Video Article

Novel Diagnostics in Revision Arthroplasty: Implant Sonication and Multiplex Polymerase Chain Reaction

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Abstract

In orthopedic patients, foreign body-associated infections, especially periprosthetic joint infections (PJIs), are a devastating complication of arthroplasty. Infection requires complex treatment, may result in long hospitalization and causes considerable costs. Multiple surgical revisions can be necessary in these patients, with a loss in function as well as in quality of life.

The routine preoperative diagnostics include blood examination for C-reactive protein (CRP) and other biomarkers, as well as joint aspirate analysis for cell count, differentiation, and culture. Intraoperative specimens for histology and microbiology are also standard procedure. The microbiological examination of removed implants with sonication, in combination with the implementation of molecular biology techniques in microbiology, represent two novel techniques currently employed to enhance the differential diagnostics of PJI.

We present here the step-wise procedure of analyzing joint aspirate and sonication fluid, using a cartridge-based multiplex polymerase chain reaction (PCR) system. Results were matched against conventional cultures and consensus criteria for PJI. Conventional microbiological cultures from tissue biopsies, joint aspirate and sonication fluid showed a sensitivity of 66.7%, 66.7%, and 88.9%, respectively, and a specificity of 82.3%, 54.6%, and 61.5%, respectively. The PCR diagnostic of the sonication fluid and the joint fluid showed a sensitivity of 50.0% and 55.6%, respectively, and both a specificity of 100.0%. Both PCR diagnostics combined had a sensitivity of 66.7% and a specificity of 100.0%. The multiplex PCR therefore presents a rapid diagnostic tool with moderate sensitivity but high specificity in diagnosing PJI.

Video Link

The video component of this article can be found at https://www.jove.com/video/55147/

Introduction

Painful arthroplasties are a diagnostic challenge in orthopedic surgery. After aseptic loosening, PJI is the second most reason for implant failure, and presents a devastating complication after arthroplasty surgery. PJI is difficult to treat and difficult to diagnose. Missed diagnosis of a PJI will likely result in recurrent infection, with major morbidity, loss of function, and loss of quality of life. Therefore, infection should be ruled out in all patients presenting with painful arthroplasty, before therapeutic measures are initiated.

Patient history, clinical examination, blood examination for CRP and white blood cell count, as well as radiography or szintigraphy of the affected joint constitute the basic diagnostics[1]. The preoperative routine should also include a joint aspiration under sterile conditions wherever possible. The aspirate acquired is a very valuable material in further diagnostics.

Besides the cell count and cell differentiation in joint aspirate as well-established assays[2], the detection of protein biomarkers may help in differential diagnostics[3,4,5]. Conventional microbiological culture remains the gold standard in pathogen detection. Biofilms, caused by both gram-positive and gram-negative bacteria, and adherent to the implant surfaces, are a major pathogenic factor in PJI. Therefore, in 2007, the sonication procedure was implemented in the diagnostics of foreign body-associated infections in orthopedic surgery, disrupting the biofilms on removed implants to allow pathogen detection. The bacteria are thereby taken from their resting form to an active form, making detection in culture possible again. Sonication of removed implants showed a higher sensitivity than cultures from tissue specimens (78.5% vs. 60.8%)[6].

Nucleic acid amplification test (NAT), such as PCR, has recently moved into the scope of clinicians and microbiologists to diagnose PJI. Especially in patients who received antibiotic therapy prior to surgery, it was shown that the PCR diagnostic is beneficial in identifying the causative organisms[7,8,9]. Lately, a new multiplex PCR system, specially designed for implant and tissue infections (ITI), was introduced. This cartridge-based system offers a variety of genomic markers for identification of pathogens and antibiotic resistance markers. Among its application range are numerous indications (prosthetic joint infections, surgical site infections, cardiology-related infections, catheter-associated

infections, diabetic foot infections, deep skin and tissue infections, implant infections, and burn-wound infections), and a broad panel of sample material can be used for this technique (sonication fluid, synovial fluid, swabs, tissue, pus, aspirate/exudate, and biofilm)[10,11,12].

The greatest advantage of the procedure, as compared to conventional microbiology, is speed: The causative pathogen can be identified within hours. Additionally, this PCR system detects a broad panel of gene-encoded resistance markers, allowing the initiation of targeted antibiotic therapy early on. With the assistance of PCR, surgeons may be able to distinguish between infected and non-infected patients at a very early stage in the diagnostic procedure[11]. Here, we present the protocol to perform this multiplex PCR, quickly diagnosing PJI from joint aspirate and sonication fluid.

Protocol

This study was approved by the local ethics committee (046/09, Rev. 3). Informed consent and data privacy statement were obtained from all patients included.

1. Joint Aspiration

NOTE: The procedure should be performed in a surgical theater or an intervention room with comparable aseptic conditions. Assistance by a nurse or doctor's assistance is helpful but not mandatory.

1. Position the patient in supine position on an examination bed. Ensure that the joint of interest is free of clothing. Shorten dense or long body hair using electric clippers. Do not remove body hair with a razor. Position a fluoroscope in anteroposterior direction if the hip joint is to be punctured. If the knee is to be punctured, place a knee roll under the patient's knee such that the knee rests in a slightly flexed position[13].
2. Prepare an instrument table with the following sterile equipment: sterile swabs, a fenestrated sterile cover drape, a disposable sterile scalpel, pointed surgical blade (type #11), and sterile 10 mL syringe with sterile cannula (20 gauge, 80 mm). Set out alcoholic skin disinfectant, blood collection tubes and a pediatric blood culture flask separately.
3. Dress in a surgical gown, hood and mask, and put on sterile gloves.
4. Thoroughly wash the patient's skin at the puncture site (*e.g.* superior recessus[14] for the knee and anterolateral portal area for the hip) with a skin disinfectant.
   NOTE: Mind the required exposure time for the disinfectant used (usually 90 s at the knee, 120 s at the hip for alcoholic disinfectants).
5. **Cover the puncture site with a fenestrated sterile cover drape. Identify the puncture site.**
   NOTE: For the knee, the correct site is 2 cm above the upper patella pole and 2 cm lateral to the lateral facet. For the hip, find the anterior superior iliac spine and move caudally until in line with the upper symphysis. Ensure that the site is lateral to the femoral artery (approximately 4 cm), which can always be palpated[15].
   1. Make a small stab incision through the skin (3 - 4 mm wide and deep) with the pointed scalpel blade at the puncture site. Do not apply local anesthetics as they may cause false negatives in the microbiological culture, due to their bacteriostatic effect.
6. **Attach the cannula to the Luer slip of the syringe. Insert the cannula through the stab incision. At the superior recess of the knee joint, aim at a 45° angle dorsal and medial, towards the recess below the top pole of the patella, and insert the needle to a depth of approximately 5 cm. Aspirate the joint fluid by drawing out the syringe's piston.**
   1. Use fluoroscope guidance when puncturing a hip joint. Insert the needle at the puncture site, aiming medially at a 60° angle. In the fluoroscope, ensure that the tip of the needle is pointed at the prosthesis neck. Advance the cannula to a depth of approximately 8 cm (deeper in adipose patients) while aspirating, until joint fluid is attained. Hold the needle in position while aspirating, to avoid scraping of the prosthesis surface.
      NOTE: Contact of the needle tip with the prosthesis ensures intraarticular positioning. Usually, no more than 5 mL of fluid can be aspirated form a hip joint, a knee joint will yield more than 10 mL.
   2. After removal of the cannula, apply a sterile wound dressing to the stab incision. To prevent hematoma formation, apply a bandage to the leg with slight compression.
7. **Transfer the collected joint aspirate into the sample containers. Ensure sterile working techniques throughout.**
   1. For inoculation of the pediatric blood culture flask with the aspirated joint fluid, disinfect the membrane of the pediatric blood culture flask with alcoholic disinfectant. Put a fresh cannula onto the syringe and inject 1 to 3 mL of aspirated joint fluid into the blood culture flask. Mix by gentle agitation or rotation[16].
   2. For preparation of a native joint aspirate specimen, remove the cannula from the syringe. Open a blood collection tube without additives and inject 1 mL of aspirate into this collection tube. Replace and fasten the lid for PCR analysis.
      NOTE: Ship the sample to the microbiology lab without delay for PCR analysis. From the same sample, conventional microbiological aerobic and anaerobic cultures can also be set up[17].
   3. Transfer 1 to 4 mL of the joint aspirate into a blood collection tube containing EDTA for cell count and differentiation[2]. Ship the sample to a biochemistry lab without any delay.

2. PCR Diagnostics

1. Make sure all three devices (the lysator, the analyzer, and the cockpit; see the Table of Materials) are up and running correctly. Set out all supplies needed for performing the test (the PCR cartridge, a master mix tube, the sample tube and sample tube cap, a 0 - 200 µL micropipette, and sterile pipette tips) on the working bench.
2. Defrost the master mix tube before starting the procedure, by setting it out at room temperature. All consumables (master mix tube, cartridge, and sample tube) are already prelabeled with a barcode.

3. Remove the cap of the sample tube. Take 180 µL of the collected specimen (either joint aspirate, see section 1; or sonication fluid, see section 4) with a pipette and transfer it into the sample tube. Close the sample tube with the sample tube cap containing protein kinase K and an internal control gene for the quality control of the entire workflow.
4. At the cockpit, open the operating software by touching the "new test" button in the lower left corner. Enter the patient's data or a sample ID via the touchscreen. To select the specimen, first choose "ITI-Cartridge" from the dropdown, then "Orthopedics" as application mode, and finally select "synovial fluid" or "sonication fluid" as sample type. Press the start button.
5. Scan the barcode on the bottom of the sample tube. Insert the sample tube into the lysator.
   NOTE: The lysis process will start automatically and take approximately 30 min to finish. Countdown of the timer on the lysator screen will indicate when the lysis is finished.
6. Select the sample on the cockpit screen to unlock the sample tube from the lysator. Remove the sample tube from the lysator and close the lysator's top cover. Transfer the sample tube into the sample tube slot of the PCR cartridge. Insert the defrosted mastermix tube into the mastermix slot of the PCR cartridge.
7. Follow the instructions on the cockpit monitor, scanning the PCR cartridge with mastermix and sample tube.
8. Insert the PCR cartridge into the indicated cartridge slot of the analyzer. When done, the analyzer releases the cartridge.
   NOTE: The PCR conditions are preset by the manufacturer and cannot be changed by the user. For further information see the manufacturer's application guide. The PCR process will start automatically and take approximately 4 h 10 min.
9. Remove and discard the cartridge. At the cockpit touchscreen, choose "current tests" in the lower left corner, then pick the correct sample ID to display the test results. Save the results on the machine's memory, and print or export (via USB drive) for further reference.
   NOTE: Report the result of the PCR, including pathogen identification and detection of resistance markers, to the surgeon without delay. A panel with 114 deoxyribonucleic acid (DNA) targets of bacterial and fungal pathogens typically found in implant infections and soft tissue infections, along with the most common antibiotic resistance markers is analyzed.

3. Surgical Procedure: Intraoperative Sample Collection

NOTE: Revision arthroplasty surgery requires an expert level of skill and expertise; for a comprehensive overview of the surgical procedures, please refer to surgeon's textbooks[18]. A full and detailed description of the surgical procedure would be well beyond the scope of this article. Here the focus is on the details of the sample collection and pre-analytics. In revision arthroplasty surgery, refrain from administration of a single shot antibiotic prophylaxis, as not to compromise the results of the microbiological samples obtained during surgery. Adhering to this rule is recommended, though this practice is controversial[19,20]. Use the existing surgical approach to the joint whenever possible. Additional surgical approaches will compromise soft tissues and increase scar formation.

1. Dissect tissue until the joint capsule is well exposed. Perform the arthrotomy with a syringe at the ready, so further joint fluid can be collected into the sterile syringe for further analysis as stated above (steps 1.7.1-1.7.3).
   NOTE: No antiseptic lavage fluids must be used until the implant is removed and tissue samples have been taken.
2. Remove the joint implants. Immediately after removal, transfer the implant parts into a sterile plastic container with an air- and water-tight lid.
   NOTE: Detailed instructions for implant removal can be found in the textbook literature (Knee, *e.g.*: Wirtz *et al.*, Chapter 16.4[21]; Hip, *e.g.*: Claes *et al.*, Chapter 14.5.1[22]). Specific instruments may be necessary, as stated by the manufacturers' instructions. The implant removal technique varies considerably between different implant types and fixation methods. A set of chisels and drills, a sliding hammer and a universal intramedullary nail removal set are helpful in removing bony integrated implants[23,24].
3. Use a sterile sharp curette, forceps and rongeur to remove the membrane tissue from the bone-implant interface. Transfer a representative sample of the tissue into a sterile plastic container with a screw-on lid, as specimens for microbiology and histology.
   NOTE: A reamer can be used to clear the medullary canal of all soft tissues. Also, take synovial tissue and articular capsule samples for examination and store them in sterile containers. At least four specimens in total must be gathered.
4. Send all samples and explanted parts to the microbiology lab instantly.
   NOTE: Antibiotics can then be given. The choice of the correct antibiotics is essential and must be an individualized decision[25,26]. Therapeutic concepts must be decided on by an interdisciplinary panel of surgeons and microbiologists beforehand.
5. Complete the debridement of the former implant site by removing any tissue that shows signs of debris (such as polyethylene wear, metal or cement particles) or infection and necrosis (purulent, avascular, fibrin-coated, membranous or "sludgy" tissues). Remove any dead or osteitic bone. Remove all foreign material such as screws, wires, cerclages, bone cement debris or non-resorbable sutures (see Claes *et al.*, Chapter 14.5.3[22]).
6. Irrigate the surgical site using a wound disinfectant of choice using a 50-mL syringe. Irrigate with ample amounts (at least 3 L) of ringer solution with a pulsed lavage system. A spacer may be necessary to stabilize the joint[27].
7. Close the wound using antimicrobial-coated resorbable deep sutures for the capsule or fascia (braided polyglactin sutures, triclosan-coated, USP 2), and subcutaneous tissue (braided polyglactin sutures, triclosan-coated, USP 0). Close the skin using non-resorbing interrupted sutures (monofilic polypropylene, USP 0 or USP 2-0).

4. Sonication

NOTE: Be careful to work strictly aseptically. Use a Class II biosafety bench with laminar air-flow for further processing of the explanted material.

1. Open the plastic container holding the explanted prosthesis from the operating room (see step 3.2) under a laminar air-flow working bench, and add sterile 0.9% sodium chloride solution until the explanted foreign body is covered at least 80% (approx. 500-800 mL).
2. Close the plastic container. Shake thoroughly or agitate the plastic container using a vortex mixer on high intensity for 30 s. Transfer the plastic container into the sonication bath. Check the fluid level in the sonication bath.
   NOTE: The sonication bath fluid level must be the same as inside the plastic container. Ensure that the plastic container cannot be flooded and if necessary add or remove liquid from the sonication bath.
3. Sonicate the specimen for 5 min with a frequency of 40 ± 2 kHz and power density 0.22 ± 0.04 W/cm^2. Shake or vortex the specimen thoroughly for 30 s.
   NOTE: Sonication times between 1-5 min are best for dissolving biofilms, without any influence on bacterial viability[28].

4. Inside the laminar flow bench, collect 50 mL of the sonication fluid and transfer it to a sterile, tightly-sealed tube.
5. To create a concentrated sonication fluid, centrifuge the tube for 10 min at 4,200 x g. Leaving a residual volume of 10 mL, discard the remaining supernatant with a pipette. Resuspend the pellet by pipetting and vortexing.
6. Inoculate suitable culture media (*e.g.* blood agar, chocolate agar, Sabouraud agar, Schaedler's agar, Kanamycin-Vancomycin agar, or thioglycolate broth) with 0.5 mL of concentrated sonication fluid each. Inoculate the pediatric blood culture flask with 2 mL as described above (see step 1.7.1). Transfer 1 mL of concentrated sonication fluid into a blood collection tube without additives for PCR analysis.
7. Transfer the inoculated culture media to the incubator (35 °C, 5% CO_2). Incubate the cultures for 14 days, and check for growth daily.
8. After 14 days, discard the cultures with no growth and report as negative. If growth is detected, carry out pathogen identification using standard identification techniques and susceptibility testing. Report the result to the surgeon without delay.
   NOTE: Here, identification of pathogens was carried out using Matrix-assisted LASER desorbtion/ionization - time of flight (MALDI-TOF) spectroscopy. Antimicrobial susceptibility testing was performed with a semiautomated analyzer[29,30,31].

Representative Results

Presence of a PJI, as defined by the consensus meeting of the Musculoskeletal Infection Society (MSIS), was considered proven when one major criteria or at least three out of five minor criteria were present. Major criteria include presence of a fistula or detection of a pathogen in two separate microbiological samples. Minor criteria include positive cell count (>3,000 leukocytes/µL) or positive cell differentiation (>85% neutrophil granulocytes) in joint aspirate, positive CRP and sedimentation rate in blood samples, positive histology in the tissue samples or detection of a pathogen in just one microbiological sample[19]. Sensitivity and specificity were calculated for nucleic acid amplification test (NAT), as compared to the MSIS gold standard. Patient details, including pathogens detected, are given in **Table 1**.

Our own results showed a moderate sensitivity for PCR diagnostic of sonication fluid and joint aspirate (50.0% and 55.6%) but a very strong specificity of 100%[11]. Whenever PCR diagnostic (total n = 62 tests) showed a positive finding in the joint aspirate (n = 31) or the sonication fluid (n = 31), the same pathogen was detected later in one of the conventional microbiological cultures. The PCR of the different samples of non-infection cases showed no false positive result (see **Figure 1**).

The PCR diagnostic failed to detect some pathogens that were proven with conventional microbiological culture methods. 6 out of 16 (37.0%) true pathogens in the sonication fluid samples and 5 out of 13 (38.0%) pathogens from the joint fluid culture were missed by PCR detection. Mostly, the PCR diagnostics missed detection of coagulase-negative staphylococci (8 out of 11). The combined PCR diagnostics of both materials (joint aspirate and sonication fluid, "pooled PCR") identified 12 out of 18 infection cases correctly (sensitivity 66.7%, 95% CI: 41.0% to 86.7%, see **Table 2**).

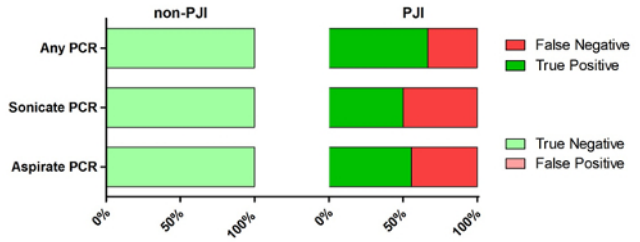

Figure 1: Summary of NAT results. The graph depicts the summarized results from the collective patient samples. On the right side is the group of patients matching PJI criteria, on the left side is the group that did not. The bars represent the nucleic acid amplification methods. False positives and false negatives are shown in red as percentage of the total. Please click here to view a larger version of this figure.

Joint	Cause of Revision	Matches MSIS Criteria?	Previous antibiotic treatment?	Sonication fluid culture	Joint aspirate culture	NAT sonication culture	NAT joint aspirate
Hip	aseptic loosening	No	No				
Knee	aseptic loosening	No	No	*Staphlococcus epidermidis*			
Knee	aseptic loosening	No	No	*Dermabacter hominis*			
Knee	aseptic loosening	No	No				
Knee	aseptic loosening	No	No	*Leifsonia aquatica*			
Hip	aseptic loosening	No	No				
Knee	aseptic loosening	No	No				
Knee	instability	No	No				
Hip	chronic luxation	No	No	*Staphylococcus haemolyticus*			
Knee	aseptic loosening	No	No				
Hip	aseptic loosening	No	No				
Hip	aseptic loosening	No	No	*Staphlococcus epidermidis*			
Knee	instability	No	No				
Knee	acute infection	Yes	Yes	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*		*Pseudomonas aeruginosa*
Knee	acute infection	Yes	No	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*	
Hip	chronic infection	Yes	No	*Corynebakterium spp.*	*Staphlococcus epidermidis*		
Knee	acute infection	Yes	No	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*
Knee	chronic infection	Yes	No	*Streptococcus agalacticae*	*Streptococcus agalacticae*	*Streptococcus agalacticae*	*Streptococcus agalacticae*
Hip	chronic infection	Yes	Yes		*Staphylococcus aureus*		
Knee	chronic infection	Yes	No	*Staphlococcus epidermidis*			
Knee	chronic infection	Yes	Yes	*Staphlococcu epidermidis*	*Staphlococcus epidermidis*		
Hip	chronic infection	Yes	No	*Staphlococcus epidermidis*	*Staphlococcus epidermidis*		
Knee	chronic infection	Yes	No	*Staphlococcus epidermidis*		*Coagulase-negative Staphylococci*	*Coagulase-negative Staphylococci*
Hip	chronic infection	Yes	No	*Staphylococcus aureus*		*Staphylococcus aureus*	
Knee	acute infection	Yes	No	*Staphlococcus epidermidis*	*Staphlococcus epidermidis*	*Coagulase-negative Staphylococci*	*Coagulase-negative Staphylococci*
Hip	acute infection	Yes	No		*Staphlococcus epidermidis*		*Coagulase-negative*

							Staphylococci
Hip	chronic infection	Yes	No	*Staphlococcus epidermidis*	*Staphlococcus epidermidis*		
Hip	acute infection	Yes	No		*Staphlococcus epidermidis*	*Coagulase-negative Staphylococci*	
Knee	chronic infection	Yes	No	*Staphylococcus aureus*	*Staphylococcus aureus*		*Staphylococcus aureus*
Hip	chronic infection	Yes	No	*Enterococcus faecialis*	*Enterococcus faecialis*	*Enterococcus spp.*	*Enterococcus spp.*
Knee	chronic infection	Yes	Yes	*Enterocuccus faecalis*	*Enterocuccus faecalis*	*Enterococcus spp.*	*Enterococcus spp.*

Table 1: Patient details and detected pathogens. Tabular results of the patient details, including the cause of revision surgery, the MSIS criteria matching and the detected pathogens in conventional microbiology and NAAT analysis.

Criteria	PCR Sonicate	PCR Aspirate	Pooled PCR
P value	0.0036	0.0013	0.0001
Sensitivity	50.0%	55.6%	66.7%
Specificity	100.0%	100.0%	100.0%
Positive Predictive Value	100.0%	100.0%	100.0%
Negative Predictive Value	59.1%	61.9%	68.4%

Table 2: Results of microbiological methods. Tabular results of the evaluation of the PCR diagnostic of sonication fluid, joint aspirate and pooled PCR. N = 31 specimen for aspirate and sonication, respectively, n = 62 for the pooled PCR data.

Discussion

Foreign body infection is an emerging problem in orthopedic and trauma surgery with costly treatment, long hospitalization and functional deficiency of the affected joints. The differential diagnostic is challenging. Many researchers and clinicians are giving attention to this topic, striving to find more precise and reliable methods to diagnose foreign body associated infections. To date, many different diagnostic tools are being evaluated and implemented in the diagnostic path[32].

The sonication procedure is an invaluable method, showing a better sensitivity than conventional cultures from tissue specimens[6]. In our study, we showed that sonication fluid cultures have a sensitivity of 88.9% with a specificity of 61.5%. Conventional cultures from tissue specimens and joint aspirates both had a sensitivity of 66.7% with a specificity of 82.3% and 84.6%, respectively. Other researchers show similar results for these conventional microbiological procedures[6,33,34,35]. It is often criticized that sonication procedure is prone to contamination, so special care must be taken in the handling of the samples.

The possibility of detecting DNA of a causative pathogen in tissue specimens or fluids is intriguing. NAT is a rapid procedure which can deliver results within a couple of hours, as compared to the time-consuming microbiological culture methods. Without doubt, NAT has a good sensitivity in detecting pathogens, but hold the risk of detecting contaminants, thus lacking specificity[36]. The great benefit of this PCR technique in diagnosing PJI was documented especially in patients who received antibiotic therapy close to surgery or for a longer period[7,8]. Studies suggest that NAT of sonication fluid may further increase sensitivity and specificity[37]. Unfortunately, this technique is not routinely available in laboratories, due to its time-consuming workflow.

There are certain limitations to the PCR technique in general: NAT detects DNA with no differentiation between viable and non-viable bacteria, making interpretation of the results difficult. Broad-range PCR will only detect ribosomal 16S ribonucleic acid (16S rRNA), not differentiating between pathogens[37]. More specific systems do not routinely detect gene-encoded antibiotic resistance markers. A targeted antibiotic therapy may therefore be limited without further susceptibility testing.

The system applied in this study overcomes some of these limitations, differentiating between specific bacteria and identifying gene-encoded resistance markers. Overall, NAT assays may be considered as a fast and useful complementation to confirm PJI[7,8,9,38].

It is necessary to plan ahead in joint aspiration and in surgery: Sample containers must be sterile and ready, and prompt sample transport must be available. Surgeons should brief their microbiologists on planned procedures and what sample material to expect. When performing the joint aspiration, strictly sterile conditions must be ensured, to prevent iatrogenic infection of the joint. In adipose patients, joint puncture can be challenging, and fluoroscopic guidance can be helpful. Revision arthroplasty surgery requires a very high level of expertise, and should only be performed by a skilled surgeon. Explanted material should not be kept in the operating room any longer than necessary, but be sent to the microbiologist as soon as possible. A very critical part within this protocol is the potential risk of contamination. Not only while gathering the samples, but also while handling the samples in the microbiology lab, all personnel involved (orthopedic surgeon, nurses, technicians, microbiologists) must work quickly and accurately, and have proper training in the procedures.

Sonication and NAT are valuable tools in diagnosing implant-associated infections. Nevertheless, the results should always be questioned carefully. We recommend discussing the results in a round table discussion of orthopedic surgeons, microbiologists and infectious disease specialists, and pathologists to agree on the individualized therapeutic strategy.

To implement this technique in the diagnostic path of PJI can have several advantages. It is a rapid diagnostic with a result within hours. Due to the analysis of several gene encoded resistance markers, a targeted antibiotic therapy can take place at a very early stage in the clinical course. As a side effect, broad-range antibiotics can be saved for the indications where they are truly needed. Other sample types (*e.g.* tissue specimens, swabs, hematoma, *etc.*) can also be investigated according to our protocol. Since the PCR cartridge is a closed system, no troubleshooting is necessary or possible on the user side. Any adaptation of the protocol must be implemented by the manufacturer. Changes in both software and hardware (cartridge) are continuously made by the manufacturer to enhance the system, however no details are made public on the exact changes in newer versions.

Disclosures

The authors have nothing to disclose.

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References

1. Wimmer, M. D., *et al.* Evaluation of an interdisciplinary therapy algorithm in patients with prosthetic joint infections. *Int Orthop* **37** (11), 2271-2278 (2013).
2. Trampuz, A., *et al.* Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. *Am J Med* **117** (8), 556-562 (2004).
3. Randau, T. M., *et al.* Interleukin-6 in serum and in synovial fluid enhances the differentiation between periprosthetic joint infection and aseptic loosening. *PLoS One* **9** (2), e89045 (2014).
4. Deirmengian, C. A., Wongworawat, M. D. Editor's spotlight/take 5: diagnosing periprosthetic joint infection: has the era of the biomarker arrived? *Clin Orthop Relat Res.* **472** (111), 3250-3253 (2014).
5. Gollwitzer, H., *et al.* Antimicrobial peptides and proinflammatory cytokines in periprosthetic joint infection. *J Bone Joint Surg Am* **95** (7), 644-651 (2013).
6. Trampuz, A., *et al.* Sonication of removed hip and knee prostheses for diagnosis of infection. *N Engl J Med* **357** (7), 654-663 (2007).
7. Achermann, Y., Vogt, M., Leunig, M., Wust, J., Trampuz, A. Improved diagnosis of periprosthetic joint infection by multiplex PCR of sonication fluid from removed implants. *J Clin Microbiol*. **48** (40), 1208-1214 (2010).
8. Portillo, M. E., *et al.* Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure. *J Infect* **65** (6), 541-548 (2012).
9. Qu, X., *et al.* PCR-based diagnosis of prosthetic joint infection. *J Clin Microbiol* **51** (8), 2742-2746 (2013).
10. Borde, J. P., *et al.* Diagnosis of prosthetic joint infections using UMD-Universal Kit and the automated multiplex-PCR Unyvero i60 ITI((R)) cartridge system: a pilot study. *Infection* **43** (5), 551-560 (2015).
11. Hischebeth, G. T., *et al.* Unyvero i60 implant and tissue infection (ITI) multiplex PCR system in diagnosing periprosthetic joint infection. *J Microbiol Methods* **121**, 27-32 (2016).
12. Prieto-Borja, L., *et al.* Evaluation of a commercial multiplex PCR (Unyvero i60(R)) designed for the diagnosis of bone and joint infections using prosthetic-joint sonication. *Enferm Infecc Microbiol Clin.* (2016).
13. Bernau, A., Heeg, P. [Intraarticular punctures and injections: indications--prevention of infection--technique--complications]. *Orthopade*. **32** (6), 548-569; quiz 569-570 (2003).
14. Courtney, P., Doherty, M. Joint aspiration and injection and synovial fluid analysis. *Best Pract Res Clin Rheumatol*. **27** (2), 137-169 (2013).
15. Arnold, S., Meurer, A. [Intra-articular punctures and injections]. *Orthopade*. **42** (12), 1075-1086 (2013).
16. Geller, J. A., *et al.* Prospective Comparison of Blood Culture Bottles and Conventional Swabs for Microbial Identification of Suspected Periprosthetic Joint Infection. *J Arthroplasty* **31** (8), 1779-1783 (2016).
17. Cross, M. C., *et al.* Utility of percutaneous joint aspiration and synovial biopsy in identifying culture-positive infected hip arthroplasty. *Skeletal Radiol* **43** (2), 165-168 (2014).
18. Berry, D. J. *Revision total hip and knee arthroplasty.* Wolters Kluwer Health/Lippincott Williams & Wilkins. Philadelphia. (2012).
19. Parvizi, J., Gehrke, T., International Consensus Group on Periprosthetic Joint, I. Definition of periprosthetic joint infection. *J Arthroplasty.* **29** (7), 1331 (2014).
20. Ghanem, E., *et al.* Perioperative antibiotics should not be withheld in proven cases of periprosthetic infection. *Clin Orthop Relat Res.* **461**, 44-47 (2007).
21. Wirtz, D. C. *AE-Manual der Endoprothetik - Knie.* Springer. (2011).
22. Claes, L. K., Peter; Perka, Carsten; Rudert, Maximilian. *AE-Manual der Endoprothetik - Hüfte und Hüftrevision.* Springer. (2012).
23. Herrmann, P., Thoele, P., Heppert, V. [Infected knee prostheses. Part 2: chronic late infections]. *Oper Orthop Traumatol*. **25** (3), 242-250 (2013).
24. Zweymuller, K. A., Steindl, M., Melmer, T. Anterior windowing of the femur diaphysis for cement removal in revision surgery. *Clin Orthop Relat Res.* **441**, 227-236 [pii] (2005).
25. Osmon, D. R. Microbiology and Antimicrobial Challenges of Prosthetic Joint Infection. *J Am Acad Orthop Surg.* **25 Suppl 1**, S17-S19 (2017).
26. Renner, L., Perka, C., Trampuz, A., Renz, N. [Treatment of periprosthetic infections]. *Chirurg*. **87** (10), 831-838 (2016).
27. Kuzyk, P. R., *et al.* Two-stage revision arthroplasty for management of chronic periprosthetic hip and knee infection: techniques, controversies, and outcomes. *J Am Acad Orthop Surg*. **22** (3), 153-164 (2014).

28. Kobayashi, N., Bauer, T. W., Tuohy, M. J., Fujishiro, T., Procop, G. W. Brief ultrasonication improves detection of biofilm-formative bacteria around a metal implant. *Clin Orthop Relat Res*. **457**, 210-213 (2007).
29. Dubois, D., *et al.* Performances of the Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for rapid identification of bacteria in routine clinical microbiology. *J Clin Microbiol*. **50** (8), 2568-2576 (2012).
30. Cherkaoui, A., *et al.* Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. *J Clin Microbiol*. **48** (4), 1169-1175 (2010).
31. Barenfanger, J., Drake, C., Kacich, G. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. *J Clin Microbiol*. **37** (5), 1415-1418 (1999).
32. Tande, A. J., Patel, R. Prosthetic joint infection. *Clin Microbiol Rev.* **27** (2), 302-345 (2014).
33. Portillo, M. E., *et al.* Advantages of sonication fluid culture for the diagnosis of prosthetic joint infection. *J Infect*. **69** (1), 35-41 (2014).
34. Scorzolini, L., *et al.* Sonication technique improves microbiological diagnosis in patients treated with antibiotics before surgery for prosthetic joint infections. *New Microbiol.* **37**, 321-328 (2014).
35. Zhai, Z., *et al.* Meta-analysis of sonication fluid samples from prosthetic components for diagnosis of infection after total joint arthroplasty. *J Clin Microbiol*. **52** (5), 1730-1736 (2014).
36. Panousis, K., *et al.* Poor predictive value of broad-range PCR for the detection of arthroplasty infection in 92 cases. *Acta Orthop.* **76** (3), 341-346 (2005).
37. Rak, M., Kavclc, M., Trebse, R., Co, R. A. Detection of bacteria with molecular methods in prosthetic joint infection: sonication fluid better than periprosthetic tissue. *Acta Orthop*. **87** (4), 339-345 (2016).
38. Li, Z., Yu, A. Diagnostic value of a PCR-based technique for prosthetic joint infection. *J Clin Microbiol*. **52** (6), 2281-2282 (2014).