Despite different criteria to diagnose a prosthetic joint infection (PJI), aetiological diagnosis of the causing microorganism remains essential to guide treatment.

Molecular-biology-based PJI diagnosis is progressing (faster, higher specificity) in different techniques, from the experimental laboratory into clinical use.

Multiplex polymerase chain reaction techniques (custom-made or commercial) provide satisfactory results in clinical series of cases, with specificity close to 100% and sensitivity over 70–80%.

Next-generation metagenomics may increase sensitivity while maintaining high specificity.

Molecular biology techniques may represent, in the next five years, a significant transformation of the currently available microbiological diagnosis in PJI.

Keywords: microbiological cultures; molecular diagnosis of PJI; prosthetic joint infection (PJI)

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Introduction

Orthopaedic prosthetic joint infection (PJI) is a specific type of infection related to joint replacement and associated with biofilm formation on the surface of the inert implant. With an overall incidence between 1% and 5% depending on the joint, PJI is a complex entity that differs from bone and joint infections because the colonized implant becomes a persistent reservoir of microorganisms, increasing the difficulty to successfully diagnose and treat the infection.1

Microbiological analysis is currently the most reliable tool to orient PJI treatment. The etiological diagnosis, based on microbiology, leads to the specific antibiotic treatment, which is the most important coadjuvant treatment to surgery in PJI since the early days of implant arthroplasty in orthopaedics. More than 40 years ago, problems in the diagnosis of PJI were already recognized.2,3 Particularly, adequate sampling and processing was praised as a method to obtain the most effective diagnosis,4 while Gram staining, microbiological cultures and histopathology were the most recognized diagnostic techniques.5

Bacterial colonization of orthopaedic implants was also identified as a major challenge in the prevention, diagnosis and treatment of PJI. The ‘race for the surface’ and the recognized pathophysiological role of the biofilm were fundamental steps towards today’s conception of PJI.6

The prominent role of microorganism identification to confirm infection also prevails in PJI, and different diagnosis guidelines have been discussed and proposed7–10 by different organizations (Infectious Diseases Society of America – IDSA; Musculoskeletal Infection Society – MSIS; International Consensus Meeting of Philadelphia – ICM; European Bone and Joint Infection Society – EBJIS). Widely accepted major criteria of PJI include sinus tract communicating with the prosthesis or the identification of the same microorganisms isolated from two or more cultures, although some differences are found in the minor criteria, under continuous revision and improvement.9

Even if repeated twice to confirm, conventional culture techniques have limitations in terms of identifying microorganisms in prosthetic infection. False negatives (negative cultures) range from 5% to 42% of PJI, as seen in a recent systematic review,11 due to sampling, prior antibiotics, insufficient culture time, chronic infections and probably other unknown causes,11–13 jeopardizing the diagnosis and treatment of PJI. But cultures and diagnostic tests also produce false positives, detecting contaminant and secondary microorganisms or indirect infection signs that may not guide the most adequate treatment. Sampling may compromise the primary causing microorganism and alter the priorities to adequately treat each case. Without an appropriate aetiological diagnosis identifying the causing microorganism in PJI, the adequate treatment may not be established, including timely surgery and a precisely oriented antibiotic chemotherapy. Sensitivity and specificity have substantially improved, particularly...
with sonicate fluid after centrifugation\textsuperscript{14} and samples in blood culture bottles.\textsuperscript{15} However, the inherent delays of these diagnostic techniques still suppose a limitation to accurately guide decisions on antibiotics and surgery for an appropriate management of these patients. As the diagnosis of the microorganism causing PJI is frequently delayed and incomplete, with variable false negatives that limit the treatment orientation and its efficacy, this review will focus on molecular diagnosis methods claimed to potentially improve this aetiopathological diagnosis.

From culture-based methods to new strategies

Almost all microorganisms can be the cause of PJI. Despite Gram-positive bacteria (especially \textit{Staphylococci}) being the most frequently isolated organisms, an increase of infections caused by Gram-negative has been described in recent years.\textsuperscript{16,17} Another special concern today is the growing development of antimicrobial resistance among these organisms, definitively impacting on the selection of antibiotics or treatment strategies, but also compromising the outcome.\textsuperscript{18,19} Furthermore, polymicrobial infection, often underdiagnosed because of technical limitations and competing growth in cultures, poses a supplementary problem in understanding the course of some recalcitrant infections. The presence of biofilm as an essential pathogenic factor is also an important issue for the selection of microbiological diagnostic methods.

To diagnose biofilm-related infections, novel techniques have been proposed to improve bacteria detection. These include modified culturing techniques (from samples obtained through sonication or other methods), visualization of biofilm through different microscopy techniques (basically for experimental studies), and finally, molecular diagnosis (Table 1). Some of these sophisticated techniques mostly rely on experimental data, and thus are not, or may not be, appropriate for standard clinical application. Others are just not well-known or have not been broadly introduced into hospitals because of logistics or organizational issues. New techniques have direct associated costs that may limit their expansion, although in the medium term, increased diagnostic accuracy will probably prove cost-effective if it helps to avoid direct and indirect extra costs due to overtreatment when the specific infection is not ascertained, or delayed treatment when infection is not detected. As effectiveness is further improved and knowledge about the clinical meaning increases, these novel techniques will spread and cost-benefit analysis will probably confirm their interest.\textsuperscript{20} A better understanding of those that can be clinically applied may offer the clinician more grounds to decide what is to be expected from new diagnostic techniques and eventually decide on their application.

| Table 1. Techniques applied in prosthetic joint infection novel diagnostic strategies |
|-------------------------------------------------|
| Cultures\textsuperscript{21–26} |
| – Culturing after sonication/rinsing |
| – Culturing in blood-culture enriched media |
| Imaging techniques\textsuperscript{27–29} |
| – Fluorescent in situ hybridization (FISH) |
| – Confocal laser scanning microscopy (CLSM) |
| – Scanning electron microscopy (SEM) |
| Biomolecular techniques\textsuperscript{30–36} |
| – PCR-based methods, including multiplex PCR and DNA microarrays |
| – Electrospray ionization (ESI-TOF) and matrix-assisted laser desorption ionization (MALDI-TOF) time of flight mass spectrometry |
| – Fourier transformed near infrared (FT-NIR) spectroscopy |
| – Next-generation sequencing (NGS) based on shotgun metagenomics |

\textit{Note.} PCR, polymerase chain reaction.

\textbf{Biomolecular techniques}

The use of molecular biology techniques, perhaps the most important advance for decades in microbiological diagnosis, is quickly spreading for PJI diagnosis, as in other areas of clinical microbiology and particularly in virology. Ideally, all infections could be detected with the highest sensitivity and specificity of these techniques, but the reality is not so clearly positive. Current polymerase chain reaction (PCR)-based techniques detect traces of microorganism nucleic acids. When applied in sonicate fluid,\textsuperscript{33,34} these techniques have shown notable effectiveness in diagnosing the infective microorganisms from biofilm, with higher sensitivity than PCR from periprosthetic tissue,\textsuperscript{37} but mostly under experimental conditions. Two different approaches (custom-made or commercial) can be described.

\textbf{Custom-made PCR}

Custom-made methodologies, based mainly in 16S rDNA amplification and sequencing, have high sensitivity and specificity,\textsuperscript{38,39} and even high reproducibility is described in some reports.\textsuperscript{40,41} These latter studies are extremely important, because a well-known claimed limitation of this technology is the lack of reproducibility. In the study by Plouzeau et al\textsuperscript{40,41} a control was submitted to different laboratories that have previously published the multicentre study.\textsuperscript{40,41} The results of reproducibility showed that, in well-trained experienced laboratories, this approach could be very useful and reproducible. Moreover, because this method could identify almost all existing bacteria, it has a very high potential for diagnosis. However, a high-quality molecular biology laboratory with experimented technicains is needed, and most medium and small-sized hospitals may not encompass these facilities. Besides, in case of polymicrobial infections, a custom-made technique
could have problems identifying different pathogens. And finally, the arrival of commercial multiplex PCR has limited the use of custom-made techniques, although commercial kits initially conceived for blood-borne microorganisms have also been adapted for PJI and published from many laboratories (Table 2).

**Commercial multiplex PCR**

Commercial techniques may be more robust, and do not require special infrastructures. Table 2 offers a comparison of studies with commercially available multiplex PCR kits. Early studies were based on customized kits already designed for the identification of microorganisms isolated from blood culture bottles, such as SeptiFast™ (Roche, Switzerland), GenoType™ (Hain, Germany), Xpert™ (Cepheid, USA), or Filmarray™ (Biofire, USA).33,42,43,45,55 These include most of the microorganisms causing PJI. Despite relatively good results (high specificity in all cases), they are not used in most laboratories beyond the experimental studies. The reasons for this may include the cost, logistics for more cumbersome procedures, worries about the clinical significance for diagnosis, or even concerns about false positives. Recently, a commercial test was especially designed for the diagnosis of bone and joint infections (Unyvero i60 ITI (Curetis AG, Germany),1 Mobidiag (Mobidiag, Finland).2

| Reference | Kits in use (bone and joint infection specific††, or adapted ††) | Type of samples for PCR | Patients total and PJI | Sensitivity | Specificity | PPV | NPV |
|-----------|---------------------------------------------------------------|------------------------|-----------------------|-------------|------------|-----|-----|
| Esteban et al43 | Adapted4 | Sonicate fluid | 126 pt (47 PJI) | 71.6 | 81.9 | 74.3 | 79.7 |
| Achermann et al42 | Adapted4 | Sonicate fluid | 47 pt (37 PJI) | 78.4 | 100.0 | 100.0 | 55.5 |
| Portillo et al44 | Adapted4 | Sonicate fluid | 86 pt (24 PJI) | 96.0 | 100.0 | 100.0 | 98.4 |
| Metso et al45 | Specific4 | Synovial fluid, tissue | 81 pt (38 PJI) | 81.6 | 100.0 | 100.0 | 74.1 |
| Vasco et al46 | Adapted4 | Sonicate fluid | 216 pt (98 PJI) | 53.0 | 99.0 | — | — |
| Borde et al46 | Specific4 | Tissue | 28 pt (7 PJI) | 42.8 | 95.2 | 75.0 | 80.0 |
| Hischebeth et al47 | Specific4 | Sonicate, synovial fluid | 31 pt (18 PJI) | 66.7 | 100.0 | 100.0 | 68.4 |
| Renz et al48 | Specific4 | Sonicate fluid, tissue | 111 pt (78 PJI) | 53.3 | 94.0 | 95.0 | 47.0 |
| Prieto-Borja et al49 | Specific4 | Sonicate fluid | 68 pt (29 PJI) | 60.5 | 98.0 | 95.8 | 76.6 |
| Mandalain et al50 | Specific4 | Tissue, synovial fluid | 239 pt | 49.1 | 99.4 | 99.3 | 51.5 |
| Morenstern et al51 | Specific4 | Synovial fluid | 142 pt (77 PJI) | 65.8 | 92.1 | 91.2 | 65.2 |
| Renz et al52 | Specific4 | Tissue, sonicate, synovial fluid | 51 pt (38 PJI) | 77.0 | 92.0 | 96.0 | 60.0 |
| Sigmund et al53 | Specific4 | Tissue, sonicate, synovial fluid | 90 pt (38 PJI) | 71.1 | 96.2 | 93.1 | 82.0 |
| Suren et al54 | Specific4 | Synovial fluid | 26 pt (15 PJI) | 78.6 | 100.0 | 91.7 | 84.6 |

*Note: PCR, polymerase chain reaction; PJI, prosthetic joint infection; PPV, positive predictive value; NPV, negative predictive value.
†Bone and joint specific kits used in these studies: Unyvero i60 ITI (Curetis AG, Germany),1 Mobidiag (Mobidiag, Finland).2
††Adapted (general kits initially conceived for blood-borne microorganisms), used in these studies for PJI: SeptiFast™ (Roche, Switzerland),3 GenoType™ (Hain, Germany),4 or Filmarray™ (Biofire, USA).5
*81 pt (only 38 confirmed PJI, only 20 confirmed controls, six false positive PCR in non-confirmed PJI, no false positives in controls).
**53% overall sensitivity, improved to 58% when considering only microorganisms included in the panel (non-specific test).
Employed on periprosthetic tissue, this method has shown higher sensitivity and specificity than microbial cultures (95% to 72%, and 90% to 77%). However, optimization of the whole process is necessary to implement its use, and would be of great interest if the process could be shortened to just some minutes after starting sequencing. In another study, shotgun metagenomics could diagnose usual pathogens in 43% of culture-negative PJI, with a percentage of positive samples in non-infected patients of 3.6%, even with the use of a threshold. Finally, this technology has been employed in shoulder surgery, where infections usually have a different pattern than in knee or hip prosthesis. They obtained also good results, but detected a higher number of polymicrobial infections whose clinical meaning needs further evaluation.

The authors of these studies expressed their concerns about the potential contaminants, because even unculturable, unviable pathogens may be detected, and a strict methodology is recommended to avoid these undesired results. However, the detection of these pathogens cannot be considered automatically as a contaminant, and the potential existence of a ‘synovial microbiome’ opens many questions that need further research. In this sense, a recent study showed that antibiotic therapy guided by the results of metagenomic next-generation sequencing was associated with a similar outcome to empirical therapy, with fewer undesired side effects.

Implementing new diagnostic techniques in clinical practice

Only limited evidence is available from level I and II diagnostic clinical studies regarding novel techniques to diagnose prosthetic joint biofilm infection. The use of PCR-based molecular biology methods poses questions regarding not only how many samples but also what an appropriate sample is. This remains to be clarified, as samples are obtained usually from synovial fluid, sonicate fluid after implant removal, and tissues where microorganisms are suspected to be present. Considering the high cost of PCR and the required time to deliver results for a large battery of potential pathogens, sample and patient selection is essential. Patients with negative cultures are those who immediately benefit from PCR, but the high specificity of PCR may also enable diagnosis from isolates with doubtful significance. The new metagenomics methodology is a step forward, so far experimental, that still requires understanding the relevance of all the microorganisms detected in a single sample. But a definite gap in these techniques is the time to obtain accurate information, currently unavailable within operative time. Technical progress will hopefully solve these issues.

As identification of microorganisms in periprosthetic tissue samples is enhanced through molecular biology techniques, despite potential low bacterial load, different techniques are progressing towards higher sensitivity and specificity. Intraoperative samples inoculated in blood culture bottles allowed an increased identification of bacterial reads, although the need for a positive blood culture may slow the diagnosis. Of considerable interest is the clinically relevant preoperative diagnosis. Some techniques proved high sensitivity and specificity not only in periprosthetic tissue samples but also in synovial fluids that can be preoperatively analysed. This may open an interesting approach for a presurgical diagnosis of these patients.

Many questions still need to be solved in clinical practice. First of all, how to obtain the sample is key. Clinical suspicion during surgery leading to adequate sample collection may be determinant. Increased accuracy in synovial fluid sampling has been obtained when guided through computerized tomography (CT), particularly when combined with image findings. Percutaneous synovial biopsy is a good alternative to synovial fluid samples, although its real value is still debated. Periprosthetic surgical biopsies prior to index surgery appear a reasonable alternative to improve sensitivity, although this supplementary surgical procedure adds considerable burden to the case, because a revision procedure will be required anyhow.

Other problems about molecular diagnosis still to be solved include the meaning of all detected organisms, the necessity to treat and what organisms must be treated. Moreover, this diagnosis requires standardization, highly prepared laboratories, specialized personnel, adequate surgical sampling and planned workflows for samples from the operating rooms. All these issues may be difficult to implement in the current routine of the hospital, and specifically in a clinical microbiology laboratory. Cost containment, above the costs of unsolved prosthetic joint infection, may not justify the barriers to spread the technology. However, confidence in the techniques and adequate training may be required to spread in clinical diagnosis. In the near future, probably all these questions can be answered and molecular-biology-based technologies may be added to the available microbiological tools for the diagnosis of PJI. Further research, especially aimed to avoid the contamination of samples and the implementation of standardized thresholds, will be necessary prior to its wide use in clinical laboratories. The evaluation of this methodology under routine conditions will be also of extreme importance.

Variability of the microorganisms and patients are difficult barriers in the precise diagnosis of PJI. Multicentric studies are probably required to standardize new techniques and diagnostic protocols in clinical routine.
Sampling protocols, understanding the best sensitivity for each technique, may reinforce the need of surgical standardization in the microbiological sampling. Comparing the effectiveness of each sampling (fluid or solid, from implant or tissue, preoperative and intraoperative) may help to establish these protocols. The specific request of a technique for a specific sample may expedite intraoperative diagnosis, while other samples (including the retrieved implant) may help to confirm, validate or reorient the associated treatment.

Future research

Classic microbiological culture alone has probably reached its maximum effectiveness. An adequate combination of available technologies, implemented in a high number of hospitals, will increase our protocol experience to complete inter-centre comparisons and to develop multicentric studies. This evidence-based methodology is obviously slow, but will develop the next gold standard.

The combination of adequately prioritized and evaluated novel techniques will improve PJi diagnosis in the next five years. Both biofilm models and surgical sampling studies on biofilm developed on prosthesis will facilitate the earlier isolation of biofilm-forming microorganisms, guiding new and established treatment options. Molecular biology seems well placed for the future. Its high specificity unfortunately involves high costs and time, barriers to clinical routine and intraoperative use. But this technology is rapidly evolving. A new multiplex PCR assay under evaluation, based on the cartridge technology, may give results in one hour (within intraoperative time frame). Good preliminary results for the microorganisms included in the kit are already being shown. Moreover, shotgun metagenomics are experiencing impressive advances and probably could be the next tool to be added to the microbiology lab. Other experimental molecular tools, such as electrospay ionization time of flight mass spectrometry (ESI-TOF) or Fourier transformed near infrared (FT-NIR) spectroscopy have been also used for the experimental diagnosis of these infections, although these are still not ready to access the clinical diagnosis. On the contrary, matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF) has become an essential tool for bacterial and fungal identification in isolates from cultures, but not from direct clinical samples. Expected technical advances, particularly when PCR has become a popular word outside laboratories, may offer significant opportunities in these next years.

However, with the increasing number of techniques, a closer relationship between clinicians and microbiologists is still the best approach in the final aim that has not changed for decades: to cure orthopaedic patients with prosthetic infections.

**REFERENCES**

1. Gomez-Barrena E. Infection. In: Blom AW, Warwick D, Whitehouse MR, eds. Apley & Solomon’s system of orthopaedics and trauma. 10th ed. Boca Raton, FL: CRC Press-Taylor & Francis Group, 2018:31–63.
2. Petty W, Bryan RS, Coventry MB, Peterson LF. Infection after total knee arthroplasty. Orthop Clin North Am 1975;6:1115–1128.
3. Wilson PD Jr, Salvati EA, Aglietti P, Kutner LJ. The problem of infection in endoprosthetic surgery of the hip joint. Clin Orthop Relat Res 1973;86:213–221.
4. Washington JA II. The microbiology of musculoskeletal infection. Orthop Clin North Am 1975;6:1115–1128.
5. Brause BD. Infected total knee replacement: diagnostic, therapeutic, and prophylactic considerations. Orthop Clin North Am 1982;13:245–249.
6. Cristina AG, Costerton JW. Bacterial adherence to biomaterials and tissue: the significance of its role in clinical sepsis. J Bone Joint Surg Am 1985;67:264–273.
7. Osmon DR, Berbari EF, Berendt AR, et al. Infectious Diseases Society of America. Executive summary: diagnosis and management of prosthetic joint infection:
clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 2013;56:1–10.

8. Parvizi J, Zmistowski B, Berbari EF, et al. New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. Clin Orthop Relat Res 2011;469:2992–2994.

9. Parvizi J, Tan TL, Goswami K, et al. The 2018 definition of periprosthetic hip and knee infection: an evidence-based and validated criteria. J Arthroplasty 2018;33:1309–1314.e2.

10. Izakovitova P, Borens O, Trampuz A. Periprosthetic joint infection: current concepts and outlook. EFORT Open Rev 2019;4:482–494.

11. Kalbían I, Park JW, Goswami K, Lee YK, Parvizi J, Koo KH. Culture-negative periprosthetic joint infection: prevalence, aetiology, evaluation, recommendations, and treatment. Int Orthop 2020;44:1255–1261.

12. Yoon HK, Cho SH, Lee DY, et al. A review of the literature on culture-negative periprosthetic joint infection: epidemiology, diagnosis and treatment. Knee Surg Relat Res 2017;29:155–164.

13. Tan TL, Kheir MM, Shohat N, et al. Culture-negative periprosthetic joint infection: an update on what to expect. JBJS Open Access 2018;3:e0060.

14. Zhai Z, Li H, Qin A, et al. Prostheses for diagnosis of infection after total joint arthroplasty. Sonications of removed hip and knee joint infections: easy and fast. Use of an automated blood culture system (BD BACTEC™) for diagnosis of prosthetic joint infections. BMC Med 2014;12:233.

15. Benito N, Franco M, Ribera A, Soriano A, Rodriguez-Pardo D, Sorli I, et al. Temporal trends in the aetiology of prosthetic joint infections: a multicentre cohort study. Clin Microbiol Infect 2016;22:732–739.

16. Benito N, Mur I, Ribera A, et al. REPI (Spanish Network for Research in Infectious Disease) Group for the Study of Prosthetic Joint Infections / GEIO (Group for the Study of Osteoarticular Infections), SEIMC (Spanish Society of Infectious Diseases and Clinical Microbiology). The different microbial etiology of prosthetic joint infections according to route of acquisition and time after prosthesis implantation, including the role of multidrug-resistant organisms. J Clin Med 2019;8:E673.

17. Pfang BG, García-Cañete J, García-Lasheras J, et al. Orthopedic implant-associated infection by multidrug resistant Enterobacteriaceae. J Clin Med 2019;9:8:220.

18. Papadopoulos A, Ribera A, Mavrogenis AF, et al; ESCMID Study Group for Implant-Associated Infections (ESGIAI). Multidrug-resistant and extensively drug-resistant Gram-negative prosthetic joint infections: role of surgery and impact of colistin administration. Int J Antimicrob Agents 2019;53:294–301.

19. Romanò CL, Trentinaglia MT, De Vecchi E, et al. Cost-benefit analysis of antibiotic microbiological techniques for peri-prosthetic joint infection diagnosis. BMC Infect Dis 2018;18:154.

20. Minassian AM, Newnham R, Kalimeris E, Bejon P, Atkins BL, Bowler IC. Use of an automated blood culture system (BD BACTEC™) for diagnosis of prosthetic joint infections: easy and fast. BMC Infect Dis 2014;14:233.

21. Shen H, Tang J, Wang Q, Jiang Y, Zhang X. Sonication of explanted prosthesis combined with incubation in BD bactec bottles for pathogen-based diagnosis of prosthetic joint infection. J Clin Microbiol 2015;53:777–781.

22. Trampuz A, Piper KE, Jacobson MJ, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 2007;357:654–663.

23. Bémer P, Plouzeau C, Tande D, et al. Centre de Référence des Infections Ostéo-articulaires du Grand Ouest (CROGO) Study Team. Evaluation of 16S rRNA gene PCR detection of bacteria with molecular methods from prosthetic joint infections using MALDI-TOF mass spectrometry. Int J Artif Organs 2010;33:568–574.

24. Esteban J, Gomez-Barrena E, Cordero J, Martin-de-Hijas NZ, Kinnari TJ, Fernandez-Roblas R. Evaluation of quantitative analysis of cultures from sonicated retrieved orthopedic implants in diagnosis of orthopedic infection. J Clin Microbiol 2008;46:488–492.

25. Tani S, Lepetsos P, Stylianakis A, Vlamis J, Birbas K, Kaklamanos I. Superiority of the sonication method against conventional periprosthetic tissue cultures for diagnosis of prosthetic joint infections. Eur J Orthop Surg Traumatol 2018;28:51–57.

26. Prieto-Borja L, Auxón A, Blanco A, et al. Evaluation of the use of sonication of retrieved implants for the diagnosis of prosthetic joint infection in a routine setting. Eur J Clin Microbiol Infect Dis 2018;37:715–722.

27. Birlutiu RM, Birlutiu V, Cismasiu RS, Mihalache M. bbbFISH-ing in the sonication fluid. Medicine (Baltimore) 2019;58(29):e16501.

28. Karygianni L, Hellwig E, Al-Ahmad A. Multiplex fluorescence in situ hybridization (M-FISH) and confocal laser scanning microscopy (CLSM) to analyze multispecies oral biofilms. Methods Mol Biol 2014;1147:65–72.

29. Lopes SP, Carvalho DT, Pereira MO, Azevedo NF. Discriminating typical and atypical cystic fibrosis-related bacteria by multiplex PNA-FISH. Biotechnol Bioeng 2017;114:355–367.

30. Swearingen MC, DiBartola AC, Dusane D, Granger J, Stoodley P. 16S rRNA analysis provides evidence of biofilms on all components of three infected periprosthetic knees including permanent branded -ure. Pathog Dis 2016;74:fxn083.

31. Jacovides Cl, Kreft R, Adeli B, Hozack B, Ehrlich GD, Parvizi J. Successful identification of pathogens by polymerase chain reaction (PCR)-based electron spray ionization time-of-flight mass spectrometry (ESI-TOF-MS) in culture-negative periprosthetic joint infection. J Bone Joint Surg Am 2012;94:2247–2254.

32. Gaudreau AM, Labrie J, Goetz C, Dufour S, Jacques M. Evaluation of MALDI-TOF mass spectrometry for the identification of bacteria growing as biofilms. J Microbiol Methods 2018;145:79–81.

33. Esteban J, Alonso-Rodríguez N, del-Prado G, et al. PCR-hybridization after sonication improves diagnosis of implant-related infection. Acta Orthop 2012;83:299–304.

34. Rak M, Kavčič M, Trebše R, Gör A. Detection of bacteria with molecular methods in prosthetic joint infection: sonication fluid better than periprosthetic tissue. Acta Orthop 2016;87:339–345.

35. Tidwell JE, Dawson-Andoh B, Adedipe EO, Nkansah K, Dietz MJ. Can near-infrared spectroscopy detect and differentiate implant-associated biofilms? Clin Orthop Relat Res 2015;473:3658–3666.

36. Harris LG, El-Bouri K, Johnston S, et al. Rapid identification of staphylococci from prosthetic joint infections using MALDI-TOF mass-spectrometry. Int J Antimicrob Agents 2010;33:486–491.

37. Karygianni L, Hellwig E, Al-Ahmad A. Multiplex fluorescence in situ hybridization (M-FISH) and confocal laser scanning microscopy (CLSM) to analyze multispecies oral biofilms. Methods Mol Biol 2014;1147:65–72.

38. Marín M, García-lechuz JM, Alonso P, et al. Evaluation of MALDI-TOF mass spectrometry for the identification of bacteria growing as biofilms. J Microbiol Methods 2018;145:79–81.

39. Esteban J, Alonso-Rodríguez N, del-Prado G, et al. PCR-hybridization after sonication improves diagnosis of implant-related infection. Acta Orthop 2012;83:299–304.

40. Rak M, Kavčič M, Trebše R, Gör A. Detection of bacteria with molecular methods in prosthetic joint infection: sonication fluid better than periprosthetic tissue. Acta Orthop 2016;87:339–345.

41. Tidwell JE, Dawson-Andoh B, Adedipe EO, Nkansah K, Dietz MJ. Can near-infrared spectroscopy detect and differentiate implant-associated biofilms? Clin Orthop Relat Res 2015;473:3658–3666.

42. Harris LG, El-Bouri K, Johnston S, et al. Rapid identification of staphylococci from prosthetic joint infections using MALDI-TOF mass-spectrometry. Int J Antimicrob Agents 2010;33:486–491.
sensitivity and specificity for diagnosis of prosthetic joint infection: a prospective multicenter cross-sectional study. *J Clin Microbiol* 2014;52:3583–3589.

41. Plouzeau C, Bémer P, Valentin AS, et al. Centre de Référence des Infections Ostéo-articulaires du Grand Ouest (CRIOGO) Study Team. First experience of a multicenter external quality assessment of molecular 16S rRNA gene detection in bone and joint infections. *J Clin Microbiol* 2015;53:419–424.

42. Achermann Y, Vogt M, Leunig M, Wüst J, Trampuz A. Improved diagnosis of periprosthetic joint infection by multiplex PCR of sonication fluid from removed implants. *J Clin Microbiol* 2019;58:1206–1214.

43. Portillo ME, Salvador M, Sorli L, et al. Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure. *J Infect* 2012;65:541–548.

44. Metso L, Mäki M, Tissari P, et al. Efficacy of a novel PCR- and microarray-based method in diagnosis of a prosthetic joint infection. *Acta Orthop* 2014;85:165–170.

45. Vasoo S, Cunningham MA, Greenwood-Quaintance KE, et al. Evaluation of the FilmArray Blood Culture ID Panel on biofilms dislodged from explanted arthroplasties for prosthetic joint infection diagnosis. *J Clin Microbiol* 2015;53:2790–2792.

46. Borde JP, Häcker GA, Guschl S, et al. Diagnosis of prosthetic joint infections using UMD-Universal Kit and the automated multiplex-PCR Unyvero 160 ITI(®) cartridge system: a pilot study. *Infection* 2015;43:551–560.

47. Hischebeth GTR, Gravius S, Buhr JK, Molitor E, Wimmer MD, Hoerauf A, et al. Novel diagnostics in revision arthroplasty: implant sonication and multiplex polymerase chain reaction. *J Vis Exp* 2017;130:55147.

48. Renz N, Feihl S, Cabric S, Trampuz A. Performance of automated multiplex PCR using sonication fluid for diagnosis of prosthetic joint infection: a prospective cohort. *Infection* 2017;45:877–884.

49. Prieto-Borja L, Rodriguez-Sevilla G, Auñon A, et al. Evaluation of a commercial multiplex PCR (Unyvero 160™) designed for the diagnosis of bone and joint infections using prosthetic-joint sonication. *Enferm Infecc Microbiol Clin* 2017;35:236–242.

50. Malandain D, Bemer P, Leroy AG, Leger J, Plouzeau C, Valentin AS, et al. Assessment of the automated multiplex-PCR Unyvero 160 ITI(R) cartridge system to diagnose prosthetic joint infection: a multicentre study. *Clin Microbiol Infect* 2018;24:83 ei–e6.

51. Morgenstern C, Cabric S, Perka C, Trampuz A, Renz N. Synovial fluid multiplex PCR is superior to culture for detection of low-virulent pathogens causing periprosthetic joint infection. *Diagn Microbiol Infect Dis* 2018;90:115–119.

52. Renz N, Cabric S, Morgenstern C, Schuetz MA, Trampuz A. Value of PCR in sonication fluid for the diagnosis of orthopedic hardware-associated infections: has the molecular era arrived? *Injury* 2018;49:806–811.

53. Sigmund IK, Windhager R, Sevelde F, et al. Multiplex PCR Unyvero 160 ITI application improves detection of low-virulent microorganisms in periprosthetic joint infections. *Int Orthop* 2019;43:1891–1898.

54. Suren C, Feihl S, Cabric S, et al. Improved pre-operative diagnostic accuracy for low-grade prosthetic joint infections using second-generation multiplex polymerase chain reaction on joint fluid aspirate. *Int Orthop* 2020;44:1609–1617.

55. Dubouix-Bourand A, de Ladoucette A, Pietri V, et al. Direct detection of Staphylococcus osteoarticular infections by use of Xpert MRSA/SA SSTI real-time PCR. *J Clin Microbiol* 2011;49:4225–4230.

56. Zannoli S, Sambri A, Morotti M, et al. Unyvero ITI® system for the clinical resolution of discrepancies in periprosthetic joint infection diagnosis. *Musculoskelet Surg* 2019.

57. Aamot HV, Johnsen BO, Skråmm I. Rapid diagnostics of orthopedic implant-associated infections using Unyvero ITI implant and tissue infection application is not optimal for Staphylococcus species identification. *BMC Res Notes* 2019;12:725.

58. Hischebeth GT, Randau TM, Buhr JK, et al. Unyvero 160 implant and tissue infection (ITI) multiplex PCR system in diagnosing periprosthetic joint infection. *J Microbiol Methods* 2018;121:27–32.

59. Goswami K, Parvizi J. Culture-negative periprosthetic joint infection: is there a diagnostic role for next-generation sequencing? *Expert Rev Mol Diagn* 2020;20:269–272.

60. Namdari S, Nicholson T, Abboud J, et al. Comparative study of cultures and next-generation sequencing in the diagnosis of shoulder prosthetic joint infections. *J Shoulder Elbow Surg* 2019;28:1–8.

61. Goswami K, Parvizi J, Maxwell Courtney P. Current recommendations for the diagnosis of acute and chronic PJI for hip and knee-cell counts, alpha-defensin, leukocyte esterase, next-generation sequencing. *Curr Rev Musculoskelet Med* 2018;11:428–438.

62. Tarabichi M, Alvand A, Shohat N, Goswami K, Parvizi J. Diagnosis of *Streptococcus canis* periprosthetic joint infection: the utility of next-generation sequencing. *Arthroplast Today* 2017;4:20–23.

63. Tarabichi M, Shohat N, Goswami K, Parvizi J. Can next generation sequencing play a role in detecting pathogens in synovial fluid? *Bone Joint J* 2018;100-B:127–133.

64. Tarabichi M, Shohat N, Goswami K, et al. Diagnosis of periprosthetic joint infection: the potential of next-generation sequencing. *J Bone Joint Surg Am* 2018;100:147–154.

65. Yan Q, Wi YM, Thoendel MJ, et al. Evaluation of the CosmosID bioinformatics platform for prosthetic joint: associated sonicate fluid shotgun metagenomic data analysis. *J Clin Microbiol* 2019;57:e00182-18.

66. Ivy MJ, Thoendel MJ, Jeraldo PR, et al. Direct detection and identification of prosthetic joint infection pathogens in synovial fluid by metagenomic shotgun sequencing. *J Clin Microbiol* 2018;56:e00402-18.

67. Thoendel M, Jeraldo P, Greenwood-Quaintance KE, et al. A novel prosthetic joint infection pathogen, Mycoplasma salivarium, identified by metagenomic shotgun sequencing. *Clin Infect Dis* 2017;65:332–335.

68. Sanderson ND, Street TL, Foster D, et al. Real-time analysis of nanopore-based metagenomic sequencing from infected orthopaedic devices. *BMC Genomics* 2018;19:714.

69. Street TL, Sanderson ND, Atkins BL, et al. Molecular diagnosis of orthopedic-device-related infection directly from sonication fluid by metagenomic shotgun sequencing. *Bone Joint J* 2018;100-B:134–138.

70. Cai Y, Fang X, Chen Y, et al. Detection of prosthetic joint infection pathogens using a shotgun metagenomics approach. *Clin Infect Dis* 2018;67:1333–1338.

71. Thoendel MJ, Jeraldo PR, Greenwood-Quaintance KE, et al. Identification of prosthetic joint infection pathogens using a shotgun metagenomics approach. *Bone Joint Res* 2019;8:367–377.
73. Wang C, Huang Z, Li W, Fang X, Zhang W. Can metagenomic next-generation sequencing identify the pathogens responsible for culture-negative prosthetic joint infection? BMC Infect Dis 2020;20:253.

74. Sanabria A, Hjerde E, Johannessen M, Sollid JE, Simonsen GS, Hanssen AM. Shotgun-metagenomics on positive blood culture bottles inoculated with prosthetic joint tissue: a proof of concept study. Front Microbiol 2020;11:1687.

75. Huang Z, Li W, Lee GC, et al. Metagenomic next-generation sequencing of synovial fluid demonstrates high accuracy in prosthetic joint infection diagnostics: mNGS for diagnosing PJI. Bone Joint Res 2020;9:440–449.

76. Tomas X, Bori G, Garcia S, et al. Accuracy of CT-guided joint aspiration in patients with suspected infection status post-total hip arthroplasty. Skeletal Radiol 2011;40:57–64.

77. Isern-Kebschull J, Tomas X, Garcia-Díez AI, Morata L, Ríos J, Soriano A. Accuracy of computed tomography-guided joint aspiration and computed tomography findings for prediction of infected hip prosthesis. J Arthroplasty 2019;34:1775–1782.

78. Fink B, Gebhard A, Fuerst M, Berger I, Schäfer P. High diagnostic value of synovial biopsy in periprosthetic joint infection of the hip. Clin Orthop Relat Res 2013;471:956–964.

79. Cross MC, Kransdorf MJ, Chivers FS, et al. Utility of percutaneous joint aspiration and synovial biopsy in identifying culture-positive infected hip arthroplasty. Skeletal Radiol 2014;43:165–168.

80. Fink B, Schuster P, Braun R, Tagtalianidou E, Schlumberger M. The diagnostic value of routine preliminary biopsy in diagnosing late prosthetic joint infection after hip and knee arthroplasty. Bone Joint J 2020;102-b:329–335.

81. Miller S, Chiu C, Rodino KG, Miller MB. Point-counterpoint: should we be performing metagenomic next-generation sequencing for infectious disease diagnosis in the clinical laboratory? J Clin Microbiol 2020;58:e01739-19.

82. Pons B, Jay C, Martin T, Sothier I, Savelli H, Kensingr B, et al. Identification of pathogens in synovial fluid samples with an automated multiplexed molecular detection system. San Diego, CA: IDWeek, 2018.