50[i] | 58 | 51 | 100 | 100 |
| Fernandez-Sampedro 2017[29] | PJI | Modified IDSA | 130 | 368 | 86 | 19 | 3 to 6 | | Not stated | 10[m] | 85 | 62 | 100 | 100 |
| Rothenberg 2017[30] | PJI | MSIS[13] | 178 | 325 | | | ≥2 | | Yes | 10 to 250 | 93 | 70 | 93 | 98 |

Table footnotes:

a Duration of symptoms < 3 months

b Synovial fluid included as a tissue specimen by most studies; median number of specimens not reported

c Colony-forming units per millilitre of unconcentrated sonication fluid

d More than one prosthetic component per patient submitted; number of components reported

e Proportion receiving antibiotics in preceding 4 weeks

f Proportion receiving antibiotics in preceding 7 days

g Mean duration of infection >12 months

h Proportion of infections of more than 12 months' duration

i Variable diagnostic threshold according to clinical or microbiologic criteria

j For non-clinical definitions of infection, positive culture led to inclusion of patients with no additional diagnostic criteria for infection

k PJI: Prosthetic Joint Infection; DRI: Device-related orthopaedic infection (other than prosthetic joints)

m Theoretical limit of detection

n Colony-forming units per millilitre of concentrated sonication fluid; concentration ratio not described

p Automated liquid mycobacterial culture not included in definition

## Supplementary file 2: Overview of used media in different studies

| Study | Sample type | Aerobic | Incubation time | Anaerobic | Incubation time | Enrichment broth | Incubation time | Blood cultures | Incubation time |
|---|---|---|---|---|---|---|---|---|---|
| (3) Fink et al. | Synovial fluid | | | | | | | Bactec Peds Plus/F + FOS | 14 days |
| | Tissue samples | Not specified | | Not specified | | Not specified | | Not specified | |
| (14) DeHaan et al. | Synovial fluid | | | ? | ? | Thioglycolate | 10 days | | |
| | Tissue samples | | | | | | | Blood culture bottles | 5 days |
| (17) Atkins et al. 1998 | Tissue samples<br>Sterile diluent<br>Sterile glass beads | Chocolate agar<br>Blood agar | 7 days | Blood agar | 7 days | Robertson's cooked meat broth | 5 days | | |
| (18) Font vicarza et al. 2010 | Synovial fluid | | | | | | | Bactec aerobic 1-3 ml<br>Bactec anaerobic 1-3 ml | 5 days |
| | Tissue samples<br>Swabs | Blood agar | 5 days | Schaedler agar | 5 days | Thio | 5 days | | |
| (19) Trampuz et al. 2007 | Tissue samples<br>BHI 3ml | Blood agar 0,5 ml<br>Chocolate agar 0,5 ml | 5 days | Blood agar 0,5 ml | 7 days | Thio 0,5 ml | ? | | |
| | Synovial fluid | Blood Agar 0,1 ml<br>Chocolate agar 0,1 ml | 5 days | Blood agar 0,1 ml | 7 days | Thio 0,1 ml | ? | Bactec Peds Plus/F > 0,5 ml | 5 days |
| | Sonication fluid | Blood agar 0,5 ml | 5 days | Blood agar 0,5 ml | 7 days | | | | |
| (34) Peel et al. | Tissue samples<br>BHI 5 ml<br>Seward Stomacher | Chocolate agar 0,1 ml<br>Blood agar 0,1 ml | 5 days | CDC anaerobic bloodagar 0,1 m | 14 days | Thioglycolate 1ml | 14 days | Bactec Plus Aerobic/F 1ml<br>Bactec Lytic/10 Anaerobic/F 1ml | 14 days |
| | Synovial fluid | | | CDC anaerobic bloodagar | 14 days | Thioglycolate | 14 days | Bactec Plus Aerobic/F | 5 days |
| | Sonication fluid | Blood agar | 5 days | Blood agar | 7 days | | | | |
| (38) Bemer et al.2016 | Biopsies (bone or tissue)<br>Sterile water<br>Stainless beads<br>Synovial fluid | Chocolate agar 50 µL<br>Blood agar 50 µL | 7 days | Blood agar 50 µL | 7 days | Schaedler broth 1 ml | 14 days | Pediatric blood culture bottle 1 ml | 14 days |
| (39) Portillo et al. 2015 | Tissue samples<br>Thio<br>Mortar and pestle | Chocolate agar 0,5 ml | 7 days | Schaedler agar 0,5 ml | 14 days | Thio 0,5 ml | 14 days? | | |
| | Synovial fluid | Chocolate agar 0,1 ml | 7 days | Schaedler agar 0,1 ml | 14 days | Thio 0,5 ml | ? | BacT/Alert anaerobic | 5 days |
| | Sonication fluid | Chocolate agar 0,5 ml | 7 days | Schaedler agar 0,5 ml | 14 days | Thio 0,5 ml | 14 days? | BacT/Alert Aerobic 10 ml<br>BacT/Alert Anaerobic 10 ml | |
| (40) Hischebeth et al. 2016 | Tissue samples | Blood agar 0,5 ml<br>Chocolate agar 0,5 ml<br>MacConkey agar 0,5 ml<br>Sabouraud 0,5 ml | 14 days | ? | ? | Thio 1 ml | 14 days | | |
| | Sonication fluid | Blood agar 0,5 ml<br>Chocolate agar 0,5 ml<br>MacConkey agar 0,5 ml<br>Sabouraud 0,5 ml | 14 days | Schaedler agar 0,5 ml<br>KV agar 0,5 ml | 14 days | Thio 1 ml | 14 days | Bactec Peds Plus/F | 14 days |
| (42) Dudereva et al. 2018 | Tissue samples<br>Sterile saline<br>Sterile Glass beads | | | | | | | Bactec Plus Aerobic/F<br>Bactec Lytic/10 Anaerobic/F | 10 days |
| | Sonication fluid | Blood Agar 0,1 ml<br>Chocolate agar 0,1 ml | 5 days | Blood agar | 10 days | | | | |

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|---|---|---|---|---|---|---|---|---|---|
| **(43) Yan t al. 2018** | Tissue samples<br>BHI 5ml<br>Seward Stomacher | | | | | | | Bactec Plus Aerobic/F 1 ml<br>Bactec Lytic/10 Anaerobic/F 1 ml | 14 days |
| | Sonication fluid | Blood Agar 0,1 ml<br>Chocolate agar 0,1 ml | 5 days | Blood agar 0,1 ml | 14 days | | | | |
| **(46) Larsen et al. 2018** | Synovial fluid<br>Tissue samples<br>Bone biopsies<br>Swabs<br>Sonication fluid | Chocolate agar<br>Blood agar<br>Chocolate agar +<br>fosfomycine<br>ChromID CPS | 14 days | Chocolate agar + Vit k<br>(+ metronidazole + kanamycin)<br>Blood agar<br>(+ metronidazole +<br>kanamycine) | 14 days | Thio + glycerol<br>Serum Broth | 14 days | | |
| **(47) Huges et al. 2001** | Synovial fluid | Chocolate agar<br>Blood agar | 2 days | | | Thioglycolate 0,25 ml | 5 days | Bactec Peds Plus/F 0,5-3ml | 5 days |
| **(48) Minassian et al.** | Tissue samples<br>Sterile saline 3 ml<br>Sterile glass beads | | | | | | | Bactec PLUS Aerobic/F 0,5 ml<br>Bactec Lytic/10 Anaerobic/F 0,5 ml<br><br>IF NEGATIVE AFTER 14 DAYS:<br>1000 bottles were selected and subcultured<br>*CHOCO (CO2)<br>*Fastidious anaerobic agar | 14 dagen<br><br>5 days |
| **(49) Van Den Bijlaardt et al. 2019** | Tissue samples<br>BHI 5ml<br>Seward Stomacher | Blood agar 0,1 ml<br>Choco 0,1 ml<br>MacConkey 0,1 ml | 4 days | Wilkins-Chalgren 0,1 ml | 4 days | Thio 0,2 ml<br>BHI 0,2 ml | 14 days | Bactec Plus Aerobic/F + FOS<br>Bactec Plus Anaerobic/F + FOS | 7 days |
| | Synovial fluid | Blood agar 0,1 ml<br>Choco 0,1 ml<br>MacConkey 0,1 ml | 4 days | Wilkins-Chalgren 0,1 ml | 4 days | | | | |
| **(50) Schafer et al. 2008** | Tissue samples<br>Minced | Chocolate Agar<br>Blood agar<br>MacConkey Agar | 14 days | Schaedler agar + vit K | 14 days | BHI<br>Schaedler Broth | 14 days | | |
| **(51) Butler-Wu et al. 2011** | Tissue samples<br>Sterile saline 3 ml<br>Seward Stomacher<br>Synovial fluid | Chocolate agar<br>Blood agar | 28 days | Brucella agar | 28 days | BHI | 28 days | | |
| **(55) Renz et al. (46)** | Synovial fluid | Not specified 0,1 ml | 7 days | Not specified 0,1 ml | 14 days | Thioglycolate | ? | Bactec Peds Plus/F 1ml | ? |
| **(57) Hughes et al.** | Tissue samples<br>Sterile saline 5ml<br>Sterile glass beads | Chocolate agar 0,25 ml<br>Blood agar 0,25 ml | 5 days | Blood agar 0,25 ml<br>Blood agar<br>+ metronidazole disk 0,25 ml | 5 days | Robertson's cooked meat broth 0,1 ml<br>Fastidious anaerobic broth 0,1 ml | 5 days | Bactec standard Anaerobic/F 0,1 ml<br>Bactec Plus aerobic/F 0,1 ml | 5 days |
| **(59) Roux et al. 2011** | Tissue samples<br>Sterile water 20ml<br>Glass beads | Chocolate agar<br>Sheep blood agar | 5 days<br>24-48h | Blood agar | 5 days | BHI<br>Rosenow broth | 48h<br>14 days | | |

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