

fhg – Center for Health Professions Tyrol GmbH FH
Bachelor's Degree Program in Biomedical Analysis

Comparison of microbiological diagnostics of tissue samples using solid cultures and blood cultures in periprosthetic and implant infections

Bachelor thesis

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Abstract

This study compares the effectiveness of microbiological diagnostics of tissue samples using agar plates and blood culture methods in the context of periprosthetic and implant infections. A total of 64 tissue samples from 24 patients were processed. Of the 24 patients, 13 samples without infection and 11 samples with infection were identified using both the solid culture method and the liquid culture method. However, four additional contaminated samples were identified in the solid culture method. The results show that the incubation period for blood cultures can be reduced from 14 to seven days based on the average time to positivity (TTP). The most frequently identified pathogens were *S. aureus*, followed by *S. epidermidis* and *E. faecium*. In addition, 74% of all blood culture bottles were positive after 24 hours of incubation. The study emphasizes the advantages of the blood culture method, including high sensitivity and specificity as well as its potential for routine use with a shorter incubation period. Further research is needed to optimize diagnostic techniques and reduce contamination rates in general.

Keywords (PubMed): periprosthetic infections, microbiological diagnostics, blood culture, agar plates, time to positivity, *C. acnes*, *S. aureus*

Abstract

This study compares the effectiveness of microbiological diagnostics of tissue samples using agar plates and blood culture methods in the context of periprosthetic and implant infections. A total of 64 tissue samples from 24 patients were processed. Of the 24 patients, 13 samples were identified as non-infected and 11 samples as infected using both the solid culture method and the liquid culture method. However, the solid culture method also yielded four contaminated samples. The results show that the incubation time for blood cultures can be reduced from 14 to seven days based on the average *time to positivity* (TTP). The most frequently identified pathogens were *S. aureus*, followed by *S. epidermidis* and *E. faecium*. In addition, 74% of all blood culture bottles were positive after 24 hours of incubation. The study highlights the advantages of the blood culture method, including high sensitivity and specificity, as well as its potential for routine use with a shortened incubation period. Further research is needed to optimize diagnostic techniques and reduce contamination rates in general.

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1. Introduction

Arthroplasty-associated infection or periprosthetic joint infections (PJI) are a rare condition involving interactions between various microorganisms, the implant, and the immune system. In addition to classic pathogenic bacteria such as Enterobacterales or *S. aureus*, microorganisms that can cause such infections also include apathogenic bacteria from the skin flora, such as coagulase-negative staphylococci, corynebacteria, or *Cutibacterium acnes*, and, in rare cases, fungi. Only a small number of microorganisms are necessary for the manifestation of PJI. The bacteria and fungi, see *chapter 1.3* for examples, adhere to the surface of the prosthesis and form biofilms there, which protect them from antibiotics and antifungal agents as well as the immune system. (Patel, 2023, p. 251)

The idea for this work arose from the problem that, in cases of suspected implant infections, the wide range of possible pathogens in microbiological diagnostics means that the solid cultures inoculated with tissue samples must be incubated for at least seven days as part of routine procedures and inspected manually by laboratory staff on a daily basis. This carries the risk of laboratory contamination by skin germs such as coagulase-negative staphylococci or cutibacteria, which can lead to false positive results or, in case of doubt, unnecessary treatment. By inoculating the blood culture bottles with the patients' tissue samples, incubation takes place in a closed system and further processing only occurs if the blood culture incubator detects growth in the bottle. The aim of this work is therefore to prove that blood culture bottles inoculated with tissue samples detect the same pathogens as the conventional solid culture method, as well as to show

that the risk of contamination by laboratory personnel is minimized or completely prevented.

The following chapters will explain in more detail the most common joint prostheses, the symptoms of PJI, the causative microorganisms, the diagnosis, and the treatment of PJI.

1.1 Types of prostheses

The most common joint prostheses include knee and hip joint prostheses, shoulder and elbow joint prostheses, and ankle, hand, metacarpal, and interphalangeal joint prostheses. (Patel, 2023, p. 252)

In the US, knee joint prostheses are the most commonly used, followed by hip joint prostheses. The other joints listed above are replaced less frequently. (Patel, 2023, pp. 251-253)

1.2 Symptoms

As with any infection, a distinction can be made between acute and chronic infections. Chronic infections are those that persist over a longer period of time and typically have slower and less intense symptoms. In contrast, acute infections are characterized by a sudden onset and rapid deterioration of clinical signs. Both types of infection require different diagnostic and therapeutic approaches. Acute PJIs are often characterized by intense signs of inflammation such as redness, swelling, joint warmth, fistula formation, and pain, while chronic PJIs may cause more subtle clinical symptoms such as persistent pain or loss of function. In addition, the treatment of chronic PJIs is more complex and protracted, often due to the formation of biofilms that can impair the effectiveness of antibiotics. (Patel, 2023, p. 251) (McNally, et al., 2021, p. 21)

1.3 Common microorganisms causing implant infections

Table 1. Microorganisms Identified in Hip and Knee Periprosthetic Joint Infection (PJI).*

Microorganism	Frequency (%)
Aerobic gram-positive bacteria	82
Coagulase-negative staphylococcus species (other than <i>S. lugdunensis</i>)	37
<i>S. aureus</i>	24
<i>S. lugdunensis</i>	4
Streptococcus species	14
Enterococcus species	8
Corynebacterium species	5
Aerobic gram-negative bacteria	11
Enterobacterales	7
Pseudomonas species	3
Anaerobic bacteria	13
Cutibacterium species	8
Other species	5
Fungi	3
Mycobacteria	0.5

* Data are from a single tertiary referral center; 70% of the PJIs were monomicrobial, and 25% were polymicrobial.[†] Pathogen distributions may vary from institution to institution.

(Patel, 2023, p. 253)

Figure 1: Microorganisms frequently detected in implant infections. These pathogens were most commonly detected in knee and hip joint prostheses.
(Patel, 2023, p. 253)

Figure 1 shows a table listing the most common bacteria found in hip and knee joint infections. It shows that aerobic Gram-positive bacteria are the most common (82%), followed by coagulase-negative staphylococci (37%) and *S. aureus* (24%). Other common pathogens are streptococci (14%), enterococci (8%), and *Corynebacterium* species (5%). Aerobic Gram-negative bacteria cause 11% of cases. The most common are enterobacteria and *Pseudomonas* species. Anaerobic bacteria are involved in 13% of cases. *Cutibacterium* occurs in 8% of infections. Other pathogens are less common. The distribution of pathogens may vary depending on the facility. (Patel, 2023, p. 253)

1.4 Risk factors

Table 2. Risk Factors for PJI.
Potentially modifiable presurgical risk factors
Anemia
Injection-drug use
Malnutrition
Obesity
Receipt of intraarticular injection in prior 3 mo
Tobacco use
Nonmodifiable presurgical risk factors
Cardiovascular disease (arrhythmia, coronary artery disease, pulmonary hypertension, congestive heart failure, or peripheral vascular disease)
Diabetes (especially with poor glycemic control)*
Immunocompromised status (owing to cancer or receipt of a transplant)
Inflammatory arthritis
Kidney or liver disease (hepatitis or cirrhosis)
Male sex
Medicaid as primary payer
Mental health disorder (depression or alcohol use)
Relative with PJI
Patellar resurfacing and post-traumatic arthritis (knees)
Prior native joint infection
Prior PJI of same or different joint
Prior revision arthroplasty
Younger age
Operative risk factors
Allogeneic blood transfusion
Prolonged operative time
Simultaneous bilateral arthroplasty
Postoperative risk factors
Discharge to rehabilitation or convalescent care
Prolonged hospitalization
<i>S. aureus</i> bacteremia
Wound-healing complications (including superficial skin infection)

* Improved glycemic control can be achieved presurgically.

(Patel, 2023, p. 254)

Figure 2: Risk factors for PJI. These factors, some of which are preventable, increase the risk of PJI. (Patel, 2023, p. 254)

Figure 2 shows a table of risk factors for PJI. These risk factors are in different categories : potentially

Modifiable preoperative risk factors, non-modifiable preoperative risk factors, operative risk factors, and postoperative risk factors. Potentially modifiable preoperative risk factors include anemia, intravenous drug use, malnutrition, obesity, intra-articular injections in the last three months, and tobacco use. Non-modifiable preoperative risk factors include cardiovascular disease, diabetes, immunodeficiency, inflammatory arthritis, kidney or liver disease, male gender, mental illness, previous joint infections and previous revision arthroplasties. Surgical risk factors include allogeneic blood transfusions, prolonged operating times, and simultaneous bilateral arthroplasties. Postoperative risk factors include discharge in a rehabilitation or nursing facility, prolonged hospital stays, *S. aureus* bacteremia and wound healing complications. (Patel, 2023, pp. 253-254)

1.5 Prevention

There are not only risk factors that can be reduced, but also other ways to prevent PJI. In general, it is recommended that treatment be carried out in specialized centers. Preoperative screening of *S. aureus* carriers with appropriate decolonization or universal decolonization is beneficial. Patients with knee or hip joint prostheses also have an increased risk of other general infections. The type of decolonization is irrelevant in this case. There are several different antibiotics that serve as prophylaxis. Cefazolin, for example, should be administered within 60 minutes before surgery. Compared to vancomycin and clindamycin, cefazolin is an antibiotic with a rather lower effect. In patients with MRSA, cefazolin is replaced by the active ingredient vancomycin. The duration of antibiotic prophylaxis varies. Nevertheless, it is recommended that antibiotic prophylaxis be discontinued after the closure of the incision.

The primary source and associated risk of contamination is the operating room. To minimize the risk of such contamination, it is necessary to keep traffic in the operating room to a minimum. This means that only essential personnel should be present and unnecessary movement or activity should be avoided. Reducing the number of people present in the operating room and the movements taking place there leads to a significant reduction in the risk of contamination. This results in improved outcomes and a reduction in the infection rate. Aggressive anticoagulation should also be avoided. Tranexamic acid, a drug used to inhibit the fibrinolysis system, reduces the rate of PJI in the hip and knee. (Patel, 2023, pp. 254-255)

1.6 Diagnostics

The diagnosis of joint prosthesis infection is very important, as the treatment of PJI differs from that of a non-infected implant, such as a loosened implant. Microbiological diagnosis plays a major role in this. The microbiological findings are a building block and provide surgeons with the results they need to decide on the further course of action and, if necessary, select the appropriate antimicrobial agents. (McNally, et al., 2021, pp. 19-21)

1.6.1 Blood biomarkers

The most important and widely used blood markers are the erythrocyte sedimentation rate (ESR), the white blood cell count, the percentage of polymorphonuclear neutrophils (PMN), and C-reactive protein (CRP). The percentage of PMN refers to the proportion of these specific white blood cells in relation to the total number of white blood cells in a blood test. All of these tests indicate general inflammation and are not specific to bacterial PJI. CRP can be helpful in determining the severity of an infection. If the CRP

has a value above 10 mg/ml, PJI cannot be ruled out. Nevertheless, the CRP value is not sufficient for a definitive diagnosis. It is important to note that a normal CRP does not rule out infection. (McNally, et al., 2021, p. 21)

1.6.2 Cytology of synovial fluid

Synovial fluid aspiration is a viscous fluid found in the cavities of movable joints. This aspiration is an important part of the diagnosis of PJI. It is important for assessing the extent of inflammation. When performing aspiration analyses, both sensitivity and specificity are close to 90%. If the leukocyte count and PMN percentage from the synovial fluid are below the detection limit, infection is unlikely. On the other hand, values exceeding this limit indicate infection, especially in PJI of the knee. The detection limits for leukocytes are between 1,500 and 4,000 cells/ μ l. Those for the PMN percentage are between 65% and 80%. A cut-off value of 3,000 cells/ μ l and a PMN above 80% indicate a periprosthetic infection of the hip and knee. The limits mentioned may be influenced by other diseases and are only relevant for knee and hip prostheses. (McNally, et al., 2021, p. 21) (Patel, 2023, pp. 256-257)

1.6.3 Synovial fluid biomarkers

Alpha defensin tests are very accurate and can be beneficial, for example, after prior antibiotic administration or in the case of microorganisms with low virulence. The alpha defensin result is not altered or influenced by other pathogens. Due to its high specificity, infections can be suspected using this biomarker. Nevertheless, it is not possible to rule out non-infected prostheses. (McNally, et al., 2021, pp. 21-22)

1.6.4 Microbiology

Examination of joint fluid prior to surgery is not suitable for ruling out joint infection. However, a positive result indicates that the joint is infected. During intraoperative surgery, at least five reliable tissue samples should be taken and sent to the laboratory immediately. It is particularly important to take tissue samples from the membrane at the interface between the bone and the implant. The patient should not take antibiotics for at least two weeks prior to surgery. Antibiotic prophylaxis prior to surgery is not necessary. Blood cultures should be incubated for at least 14 days. If the same bacteria with the same antibiotic resistance pattern are found in all positive samples or in at least two different samples, this is an indication of infection. A single positive culture result must be considered in conjunction with other findings. If rare bacteria or viruses such as *S. aureus* or Gram-negative rods are detected, this is an indication of infection. A single positive culture result does not necessarily mean that patients have been infected with a pathogen. Further investigations must be carried out. It is best to examine the implant as well, using sonication (see *section 2.6*). This can reveal whether a biofilm has formed there. Any positive culture from the sonication fluid must be considered a potential infection. More than 50 colony-forming units per milliliter (CFU/mL) confirm an infection. If a concentration technique is used, the threshold for confirming an infection is 200 CFU/mL. Histologically, inflammatory cells can be seen in the tissue if PJI is present. This can be determined from biopsies or tissue samples. At least three deep samples must be taken for this purpose. Certain stains in the tissue can also be used to detect microorganisms that are otherwise invisible. This is particularly important for bacteria that cannot otherwise be detected. (McNally, et al., 2021, p. 22) (Patel, 2023, pp. 255-257)

1.6.5 Errors in diagnosis

It is very important to treat an infection quickly. No time should be wasted by waiting and hoping that the clinical symptoms will improve or even disappear on their own. The DAIR method (see *section 1.7*) should be performed surgically as soon as possible, as the effectiveness of this form of therapy decreases with increasing waiting time. Microorganisms attach themselves to the implants within seconds and begin to surround themselves with a polysaccharide matrix. After about three weeks, this biofilm is difficult to break down using antimicrobial substances. (Li, Renz, Trampuz, & Ojeda-Thies, 2019, pp. 3-4)

Intraoperative swabs of periprosthetic tissue should be avoided. Previous reports have shown that cultures from swabs have a low sensitivity of 53-76%. (Li, Renz, Trampuz, & Ojeda-Thies, 2019, p. 4)

Since CRP and ESR can be analyzed quickly, some surgeons prefer these parameters when PJI is suspected. These two analytes are highly recommended in the 2010 guidelines of *the American Association of Orthopaedic Surgeons* (AAOS). Nevertheless, as already explained in *section 1.6.1*, these inflammatory markers have low sensitivity, as values within the normal reference range do not rule out infection. In particular, infections caused by low-virulence pathogens (mainly skin bacteria) do not necessarily go hand in hand with elevated CRP. (Li, Renz, Trampuz, & Ojeda-Thies, 2019, p. 4)

1.7 Therapy

The treatment of PJI is both complicated and expensive. Where possible, such treatment should be carried out in specialist centers. The objectives of PJI therapy include maintaining the function of the joint prostheses, alleviating pain, and, in the best case, restoring joint function.

Curing the infection. Antibiotic therapies are usually unsuccessful without surgical intervention. One well-known form of therapy is the DAIR method. DAIR stands for *debridement, antibiotics, and implant retention*. This refers to the removal of dead material and contaminated tissue from a wound, antibiotic therapy, and implant retention. Performing a DAIR in cases of acute PJI of hip and knee prostheses is important. In cases of chronic PJI, the affected implant is removed. This can be done in a single stage, i.e., removal of the infected prosthesis and reimplantation of a new prosthesis in the same session, or in a two-stage revision. In a two-stage revision, antibiotic-releasing joint spacers are used to maintain function during the prosthesis-free interval. If patients do not want surgical intervention, antimicrobial suppression can be performed. However, this method does not usually cure the infection. For this reason, patients often have to take the prescribed antibiotics for the rest of their lives. If all these different methods have no positive effect, resection arthroplasty with the creation of pseudoarthrosis can be performed for hip infections, or arthrodesis or amputation (as a last resort) for knee joint infections. The treatment is lengthy and usually lasts at least 12 weeks. (Patel, 2023, pp. 257-258)

1.8 Overview of future steps

Over the past two decades, significant progress has been made in the diagnosis of PJI, but most of the data available relates only to hip and knee prostheses. More data is needed for other joints. Further research is needed to determine whether genetic predisposition plays a role in joint infections. It is important to identify pathogens that form biofilms on prostheses. It is also often mentioned that surgery for joint prostheses or the treatment of a

PJI should be treated at specialized centers. It is also important to determine the best surgical approaches and to conduct a medical assessment. Both antibiotic and non-antibiotic therapies, as well as the duration and method of administration, still need to be investigated in more detail. Nevertheless, antibiotics are always administered for diagnosed infections. (Patel, 2023, p. 259)

1.9 Research question and hypotheses

The guiding principle of this work is the question of whether there is a difference in the diagnosis of periprosthetic infections using blood culture or conventional solid culture incubation and whether blood culture bottles can be used to better differentiate between contamination and infection.

My research questions are as follows: Which of the two methods, blood culture or solid culture incubation, is better suited for differentiating between contamination and actual infection in patients with suspected periprosthetic and implant infections? And can the incubation time of blood culture bottles be reduced from a maximum of 14 days to seven days?

The expected results include, on the one hand, the finding that both cultures, aerobic and anaerobic bottles, will have approximately the same TTP (see *section 2.3*). In most cases, both bottles will be positive. With the exception of anaerobes, as these can only be cultivated in anaerobic blood culture bottles. The same applies to aerobic pathogens, which can only be cultivated in aerobic bottles. It is also to be expected that the results of the blood culture method will be similar or identical to those of the conventional method.

2 Materials and methods

This chapter explains the equipment used and describes the technical background. It also outlines the process from sample collection to the results obtained using the methods employed. In addition, it lists the materials used that are relevant to this work. Finally, it explains the conventional method currently used by the Feldkirch microbiology laboratory.

2.1 Equipment

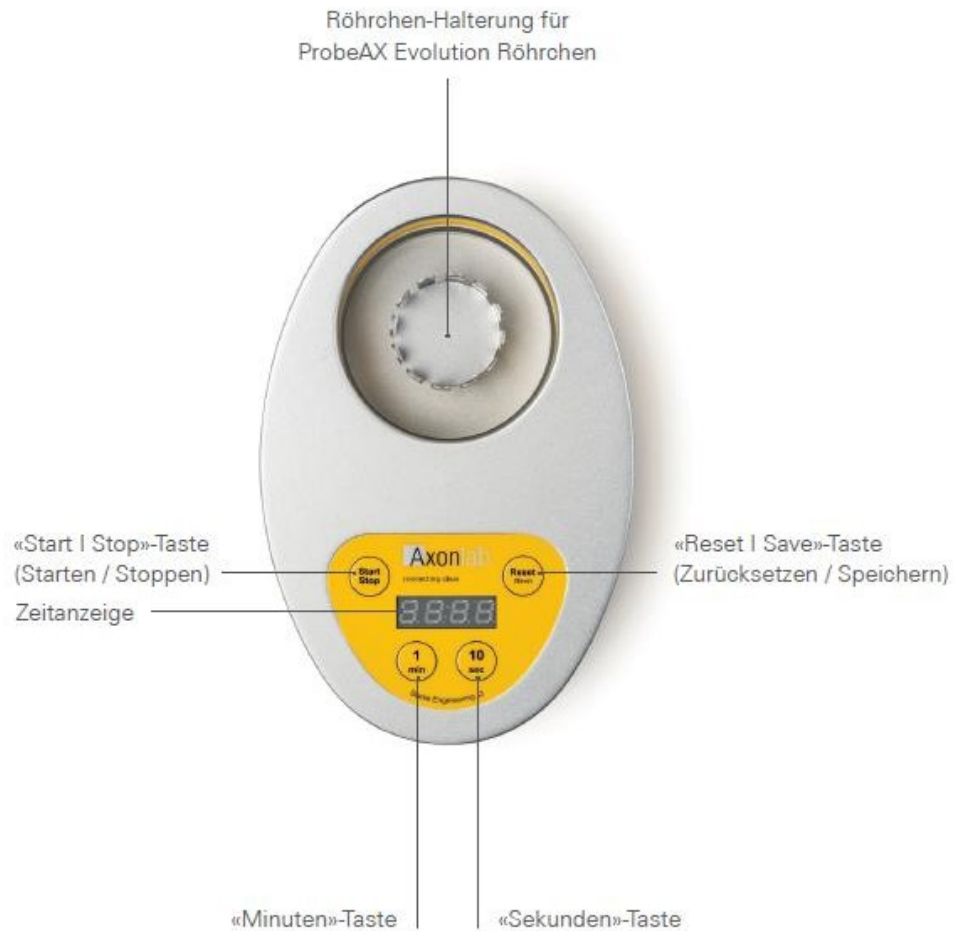
Table 1 provides information about the device names and respective manufacturers used in this study. A technical explanation of the devices mentioned is provided in the following chapters. It should be noted that the BMAs in the pathology laboratory do not require SpinAX or BACTEC-FX for the original method.

Table 1: List of devices used

Device	Manufacturer	Reference number
SpinAX	AxonLab, Baden-Dättwil, Switzerland	22553

Bactec-FX	Becton, Dickinson and Company, Sparks, USA	Top FT4952 Top + Bottom FT4939
Incubator INC-246	MEMMERT, Schwabach, Germany	O3070015
Incubation chamber XR110C	Smeva B.V., Valkenswaard, Netherlands	411812
MALDI Biotyper	Becton, Dickinson and Company, Sparks, USA; Bruker Daltonik GmbH, Bremen, Germany	860467404819
Previ Color V2 Gram	Biomérieux, Maryy-L'Etoile, France	414292212875
Bactosonic BS14	Bandelin Electronic GmbH & Co. KG, Berlin, Germany	277003.000062174.009

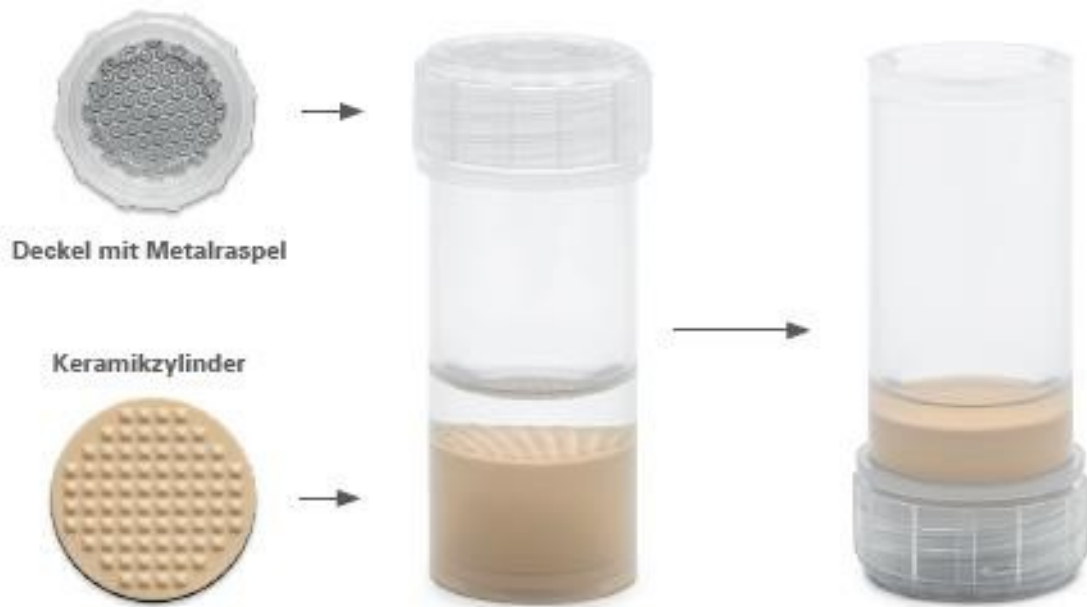
2.1.1 SpinAX



(AxonLab AG, n.d., p. 7)

Figure 3: SpinAX. The structure and function keys of the SpinAX are shown here. (AxonLab AG, n.d., p. 7)

The SpinAX (see *Figure 3*) consists of a tube holder for ProbeAX Evolution tubes (see *Figure 4*), a start/stop button, a reset/save button, a time display, and separate minute and second buttons. (AxonLab AG, n.d., p. 9)



(AxonLab AG, n.d., p. 9)

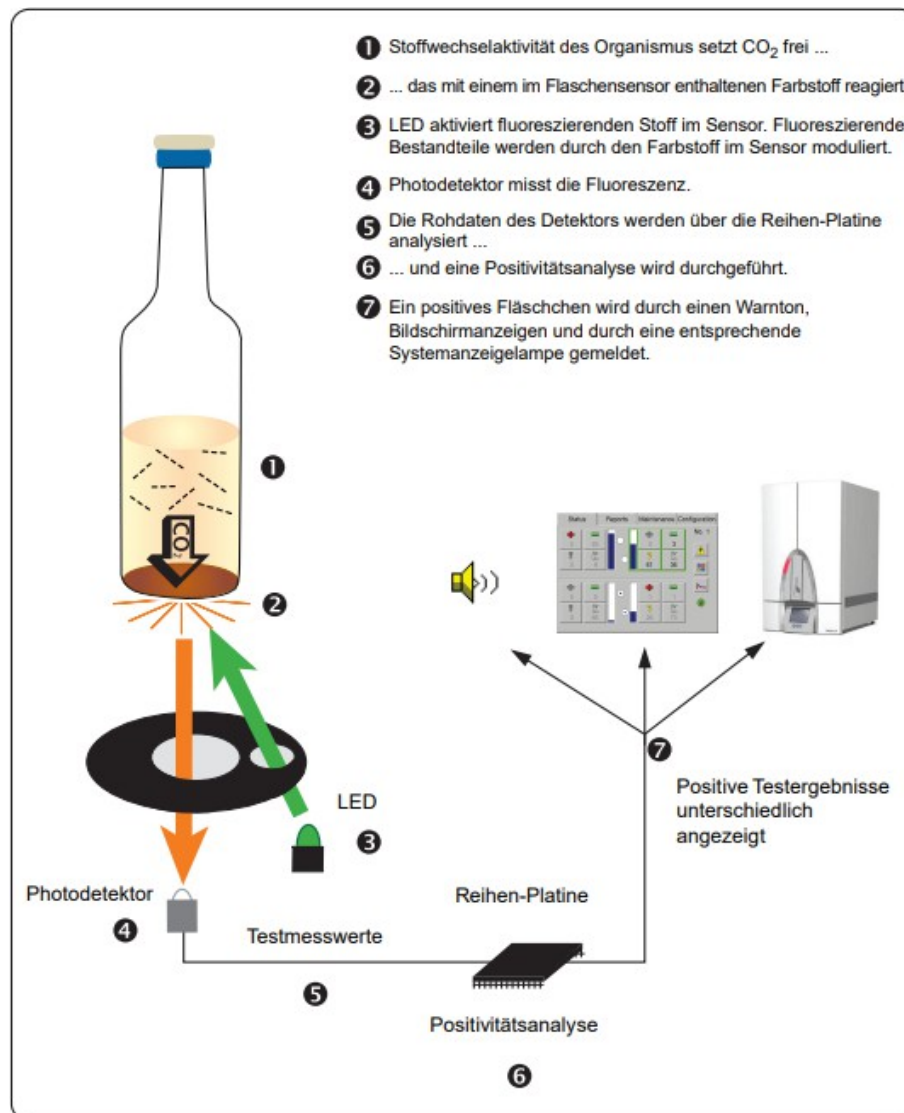
Figure 4: ProbeAX Evolution Tubes. The ProbeAX Evolution tube consists of a rasp and a cylinder. (AxonLab AG, n.d., p. 9)

The ProbeAX Evolution Tubes (see *Figure 4*) are equipped with a lid containing a metal rasp and an exposed ceramic cylinder. (AxonLab AG, n.d., p. 9)

These tubes are filled with 5 ml of 0.85% sodium chloride (NaCl). Various tissue types can be dispersed and homogenized using these tubes in the SpinAX. To do this, the sample is placed in the tube and, after carefully closing the tube with the cap, placed upside down in the SpinAX tube holder. Within one minute, the tissue pieces are dispersed and homogenized by a grinding motion between the ceramic block and the metal rasp. (AxonLab AG, n.d., p. 9)

2.1.2 Bactec-FX

The BACTEC-FX from BD is used for the rapid detection of bacteria and fungi from blood culture bottles and is suitable for the incubation and detection of microorganisms. (Becton, Dickinson and Company, 2021, p. 9)



(Becton, Dickinson and Company, 2021, p. 10)

Figure 5: BACTEC-FX operating principles. This figure shows how the BACTEC-FX checks cultures for microorganisms. (Becton, Dickinson and Company, 2021, pp. 9–10)

Figure 5 illustrates how the BACTEC-FX works. After the blood culture bottles have been inoculated with a sample, they are loaded horizontally into the devices. This causes the blood culture bottles to dock with the bottom of the device. If microorganisms are present in the bottles, the nutrients in the culture medium of the bottle are metabolized and carbon dioxide (CO_2) is released. When CO_2 is released, the dyes at the bottom of the bottles react with the CO_2 . The device contains light-emitting diodes (LEDs). These activate the light sensors located at the bottom of the bottles so that the photodetectors can measure the fluorescence. The light value corresponds to the amount of CO_2 released. These text analyses are performed every ten minutes. Positive blood culture bottles appear in red on the indicator light, a warning tone is emitted, and they also appear on the LCD display. (Becton, Dickinson and Company, 2021, pp. 9-10)

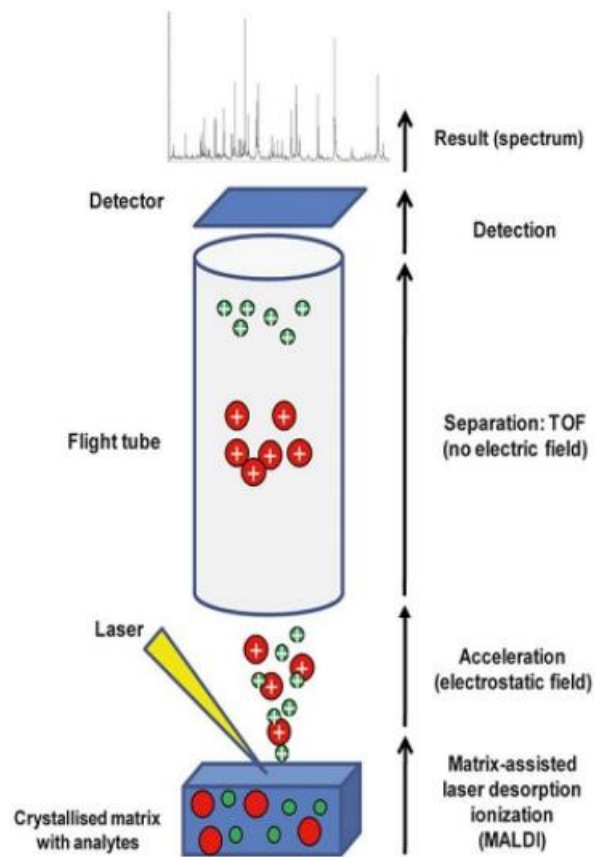
2.1.3 Incubator and incubation room

The incubator and incubation room are used daily at the Institute of Pathology in Feldkirch and are an important part of the laboratory's inventory. They are heated by all-round heating and CO_2 or nitrogen (N_2) is introduced via sterile filters. These are fed into the interior from above. Turbulence-free interior ventilation ensures even distribution of the gases. (Mettler GmbH + Co KG., 2014, p. 11)

2.1.4 MALDI-TOF mass spectrometry

The principle of *matrix-assisted laser desorption/ionization time-of-flight* (MALDI-TOF) is based on the mass-to-charge ratio (m/z) of the molecular ions of microorganisms. This ratio can be used to identify all ionized biological molecules. The mass spectrum is interpreted in the spectrometer and molecules are identified. (Calderaro & Chezzi, 2024, p. 3)

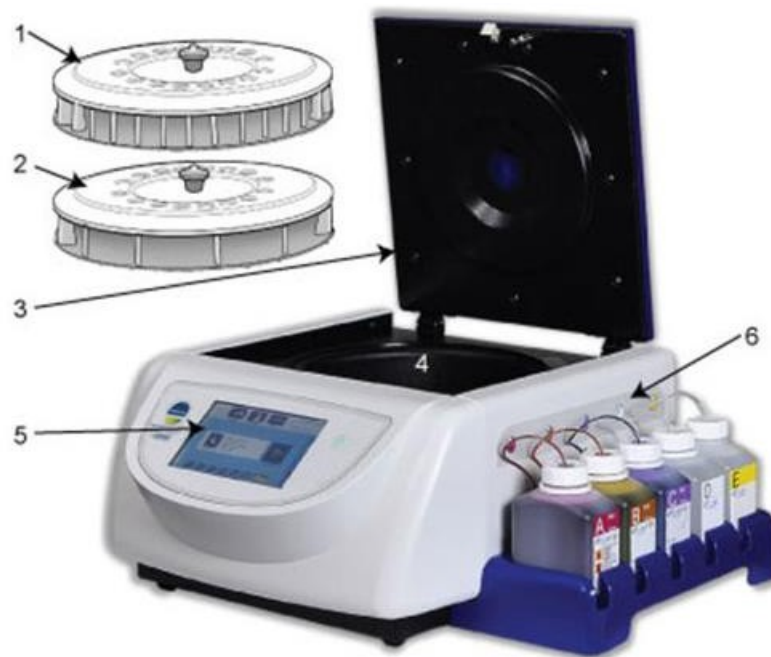
MALDI requires samples that have been incubated on agar plates, a lyophilized matrix, diluted formic acid, and a biotarget. All materials required for MALDI can be found in *Table 6*. The preparation steps for these materials can be found in *Chapter 2.5*. First, part of a colony is applied to a free position on the biotarget using the tip of a toothpick, dried at room temperature, and covered with 1 µl of 70% formic acid. After drying, 1 µl of matrix is added to each position. The samples crystallize together with the matrix. After drying, the biotarget is placed in the MALDI and a vacuum is created in the device, as shown in *Figure 6*. The molecules are then ionized using short laser pulses. The matrix protects the samples during this process. This process creates ions that fly through a flight tube. The ions are accelerated by electromagnetic fields. The *time of flight* is measured in this tube. This flight time then produces a spectrum that is compared with other spectra from various databases. The database used in the microbiology laboratory is MBT Compass HT IVD, version 5.2.300. The software with reference number 1877017 contains Library V12. The additional MBT HT Subtyping IVD module with reference number 1877010 is part of this database. The score determined from this, a number of points corresponding to the number of similar spectra found, enables the species to be identified. (Calderaro & Chezzi, 2024, pp. 3-4)



(Calderaro & Chezzi, 2024, p. 5)

Figure 6: Principle of MALDI-TOF. Laser pulses ionize the molecules. These ions are accelerated in the flight tube, producing spectra that are necessary for the identification of microorganisms. (Calderaro & Chezzi, 2024, pp. 3-4)

2.1.5 Previ Color Gram



1. Karussell für 30 Objektträger
2. Karussell für 12 Objektträger
3. Deckel mit Sicherheitsverschluss
4. Schale
5. Vorderseite
6. Rechte Seite mit Etiketten für das korrekte Einsetzen der Reagenzien

(bioMérieux SA, 2021, pp. 3-6)

Figure 7: Structure and components of the Previ Color Gram. The Previ Color Gram consists of several components and is used for the efficient and environmentally friendly staining of sample smears. (bioMérieux SA, 2021, pp. 1-1, 1-2, 3-1, 3-6)

The Previ Color Gram is an automated Gram staining device for staining and analyzing samples. *Figure 7* shows that the Previ uses various reagents **(6)** and has a spray mechanism that distributes the staining solution evenly on the slides. This ensures that the samples are stained uniformly and reproducibly. The centrifuge also has a high volume staining capacity **(1 + 2)**. This allows many samples (12 to 30 samples) to be stained quickly. The device has a cytocentrifuge **(4)** that prepares the samples, and a screen **(5)**, with the device

can be controlled. In addition, different staining programs can be created, edited, and saved on the Previ. The system works according to these preprogrammed protocols. This means that the staining and destaining times are always identical. This leads to clearly differentiated bacterial images and reliable diagnoses. Special nozzles and pumps distribute the reagents evenly. A barcode reader helps to manage the reagents listed in *Table 2* and use them optimally to minimize waste. The system is not only more efficient but also more environmentally friendly than manual staining methods. (bioMérieux SA, 2021, pp. 1-1, 1-2, 3-1, 3-6)

Table 2: List of reagents for the Previ Color Gram

Material	Manufacturer	Reference number
Reagent A – Fuchsin or Safranin (with or without acetone)	Biomérieux, Maryy-L'Etoile, France	Acetone fuchsin/safranin: 295521/29582 Fuchsin/safranin: 29585/29583
Reagent B – Iodine	Biomérieux, Maryy-L'Etoile, France	29586
Reagent C - Crystal violet	Biomérieux, Maryy-L'Etoile, France	29587
Reagent D – Distilled water		
Reagent E – approved alcohol	SAV Liquid Production GmbH, Flinntsbach am Inn, Germany	ETO-10000-99-1

2.1.6 BactoSonic

BactoSonic ultrasonic baths utilize the effect of cavitation. Piezoelectric vibration systems are mounted under the bottom of the vibration tank, whose energy is transferred to the bath liquid in the form of mechanical vibrations at ultrasonic frequency. This constantly generates microscopic bubbles in the bath liquid, which release energy when they implode and create local microcurrents. This process is called cavitation. Compared to other ultrasonic baths, the sonication in the BactoSonic is based on low-frequency ultrasound, whose low intensity is at the cavitation threshold. The adhesion of the biofilm to the implant surface is reduced to such an extent by the microcurrents, shear forces, and oscillating cavitation bubbles that it detaches. However, the cavitation events that occur are still so low in energy that there is no significant destruction of cell structures and the detached microorganisms are available as sample material for subsequent analysis. (Bandelin electronic GmbH & Co. KG, 2020, p. 6)

2.2 Preparation of tissue samples

If prosthetic infections are suspected, tissue samples are taken intraoperatively from the affected patients. These are delivered from all over Vorarlberg to the microbiology laboratory of the Feldkirch pathology department. The tissue samples relevant to this work come exclusively from orthopedics and trauma surgery. Three tissue samples are always taken per patient and removal, which arrive at the laboratory from Monday to Friday. Samples that arrived over the weekend were not used for this work. If more than three tissue samples were available, three were selected at random and then used for this work. Care was taken to ensure that the samples had a maximum diameter of one centimeter. Not only were samples from patients used, at those a suspicion of an

infection, but also from patients who have, for example, a loose prosthesis. The analysis of these tissue samples also serves as a follow-up during or after therapy.

The tissue was processed by the BMAs in the microbiology laboratory under a fume hood using sterile scissors, sterile tweezers, or a disposable scalpel in a sterile mortar. After the tissue had been cut to size, one piece was placed in each ProbeAX Evolution tube. In the best case scenario, three ProbeAX tubes were filled per patient. These tubes were further processed as described in *section 2.3* below. The rest of the sample was ground in a mortar for routine processing. Two different plates were smeared in a circular motion using the mortar itself and incubated overnight. The rest was mixed with the broth liquid in the mortar and added to the broth tubes as a whole. The materials required for processing are listed in *Table 3*.

Table 3: List of materials used for the preparation of tissue samples

Material	Manufacturer	Reference number
SampleAX Evolution Tubes	AxonLab, Baden-Dättwil, Switzerland	AL0510
<i>disposable scalpels</i>	Troge Medical GmbH, Hamburg, Germany	146210
Sterile mortar		
Sterile scissors		
Sterile tweezers		

2.3 Incubation of liquid culture

Further processing was carried out independently. After the ProbeAX Evolution tubes had been filled by the BMAs, they were then homogenized and dispersed in the SpinAX for one minute at 2200 rpm. Meanwhile, the membrane of the aerobic and anaerobic blood culture bottles was disinfected with a cellulose swab and Sterillium. Using a syringe, one milliliter from each ProbeAX vessel was injected into one aerobic and one anaerobic bottle. Finally, three sets consisting of three aerobic and three anaerobic blood culture bottles were available for each patient. These were incubated in the BACTEC-FX for a maximum of 14 days. In the case of positive blood cultures, the *time-to-positivity* (TTP) is important. The TTP refers to the time span in which the bottles become positive, namely the period between inserting the bottle into the BACTEC-FX and the time at which the device was able to detect microorganisms. The positive blood culture bottles are removed and further processed as described in *section 2.4* below. The materials used for these steps can be found in *Table 4*.

Table 4: List of materials used for the BACTEC-FX

Material	Manufacturer	Reference number
BACTEC Plus Aerobic/F Culture Vials	Becton, Dickinson and Company, Sparks, USA	442023
BACTEC Lytic/10 Anaerobic/F Culture Vials	Becton, Dickinson and Company, Sparks, USA	442021
Injekt Luer Solo (10 ml)	B Braun, Melsungen, Germany	4606108N

Blunt Fill Needle	Becton, Dickinson and Company, Sparks, USA	303129
Sterillium pure	BODE Chemie GmbH, Hamburg, Germany	981534
Zelletten	Lohmann & Rauscher International GmbH, Rengsdorf, Germany	13 356

2.4 Transfer from liquid culture to solid culture

Since the BACTEC-FX cannot accurately identify microorganisms, positive blood culture bottles must be processed manually. The materials required for this are listed in *Table 5*. Mueller-Hinton chocolate agar (PVX) and Schaedler agar with vitamin K1 (SK1) are required for further analysis.

Mueller-Hinton chocolate agar (PVX)

PVX plates are enriched, non-selective media used for the isolation and cultivation of both fastidious and non-fastidious bacteria, as well as bacteria from normal flora. Fastidious bacteria are those that do not grow, or grow poorly, on sheep blood-based primary isolation media. These plates are incubated at 35-37°C. According to the manufacturer, the culture plates can be read for the first time after 18-24 hours. They are then incubated further and read again after 42-48 hours. PVX plates are also suitable for sensitivity testing of *N. gonorrhoeae*, *H. influenzae*, *N. gonorrhoeae*, and *N.*

meningitidis grow on this medium. (Becton, Dickinson and Company, 2011, pp. 1-5)

Schaedler agar with vitamin K1 and 5% sheep blood (SK1)

SK1 medium is nutrient-rich and is used for the non-selective isolation of anaerobes and the selective isolation of both Gram-negative and Gram-positive anaerobic rods. The addition of vitamin K1 and hemin forms the basis for several selective media. These plates are incubated anaerobically for 48–72 hours at 35–37°C. *B. fragilis*, *B. thetaiotaomicron*, *C. perfringens*, *F. nucleatum*, and others grow on SK1 plates.

P. anaerobius, *P. levii*, but also facultative anaerobes such as *E. coli*, *S. aureus*, and *P. mirabilis*. (Becton, Dickinson and Company, 2016, pp. 1–5)

One drop from each positive blood culture bottle was applied to a PVX and SK1 plate. Before the applicator with the adapter was placed on the membrane of the bottle, it had to be disinfected with a cellulose swab and Sterillium to prevent contamination. All the materials required can be found in *Table 4*. The positive culture bottles were streaked using the three-eyelet method with a swab. As already mentioned, the SK1 plate must be incubated anaerobically. For this purpose, the plate was placed in a plastic bag with a gas-generating bag. While the PVX plate was incubated in the incubator, the SK1 medium was incubated in the incubation chamber.

Table 5: List of materials used for solid culture incubation

Material	Manufacturer	Reference number
Mueller-Hinton chocolate agar (PVX)	Biomérieux, Maryy-L'Etoile, France	43109
Schaedler agar with vitamin K1 (SK1)	Becton, Dickinson and Company, Sparks, USA	254084
AnaeroGen Compact	Thermo Fischer Scientific, Massachusetts, USA	AN0020D
Applicator plastic, viscose tip, 2.5x150mm	Becton, Dickinson and Company, Sparks, USA	09-812-8052
Vacutainer Multiple Sample Luer Adapter	Becton, Dickinson and Company, Sparks, USA	367300
Inoculation loop	Nerbe Plus, Winsen/Luhe, Germany	09-401-5002
PS stick with small viscose tip	Nerbe Plus, Winsen/Luhe, Germany	09-812-8052

2.5 Identification

Ideally, colonies were visible on the culture plates the next day. If this was not the case, they had to be incubated until colonies were present. Both the conventional method and the liquid culture method require MALDI for pathogen identification

. It should be noted that the laboratory's BMAs did not create liquid cultures using the conventional routine method. Therefore, they performed a MALDI directly after streaking and incubating the agar plates.

As already mentioned, various solutions are required for MALDI. The *organic solvent* (OS) is enriched so that it can be used immediately. The matrix is lyophilized and was dissolved with 250 µl OS. The 100% formic acid was diluted to 70% with ultrapure water. After preparing the solutions, the colonies were removed with a toothpick and smeared onto a Biotarget. After further processing steps, which are explained in more detail in *section 2.1.4*, a wide variety of microorganisms that have grown on the plates can be identified using mass spectrometry. For personal verification, Gram stains (see *section 2.1.5*) were prepared and viewed under a microscope. The materials required for the identification of microorganisms are listed in *Table 6*.

Table 6: List of materials used for pathogen identification

Material	Manufacturer	Reference number
Menzel Superfrost Plus slides <i>microscope slides</i>	Gerhard Menzel, Braunschweig, Germany	J1800AMNZ
<i>Solution Organic Solvent</i> (OS)	VWR International Chemicals, Pennsylvania, USA	89449.180
Formic acid 98/100% for analysis ACS	Bernd Kraft, Duisburg, Germany	05314.2010
IVD Matrix HCCA lyophilized portioned	Bruker Daltonik GmbH, Bremen, Germany	8290200

Water, Chromasolv LC-MS	Honeywell Riedel-de Haen, Seelze, Germany	39253-1L
MBT Biotarget 96	Bruker Daltonik GmbH, Bremen, Germany	8267615
10µl sterile filter tips	Greiner Bio-One, Kremsmünster, Austria	771261

2.6 Sonication with BactoSonic

Sonification is an ultrasonic treatment in which the biofilm on the surface of the implant is removed by high-frequency sound waves. This procedure was performed by the BMAs in the laboratory. There are a few things to keep in mind when preparing for sonification. An oscillating tank was filled 1/3 full with water. A surfactant-containing preparation was added and the tank was refilled with water up to the mark. In the next step, the liquid had to be degassed by turning on the ultrasound for 10 minutes. After all the materials listed in *Table 7* had been prepared and the sterile workbench was ready for use, ear protection and sterile gloves had to be put on. The bag filled with physiological saline solution was opened and a transfer device was inserted into the bag as far as it would go. The sample, which was in a box, had to be covered with sterile saline solution to at least 90%. The box was then closed and shaken vigorously for at least 30 seconds. The sonication itself runs at 100% power in the BactoSonic for one minute. It is important to note that the box must not touch the bottom and the walls of the bath not. After the

Sonification: The box was shaken again. Then, 50 ml of the sonicate had to be transferred to a test tube. From this liquid, 1 ml each was placed on a cooked blood, Columbia, and Schädler plate. Likewise, 6 ml each was injected into an aerobic and anaerobic blood culture bottle. The rest of the sonicate was then emptied from the box and the sample was shaken vigorously in the BactoSonic again for at least 30 seconds. Finally, the box was sealed and sent to the central reception. After 24 hours of incubation, the culture plates were examined. The results were evaluated as colony-forming colonies (CFU). The number of CFU per plate was given in CFU/ml of sonication fluid. A significant result is obtained when the number is greater than or equal to 50 CFU/ml. The result of the sonication is decisive and confirms the presence of an infection. (Bandelin electronic GmbH & Co. KG, 2020, pp. 16-18)

Table 7: List of materials used for the BactoSonic

Material	Manufacturer	Reference number
free flex, 0.9% physiological saline solution, 500 ml (infusion solution) + 250 ml bag	Fresenius Kabi, Austria	Ref. no.: 1-16417
Transfer device	Fresenius Kabi, Austria	8500432

2.7 Evaluation

The results of both the agar plate and blood culture methods, as well as the names and dates of birth of the patients, were collected in an Excel file. The date of submission and the date of processing were documented. The results of the conventional method used by the Feldkirch pathology department were recorded and compared with the method used in this study. In *the following chapter 3*, all results are presented graphically and discussed.

3 Results

This chapter presents all results in graphical and tabular form. Both methods will be compared. The tissue types used for this work, the results of both methods, and their differences will be explained. In addition, the positive samples will be listed in more detail. Furthermore, meaningful data on the TTPs will be presented and conspicuous samples will be discussed.

In the period from February 19, 2024, to April 12, 2024, a total of 64 tissue samples from the immediate vicinity of implants from 21 different patients were processed and analyzed for this work. Tissue samples were taken twice at different times from three of these patients. Nevertheless, these samples are considered individual samples. The results of this study therefore refer to 24 patients or 24 samples. Three tissue samples were used for the blood culture method from each patient in most cases. With the exception of six patients, from whom only two or one tissue sample was received in some cases.

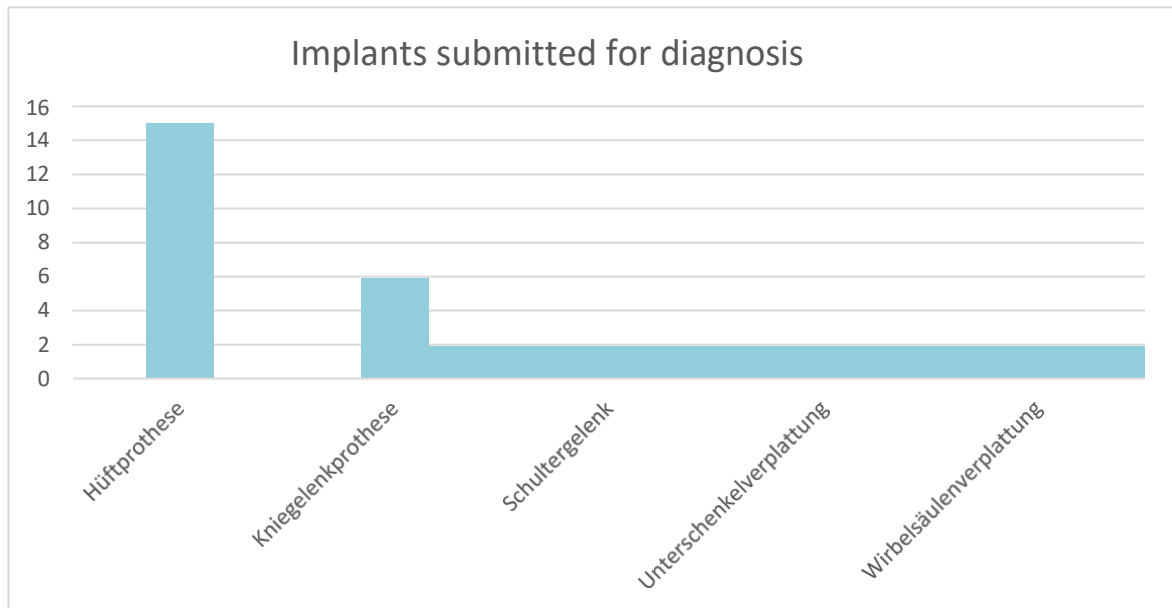


Figure 8: Implants submitted for diagnosis. This diagram shows the types of joint tissue used for this study.

The state hospitals in Vorarlberg sent in the most tissue samples from hip joints, followed by knee joints. The exact data can be found in *Figure 8*. One sample each from a shoulder, lower leg, and lumbar spine was also processed.

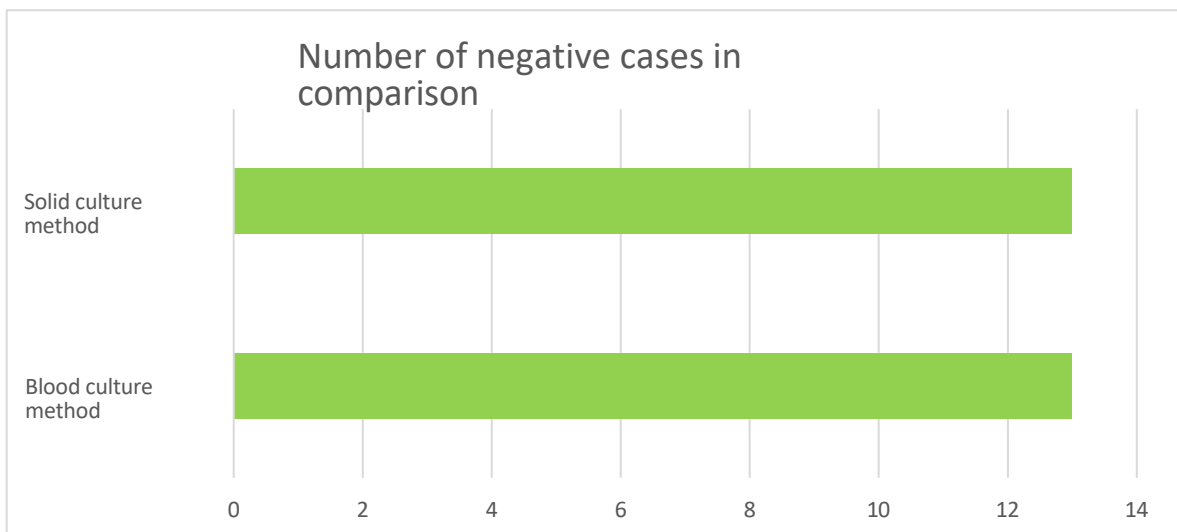


Figure 9: Number of negative cases in comparison. This bar chart shows the number of negative samples from both the solid culture method and the blood culture method.

As can be seen in *Figure 9*, there is no difference in the number of negative samples. The results match 100%. Both in routine and in the blood culture method, the negative results always match. It follows that both methods have identical specificity.

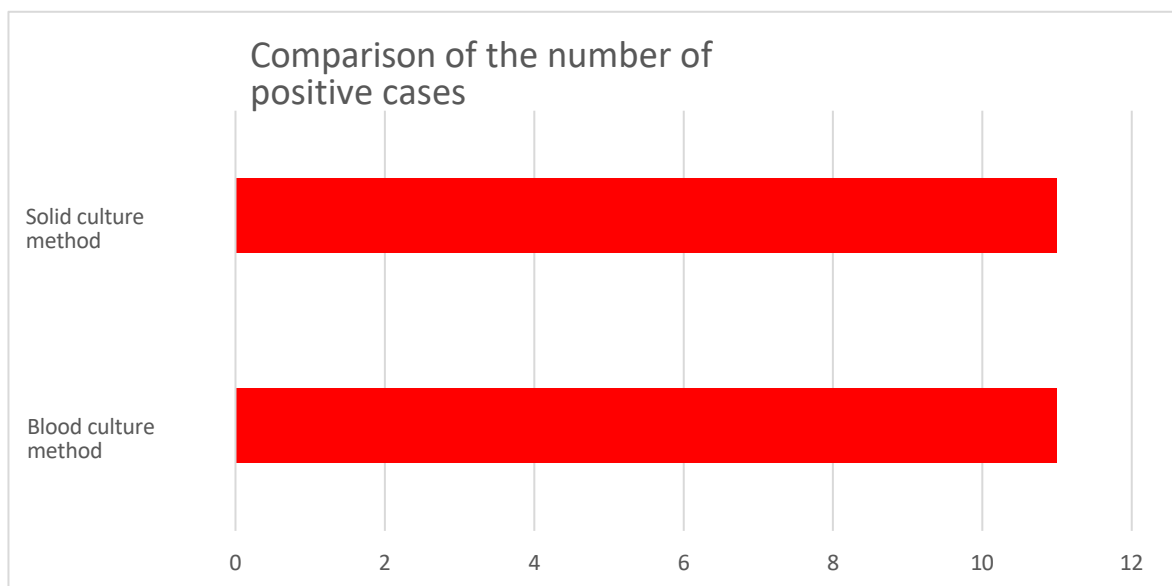


Figure 10: Comparison of the number of positive cases. These two bars show the number of positive samples obtained using the conventional method and the blood culture bottle method.

Similarly, the positive results of both methods in *Figure 10* match 100%, and there were no false positive results. Thus, the sensitivity is identical for both methods.

To explain the results of this study, *Table 8* is probably the most informative. All samples are listed in ascending order. The order was determined based on the date of submission of the samples and in the following tables

continued to be handled in this way. Each patient has his or her own number. The three columns next to the sample number are for the results of the conventional method and the blood culture method, respectively. In addition, the last column is for comments.

Table 8: The results of both methods and comments

Sample number	Solid culture method	Liquid culture method	Comments
Sample 1	Negative	Negative	-
Sample 2	Negative	Negative	-
Sample 3	Positive	Positive	Patient double (see sample 13)
Sample 4	Negative	Negative	-
Sample 5	Negative	Negative	-
Sample 6	Positive	Positive	-
Sample 7	Negative	Negative	-
Sample 8	Negative	Negative	Contaminants on solid culture method
Sample 9	Negative	Negative	-
Sample 10	Negative	Negative	Contaminants on solid culture method
Sample 11	Negative	Negative	-
Sample 12	Negative	Negative	-
Sample 13	Positive	Positive	-
Sample 14	Positive	Positive	-
Sample 15	Positive	Positive	-
Sample 16	Positive	Positive	Contaminants on solid culture method
Sample 17	Positive	Positive	Patient double (see sample 21)

Sample 18	Negative	Negative	Contaminant using solid culture method and patient double (see Sample 20)
Sample 19	Negative	Negative	-
Sample 20	Positive	Positive	-
Sample 21	Negative	Negative	-
Sample 22	Positive	Positive	-
Sample 23	Positive	Positive	-
Sample 24	Positive	Positive	-

Samples numbered three, 17, and 18 are from patients from whom tissue samples were taken twice at different times. While several contaminations, four to be precise, occurred with the conventional solid culture method, there were no false positive cases with the blood culture method. Contamination occurs when, in addition to the main pathogen with a high bacterial count, additional microorganisms with a low bacterial count are detected. It can therefore be concluded that the blood culture method did not produce any false positive results. Samples that show a further discrepancy between the methods are cases 14 and 16. The two samples produced different results in both methods. *Table 9* shows that two of four tissue samples from sample 14 and two of three tissue samples from sample 16 were positive. In both cases, sonication yielded a positive result. The liquid culture method showed a positive result in only one of three sample sets. However, sonication must be taken into account. It can therefore be concluded that both samples confirm an infection in both methods.

Table 9 lists all positive samples from the conventional method, the number of tissue samples submitted, and the number of positive tissue samples. For a case to be classified as positive

, the same pathogen must be detectable in at least two samples. With the liquid culture method, the same pathogen also had to be present in two out of three sets in order to be classified as positive. However, the sonification result is decisive in both methods. For example, if fewer than two out of three tissue pieces or sets were positive, the result of sonication determined whether the case had to be evaluated as positive or negative. Sonication was not performed on most samples. Four sonications (samples 14, 16, 22, and 23) yielded a positive result.

Table 9: Number of positive tissues and results of sonication of the solid culture method

Sample number	Number of tissues	Of which positive	Sonification
Sample	4	4	-
Sample 6	1	1	-
Sample 13	4	4	-
Sample 14	4	2	Positive
Sample 15	2	2	-
Sample 16	3	2	Positive
Sample 17	5	5	-
Sample 20	3	3	-
Sample 22	2	2	Positive
Sample 23	3	2	Positive
Sample 24	5	4	-

Table 10 below lists both the number of blood culture bottle sets used and the number of positive sets. One set corresponds to one aerobic and one anaerobic bottle. The same table also shows the shortest times to a positive result. The minimum time is only two hours, 59 minutes, and ten seconds, while the maximum time is four days, 22 hours, and 20 minutes. This illustrates that the time span to a positive result

Bottle is highly variable. However, it is noteworthy that none of the cases reached the 14-day mark. It is also striking that all samples became positive within less than seven days, regardless of whether they were anaerobic or aerobic bottles. Even with anaerobes, such as sample 23, positive results were achieved within four days, 22 hours, and 20 minutes. Data from *Table 10* show that aerobic bottles tended to turn positive faster than anaerobic ones. After 24 hours of incubation, the BACTEC-FX evaluated 60.87% of anaerobic bottles and 90% of aerobic bottles as positive. After 24 hours of incubation, 74% of all sets were evaluated as positive.

Table 10: Number of positive sets using the blood culture bottle method, shown with the shortest TTP

Sample number	Sets	of which positive	Positive bottles	TTP anaerobic	TTP aerobic
Sample 3	3	3	6/6	00d 06h 14 min 16 sec	-
Rehearsal 6	1	1	1/1	00d 10h 06min 00sec	-
Sample 13	3	3	5/6	-	00d 15h 15 min 44 sec
Rehearsal 14	3	1	2/6, Sonification positive	-	00d 16h 38min 27sec
Sample 15	2	2	4/4	00d 12h 13min 42sec	-
Sample 16	3	1	2/6, Sonification positive	-	01d 03h 44min 58sec
Sample 17	3	3	6/6	-	00d 07h 38min 39 sec

Rehearsal 20	3	3	6/6	-	00d 02h 59 min 10 sec
Rehearsal 22	1	1	1/1	-	00d 17h 03min 11 sec
Rehearsal 23	3	3	3/6	04d 22h 20min 00sec	Negative
Sample 24	3	3	5/6	00d 19h 07min 34sec	-

The results of the MALDI are shown in the last *table 11*. The results of both methods are consistent.

Table 11: Microorganisms identified in the positive samples using both methods

Sample number	Solid culture method	Blood culture method
Sample 3	<i>E. faecium</i>	<i>E. faecium</i>
Sample 6	<i>S. aureus</i>	<i>S. aureus</i>
Sample 13	<i>E. faecium</i>	<i>E. faecium</i>
Sample 14	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>
Sample 15	<i>S. epidermidis</i>	<i>S. epidermidis</i>
Sample 16	<i>S. lugdunensis</i>	<i>S. lugdunensis</i>
Sample 17	<i>S. aureus</i>	<i>S. aureus</i>
Sample 20	<i>E. clocae</i> complex	<i>E. clocae</i> complex
Sample 22	<i>S. epidermidis</i>	<i>S. epidermidis</i>
Sample 23	<i>C. acnes</i>	<i>C. acnes</i>
Sample 24	<i>S. aureus</i>	<i>S. aureus</i>

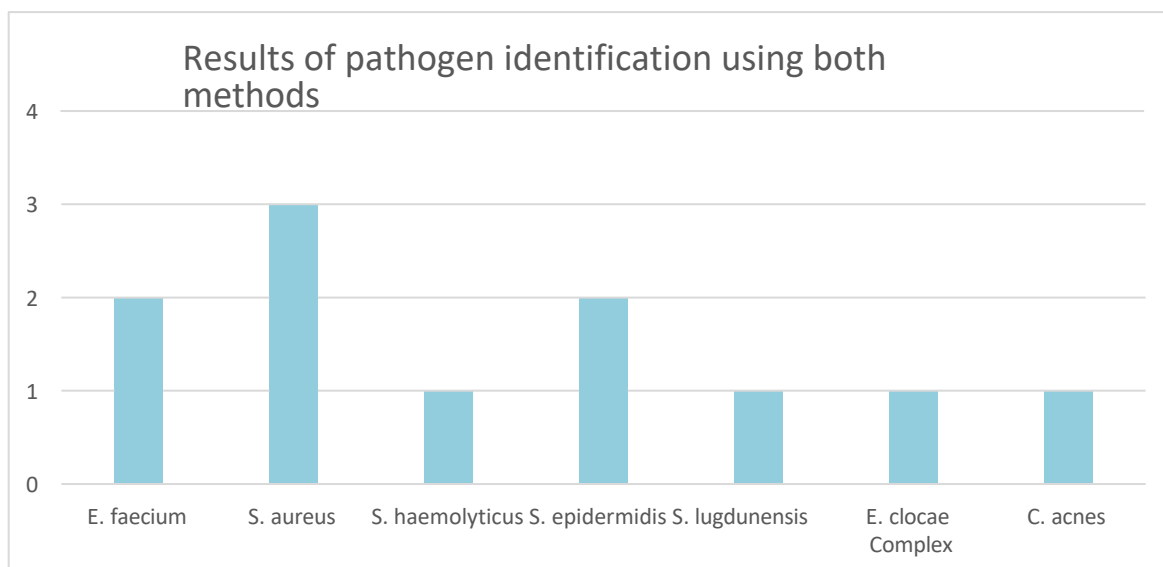


Figure 11: Frequency of pathogens identified by both methods. This bar chart shows all pathogens identified using MALDI.

The most frequently detected bacterium is *S. aureus*. *S. epidermidis* and *E. faecium* bacteria occur with equal frequency. All other bacteria were detected only once in samples. These results are shown in *Figure 11*.

The results of sample 23 allow us to conclude that the initially recommended incubation period of 14 days can be reduced to a maximum of seven days.

4 Conclusion

This study examined 24 patients, analyzing a total of 64 tissue samples. Using the blood culture method, 13 of the 24 samples tested were negative and 11 were confirmed infections, which was also confirmed by the conventional solid culture method. However, four contaminations occurred with the solid culture method.

The TTPs achieved indicate that the incubation period can be reduced from a maximum of 14 days to a maximum of 7 days. The most frequently identified pathogen was *S. aureus*, followed by *E. faecium* and *S. epidermidis*.

It should be noted that the blood culture method has several positive aspects. On the one hand, no samples were contaminated, which increases the reliability of the results. The liquid culture method enabled rapid diagnosis, as approximately 74% of samples were positive after just 24 hours. The blood culture method is very sensitive, meaning that infections are not overlooked. No false-negative or false-positive results occurred in the present study. The results of the blood culture method are consistent with the results of routine tests, confirming its reliability. The diagnostic process can be accelerated with the blood culture method, as it would generally be sufficient to incubate the BC bottles for a maximum of seven days instead of 14 days.

The results of this work provide valuable insights into optimizing incubation times and improving methods for identifying pathogens in clinical samples.

The examination of blood cultures will be important for further development . It requires further research. A central

focus should be on shortening incubation times, especially for anaerobic bacteria. New technologies should also be developed to improve sampling and thus prevent contamination. Overall, different areas of research offer opportunities to improve the diagnosis and treatment of infections.

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Abbreviations

on

AAOS	<i>Association of Orthopedic Surgeons</i>
BSG	Blood sedimentation rate
CFU	<i>colony forming units</i>
CNA	Columbia nalidixic acid agar
CO ₂	carbon dioxide
CRP	C-reactive protein
DAIR	<i>Debridement, antibiotics, and implant retention</i>
LED	Light-emitting diodes
MALDI-TOF	<i>matrix-assisted laser desorption/ionization time-of-flight</i>
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
NaCl	sodium chloride
OS	<i>Organic solvent solution</i>
PJI	<i>Periprosthetic joint infection</i>
PMN	<i>polymorphonuclear cell</i>
PVX	Mueller-Hinton chocolate agar
SK1	Schaedler agar with vitamin K1
TTP	<i>Time-to-positivity</i>

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9 Affidavit

Student data

Last name	First name	Özgün, Rana Ebru
Title		
Personal identification number		2110496015
Degree program/course		Biomedical Analysis
Year, class		BMABsc_2021

I hereby declare that I have written my bachelor's thesis on the topic

Comparison of microbiological diagnostics of tissue samples using solid cultures and blood cultures in periprosthetic and implant infections

.....

Prepared as part of the course

FLB6P - Research-based learning in the professional field, bachelor's thesis

independently and have not used any sources or aids other than those indicated, and have identified all passages taken verbatim or in substance from other texts as such. This applies to printed texts as well as to texts taken from the Internet, audiovisual media, audiobooks, and image credits.

Innsbruck, June 7, 2024

Place, date

Signature of the student

