JCM
Journals.ASM.org

Detection of Prosthetic Joint Infection by Use of PCR-Electrospray Ionization Mass Spectrometry Applied to Synovial Fluid

Dante P. Melendez,[a] James R. Uhl,[b] Kerryl E. Greenwood-Quaintance,[b] Arlen D. Hanssen,[c] Rangarajan Sampath,[d] Robin Patel[a,b,c]

Division of Infectious Diseases, Department of Medicine,[a] Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,[b] and Department of Orthopedic Surgery,[c] Mayo Clinic, Rochester, Minnesota, USA; Ibis Biosciences, Abbott Company, Carlsbad, California, USA[d]

PCR coupled with electrospray ionization mass spectrometry applied to synovial fluid specimens had an 81% sensitivity and a 95% specificity for the diagnosis of prosthetic joint infection.

The number of cases of prosthetic joint infection (PJI) is increasing. Microbiologic diagnosis of PJI has traditionally been made by culture of synovial fluid, periprosthetic tissue, and/or the implant itself. However, cultures are not universally positive (1–6); this has spawned an interest in using molecular strategies to diagnose PJI (7–13). We have recently shown that a technique that couples PCR with electrospray ionization mass spectrometry (PCR-ESI/MS), used previously in a variety of settings (14–18), can be applied to materials dislodged from explanted orthopedic implants (sonicate fluid) to diagnose PJI with increased sensitivity compared with culture (19). Jacovides et al. used an older version of this technology than we used and detected organisms in synovial fluid in 88% of presumed noninfectious arthroplasty failures (20). Although we also found a lower specificity of PCR-ESI/MS (94%) than culture (99%) when applied to sonicate fluid (21), we did not find nearly the proportion of positive specimens in presumed noninfectious failure reported by Jacovides et al. (20). Herein, we evaluated synovial fluid specimens collected in containers treated to minimize background DNA using the same version of the PCR-ESI/MS assay we used to study sonicate fluid (21).

(This study was presented in part at the 113th General Meeting of the American Society for Microbiology, 18 to 21 May 2013, Denver, CO.)

The PCR-ESI/MS BAC protocol (Ibis Biosciences, Carlsbad, CA) was used to test synovial fluid collected by sterile arthrocentesis from subjects with knee arthroplasty failure at the Mayo Clinic, Rochester, MN. The research application PCR-ESI/MS BAC assay studied detects more than 3,400 species of bacteria, 40 species of *Candida*, and four antibiotic resistance markers, bla_{KPC}, *vanA*, *vanB*, and *mecA*. Syringes, Vacutainer collection tubes, and freezer storage vials used for collection and storage of specimens were pretreated to minimize contaminating DNA by irradiation in a self-contained ^{137}Cs gamma irradiator with a total dose of 1 Gy (22). Specimens were collected between 2001 and 2012 and stored at −70°C until PCR-ESI/MS testing in 2012. At the time of specimen collection, cultures had been performed using previously described methods (23). Subjects were classified as having PJI or aseptic failure (AF) using the 2011 Musculoskeletal Infection Society (MSIS) criteria, a combined clinical/laboratory classification system (24). Accordingly, a subject was considered to have PJI under the following conditions: if a sinus tract communicating with the prosthesis was found; if the same organism was isolated from two non-synovial fluid samples (i.e., periprosthetic tissue, sonicate fluid) obtained from the affected joint at the time of revision surgery; or if four of the following six minor criteria were present: elevated erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP); elevated synovial fluid white blood cell (WBC) count; elevated synovial neutrophil percentage (percent polymorphonuclear leukocytes [PMNs]); presence of purulence in the affected joint; isolation of a microorganism from a single periprosthetic tissue specimen or the implant itself; and acute inflammation on periprosthetic tissue histologic examination. Cases that did not meet the above criteria were considered to have AF.

DNA was extracted from 1 ml of synovial fluid using magnetic bead beating with the KingFisher DNA extraction instrument (Thermo Scientific, Waltham, MA) as previously described (17). Following PCR amplification, the molecular mass of the amplified DNA was determined by mass spectrometry. The organism detected (assessed based on the PCR assay that was positive and the base composition of the amplified product inferred from its molecular mass) was determined by the system's software with two parameters additionally reported, Q-score and level of detection (25). The Q-score, a rating between 0 (low) and 1 (high), represents a relative measure of the strength of the data supporting identification. Per the system software, only organisms with Q scores of ≥0.90 were reported. The level of detection is a semiquantitative measure of the amount of amplified DNA calculated relative to an internal calibrant and is reported in genome equivalents (GE)/well. We performed a receiver operating characteristic (ROC) curve analysis to determine the level of detection that would best discriminate PJI from AF. The sensitivity and specificity for synovial fluid culture and PCR-ESI/MS were compared using McNemar's test of paired proportions.

One hundred three synovial fluid samples from 21 PJI and 82 AF cases were evaluated. No differences in age, gender, time from synovial fluid collection to revision surgery or specimen storage time were found between the groups (Table 1). A cutoff of ≥24

Received 28 February 2014 **Returned for modification** 25 March 2014
Accepted 31 March 2014
Published ahead of print 9 April 2014
Editor: G. V. Doern
Address correspondence to Robin Patel, patel.robin@mayo.edu.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.00570-14.

doi:10.1128/JCM.00570-14

TABLE 1 Demographic and laboratory characteristics

Characteristic	Value for group [mean (SD)][a]		
	Aseptic failure ($n = 82$)	Prosthetic joint infection ($n = 21$)	*P* value
Age (yr)	68.6 (11.7)	67.76 (10.1)	0.62
Gender			
Male (%)	57.2	76.2	0.11
Female (%)	42.9	53.8	
Synovial fluid			
Cell count (cells/μl)	1,498.1 (2,579.3)	39,263 (37,574)	<0.0001
% neutrophils	18.4 (21.7)	88.9 (7.9)	<0.0001
Erythrocyte sedimentation rate (mm/h)	13.4 (14.7)	69.5 (34.8)	<0.0001
C-reactive protein (mg/dl)	0.79 (1.1)	8.26 (5.5)	<0.0001
Time from synovial fluid collection to revision surgery (days)	45.8 (65.1)	45.1 (71.8)	0.96
Synovial fluid storage time (from collection to PCR-ESI/MS analysis) (mo)	88.2 (30.3)	88.7 (39.6)	0.95

[a] All values with the exception of the values for gender are means (standard deviations [SDs]).

TABLE 2 Microbiology of PCR-ESI/MS and culture of synovial fluid

Organism	No. of positive results in patients with PJI or AF by[a]:					
	PCR-ESI/MS (at any level of detection)			Synovial fluid culture		
	PJI	AF	Total	PJI	AF	Total
Coagulase-negative *Staphylococcus* spp.	8	3[e]	11[b]	10	0	10
Staphylococcus aureus	4	1[e]	5	4	0	4
Viridans group *Streptococcus* species	1[c]	0	1	1	0	1
Propionibacterium acnes	0	1	1	0	0	0
Methylobacterium mesophilium	0	1[e]	1	0	0	0
Micrococcus lylae/luteus	0	2[e]	2	0	0	0
Capnocytophaga canimorsus	1	0	1	1	0	1
Candida albicans	1	0	1	1	0	1
Enterococcus faecalis	1	0	1	1	0	1
Polymicrobial	1[d]	2[d,e]	3	0	0	0

[a] PJI, prosthetic joint infection; AF, aseptic failure.
[b] The 11 positive results were broken down as follows: *Staphylococcus epidermidis* (7 positive results), *Staphylococcus epidermidis/caprae* (1 positive result), *Staphylococcus capitis* (1 positive result), *Staphylococcus warneri* (1 positive result), and *Staphylococcus haemolyticus* (1 positive result). Six (all *S. epidermidis*) were *mecA* positive.
[c] *Streptococcus salivarius/thermophilus.*
[d] Polymicrobial detections by PCR-ESI/MS included *Propionibacterium acnes* plus *Candida tropicalis* (PJI subject), *P. acnes* plus *Streptococcus salivarius* species group plus *Streptococcus mitis* species group (AF subject), and *Enterobacter cloacae* complex plus *Acinetobacter baumannii* (AF subject).
[e] The PCR-ESI/MS result was positive but below the 24-GE/well level of detection for *Staphylococcus haemolyticus* (1 result), *Staphylococcous aureus* (1 result), *Methylobacterium mesophilium* (1 result), and *Micrococcus lylae/luteus* (2 results) and in both polymicrobial cases (except for *Propionibacterium acnes*).

GE/well was determined to be the ideal discriminator to separate infected and noninfected cases (area under the curve [AUC], 0.88). In 6 cases, PCR-ESI/MS detected an organism but with levels of detection below the cutoff of 24 GE/well; these cases were thus considered negative for the calculation of sensitivity and specificity.

In the PJI group, PCR-ESI/MS gave positive results for 17 subjects, and synovial fluid culture gave positive results for 18 subjects (sensitivities of 81 and 86%, respectively; $P = 0.56$), and in the AF group, PCR/ESI-MS gave negative results for 78 subjects, while synovial fluid culture gave negative results for all 82 subjects (specificities of 95% and 100%, respectively; $P = 0.045$).

Four PJI cases had negative PCR-ESI/MS results, two of which had positive synovial fluid cultures (cases 180 and 181 [see Table S1 in the supplemental material]). The other two had negative synovial fluid PCR-ESI/MS and culture results; in one, 10 months had elapsed between synovial fluid collection and surgery (i.e., periprosthetic tissue specimen collection), suggesting that this subject may not have been infected at the time of synovial fluid collection. In the fourth case, only 1 month had elapsed between synovial fluid collection and surgery, the patient had not previously received antibiotics, periprosthetic tissue and synovial fluid cultures were negative, but prosthesis sonication fluid was positive for >100 CFU/ml *Propionibacterium acnes*. During surgery, purulence was encountered, although pathological review of the periprosthetic tissue specimen revealed synovial hyperplasia with chronic inflammation.

Table S2 in the supplemental material shows clinical follow-up information on all subjects with aseptic failure who had microorganisms detected at any level by PCR-ESI/MS ($n = 10$). Six (cases 221, 277, 285, 331, 332, and 350) had levels of detection below what was considered positive (<24 GE/well). The remaining four positive PCR-ESI/MS cases had levels of detection ranging from 24 GE/well (*P. acnes* and *Staphylococcus warneri*) to 160 GE/well (*mecA*-positive *Staphylococcus epidermidis*). No evidence of infection was found in any of these 10 cases.

The most common organism detected by PCR-ESI/MS was *Staphylococcus epidermidis* (Table 2). In the PJI group, there was microbiologic concordance between PCR-ESI/MS and synovial fluid culture in 15 cases. For one discordant case, oxacillin-resistant coagulase-negative *Staphylococcus* species (CoNS) were grown from synovial fluid culture as well as from all three periprosthetic tissue specimens cultured, while *Propionibacterium acnes* and *Candida tropicalis* were detected by PCR-ESI/MS. Of the remaining three discordant cases, one was positive by PCR-ESI/MS and negative by synovial fluid culture, and two were negative by PCR-ESI/MS and positive by culture (see Table S1 in the supplemental material).

We also compared results of PCR-ESI/MS with results of cultures of non-synovial fluid specimens. Seven subjects with PJI had undergone implant culture using sonication (3, 4, 6), five of whom had concordant results with PCR-ESI/MS, one of whom had an additional organism (CoNS; 51 to 100 CFU/10 ml) detected by culture, and one of whom was positive only by sonicate fluid culture (*P. acnes*; >100 CFU/10 ml). Eighteen PJI subjects had had specimens submitted for periprosthetic tissue culture. Of these 18 subjects, complete microbiologic concordance was found for 12 subjects. PCR-ESI/MS was positive and culture negative for one subject, and culture was positive and PCR-ESI/MS negative for

two subjects. Tissue culture grew two additional organisms in one case (PCR-ESI/MS detected only *S. epidermidis*, while culture was positive for CoNS, *Lactobacillus* species, and *Klebsiella* species—all grew only from broth). For one case, CoNS grew in tissue culture, while PCR-ESI/MS and synovial fluid culture were positive for *Enterococcus faecalis*, and in the last case, mentioned above, PCR-ESI/MS was positive for *P. acnes* and *C. tropicalis*, whereas periprosthetic tissue and synovial fluid culture grew CoNS.

Twelve subjects (9 subjects with PJI and 3 subjects with AF) had received antibiotics within 30 days prior to synovial fluid aspiration. Of the nine PJI cases, PCR-ESI/MS gave positive results for eight cases, and synovial fluid culture gave positive results for nine cases; PCR-ESI/MS and culture gave negative results for all three AF cases.

There was a high level of concordance between synovial fluid PCR-ESI/MS detection of antibiotic resistance markers *mecA* and *vanA* or *vanB* and traditional phenotypic antimicrobial susceptibility testing of cultured isolates. Out of the 11 staphylococci detected by both synovial fluid culture and PCR-ESI/MS, 10 had concordant results between detection of *mecA* by PCR-ESI/MS and resistance to oxacillin by phenotypic antimicrobial susceptibility testing of the isolated organism. The single discordant case was a CoNS isolated from synovial fluid culture; the isolate had an oxacillin MIC of 1 μg/ml (interpreted as resistant by current Clinical and Laboratory Standards Institute [CLSI] guidelines [26]), but the synovial fluid was *mecA* negative but positive for *S. epidermidis/caprae* by PCR-ESI/MS. Oxacillin resistance in the isolate may have been mediated by a mechanism other than *mecA*, more than one CoNS strain may have been present in the specimen, or the oxacillin susceptibility breakpoint may not correlate with the presence of *mecA*. Only one subject had *E. faecalis* detected in synovial fluid by culture; *vanA* or *vanB* testing was negative by PCR-ESI/MS, and the isolate was vancomycin susceptible by phenotypic antimicrobial susceptibility testing.

Twenty-two subjects (5 subjects with PJI and 17 subjects with AF) had PCR-ESI/MS done on prosthesis sonicate fluid samples as part of our prior study (21). The five PJI cases had concordant positive results by PCR-ESI/MS in both specimen types, and 15 of 17 AF cases were PCR-ESI/MS negative in both specimen types. In one case, PCR-ESI/MS detected *Micrococcus lylae/luteus* below the cutoff of 24 GE/well (level, 15; Q-score, 0.90) in synovial fluid but not in sonicate fluid; the final case was PCR-ESI/MS positive for *Hyphomicrobium denitrificans* (level, 31; Q-score, 0.95) in sonicate fluid and negative in synovial fluid.

As mentioned above, review of the medical records of all presumed noninfected cases with a positive PCR-ESI/MS result with follow-up as long as 9 years after synovial fluid collection (see Table S2 in the supplemental material) did not reveal any evidence of PJI. In the study by Jacovides et al. (20), a high rate of false positivity was reported, with 49 out of 57 AF subjects having a positive PCR-ESI/MS result. The use of different PCR-ESI/MS system criteria for considering results positive and/or collection of specimens in such a way as to eliminate contaminating DNA may account for the differences found.

A finding common to both our study and that of Jacovides et al. (20) is that some of the AF subjects with a positive PCR-ESI/MS signal had a history of prior infection. In our study, two such cases were found (cases 214 and 285 [see Table S2 in the supplemental material]), one of which was a subject with a history of *Staphylococcus aureus* PJI 3 years prior to synovial fluid collection for whom PCR-ESI/MS detected *S. aureus* below the cutoff of 24 GE/well (level, 3; Q-score, 0.9). This suggests that microbial DNA may persist in synovial fluid following successful treatment, and therefore, its presence may not always indicate persistent infection.

Limitations of our study include the relatively small sample size and prolonged storage of some of our specimens prior to PCR-ESI/MS testing. In addition, because of the retrospective nature of the medical record review, some data, such as antecedent use of antimicrobial agents, may be incomplete. PCR-ESI/MS and culture may complement one another in microorganism detection in PJI, as PCR-ESI/MS detected a microorganism in one culture-negative PJI case and synovial fluid culture was positive in two PCR-ESI/MS-negative PJI cases. Failure of PCR-ESI/MS to detect an organism in four PJI cases may be explained by the time elapsed between specimen collection and the surgical procedure in one case, the low organism burden of chronic infection, and/or prolonged storage of synovial fluid specimens in the other cases.

In summary, our study shows that PCR-ESI/MS of synovial fluid has a similar sensitivity to synovial fluid culture albeit a lower specificity. PCR-ESI/MS can be performed in approximately 12 to 16 h and provides not just microbial identification but also information on selected antimicrobial resistance markers.

ACKNOWLEDGMENTS

Abbott Laboratories provided reagents and loaned a PLEX-ID PCR-ESI/MS instrument to the Mayo Clinic for this study. We thank Elie F. Berbari and Douglas R. Osmon for their input into the design of the study. We thank Melissa J. Karau and Suzannah M. Schmidt-Malan for collection and storage of synovial fluid specimens. We thank the outstanding technologists in the Mayo Clinic clinical bacteriology and initial processing laboratories for performing the cultures.

Research grant support was provided to R.P. by the National Institutes of Health (R01 AR056647 and R01 AI91594).

R.P. has patents on a PCR assay for detection of *Bordetella pertussis/parapertussis*, an antibiofilm substance, and a method/device for sonication. She has relinquished her rights to receive royalties for the sonication method/device.

REFERENCES

1. **Font-Vizcarra L, Garcia S, Martinez-Pastor JC, Sierra JM, Soriano A.** 2010. Blood culture flasks for culturing synovial fluid in prosthetic joint infections. Clin. Orthop. Relat. Res. **468:**2238–2243. http://dx.doi.org/10.1007/s11999-010-1254-3.
2. **Larsen LH, Lange J, Xu Y, Schonheyder HC.** 2012. Optimizing culture methods for diagnosis of prosthetic joint infections: a summary of modifications and improvements reported since 1995. J. Med. Microbiol. **61:**309–316. http://dx.doi.org/10.1099/jmm.0.035303-0.
3. **Vergidis P, Greenwood-Quaintance KE, Sanchez-Sotelo J, Morrey BF, Steinmann SP, Karau MJ, Osmon DR, Mandrekar JN, Steckelberg JM, Patel R.** 2011. Implant sonication for the diagnosis of prosthetic elbow infection. J. Shoulder Elbow Surg. **20:**1275–1281. http://dx.doi.org/10.1016/j.jse.2011.06.016.
4. **Piper KE, Jacobson MJ, Cofield RH, Sperling JW, Sanchez-Sotelo J, Osmon DR, McDowell A, Patrick S, Steckelberg JM, Mandrekar JN, Fernandez Sampedro M, Patel R.** 2009. Microbiologic diagnosis of prosthetic shoulder infection by use of implant sonication. J. Clin. Microbiol. **47:**1878–1884. http://dx.doi.org/10.1128/JCM.01686-08.
5. **Holinka J, Bauer L, Hirschl AM, Graninger W, Windhager R, Presterl E.** 2011. Sonication cultures of explanted components as an add-on test to routinely conducted microbiological diagnostics improve pathogen detection. J. Orthop. Res. **29:**617–622. http://dx.doi.org/10.1002/jor.21286.
6. **Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, Mandrekar JN, Cockerill FR, Steckelberg JM, Greenleaf JF, Patel R.** 2007. Sonication of removed hip and knee prostheses for diagnosis of infection. N. Engl. J. Med. **357:**654–663. http://dx.doi.org/10.1056/NEJMoa061588.

7. **Vandercam B, Jeumont S, Cornu O, Yombi JC, Lecouvet F, Lefevre P, Irenge LM, Gala JL.** 2008. Amplification-based DNA analysis in the diagnosis of prosthetic joint infection. J. Mol. Diagn. **10:**537–543. http://dx.doi.org/10.2353/jmoldx.2008.070137.
8. **De Man FH, Graber P, Luem M, Zimmerli W, Ochsner PE, Sendi P.** 2009. Broad-range PCR in selected episodes of prosthetic joint infection. Infection **37:**292–294. http://dx.doi.org/10.1007/s15010-008-8246-1.
9. **Gallo J, Kolar M, Dendis M, Loveckova Y, Sauer P, Zapletalova J, Koukalova D.** 2008. Culture and PCR analysis of joint fluid in the diagnosis of prosthetic joint infection. New Microbiol. **31:**97–104.
10. **Panousis K, Grigoris P, Butcher I, Rana B, Reilly JH, Hamblen DL.** 2005. Poor predictive value of broad-range PCR for the detection of arthroplasty infection in 92 cases. Acta Orthop. **76:**341–346.
11. **Achermann Y, Vogt M, Leunig M, Wust J, Trampuz A.** 2010. Improved diagnosis of periprosthetic joint infection by multiplex PCR of sonication fluid from removed implants. J. Clin. Microbiol. **48:**1208–1214. http://dx.doi.org/10.1128/JCM.00006-10.
12. **Portillo ME, Salvado M, Sorli L, Alier A, Martinez S, Trampuz A, Gomez J, Puig L, Horcajada JP.** 2012. Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure. J. Infect. **65:**541–548. http://dx.doi.org/10.1016/j.jinf.2012.08.018.
13. **Cazanave C, Greenwood-Quaintance KE, Hanssen AD, Karau MJ, Schmidt SM, Gomez Urena EO, Mandrekar JN, Osmon DR, Lough LE, Pritt BS, Steckelberg JM, Patel R.** 2013. Rapid molecular microbiologic diagnosis of prosthetic joint infection. J. Clin. Microbiol. **51:**2280–2287. http://dx.doi.org/10.1128/JCM.00335-13.
14. **Baldwin CD, Howe GB, Sampath R, Blyn LB, Matthews H, Harpin V, Hall TA, Drader JJ, Hofstadler SA, Eshoo MW, Rudnick K, Studarus K, Moore D, Abbott S, Janda JM, Whitehouse CA.** 2009. Usefulness of multilocus polymerase chain reaction followed by electrospray ionization mass spectrometry to identify a diverse panel of bacterial isolates. Diagn. Microbiol. Infect. Dis. **63:**403–408. http://dx.doi.org/10.1016/j.diagmicrobio.2008.12.012.
15. **Wallet F, Herwegh S, Decoene C, Courcol RJ.** 2013. PCR-electrospray ionization time-of-flight mass spectrometry: a new tool for the diagnosis of infective endocarditis from heart valves. Diagn. Microbiol. Infect. Dis. **76:**125–128. http://dx.doi.org/10.1016/j.diagmicrobio.2013.02.007.
16. **Laffler TG, Cummins LL, McClain CM, Quinn CD, Toro MA, Carolan HE, Toleno DM, Rounds MA, Eshoo MW, Stratton CW, Sampath R, Blyn LB, Ecker DJ, Tang YW.** 2013. Enhanced diagnostic yields of bacteremia and candidemia in blood specimens by PCR-electrospray ionization mass spectrometry. J. Clin. Microbiol. **51:**3535–3541. http://dx.doi.org/10.1128/JCM.00876-13.
17. **Brinkman CL, Vergidis P, Uhl JR, Pritt BS, Cockerill FR, Steckelberg JM, Baddour LM, Maleszewski JJ, Edwards WD, Sampath R, Patel R.** 2013. PCR-electrospray ionization mass spectrometry for direct detection of pathogens and antimicrobial resistance from heart valves in patients with infective endocarditis. J. Clin. Microbiol. **51:**2040–2046. http://dx.doi.org/10.1128/JCM.00304-13.
18. **Kaleta EJ, Clark AE, Cherkaoui A, Wysocki VH, Ingram EL, Schrenzel J, Wolk DM.** 2011. Comparative analysis of PCR-electrospray ionization/mass spectrometry (MS) and MALDI-TOF/MS for the identification of bacteria and yeast from positive blood culture bottles. Clin. Chem. **57:**1057–1067. http://dx.doi.org/10.1373/clinchem.2011.161968.
19. **Greenwood-Quaintance KE, Uhl JR, Hanssen AD, Sampath R, Mandrekar JN, Patel R.** 2014. Diagnosis of prosthetic joint infection by use of PCR-electrospray ionization mass spectrometry. J. Clin. Microbiol. **52:**642–649. http://dx.doi.org/10.1128/JCM.03217-13.
20. **Jacovides CL, Kreft R, Adeli B, Hozack B, Ehrlich GD, Parvizi J.** 2012. 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. **94:**2247–2254. http://dx.doi.org/10.2106/JBJS.L.00210.
21. **Greenwood-Quaintance KE, Uhl JR, Hanssen AD, Sampath R, Mandrekar JN, Patel R.** 27 November 2013. Diagnosis of prosthetic joint infection (PJI) using polymerase chain reaction-electrospray ionization mass spectrometry (PCR-ESI/MS). J. Clin. Microbiol. http://dx.doi.org/10.1128/JCM.03217-13.
22. **Trampuz A, Piper KE, Steckelberg JM, Patel R.** 2006. Effect of gamma irradiation on viability and DNA of *Staphylococcus epidermidis* and *Escherichia coli*. J. Med. Microbiol. **55:**1271–1275. http://dx.doi.org/10.1099/jmm.0.46488-0.
23. **Cazanave C, Greenwood-Quaintance KE, Hanssen AD, Karau MJ, Schmidt SM, Gomez Urena EO, Mandrekar JN, Osmon DR, Lough LE, Pritt BS, Steckelberg JM, Patel R.** 2013. Rapid molecular microbiologic diagnosis of prosthetic joint infection. J. Clin. Microbiol. **51:**2280–2287. http://dx.doi.org/10.1128/JCM.00335-13.
24. **Parvizi J, Zmistowski B, Berbari EF, Bauer TW, Springer BD, Della Valle CJ, Garvin KL, Mont MA, Wongworawat MD, Zalavras CG.** 2011. New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. Clin. Orthop. Relat. Res. **469:**2992–2994. http://dx.doi.org/10.1007/s11999-011-2102-9.
25. **Hofstadler SA, Sampath R, Blyn LB, Eshoo MW, Hall TA, Jiang Y, Drader JJ, Hannis JC, Sannes-Lowery KA, Cummins LL, Libby B, Walcott DJ, Schink A, Massire C, Ranken R, Gutierrez J, Manalili S, Ivy C, Melton R, Levene H, Barrett-Wilt G, Li F, Zapp V, White N, Samant V, McNeil JA, Knize D, Robbins D, Rudnick K, Desai A, Moradi E, Ecker DJ.** 2005. TIGER: the universal biosensor. Int. J. Mass Spectrom. **242:**23–41. http://dx.doi.org/10.1016/j.ijms.2004.09.014.
26. **CLSI.** 2013. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement M100S24E. Clinical and Laboratory Standards Institute, Wayne, PA.