

# Efficacy of a novel PCR- and microarray-based method in diagnosis of a prosthetic joint infection

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**Background and purpose** — Polymerase chain reaction (PCR) methods enable detection and species identification of many pathogens. We assessed the efficacy of a new PCR and microarray-based platform for detection of bacteria in prosthetic joint infections (PJIs).

**Methods** — This prospective study involved 61 suspected PJIs in hip and knee prostheses and 20 negative controls. 142 samples were analyzed by Prove-it Bone and Joint assay. The laboratory staff conducting the Prove-it analysis were not aware of the results of microbiological culture and clinical findings. The results of the analysis were compared with diagnosis of PJIs defined according to the Musculoskeletal Infection Society (MSIS) criteria and with the results of microbiological culture.

**Results** — 38 of 61 suspected PJIs met the definition of PJI according to the MSIS criteria. Of the 38 patients, the PCR detected bacteria in 31 whereas bacterial culture was positive in 28 patients. 15 of the PJI patients were undergoing antimicrobial treatment as the samples for analysis were obtained. When antimicrobial treatment had lasted 4 days or more, PCR detected bacteria in 6 of the 9 patients, but positive cultures were noted in only 2 of the 9 patients. All PCR results for the controls were negative. Of the 61 suspected PJIs, there were false-positive PCR results in 6 cases.

**Interpretation** — The Prove-it assay was helpful in PJI diagnostics during ongoing antimicrobial treatment. Without preceding treatment with antimicrobials, PCR and microarray-based assay did not appear to give any additional information over culture.

Of the patients with a total hip or knee arthroplasty, 1–2% suffer from a prosthetic joint infection (PJI) (Blom et al. 2004, Kurtz et al. 2012). Diagnosis of a PJI is often a challenge. To successfully cure a PJI, an exact microbiological diagnosis is crucial. All the contemporary investigation methods have their own strengths and weaknesses.

Polymerase chain reaction (PCR) can be faster than the time-consuming traditional culture of bacteria. The novel Prove-it Bone and Joint assay can provide results in 6 hours, including the time required for sample preparation. The Prove-it PCR and microarray-based platform, targeted for over 60 bacterial species, has proven to be faster in identifying bacterial species in positive blood cultures than the conventional culture-based methods in sepsis diagnostics. Clinical sensitivity and specificity in blood cultures have been high, 95% and 99% (Tissari et al. 2010).

We investigated whether the novel broad-range PCR and microarray-based platform efficiently detects bacterial infections in suspected PJIs and whether it offers advantages over routine culture.

## Material and methods

### Sample collection

This was a prospective cohort study of 61 patients with suspected PJI (Table 1). All the patients in a single tertiary care hospital in Helsinki who were examined or operated because of a suspicion of PJI in their total hip or knee prosthesis and who gave their informed consent, and from whom the deep samples were successfully obtained, were recruited to this study from October 3, 2010 through December 19, 2011. The study samples were obtained by needle aspiration (40 samples for PCR) or during an operation (62 samples for PCR). At each operation, the surgeons were asked to take 5 or more tissue samples for culture and 2 tissue samples for PCR. The 29 operations were: 14 prosthesis removals (13 first operations for 2-stage exchange and 1 girdlestone), three 1-stage exchanges, and 12 debridements with implant retention.

Fever, pain, discharge from the wound, signs of a local infection, and the presence of a fistula were noted. Blood samples (for leukocyte count, erythrocyte sedimentation rate (ESR),

**Table 1.** Demographic data on 61 patients with suspected prosthetic joint infection and 20 negative controls, and on the number of samples analyzed

	Study group	Control group	p-value
Samples, n	102	40	
Patients	61	20	
Male	30	12	0.5
Female	31	8	0.5
Mean (SD) age, years	68 (12.8)	69 (8.5)	
Hips (%)	22 (36%)	12 (60%)	0.07
Knees (%)	39 (64%)	8 (40%)	0.07
No. of operations	29	20	
Mean (range) no. of			
PCR-based samples/operation	2.1 (1–4)	2.0	
culture samples/operation	5.6 (3–11)	4.3 (2–6)	
needle aspirations/patient	0.65 (0–2)	0	
No. of needle aspirations	40	0	

and C-reactive protein (CRP)) were taken and radiographs of the affected joint were obtained. Leukocyte count and percentage neutrophils were determined from synovial fluid aspirates. Data on any previous or ongoing antimicrobial treatment were collected.

The control group consisted of 20 patients in whom revision hip or knee arthroplasty was performed for aseptic, mechanical complications (Table 1). All the patients in the control group fulfilled the following criteria: (1) the indication for revision was a mechanical complication (liner wear, dislocation) or aseptic loosening in a previously well-functioning joint more than 5 years after the index operation, (2) CRP below 10 mg/L and ESR less than 30 mm/h, and (3) the orthopedic surgeon had no suspicion of an infection. All the control samples were taken during the operation.

### Ethics

Each patient gave written informed consent to participate in the study. Ethical committee approval was obtained from our hospital district (no. 153/2010).

### Definition of infection

The results of the PCR and microarray analysis were compared to the PJI diagnosis defined according to the Musculoskeletal Infection Society (MSIS) criteria and to the results of bacterial culture (Parvizi et al. 2011).

Based on the MSIS criteria, a diagnosis of PJI is made when one or more of the following conditions are met: (1) a sinus tract communicating with the prosthesis; or (2) a pathogen is isolated by culture from 2 separate tissue or fluid samples obtained from the affected prosthetic joint; or (3) 4 of the following 6 criteria exist: (a) elevated serum ESR (> 30 mm/h) or CRP concentration (> 10 mg/L), (b) elevated synovial white blood cell count, (c) elevated synovial neutrophil percentage, (d) presence of purulence in the affected joint, (e) isolation of a microorganism in 1 culture of periprosthetic tissue or fluid,

and (f) greater than 5 neutrophils per high-power field in 5 high-power fields observed from histological analysis of periprosthetic tissue at 400× magnification. In those cases that did not fulfill the MSIS criteria, the appearance of possible signs of PJI was evaluated after a follow-up time of 14–28 months.

PJIs were categorized as (1) early infection (less than 3 months after surgery), (2) delayed infection (3–24 months after surgery), and (3) late infection (more than 24 months after surgery) (Zimmerli et al. 2004).

The bacterial culture was performed according to Clinical and Laboratory Standards Institute standards. Aerobic growth was performed on non-selective blood agar and chocolate agar at 35°C, in an atmosphere of 5% CO<sub>2</sub>. Anaerobic growth was performed with non-selective Fastidious Anaerobe Agar. Thioglycollate broth was used for the enrichment culture. The samples were in culture for 7 days and were inspected after 1 day, 2 days, and 7 days.

Bacterial cultures were regarded as positive if bacterial growth was noted from 2 or more tissue samples or synovial fluid samples. In cases in which only 1 synovial fluid aspirate was taken for microbiological analysis, bacterial growth in this aspirate was regarded as a positive result. The PCR and microarray result was regarded as positive if bacteria were detected in any of the samples.

### Samples

All 142 samples in the study were delivered to the investigating laboratory in blind fashion. Sample material included bone and other tissue samples, synovial fluid, and pus.

### Pretreatment of samples

Bone biopsies, tissue, and viscous pus samples were homogenized using the MagNA Lyser instrument and MagNA Lyser Green Beads (Roche Applied Science, Germany) according to a modification of the manufacturer's protocol. Each sample was transferred to a MagNA Lyser Green Beads tube and 350 µL of MagNA Pure Bacteria Lysis Buffer (BLB) was added. A disruption cycle of 7,000 rpm for 60 sec was performed followed by a cooling step, in which the samples were cooled in the block at 2–8°C for 90 sec. The steps were repeated 3 times and after the final cooling, formed foam was removed by centrifugation at 16,060 × g for 1–3 min. 200 µL of BLB and 20 µL of PCR-grade proteinase K (14–22 mg/mL; Roche Applied Science) was added to the samples.

Bloody synovial fluid and pus samples were diluted by adding 1 part of the sample to 9 parts of PCR-grade water (Jena Bioscience GmbH, Germany). The samples were incubated for 15 sec at room temperature before centrifugation at 2,465 × g for 10 min. The pellet was resuspended in 550 µL phosphate-buffered saline (PBS) (Jena Bioscience) or PCR-grade water. 20 µL proteinase K was added to the samples.

Viscous synovial fluid samples (volume > 300 µL) were centrifuged at 16,060 × g for 10 min. 100–200 µL of supernatant was used to resuspend the pellet. Viscous synovial fluid

samples (volume < 300 µL) were used as such. 20 µL proteinase K was added to the samples.

Non-viscous fluid samples (volume > 550 µL) were centrifuged at  $16,060 \times g$  for 10 min. The pellet was resuspended in 550 µL PBS. Swab samples were dissolved directly in 550 µL PBS. Sample volumes less than 550 µL were adjusted to a final volume of 550 µL using PBS. 20 µL proteinase K was added to the samples.

### Incubation of samples and DNA extraction

Pretreated samples were then incubated at 60°C for 2 h with agitation at 400 rpm, followed by an incubation at 95°C for 10 min, after which they were centrifuged at  $16,060 \times g$  for 1 min. 550 µL of each sample was transferred to a NorDiag Arrow instrument (NorDiag, Norway). DNA extraction was performed with Arrow Viral NA kit v.1.0 or v.2.0 according to the manufacturer's instructions, using 60 µL as elution volume.

### PCR and microarray assay

DNA extracts were analyzed with the Prove-it Bone and Joint StripArray assay (research-use-only version; Mobidiag Ltd., Helsinki, Finland). The Prove-it Bone and Joint assay is a broad-range PCR and microarray-based assay targeting over 60 bacterial species (Järvinen et al. 2009, Tissari et al. 2010, Laakso et al. 2011). Proprietary primers were used for amplification of specific regions of the bacterial topoisomerase genes *gyrB* and *parE*, and the methicillin-resistance gene *mecA*. 1.5 µL of DNA extract was used as PCR template. The PCR procedure was carried out according to the instructions of the manufacturer.

### Statistics

Positive predictive value, negative predictive value, false-positive rate, false-negative rate, and accuracy were calculated. Sensitivity, specificity, and confidence intervals (CIs) were calculated according to Clinical and Laboratory Standards Institute recommendations (National Committee on Clinical Laboratory Standards 2002). Fisher's exact test was used to calculate p-values.

### Results

Of the 61 suspected PJIs, 38 of them were a PJI according to the MSIS criteria. In the PJIs fulfilling the MSIS criteria, mean blood leukocyte count was  $12.0 \times 10^9/L$  (range 5–23), mean ESR was 56 (19–109) mm/h, and mean CRP was 177 (15–413) g/L. Mean synovial leukocyte count was  $71,090 \times 10^6/L$  (1,390–222,000) and mean percentage of polymorphonuclear leukocytes was 89% (46–100). There were 20 early infections, 12 delayed infections, and 6 late infections.

23 suspected PJIs that did not fulfill the MSIS criteria showed no new signs of infection during 14–28 months of follow-up after the sample collection.

Table 2. The results of PCR and microarray assay in 61 patients with suspected prosthetic joint infection

Bacteria	No. of patients
Prosthetic joint infection fulfilling MSIS criteria (n = 38)	
<i>Staphylococcus aureus</i>	8
<i>Staphylococcus aureus</i> , <i>mecA</i>	1
<i>Staphylococcus epidermidis</i> , <i>mecA</i>	4
<i>MecA</i>	3
Coagulase-negative staphylococci	2
<i>Staphylococcus epidermidis</i>	1
<i>Streptococcus agalactiae</i>	2
<i>Streptococcus dysgalactiae</i> subspecies <i>equisimilis</i>	2
<i>Streptococcus pneumoniae</i>	1
<i>Serratia marcescens</i>	1
<i>Escherichia coli</i>	1
<i>Enterobacter cloacae</i>	1
<i>Staphylococcus aureus</i> , coagulase-negative staphylococci, <i>mecA</i>	1
<i>Staphylococcus epidermidis</i> , <i>mecA</i> , <i>Enterococcus faecalis</i>	1
Coagulase-negative staphylococci, <i>Enterococcus faecium</i>	1
<i>Staphylococcus epidermidis</i> , <i>mecA</i> , <i>Enterococcus faecalis</i> , <i>Enterobacter cloacae</i>	1
Negative	7
No prosthetic joint infection according to MSIS criteria (n = 23)	
Enterobacteriaceae	3
<i>Klebsiella pneumoniae</i> and <i>Enterococcus casseliflavus</i>	1
<i>Acinetobacter baumannii</i> , <i>Klebsiella pneumoniae</i> , and <i>Staphylococcus epidermidis</i>	1
<i>Staphylococcus epidermidis</i> and <i>mecA</i>	1
Negative	17

MSIS: the Musculoskeletal Infection Society;  
*mecA*: the methicillin-resistance gene.

### Detection of bacteria from 61 suspected PJIs by PCR and microarray assay and by culture

The PCR and microarray assay was positive in 37 patients and 29 patients were positive by culture (Tables 2 and 3). In 25 patients, the same bacteria were identified by microbiological culture and by PCR and microarray assay (including 3 cases where PCR detected only the methicillin-resistance marker gene *mecA* of staphylococci). Some slightly different polymicrobial results were obtained in cases with a purulent open wound.

### Comparison between bacterial detection and the MSIS definition

When compared to the 38 cases with an MSIS clinical definition of PJI, the study showed 31 true-positive PCR and microarray results (Table 4). PCR was positive in 17 of the 20 early PJIs, in 8 of the 12 delayed PJIs, and in all 6 late PJIs.

In 5 cases with an MSIS definition of PJI, culture was negative but PCR detected bacteria (2 *Staphylococcus epidermidis*, 1 *Staphylococcus aureus*, 1 *Streptococcus pneumoniae*, and 1 *Enterobacter cloacae*). All of these patients had received antimicrobial treatment before sample collection.

Table 3. Culture results in 61 patients with suspected prosthetic joint infection

Bacteria	No. of patients
Prosthetic joint infection fulfilling MSIS criteria (n = 38)	
<i>Staphylococcus aureus</i>	8
<i>Staphylococcus epidermidis</i>	6
<i>Staphylococcus lugdunensis</i>	3
Coagulase-negative staphylococci	1
<i>Streptococcus agalactiae</i>	2
Group G beta-hemolytic streptococci <sup>a</sup>	2
<i>Serratia marcescens</i>	1
<i>Escherichia coli</i>	1
<i>Staphylococcus epidermidis</i> and <i>Enterococcus faecalis</i>	1
<i>Staphylococcus epidermidis</i> and <i>Streptococcus viridans</i> <sup>b</sup>	1
<i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i>	1
<i>Enterobacter cloacae</i> , <i>Enterococcus faecalis</i> , <i>Citrobacter</i>	1
Negative	10
No prosthetic joint infection according to MSIS criteria (n = 23)	
<i>Staphylococcus epidermidis</i>	1
Negative	22

<sup>a</sup> Group G beta-hemolytic streptococci includes *Streptococcus dysgalactiae* subspecies *equisimilis* detected by PCR.

<sup>b</sup> *Streptococcus viridans* was not included in the pathogen panel of the PCR assay.

MSIS: The Musculoskeletal Infection Society.

Table 4. Results of PCR and microarray assay and of culture compared against the diagnosis of prosthetic joint infection according to the Musculoskeletal Infection Society (MSIS) criteria, from 61 patients with suspected prosthetic joint infections

	PCR +	PCR –	Total
MSIS criteria +	31	7	38
Culture +	26	2	
Culture –	5	5	
MSIS criteria –	6	17	23
Culture +	1	0	
Culture –	5	17	
Total	37	24	61

### The effect of ongoing antimicrobial treatment

15 of the 38 PJI patients had ongoing antimicrobial treatment when the samples were taken. In these 15 patients, 11 had bacteria by PCR and microarray assay and 8 had bacteria by microbiological culture (Table 5). In patients with no ongoing antimicrobial treatment, the PCR-based assay and culture both detected bacteria equally in 20 PJIs that fulfilled the MSIS criteria.

### False-positive results

There were 6 false-positive PCR and microarray assay results out of 23 real negatives in the PJI suspicion group. All of these 6 false positives were the only samples from that particular

Table 5. Comparison of the effect of previous antimicrobial treatment and its length with culture results and PCR and microarray assay results from 15 prosthetic joint infections fulfilling the Musculoskeletal Infection Society criteria

Length of antimicrobial treatment	PCR		Culture	
	+	–	+	–
Up to 3 days	5	1	6	0
4 days or more	6	3	2	7
Total	11	4	8	7

patient—single aspirates. In 5 cases, the culture was negative, MSIS criteria for infection were not met, and no signs of infection were noticed in follow-up either. 4 of these false-positive samples had been analyzed in the laboratory within 10 days. The PCR and microarray assay results were as follows: 3 Enterobacteriaceae, 1 case with *Klebsiella pneumoniae* and *Enterococcus casseliflavus*, and 1 case with *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Staphylococcus epidermidis*. 1 patient was both culture-positive (with very scanty growth of *Staphylococcus epidermidis* from synovial fluid) and PCR-positive, but clinically there had been no PJI-fulfilling criteria at the time of sampling; nor was there any evidence of PJI during 15 months of follow-up.

There were 17 true-negative PCR and microarray assay results in the PJI-suspicion group. 5 negative PCR and microarray assay results matched the finding of negative cultures, but there had been a PJI according to the MSIS criteria. 2 of these cases had had previous antimicrobial treatment of 10 days and 16 days. Then there were 2 negative PCR results with positive cultures and with the MSIS criteria fulfilled, giving a final false-negative rate of 7/28.

### Results in the control group

All 40 samples from the 20 controls gave true-negative PCR and microarray assay results. All the negative controls were also negative by culture.

### Sensitivity and specificity

Compared to the MSIS criteria of PJI, the PCR and microarray assay achieved 82% (95% CI: 67–91) sensitivity and 74% (95% CI: 54–87) specificity. The predictive value of a positive test result was 84% and that of a negative result was 71%. The accuracy was 79%. If the detection of only the *mecA* gene was judged to be a false negative, the sensitivity was 74% (95% CI: 58–85).

### Discussion

We found that detection of bacteria by PCR and microarray-based assay was superior to that by culture in patients who had had antimicrobial treatment for more than 4 days. In patients

without any ongoing antimicrobial treatment, there was no difference between PCR and microarray-based assay and conventional culture in detecting bacteria.

The sensitivity of the PCR and microarray assay was 82%. With a broad-range 16S rDNA PCR, Moojen et al. (2007) reported a sensitivity of as high as 97%, when compared to clinical diagnosis of PJI. More than 90% sensitivity with the PCR assay has also been reported by Marin et al. (2012) and by Panousis et al. (2005). On the other hand, lower PCR sensitivities in diagnosing PJI have been reported by Fihman et al. (2007), at 54%, and by De Man et al. (2009), at 50%. From a technical point of view, the PCR and microarray can detect only predefined target species, whereas PCR and sequencing possibly detects all eubacterial species. However, the ability of the microarray to simultaneously detect and identify several bacterial species and also antimicrobial resistance genes in 1 sample is an advantage over PCR and DNA sequencing, where multibacterial infections or multiple targets are technically challenging. The microarray taxa do not include the *Streptococcus viridans* group, *Propionibacter acnes*, and *Mycoplasma* or other rare species. This is a limitation of the present microarray layout: the next version of the test is planned to cover some other important species. On the other hand, the *gyrB* gene used in the Prove-it Bone and Joint assay differentiates closely related bacterial species better than 16S rDNA-based PCR, which is often used with sequencing (Dauga 2002, Tayeb et al. 2008).

The design of the microarray approach tolerates the presence of traces of environmental DNA, which is often present in most reagents and consumables. Nevertheless, the interpretation of results is still difficult if the degree of contamination is not already known and if meticulous care is not taken throughout the process (Bjerkkan et al. 2012). For example, in this study the false-positive rate for the PCR and microarray assay was 26%, and the possibility of contamination was suspected as 4 out of the 6 false positives had been analyzed within 10 days, and none of these 6 patients had had any signs of PJI during follow-up. Without the 4 false-positive cases—the suspected cluster of contamination—the specificity of the PCR and microarray assay would have been higher: 91% (95% CI: 73–98). Thus, positive PCR results must always be interpreted with caution if patients do not have clinical signs and symptoms of PJI. However, in addition to false positives, PCR-based methods may also detect viable but non-cultivable (VBNC) bacteria (Trevors 2011).

In our material, the PCR and microarray assay detected the smallest proportion of infections in the group of delayed PJIs. The delayed infections are usually caused by low-virulence organisms such as coagulase-negative staphylococci or *Propionibacterium acnes* tightly that are fixed to biofilm on the surface of the prosthesis (Zimmerli et al. 2004, Trampuz et al. 2007), so they may not always be easily detected with PCR methods from periprosthetic tissue biopsies (Ince et al. 2004). Also, *Propionibacterium acnes* was not included in the

pathogen panel of the PCR assay in this study, thus reducing the yield in the delayed-PJI group. Sonication of an explanted prosthesis to dislodge adherent bacteria followed by sample culture has been shown to improve the microbiological diagnosis of PJI (Trampuz et al. 2007). PCR after sonication of the implant has also been studied, with mostly promising results (Achermann et al. 2010, Esteban et al. 2012, Gomez et al. 2012, Portillo et al. 2012).

The PCR and microarray assay was more informative than culture, if the antimicrobial treatment had already been started. Even though the aim should always be to collect microbiological samples before initiating antimicrobial treatment (Malekzadeh et al. 2010), the treatment is sometimes initiated earlier—usually due to severe systemic infection or for some other reason. In earlier studies, PCR has also been found to be useful in examining samples during antimicrobial treatment (Achermann et al. 2010) and in cultures after sonication (Trampuz et al. 2007).

As in previous studies, the most commonly cultured microorganisms in the PJIs were coagulase-negative staphylococci and *Staphylococcus aureus* (Zimmerli et al. 2004, Barberan 2006). Thus, for the treating physician, detection of the *mecA*-resistance gene by PCR is important: it can show whether or not vancomycin is needed in the treatment. Also, special rapid tests for *mecA* detection have been developed (Titécat et al. 2012).

In conclusion, in the present study a PCR and microarray-based platform—with the attractive possibility of faster bacterial diagnosis than with routine culture—was most helpful in PJI diagnostics during ongoing antimicrobial treatment. Without any preceding antimicrobial treatment, the assay did not appear to provide any additional information to that gained from routine culture.

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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* 2010; 48 (4): 1208-14.

Barberan J. Management of infections of osteoarticular prosthesis. *Clin Microbiol Infect (Suppl 3)* 2006; 12: 93-101.

Bjerkkan G, Witsø E, Nor A, Viset T, Løseth K, Lydersen S, Persen L, Bergh K. A comprehensive microbiological evaluation of fifty-four patients undergoing revision surgery due to prosthetic joint loosening. *J Med Microbiol* 2012; 61 (Pt 4): 572-81.

- Blom A W, Brown J, Taylor A H, Pattison G, Whitehouse S, Bannister G C. Infection after total knee arthroplasty. *J Bone Joint Surg (Br)* 2004; 86 (5): 688-91.
- Dauga C. Evolution of the *gyrB* gene and the molecular phylogeny of Enterobacteriaceae: a model molecule for molecular systematic studies. *Int J Syst Evol Microbiol* 2002; 52 (Pt 2): 531-47.
- De Man F H R, Graber B, Luem M, Zimmerli W, Ochsner P E, Sendi P. Broad-range PCR in selected episodes of prosthetic joint infection. *Infection* 2009; 37 (3): 292-4.
- Esteban J, Alonso-Rodríguez N, del-Prado G, Ortiz-Pérez A, Molina-Manso D, Cordero-Ampuero J, Sandoval E, Fernández-Roblas R, Gómez-Barrena E. PCR-hybridization after sonication improves diagnosis of implant-related infection. *Acta Orthop* 2012; 83 (3): 299-304.
- Fihman V, Hannouche D, Bousson V, Bardin T, Liote F, Raskine L, Riahi J, Sanson-Le Pors M J, Bercot B. Improved diagnosis specificity in bone and joint infections using molecular techniques. *J Infect* 2007; 55 (6): 510-7.
- Gomez E, Cazanave C, Cunningham S A, Greenwood-Quaintance K E, Steckelberg J M, Uhl J R, Hanssen A D, Karau M J, Schmidt S M, Osmon D R, Berbari E F, Mandrekar J, Patel R. Prosthetic joint infection diagnosis using broad-range PCR of biofilms dislodged from knee and hip arthroplasty surfaces using sonication. *J Clin Microbiol* 2012; 50 (11): 3501-8.
- Ince A, Rupp J, Frommelt L, Katzer A, Gille J, Löhr J F. Is "Aseptic" loosening of the prosthetic cup after total hip replacement due to nonculturable bacterial pathogens in patients with low-grade infection? *Clin Infect Dis* 2004; 39 (11): 1599-603.
- Järvinen A K, Laakso S, Piiparinen P, Aittakorpi A, Lindfors M, Huopaniemi L, Piiparinen H, Mäki M. Rapid identification of bacterial pathogens using a PCR- and microarray-based assay. *BMC Microbiol* 2009; 9: 161.
- Kurtz S M, Lau E, Watson H, Schmier J K, Parvizi J. Economic burden of periprosthetic joint infection in the United States. *J Arthroplasty* (8 Suppl) 2012; 27: 61-5.
- Laakso S, Kirveskari J, Tissari P, Mäki M. Evaluation of high-throughput PCR and microarray-based assay in conjunction with automated DNA extraction instruments for diagnosis of sepsis. *PLoS ONE* 2011; 6 (11): e26655.
- Malekzadeh D, Osmon D R, Lahr B D, Hanssen A D, Berbari E F. Prior use of antimicrobial therapy is a risk factor for culture-negative prosthetic joint infection. *Clin Orthop* 2010; (468) (8): 2039-45.
- Marin M, Garcia-Lechuz J M, Alonso P, Villanueva M, Alcalá L, Gimeno M, Cercenado E, Sanchez-Somolinos M, Radice C, Bouza E. The role of universal 16S rRNA gene PCR and sequencing in diagnosis of prosthetic joint infection. *J Clin Microbiol* 2012; 50 (3): 583-9.
- Moojen D J, Spijkers S N, Schot C S, Nijhof M W, Vogely H C, Fleer A, Verbout A J, Castelein R M, Dhert W J, Schouls L M. Identification of orthopaedic infections using broad-range polymerase chain reaction and reverse line blot hybridization. *J Bone Joint Surg (Am)* 2007; 89 (6): 1298-305.
- National Committee on Clinical Laboratory Standards. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline. Wayne, PA: National Committee on Clinical Laboratory Standards 2002. NCCLS document EP12-A.
- Panousis K, Grigoris P, Butcher I, Rana B, Reilly J H, Hamblen D L. Poor predictive value of broad-range PCR for the detection of arthroplasty infection in 92 cases. *Acta Orthop* 2005; 76 (3): 341-6.
- Parvizi J, Zmistowski B, Berbari E F, Bauer T W, Springer B D, Della Valle C J, Garvin K L, Mont M A, Wongworawat M D, Zalavras C G. New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. *Clin Orthop* 2011; (469) (11): 2992-4.
- Portillo M E, Salvadó M, Sorli L, Alier A, Martínez S, Trampuz A, Gómez J, Puig L, Horcajada J P. Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure. *J Infect* 2012; 65 (6): 541-8.
- Tayeb L A, Lefevre M, Passet V, Diancourt L, Brisse S, Grimont P A. Comparative phylogenies of Burkholderia, Ralstonia, Comamonas, Brevundimonas and related organisms derived from *rpoB*, *gyrB* and *rrs* gene sequences. *Res Microbiol* 2008; 159 (3): 169-77.
- Tissari P, Zumla A, Tarkka E, Mero S, Savolainen L, Vaara M, Aittakorpi A, Laakso S, Lindfors M, Piiparinen H, Mäki M, Carder C, Huggett J, Gant V. Accurate and rapid identification of bacterial species from positive blood cultures with a DNA-based microarray platform: an observational study. *Lancet* 2010; 375 (9710): 224-30.
- Titécát M, Loïez C, Senneville E, Wallet F, Dezèque H, Legout L, Migaud H, Courcol R J. Evaluation of rapid *mecA* gene detection versus standard culture in staphylococcal chronic prosthetic joint infections. *Diagn Microbiol Infect Dis* 2012; 73 (4): 318-21.
- Trampuz A, Piper K E, Jacobson M J, Hanssen A D, Unni K K, Osmon D R, Mandrekar J R, Cockerill F R, Steckelberg J M, Greenleaf J F, Patel R. Sonication of removed hip and knee prosthesis for diagnosis of infection. *N Engl J Med* 2007; 357 (7): 654-63.
- Trevors J T. Viable but non-culturable (VBNC) bacteria: Gene expression in planktonic and biofilm cells. *J Microbiol Methods* 2011; 87 (3): 263-72.
- Zimmerli W, Trampuz A, Ochsner P E. Prosthetic joint infections. *N Engl J Med* 2004; 351 (16): 1645-54.