COPYRIGHT © BY THE JOURNAL OF BONE AND JOINT SURGERY, INCORPORATED McClure et al. Application of Nucleic Acid-Based Strategies to Detect Infectious Pathogens in Orthopaedic Implant-Related Infection http://dx.doi.org/10.2106/JBJS.22.00315 Page 1

The following content was supplied by the authors as supporting material and has not been copy-edited or verified by JBJS.

Study	Country	Year published	Category	Seq type	Sequenced Nucleic Acid	Target Gene	Sequencing Technology	Infection diagnosis	Sample site	Level of Evidence
			G .:				Real-time &			II
Grif et al ¹	Austria	2012	Septic arthritis	16S & 18S rRNA gene PCR	DNA	16S & 18S	Sanger Sequencing	Positive culture	hip, knee	
Suda et al ²	Germany	2013	РЛ	16S rRNA gene PCR	DNA	16S	Endpoint PCR & Sanger Sequencing	Surgeon judgement	hip, knee	II
Suda et al	Czech	2013	131	16S rRNA gene PCR &	DIVA	105	Endpoint	judgement	hip, knee,	II
Gallo et al ³	Republic	2008	PJI	RFLP	DNA	16S	PCR	MSIS variant	elbow	
Chen et al ⁴	Taiwan	2019	РЈІ	16S rRNA gene PCR	DNA	16S	Endpoint PCR	MSIS	hip, knee	II
Cazanave et al ⁵	United States	2013	РЈІ	RT-FRET	DNA	16S	Real-time	MSIS	hip, knee	II
Gomez et al ⁶	United States	2012	РЛ	16S rRNA gene PCR	DNA	16S	Real-time & Sanger Sequencing	MSIS	hip, knee	II
Rak et al ⁷	Slovenia	2013	РЛ	16S rRNA gene PCR	DNA	16S	Real-time & Sanger Sequencing	MSIS	hip, knee	II
Omar et al ⁸	Germany	2018	РЛ	16S rRNA gene PCR	DNA	16S	Endpoint PCR & Sanger Sequencing	MSIS	hip, knee	II
Borde et al ⁹	Germany	2015	РЛ	RT-PCR, Sanger sequencing, Multiplex PCR (Unyvero- ITI)	RNA	16S	Endpoint PCR & Sanger Sequencing	IDSA	hip, knee	II
Bereza et	Germany	2013	1 11	111)	MINA	105	Sanger	Surgeon	mp, knee	II
al ¹⁰	Poland	2013	РЛ	16S rRNA gene PCR	DNA	16S	Sequencing	judgement	hip, knee	
Omar et al ¹¹	Germany	2016	Fx	16S rRNA gene PCR	DNA	16S	Endpoint PCR &	MSIS	hip, knee	II

COPYRIGHT $\ensuremath{\mathbb{C}}$ By The Journal of Bone and Joint Surgery, Incorporated McClure et al.

 $Application \ of \ Nucleic \ Acid-Based \ Strategies \ to \ Detect \ Infectious \ Pathogens \ in \ Orthopaedic \ Implant-Related \ Infection \ http://dx.doi.org/10.2106/JBJS.22.00315$

							Sanger			
							Sequencing			
Palmer et	United						Endpoint		fracture	II
al ¹²	States	2014	Nonunion	Multiplex PCR (Ibis)	DNA	many	PCR	Positive culture	nonunion	
							Real-time &		hip, knee,	II
Bemer et							Sanger		shoulder,	
al ¹³	France	2014	PJI	16S rRNA gene PCR	DNA	16S	Sequencing	MSIS	elbow	
							Endpoint			II
							PCR &			
							Sanger			
Rak et al ¹⁴	Slovenia	2016	PJI	16S rRNA gene PCR	DNA	16S	Sequencing	MSIS	hip, knee	
							Endpoint			II
							PCR &			
Stylianakis							Sanger			
et al ¹⁵	Greece	2018	PJI	16S rRNA gene PCR	DNA	16S	Sequencing	MSIS variant	hip, knee	
							Endpoint			II
							PCR &			
				16S/28S rRNA gene			Sanger			
Kuo et al ¹⁶	Taiwan	2018	PJI	sequencing	RNA	16S & 28S	Sequencing	MSIS	hip, knee	
	United			metagenomic shotgun seq		metagenom	Next			II
Ivy et al ¹⁷	States	2018	PJI	(Illumina HiSeq)	DNA	ic	Generation	IDSA	hip, knee	
Thoendel et	United			metagenomic shotgun seq		metagenom	Next			
al^{18}	States	2018	PJI	(Illumina HiSeq)	DNA	ic	Generation	IDSA	hip, knee	II
							Next			
He et al ¹⁹	China	2021	PJI	mNGS	DNA		Generation	MSIS	hip, knee	II
Huang et						metagenom	Next			
al^{20}	China	2020	PJI	mNGS	DNA	ic	Generation	MSIS	hip, knee	II
							Endpoint			
Lazic et al ²¹	Germany	2021	PJI	Multiplex PCR (Unyvero)	DNA	many	PCR	Zimmerli criteria	hip, knee	II
	_								hip, knee,	
Portillo et	Spain,			Multiplex PCR (Roche-			Endpoint		shoulder,	
al ²²	Switzerland	2012	PJI	SeptiFast)	DNA	many	PCR	MSIS variant	elbow	II
Jacobs et				Multiplex PCR (Unyvero-			Endpoint			
al^{23}	Netherlands	2021	PJI	ITI)	DNA	many	PCR	aseptic	hip, knee	II
								•	hip, knee,	
				Multiplex PCR (Unyvero-			Endpoint		shoulder,	
Renz et al ²⁴	Germany	2017	PJI	i60)	DNA	many	PCR	MSIS	elbow	II
Lausmann				Multiplex PCR (Unyvero-			Endpoint			
et al ²⁵	Germany	2017	PJI	ITI)	DNA	many	PCR	MSIS	hip, knee	П

COPYRIGHT $\ensuremath{\mathbb{C}}$ By The Journal of Bone and Joint Surgery, Incorporated McClure et al.

 $Application \ of \ Nucleic \ Acid-Based \ Strategies \ to \ Detect \ Infectious \ Pathogens \ in \ Orthopaedic \ Implant-Related \ Infection \ http://dx.doi.org/10.2106/JBJS.22.00315$

Morgenstern				Multiplex PCR (Unyvero-			Endpoint			
et al ²⁶	Germany	2018	PJI	ITI)	DNA	many	PCR	EBJIS	knee, hip	II
				Multiplex PCR (Unyvero-			Endpoint			
Suren et al ²⁷	Germany	2020	PJI	ITI)	DNA	many	PCR	MSIS	hip, knee	II
Kildow et	United						Next			
al^{28}	States	2021	PJI	NGS (MicroGen)	DNA	16S & ITS	Generation	MSIS	hip, knee	II
								Positive culture		
Tarabichi et	United						Next	and alpha		
al ²⁹	States	2018	PJI	NGS (Ion Torrent)	DNA	16S & ITS	Generation	defensin	hip, knee	II
Namdari et	United						Next	Frangiamore		
al^{30}	States	2019	PJI	NGS (454)	DNA	16S	Generation	criteria	Shoulder	II
	United						Next			
Rao et al ³¹	States	2020	PJI	NGS (MicroGen)	DNA	16S & ITS	Generation	Positive culture	Shoulder	II
Torchia et	United						Next			
al^{32}	States	2020	PJI	NGS (MicroGen)	DNA	16S & ITS	Generation	Aseptic	knee	II
	United						Next			
Flurin et al ³³	States	2021	PJI	NGS (Illumina MiSeq)	DNA	16S	Generation	IDSA	elbow	II
	United					spp.	Endpoint			
Askar et al ³⁴	Kingdom	2019	PJI	PMA PCR	DNA	Specific	PCR	MSIS	hip, knee	II
Clarke et	United						Endpoint			
al ³⁵	States	2004	PJI	PCR	DNA	16S	PCR	Aseptic	hip	II
						gyrB &	Endpoint			
Metso et al ³⁶	Finland	2014	PJI	PCR & microarray (Prove-it)	DNA	mecA	PCR	MSIS	hip, knee	II
Jacovides et	United			ESI-TOF-MS (Ibis T5000)			Next	Surgeon		
al^{37}	States	2012	PJI	& NGS (454)	DNA	16S	Generation	judgement	hip, knee	II
Melendez et	United									
al^{38}	States	2016	PJI	RT-FRET	DNA	many	Real-time	MSIS	knee	II
							Real-time &			
Moshirabadi							Endpoint			
et al ³⁹	Iran	2019	PJI	RT-PCR & RFLP	DNA	16S	PCR	MSIS	hip, knee	II
Bergin et	United									
al ⁴⁰	States	2010	PJI	RT-qPCR	RNA	16S	Real-time	MSIS variant	knee	II
	United									
Ryu et al ⁴¹	States	2014	PJI	RT-FRET	DNA	many	Real-time	MSIS variant	knee	II
Yang et al ⁴²	China	2020	PJI	RT-PCR	DNA	16S	Real-time	ICM	hip, knee	II
			_				Real-time &			
							Sanger			
Yang et al ⁴³	China	2021	PJI	RT-qPCR	RNA	16S	Sequencing	MSIS	hip, knee	II

COPYRIGHT © BY THE JOURNAL OF BONE AND JOINT SURGERY, INCORPORATED

McClure et al.

 $Application \ of \ Nucleic \ Acid-Based \ Strategies \ to \ Detect \ Infectious \ Pathogens \ in \ Orthopaedic \ Implant-Related \ Infection$

http://dx.doi.org/10.2106/JBJS.22.00315

Page 4

Fink et al ⁴⁴	Carmany	2018	PJI	UMD-Universal PCR	DNIA	160 % 100	Endpoint PCR & Sanger	Positive culture	Knee	11
rink et ai	Germany	2018	PJI	UMD-Universal PCR	DNA	16S & 18S	Sequencing	Positive culture	Knee	II
Kildow et	United						Next			
al ⁴⁵	States	2020	PJI	NGS (MicroGen)	DNA	16S & ITS	Generation	IDSA	knee hip	III
Miyamae et										
al ⁴⁶	Japan	2012	PJI	RT-PCR	DNA	16S	Real-time	Positive culture	hip, knee	III

Legend: DNA: deoxyribonucleic acid; ESI-TOF: Electrospray Ionization Time-of-Flight; IDSA: Infectious diseases society of America; mNGS: metagenomic next-generation sequencing; MS: Mass spectrometry; MSIS: Musculoskeletal infection society; NGS: Next-generation sequencing; PCR: polymerase chain reaction; PMA-PCR: propidium monoazide-based PCR; PJI: prosthetic joint infection; qPCR: Quantitative PCR; RFLP: restriction fragment length polymorphism; RNA: ribonucleic acid; rRNA: ribosomal RNA; RT-FRET: real-time fluorescence resonance energy transfer; RT-PCR: real-time PCR; UMD: Universal microbial diagnostics; 16S: small subunit of bacterial ribosome; 18S: small subunit of eukaryotic ribosome; 28S: large subunit of eukaryotic ribosome

References for Supplemental Table 1:

- 1. Grif, K. *et al.* Improvement of detection of bacterial pathogens in normally sterile body sites with a focus on orthopedic samples by use of a commercial 16S rRNA broad-range PCR and sequence analysis. *J Clin Microbiol* **50**, 2250–2254 (2012).
- 2. Suda, A. J. *et al.* Prosthetic infection: improvement of diagnostic procedures using 16S ribosomal deoxyribonucleic acid polymerase chain reaction. *Int Orthop* **37**, 2515–2521 (2013).
- 3. Gallo, J. et al. Culture and PCR analysis of joint fluid in the diagnosis of prosthetic joint infection. The new microbiologica 31, (2008).
- 4. Chen, M.-F. *et al.* Rapid analysis of bacterial composition in prosthetic joint infection by 16S rRNA metagenomic sequencing. *Bone Joint Res* **8**, 367–377 (2019).
- 5. Cazanave, C. et al. Rapid molecular microbiologic diagnosis of prosthetic joint infection. J Clin Microbiol 51, 2280–2287 (2013).

McClure et al.

APPLICATION OF NUCLEIC ACID-BASED STRATEGIES TO DETECT INFECTIOUS PATHOGENS IN ORTHOPAEDIC IMPLANT-RELATED INFECTION

http://dx.doi.org/10.2106/JBJS.22.00315

- 6. Gomez, E. *et al.* Prosthetic joint infection diagnosis using broad-range PCR of biofilms dislodged from knee and hip arthroplasty surfaces using sonication. *Journal of clinical microbiology* **50**, (2012).
- 7. Rak, M., Barlič-Maganja, D., Kavčič, M., Trebše, R. & Cőr, A. Comparison of molecular and culture method in diagnosis of prosthetic joint infection. *FEMS Microbiol Lett* **343**, 42–48 (2013).
- 8. Omar, M. *et al.* Higher sensitivity of swab polymerase chain reaction compared with tissue cultures for diagnosing periprosthetic joint infection. *J Orthop Surg (Hong Kong)* **26**, 2309499018765296 (2018).
- 9. Borde, J. P. *et al.* Diagnosis of prosthetic joint infections using UMD-Universal Kit and the automated multiplex-PCR Universal (®) cartridge system: a pilot study. *Infection* **43**, 551–560 (2015).
- 10. Bereza, P. L. *et al.* Identification of silent prosthetic joint infection: preliminary report of a prospective controlled study. *Int Orthop* **37**, 2037–2043 (2013).
- 11. Omar, M. *et al.* Diagnostic performance of swab PCR as an alternative to tissue culture methods for diagnosing infections associated with fracture fixation devices. *Injury* **47**, 1421–1426 (2016).
- 12. Palmer, M. P. et al. Can We Trust Intraoperative Culture Results in Nonunions? Journal of Orthopaedic Trauma 28, 384–390 (2014).
- 13. Bémer, P. *et al.* Evaluation of 16S rRNA gene PCR sensitivity and specificity for diagnosis of prosthetic joint infection: a prospective multicenter cross-sectional study. *J Clin Microbiol* **52**, 3583–3589 (2014).
- 14. Rak, M., KavčIč, M., Trebše, R. & CőR, A. Detection of bacteria with molecular methods in prosthetic joint infection: sonication fluid better than periprosthetic tissue. *Acta Orthop* 87, 339–345 (2016).

COPYRIGHT © BY THE JOURNAL OF BONE AND JOINT SURGERY, INCORPORATED

McClure et al.

APPLICATION OF NUCLEIC ACID-BASED STRATEGIES TO DETECT INFECTIOUS PATHOGENS IN ORTHOPAEDIC IMPLANT-RELATED INFECTION

http://dx.doi.org/10.2106/JBJS.22.00315

- 15. Stylianakis, A. *et al.* Combination of conventional culture, vial culture, and broad-range PCR of sonication fluid for the diagnosis of prosthetic joint infection. *Diagn Microbiol Infect Dis* **92**, 13–18 (2018).
- 16. Kuo, F.-C. *et al.* Comparison of molecular diagnosis with serum markers and synovial fluid analysis in patients with prosthetic joint infection.

 *Bone Joint J 100-B, 1345–1351 (2018).
- 17. Ivy, M. I. *et al.* Direct Detection and Identification of Prosthetic Joint Infection Pathogens in Synovial Fluid by Metagenomic Shotgun Sequencing. *J Clin Microbiol* **56**, e00402-18 (2018).
- 18. Thoendel, M. J. et al. Identification of Prosthetic Joint Infection Pathogens Using a Shotgun Metagenomics Approach. Clin Infect Dis 67, 1333–1338 (2018).
- 19. He, R. *et al.* Better choice of the type of specimen used for untargeted metagenomic sequencing in the diagnosis of periprosthetic joint infections.

 *Bone Joint J 103-B, 923-930 (2021).
- 20. Huang, Z. *et al.* Metagenomic next-generation sequencing of synovial fluid demonstrates high accuracy in prosthetic joint infection diagnostics: mNGS for diagnosing PJI. *Bone Joint Res* **9**, 440–449 (2020).
- 21. Lazic, I. *et al.* Diagnostic accuracy of multiplex polymerase chain reaction on tissue biopsies in periprosthetic joint infections. *Sci Rep* **11**, 19487 (2021).
- 22. Portillo, M. E. *et al.* Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure. *J Infect* **65**, 541–548 (2012).

McClure et al.

APPLICATION OF NUCLEIC ACID-BASED STRATEGIES TO DETECT INFECTIOUS PATHOGENS IN ORTHOPAEDIC IMPLANT-RELATED INFECTION

http://dx.doi.org/10.2106/JBJS.22.00315

- 23. Jacobs, A. M. E. *et al.* Ruling out underlying infection in 200 presumed aseptic knee and hip revision arthroplasties using a multiplex PCR system. *Eur J Clin Microbiol Infect Dis* **40**, 1283–1290 (2021).
- 24. Renz, N., Feihl, S., Cabric, S. & Trampuz, A. Performance of automated multiplex PCR using sonication fluid for diagnosis of periprosthetic joint infection: a prospective cohort. *Infection* **45**, 877–884 (2017).
- 25. Lausmann, C. et al. Are There Benefits In Early Diagnosis Of Prosthetic Joint Infection With Multiplex Polymerase Chain Reaction? J Bone Jt Infect 2, 175–183 (2017).
- 26. 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* **90**, 115–119 (2018).
- 27. Suren, C. *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* **44**, 1629–1637 (2020).
- 28. Kildow, B. J. *et al.* Next-generation sequencing not superior to culture in periprosthetic joint infection diagnosis. *Bone Joint J* **103-B**, 26–31 (2021).
- 29. Tarabichi, M. et al. Diagnosis of Periprosthetic Joint Infection: The Potential of Next-Generation Sequencing. *The Journal of bone and joint surgery. American volume* **100**, (2018).
- 30. Namdari, S. *et al.* Comparative study of cultures and next-generation sequencing in the diagnosis of shoulder prosthetic joint infections. *J Shoulder Elbow Surg* **28**, 1–8 (2019).
- 31. Rao, A. J. et al. Next-generation sequencing for diagnosis of infection: is more sensitive really better? J Shoulder Elbow Surg 29, 20–26 (2020).

COPYRIGHT © BY THE JOURNAL OF BONE AND JOINT SURGERY, INCORPORATED

McClure et al.

APPLICATION OF NUCLEIC ACID-BASED STRATEGIES TO DETECT INFECTIOUS PATHOGENS IN ORTHOPAEDIC IMPLANT-RELATED INFECTION

http://dx.doi.org/10.2106/JBJS.22.00315

- 32. Torchia, M., Amakiri, I., Werth, P. & Moshetti, W. Characterization of native knee microorganisms using next-generation sequencing in patients undergoing primary total knee arthroplasty. *The Knee* 27, (2020).
- 33. Flurin, L., Wolf, M. J., Greenwood-Quaintance, K. E., Sanchez-Sotelo, J. & Patel, R. Targeted next generation sequencing for elbow periprosthetic joint infection diagnosis. *Diagn Microbiol Infect Dis* **101**, 115448 (2021).
- 34. Askar, M. *et al.* Propidium monoazide-polymerase chain reaction for detection of residual periprosthetic joint infection in two-stage revision. *Mol Biol Rep* **46**, 6463–6470 (2019).
- 35. Clarke, M. T. *et al.* Polymerase chain reaction can detect bacterial DNA in aseptically loose total hip arthroplasties. *Clin Orthop Relat Res* 132–137 (2004) doi:10.1097/01.blo.0000136839.90734.b7.
- 36. Metso, L. *et al.* Efficacy of a novel PCR- and microarray-based method in diagnosis of a prosthetic joint infection. *Acta Orthop* **85**, 165–170 (2014).
- 37. Jacovides, C. L. *et al.* 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 (2012).
- 38. Melendez, D. P. *et al.* Evaluation of a Genus- and Group-Specific Rapid PCR Assay Panel on Synovial Fluid for Diagnosis of Prosthetic Knee Infection. *J Clin Microbiol* **54**, 120–126 (2016).
- 39. Moshirabadi, A. *et al.* Polymerase Chain Reaction Assay Using the Restriction Fragment Length Polymorphism Technique in the Detection of Prosthetic Joint Infections: A Multi-Centered Study. *J Arthroplasty* **34**, 359–364 (2019).

McClure et al.

APPLICATION OF NUCLEIC ACID-BASED STRATEGIES TO DETECT INFECTIOUS PATHOGENS IN ORTHOPAEDIC IMPLANT-RELATED INFECTION

http://dx.doi.org/10.2106/JBJS.22.00315

- 40. Bergin, P. F. *et al.* Detection of periprosthetic infections with use of ribosomal RNA-based polymerase chain reaction. *J Bone Joint Surg Am* **92**, 654–663 (2010).
- 41. Ryu, S. Y., Greenwood-Quaintance, K. E., Hanssen, A. D., Mandrekar, J. N. & Patel, R. Low sensitivity of periprosthetic tissue PCR for prosthetic knee infection diagnosis. *Diagn Microbiol Infect Dis* **79**, 448–453 (2014).
- 42. Yang, B. *et al.* Detecting the presence of bacterial RNA by polymerase chain reaction in low volumes of preoperatively aspirated synovial fluid from prosthetic joint infections. *Bone Joint Res* **9**, 219–224 (2020).
- 43. Yang, F. *et al.* An automated real-time PCR assay for synovial fluid improves the preoperative etiological diagnosis of periprosthetic joint infection and septic arthritis. *J Orthop Res* **39**, 348–355 (2021).
- 44. Fink, B. *et al.* Preoperative PCR analysis of synovial fluid has limited value for the diagnosis of periprosthetic joint infections of total knee arthroplasties. *Arch Orthop Trauma Surg* **138**, 871–878 (2018).
- 45. Kildow, B. J. *et al.* Commercially Available Polymerase Chain Reaction Has Minimal Utility in the Diagnosis of Periprosthetic Joint Infection. *Orthopedics* **43**, 333–338 (2020).
- 46. Miyamae, Y. *et al.* Quantitative evaluation of periprosthetic infection by real-time polymerase chain reaction: a comparison with conventional methods. *Diagn Microbiol Infect Dis* **74**, 125–130 (2012).