AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

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Comparison of Diagnostic Accuracy of Periprosthetic Tissue Culture in Blood Culture Bottles to That of Prosthesis Sonication Fluid Culture for Diagnosis of Prosthetic Joint Infection (PJI) by Use of Bayesian Latent Class Modeling and IDSA PJI Criteria for Classification

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ABSTRACT We have previously demonstrated that culturing periprosthetic tissue in blood culture bottles (BCBs) improves sensitivity compared to conventional agar and broth culture methods for diagnosis of prosthetic joint infection (PJI). We have also shown that prosthesis sonication culture improves sensitivity compared to periprosthetic tissue culture using conventional agar and broth methods. The purpose of this study was to compare the diagnostic accuracy of tissue culture in BCBs (subsequently referred to as tissue culture) to prosthesis sonication culture (subsequently referred to as sonicate fluid culture). We studied 229 subjects who underwent arthroplasty revision or resection surgery between March 2016 and October 2017 at Mayo Clinic in Rochester, Minnesota. Using the Infectious Diseases Society of America (IDSA) PJI diagnostic criteria (omitting culture criteria) as the gold standard, the sensitivity of tissue culture was similar to that of the sonicate fluid culture (66.4% versus 73.1%, $P = 0.07$) but was significantly lower than that of the two tests combined (66.4% versus 76.9%, $P < 0.001$). Using Bayesian latent class modeling, which assumes no gold standard for PJI diagnosis, the sensitivity of tissue culture was slightly lower than that of sonicate fluid culture (86.3% versus 88.7%) and much lower than that of the two tests combined (86.3% versus 99.1%). In conclusion, tissue culture in BCBs reached sensitivity similar to that of prosthesis sonicate fluid culture for diagnosis of PJI, but the two tests combined had the highest sensitivity without compromising specificity. The combination of tissue culture in BCBs and sonicate fluid culture is recommended to achieve the highest level of microbiological diagnosis of PJI.

KEYWORDS prosthetic joint infection, PJI, periprosthetic tissue culture, sonicate fluid culture, blood culture bottles

Prosthetic joint infection (PJI) is a severe complication of joint arthroplasty, with an infection rate ranging from 0.88% to 2.18% (1–3). PJI is associated with mortality, prolonged hospital stays, and high cost (2, 4). Accurate diagnosis is important in PJI disease management; however, diagnosis remains challenging because signs and symptoms of PJI are often subtle (5) and there is no diagnostic test with perfect

Received 22 February 2018 **Returned for modification** 19 March 2018 **Accepted** 2 April 2018

Accepted manuscript posted online 11 April 2018

Citation Yan Q, Karau MJ, Greenwood-Quaintance KE, Mandrekar JN, Osmon DR, Abdel MP, Patel R. 2018. Comparison of diagnostic accuracy of periprosthetic tissue culture in blood culture bottles to that of prosthesis sonication fluid culture for diagnosis of prosthetic joint infection (PJI) by use of Bayesian latent class modeling and IDSA PJI criteria for classification. J Clin Microbiol 56:e00319-18. https://doi.org/10.1128/JCM.00319-18.

Editor Sandra S. Richter, Cleveland Clinic

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accuracy (6). Periprosthetic tissue (subsequently referred to as tissue) culture results are important criteria for diagnosis of PJI as defined by the Infectious Diseases Society of America (IDSA) and the Musculoskeletal Infection Society (MSIS) (6, 7). Currently, conventional tissue culture using agar and thioglycolate broth is the most commonly used clinical microbiological test. However, it has been reported as having low sensitivity, ranging from 33% for agar cultures to 44% with combined agar and thioglycolate broth culture when using IDSA PJI criteria (8).

With the recognition of the role of bacterial biofilms on prosthesis surfaces, Tunney et al. first applied sonication to dislodge adherent bacteria from explanted prosthetic hips (9). Our group further developed a clinically useful implant sonication technique and demonstrated that using sonication to dislodge adherent bacteria for culture yielded higher sensitivity than conventional tissue culture for diagnosis of prosthetic hip and knee infection (78.5% versus 60.8%, $P < 0.001$) (10). Subsequently, we showed that the sonication method had higher sensitivity than conventional tissue culture in shoulder arthroplasty infection (66.7% versus 54.5%, $P = 0.046$) and spinal implant infection (91% versus 73%, $P = 0.046$) (11, 12). Furthermore, several other groups have demonstrated that the sonication method improves the diagnosis of orthopedic implant-associated infection (13–16).

Despite the high sensitivity of sonicate fluid culture for diagnosis of PJI, periprosthetic tissue remains the predominant sample type used for culture-based diagnosis of PJI. Recently, we compared culturing tissue in blood culture bottles (BCBs) to conventional agar and thioglycolate broth cultures for diagnosis of PJI using Bayesian latent class modeling (LCM), a statistical method that can be used to define diagnostic accuracy in the absence of a "gold standard" (17). We demonstrated culturing tissue in BCBs to be more sensitive than agar and thioglycolate broth cultures. Tissue culture in BCBs, as well as sonicate fluid culture, is now standard practice at our institution (8). However, the sensitivity of tissue culture in BCBs compared to that of prosthesis sonicate fluid culture is unknown. In particular, it is not clear whether culturing periprosthetic tissue specimens in BCBs reaches the diagnostic accuracy of prosthesis sonication culture.

Therefore, the purpose of this study was to compare the diagnostic accuracy of periprosthetic tissue culture in BCBs to that of prosthesis sonicate fluid culture for the diagnosis of PJI. We applied Bayesian LCM for our analysis, in addition to using standard nonmicrobiological IDSA PJI criteria for classification of our cases.

MATERIALS AND METHODS

Study design. This study was a prospective cohort study of consecutive patients undergoing revision or resection hip, knee, shoulder, or elbow arthroplasty between March 2016 and September 2017 at Mayo Clinic, in Rochester, MN. This study was approved by the Mayo Clinic Inquisitional Review Board. Patients were excluded if they did not provide authorization of use of their health records (Minnesota Statute 144.335), no prosthesis was submitted for sonication culture, an antibiotic-loaded cement spacer was submitted for sonication culture, or fewer than two periprosthetic tissue specimens were cultured.

Patient classification. Patients were classified as having PJI based on nonmicrobiological IDSA criteria when they had at least one of the following criteria: a sinus tract communicating with the prosthesis was present, acute inflammation was noted on histopathologic examination of periprosthetic tissue obtained during surgery, or purulence surrounding the prosthesis was documented by the surgeon (7). Microbiology culture results were not used to define PJI because diagnostic accuracy of culture-based testing was being evaluated. Aseptic failure was defined as cases not meeting criteria for PJI. Patient laboratory values for comparison were chosen based on the threshold for PJI diagnostic criteria by the Musculoskeletal Infection Society (18). Patients were also separated into a lower extremity (i.e., hip and knee) subgroup and an upper extremity (i.e., shoulder and elbow) subgroup for diagnostic accuracy analyses.

Microbiological methods. Periprosthetic tissue culture was processed as previously described (8). Briefly, periprosthetic tissue collected during surgery was placed into a sterile vial containing CO_2 (to maintain anaerobic conditions) and delivered to the clinical microbiology laboratory. Tissue specimens were homogenized for 1 min in 5 ml brain heart infusion broth (Seward Stomacher 80 Biomaster; Seward Inc., Port St. Lucie, FL), and then 1 ml of homogenized liquid was inoculated (using a 3-ml syringe with a 22-gauge [22G] needle) into each of a Bactec Plus Aerobic/F bottle (with resin) and a Bactec Lytic/10 Anaerobic/F bottle (without resin) (BD Diagnostic Systems) according to standard procedures at our institution. Bottles were incubated in a Bactec FX instrument (BD Diagnostic Systems) for 14 days. Positive bottles were subcultured to agar plates based on the results of Gram staining per standard

laboratory practice. Periprosthetic tissue cultures were defined as positive when an identical microorganism was isolated from two or more tissue specimens or if *Staphylococcus aureus*, *Enterococcus* species, or yeast was isolated from a single tissue specimen. Sonication was performed as previously described (11). In brief, prostheses were collected and placed in sterile 1-liter straight-side wide-mouth polypropylene containers (Nalgene, Lima, OH). Sterile Ringer's solution (400 ml) was added to each container. The container was vortexed for 30 s and then sonicated for 5 min (40 kHz) using an ultrasonic cleaning bath (Branson 5510; Sonics, Richmond, VA), followed by additional vortexing for 30 s. The sonicate fluid was centrifuged at 4,000 rpm (3,150 $\times$ *g*) for 5 min and concentrated 1:100 to 4 ml; 0.1 ml of the concentrated sonicate fluid was inoculated onto aerobic sheep blood and chocolate agar and incubated in 5% CO_2 for 5 days and onto anaerobic sheep blood agar and incubated anaerobically for 14 days. A single colony growing on a plate is equivalent to one colony per 10 ml sonicate fluid. A cutoff value of $\geq$20 CFU/10 ml was defined as positive for sonicate fluid cultures, in accordance with the previously established standard at our institution (11). Sonicate fluid culture was also considered positive if any *S. aureus*, *Enterococcus* species, or yeast growth was present. Organism identification was performed using routine laboratory methods, most commonly, matrix-assisted laser desorption ionization–time of flight mass spectrometry, using a Bruker Daltonics system (Billerica, MA).

Statistical analysis. Descriptive summaries were reported as medians and interquartile ranges (IQRs) for continuous variables and as frequencies and percentages for categorical variables. Due to lack of a proper gold standard, the Bayesian LCM approach was used for the analysis. This approach overcomes potential flaws of traditional analysis and is based on the assumptions that no gold standard exists and that the true disease prevalence requires estimation, both relevant to PJI (17). We used this approach in our prior research work (8, 19). Using the Bayesian LCM Joseph et al. model (17), estimates of prevalence, sensitivity, and specificity were reported along with 95% credible intervals. We used uniform prior distribution for the prevalence of each latent variable as done in our prior study (8). Input data for Bayesian LCM are shown in Table S1 in the supplemental material. Further analysis was also performed using nonmicrobiological IDSA criteria as a gold standard (7). Sensitivity, specificity, NPV, and PPV were estimated, along with 95% exact binomial confidence intervals. Comparison of sensitivities and specificities among culture-types and the combination of tissue and sonicate fluid culture were performed in a pairwise manner using McNemar's test. *P* values of less than 0.05 were considered statistically significant. Analyses were performed using SAS version 9.4 (SAS Inc., Cary, NC) and Bayesian LCM software version 1.14 (20), which is available at http://www.nandinidendukuri.com/software/blcm.

RESULTS

Patient characteristics. A total of 305 subjects undergoing revision or resection arthroplasties during the study period had prostheses sent for sonication culture. Seventy-six subjects were excluded: 24 did not have written authorization for use of their medical records, 37 had an antimicrobial spacer submitted for culture, and 15 had fewer than two periprosthetic tissue samples submitted for culture. The remaining 229 subjects were studied, and among them, 104 had PJI and 125 had aseptic failure according to IDSA nonmicrobiological PJI criteria by meeting at least one of the following criteria: a sinus tract communicating with the prosthesis, acute inflammation noted on histopathologic examination of periprosthetic tissue obtained during surgery, or purulence surrounding the prosthesis documented by the surgeon (7). Among 104 PJI subjects, 8.7% (9/104) met all three criteria, 38.4% (40/104) met two of three criteria, and 52.9% (55/104) met a single criterion. Demographic and clinical characteristics of the study subjects are shown in Table 1. Subject age and gender distributions and reasons for primary arthroplasty were similar between the two groups. Most subjects had pain without significant difference between the two groups. Fever and local signs of infection were, not unexpectedly, higher among PJI than aseptic failure subjects. PJI subjects more frequently had an elevated preoperative blood leukocyte count, C-reactive protein concentration, erythrocyte sedimentation rate, or synovial fluid leukocyte count, as well as positive synovial fluid cultures than did aseptic failure subjects ($P < 0.0001$). Eighteen percent of PJI subjects had revision or resection surgeries less than 3 months after prosthesis implantation compared to only 1% of the aseptic failure subjects ($P < 0.0001$). Eighty-four percent of aseptic failure subjects had surgeries performed >12 months after prosthesis implantation, compared to 57% of the PJI subjects ($P < 0.0001$).

Microbiology. In total, 85 of 229 subjects had microorganisms detected by tissue culture or sonicate fluid culture, and the majority were monomicrobial (74 subjects, 87.1%), with a small portion being polymicrobial (11 subjects, 12.9%). *Staphylococcus aureus* ($n = 17$) and *Staphylococcus epidermidis* ($n = 15$) were the most prevalent organisms among PJI subjects; *S. aureus* was the predominant pathogen among

TABLE 1 Characteristics of the study subjects

Characteristic	Value[a] for subjects with:		P[b]
	Prosthetic joint infection (n = 104)	Aseptic failure (n = 125)	
Median age (IQR), yr]	66.5 (25, 92)	67 (26, 87)	0.825
Male gender	61 (59)	66 (53)	0.375
Type of prosthetic joint			0.013
Hip	31 (30)	16 (13)	
Knee	55 (53)	85 (68)	
Shoulder	11 (10)	12 (10)	
Elbow	7 (7)	12 (10)	
Reason for primary arthroplasty			0.618
Osteoarthritis	66 (64)	86 (69)	
Fracture or trauma	23 (22)	28 (22)	
Rheumatoid arthritis	5 (5)	5 (4)	
Avascular necrosis	0 (0)	1 (1)	
Fibromyalgia	1 (1)	1 (1)	
Tumor	3 (3)	2 (2)	
Unknown	6 (6)	2 (2)	
Presence of sinus tract	23 (22)	0 (0)	<0.0001
Visible purulence at implant site	79 (76)	0 (0)	<0.0001
Acute inflammation in periprosthetic tissue [no./total (%)]	55/86 (64)	0/117 (0)	<0.0001
Presence of clinical symptoms or signs			
Pain	92 (89)	119 (95)	0.059
Local signs of infection[c]	48 (46)	4 (3)	<0.0001
Fever	8 (8)	2 (2)	0.025
Radiolucent lines present	33 (32)	49 (39)	0.241
Preoperative laboratory findings [no./total (%)][d]			
Blood leukocyte count >10 × 10^9/liter	21/97 (22)	15/110 (14)	<0.0001
Erythrocyte sedimentation rate >30 mm/h	58/98 (59)	16/116 (14)	<0.0001
Serum C-reactive protein concn >10 mg/liter	72/100 (72)	22/116 (19)	<0.0001
Synovial fluid leukocyte count >3 × 10^9/liter	38/50 (76)	14/56 (25)	<0.0001
Synovial fluid neutrophils >80%	31/50 (62)	6/56 (11)	<0.0001
Positive synovial fluid culture	27/57 (48)	2/63 (3)	<0.0001
Antibiotics received 4 weeks prior to surgery	38 (37)	10 (8)	<0.0001
Surgical procedure			<0.0001
Revision (including one-stage exchange)	26 (25)	102 (82)	
Resection (including insertion of a spacer)	78 (75)	23 (18)	
No. of tissue cultures [median (IQR)]	4 (2, 10)	3 (2, 10)	<0.0001
Timing from prosthesis implanted to surgery (mo)			<0.0001
<3	19 (18)	1 (1)	
3–12	26 (25)	19 (15)	
>12	59 (57)	105 (84)	

[a]Values are no. or no./total (%) unless interquartile range (IQR) is specified.
[b]Age as a continuous variable was compared using the Wilcoxon rank sum test, and categorical variables were compared using Fisher's exact test.
[c]Local signs of infection included redness and swelling around the surgical site.
[d]Blood leukocyte count was the result within 1 week preoperative; other laboratory findings were the latest results within 6 months.

prosthetic hip infections, and *S. epidermidis* was the most common organism in prosthetic knee and elbow infections. *Cutibacterium acnes* (n = 11) was the third most commonly identified organism and was the dominant organism in prosthetic shoulder infection. It took 3 to 13 days for *C. acnes* to grow in blood culture bottles and 5 to 12 days for *C. acnes* to grow in sonicate fluid culture. An unusual finding was that one PJI subject had *Mycobacterium bovis* BCG detected through periprosthetic tissue mycobacterial culture, while the aerobic and anaerobic cultures of periprosthetic tissue and sonicate fluid were both negative. Microbiological findings in periprosthetic tissue and

TABLE 2 Microbiology results of periprosthetic tissue and sonicate fluid cultures

Category and no. of patients and culture results	Microorganism(s) (no. of patients)	No. of affected prosthetic joints			
		Hip	Knee	Shoulder	Elbow
Patients with prosthetic joint infection (104)					
Positive tissue and sonicate fluid cultures (65)		20	33	7	5
Concordant (58)					
Monomicrobial (54)	*Staphylococcus aureus* (15)	8	6	1	
	Staphylococcus epidermidis (11)	1	9		1
	Streptococcus mitis group (5)		5		
	Cutibacterium acnes (5)			5	
	Staphylococcus lugdunensis (3)	1	2		
	Enterococcus faecalis (3)	1	1		1
	Staphylococcus caprae (1)		1		
	Staphylococcus simulans (1)				1
	Streptococcus agalactiae (1)	1			
	Streptococcus dysgalactiae (1)		1		
	Streptococcus salivarius group (1)		1		
	Clostridium ramosum (1)		1		
	Corynebacterium striatum (1)		1		
	Escherichia coli (1)		1		
	Lelliottia amnigena (1)		1		
	Pseudomonas aeruginosa (1)		1		
	Enterobacter cloacae (1)				1
	Prevotella bivia (1)	1			
Polymicrobial (4)	*S. epidermidis* + *Staphylococcus pseudintermedius* (1)	1			
	S. mitis + *E. cloacae* + *Finegoldia magna* (1)	1			
	S. aureus + *P. aeruginosa* (1)	1			
	Candia albicans + *E. faecalis* (1)	1			
Discordant (7) (organisms detected in tissue/sonicate fluid)	*E. cloacae* (1)/*E. cloacae* + *Finegoldia magna* (1)	1			
	S. epidermidis (1)/*S. epidermidis* + *Corynebacterium amycolatum* (1)	1			
	Aerococcus sanguinicola + *P. aeruginosa* (1)/*A. sanguinicola* + *Anaerococcus murdochii* (1)	1			
	S. aureus + *E. faecalis* + *E. coli* (1)/*E. faecalis* (1)		1		
	Candida parapsilosis + *C. acnes* (1)/*C. parapsilosis* (1)		1		
	Staphylococcus saccharolyticus + *C. acnes* (1)/*C. acnes* + *Enterococcus faecium* (1)			1	
	S. lugdunensis (1)/*S. lugdunensis* + *E. coli* (1)				1
Positive tissue and negative sonicate fluid cultures (4)		2	1	1	
	C. acnes (1)			1	
	S. epidermidis (1)	1			
	S. aureus (1)		1		
	S. aureus + *C. striatum* (1)	1			
Negative tissue and positive sonicate fluid cultures (11)		5	2	2	2
	S. epidermidis (3)	1		1	1
	S. capitis (2)	2			
	S. caprae (1)	1			
	S. aureus (1)		1		
	S. mitis group (1)		1		
	C. striatum (1)	1			
	C. acnes (1)			1	
	Parvimonas micra (1)				1
Negative tissue and sonicate fluid cultures (24)		4	19	1	
Patients with aseptic failure (125)					
Positive tissue and negative sonicate fluid cultures (5)			2	3	
	C. acnes (4)		1	3	
	S. capitis (1)		1		
Negative tissue and sonicate fluid cultures (120)		16	83	9	12

sonicate fluid culture are shown in Table 2. Sixty-five (62.5%) PJI subjects had microorganisms detected by both tissue culture and sonicate fluid culture. Fifteen (14.4%) PJI subjects had organisms detected only by tissue culture or sonicate fluid culture, and the remaining 24 (23.1%) PJI subjects had no microorganism detected with either method. One hundred twenty (96.0%) subjects with aseptic failure had negative results from both tests. However, five (4.0%) subjects with aseptic failure had tissue cultures defined as positive with an identical microorganism isolated from two or more tissue specimens. Four of these tissue cultures had *C. acnes*, and the corresponding sonicate fluid cultures had less than 20 CFU/10 ml organisms isolated (without further identification performed), which was below the threshold of positivity. The fifth subject had positive tissue cultures for *Staphylococcus capitis* without any organism growth in sonicate fluid.

Sensitivity and specificity. Using IDSA nonmicrobiological criteria as the gold standard for diagnosing PJI, the sensitivity of tissue culture was not statistically different from that of sonicate fluid culture (66.4%, 69/104 versus 73.1%, 76/104; $P = 0.07$), but was significantly lower than that of the two tests combined (66.4%, 69/104 versus 76.9%, 80/104; $P < 0.001$). The sensitivity of sonicate fluid culture alone was slightly lower than that of the two tests combined (73.1%, 76/104 versus 76.9%, 80/104; $P = 0.045$). The specificity of tissue culture was lower than that of sonicate fluid (96.0%, 120/125 versus 100%, 125/125; $P = 0.025$) and the same as that of the two tests combined (96.0%, 120/125 for both). In the lower extremity subgroup (i.e., hips and knees), the sensitivity of tissue culture was not different from that of sonicate fluid culture (65.1%, 56/86 versus 69.8%, 60/86; $P = 0.205$). In the upper extremity subgroup (i.e., shoulders and elbows), tissue culture showed lower sensitivity than sonicate fluid culture (72.2%, 13/18, versus 88.9%, 16/18), although the difference was not statistically significant ($P = 0.179$). Individual joint types were not analyzed due to the sample size.

When using Bayesian LCM analysis, the sensitivity of tissue culture was slightly lower than that of sonicate fluid culture (86.3% versus 88.7%), while the specificities were the same. In the lower extremity subgroup, the sensitivity of tissue culture was also slightly lower than that of sonicate fluid culture (88.4% versus 91.4%). In the upper extremity subgroup, tissue culture showed a trend toward lower sensitivity than sonicate fluid culture (68.4% versus 80.7%), with the combination of the two tests having the highest sensitivity, without compromising the specificity (Table 3).

Effect of preoperative antimicrobial therapy on culture sensitivity. Among 104 PJI subjects, 38 had received antimicrobial therapy within 4 weeks before surgery and 66 had not. Thirty-one of 80 PJI subjects (38.7%) with positive culture results had received antimicrobial therapy within 4 weeks before surgery, compared to 7 of 24 PJI subjects (23.1%) with negative culture results. The sensitivity of tissue culture in PJI subjects who had received antimicrobial therapy within 4 weeks before surgery was not different from that of PJI subjects who had not (71.1% versus 63.6%, $P = 0.44$). The sensitivity of sonicate fluid culture also showed no significant difference between those two groups (76.3% versus 71.2%, $P = 0.57$) (Table 4).

DISCUSSION

In this study, we compared the diagnostic accuracy of periprosthetic tissue culture in BCBs to that of sonicate fluid culture for diagnosis of PJI. Two approaches were used. First, we used IDSA nonmicrobiological PJI criteria as the "gold standard" for classifying our subjects as having PJI. Some studies have included microbiological results as a criterion for diagnosis of PJI when evaluating microbiological methods (8, 21), whereas others have not (10, 22). We chose to use the nonmicrobiological approach, as including microbiological criteria would have *de facto* yielded 100% specificity of tissue culture. However, we recognize that this may have resulted in subject misclassification. Five aseptic failure subjects would have been classified as having PJI had microbiological criteria been used for subject classification. To overcome the lack of a perfect "gold standard," and also because tissue and sonicate fluid cultures are conditionally independent, we compared test performances using Bayesian LCM analysis. Bayesian LCM is used to define diagnostic accuracy in the absence of a "gold standard" (17). It has

TABLE 3 Sensitivity and specificity of microbiological tests using IDSA PJI criteria as gold standard and Bayesian latent-class modeling[a]

Specimen or culture	Patients with PJI	Patients with aseptic failure	IDSA PJI criteria as gold standard[b]				Bayesian latent-class modeling[b]		
			Sensitivity	Specificity	PPV	NPV	Prevalence	Sensitivity	Specificity
All joints	104	125					37.2 (31.1, 43.5)		
Tissue culture	69	5	66.4 (56.4, 75.3)*	96.0 (90.9, 98.7)	93.2 (84.9, 97.8)	77.4 (70.0, 83.7)		86.3 (78.3, 92.4)	99.6 (97.7, 100.0)
Sonicate fluid culture	76	0	73.1 (63.5, 81.3)*	100.0 (97.1, 100.0)	100.0 (95.3, 100.0)	81.7 (74.7, 87.5)		88.7 (81.0, 94.3)	99.6 (97.7, 100.0)
Combination[c]	80	5	76.9 (67.6, 84.6)*	96.0 (90.9, 98.7)	94.1 (86.8, 98.1)	83.3 (76.2, 89.0)		99.1 (95.7, 100.0)	99.5 (97.6, 100.0)
Hips and knees	86	101					34.8 (28.4, 41.8)		
Tissue culture	56	2	65.1 (54.1, 75.1)*	98.0 (93.0, 99.8)	96.6 (88.1, 99.6)	76.7 (68.5, 83.7)		88.4 (79.6, 94.7)	99.5 (97.1, 100.0)
Sonicate fluid culture	60	0	69.8 (58.9, 79.2)	100.0 (96.4, 100.0)	100.0 (95.3, 100.0)	79.5 (71.5, 86.2)		91.4 (83.3, 96.5)	99.4 (97.2, 100.0)
Combination	63	2	73.3 (62.6, 82.2)*	98.0 (93.0, 99.8)	96.9 (89.3, 99.6)	81.2 (73.1, 87.7)		98.9 (94.7, 100.0)	99.4 (96.9, 100.0)
Shoulders and elbows	18	24					50.9 (36.1, 65.5)		
Tissue culture	13	3	72.2 (46.5, 90.3)*	87.5 (67.6, 97.3)	81.3 (54.4, 96.0)	80.8 (60.7, 93.5)		68.4 (48.2, 84.3)	96.8 (84.3, 99.9)
Sonicate fluid culture	16	0	88.9 (65.3, 98.6)	100.0 (85.8, 100.0)	100.0 (79.4, 100.0)	92.3 (74.9, 99.1)		80.7 (62.8, 93.1)	96.8 (84.7, 99.9)
Combination	17	3	94.4 (72.7, 99.9)*	87.5 (67.6, 97.3)	85.0 (62.1, 96.8)	95.5 (77.2, 99.9)		97.0 (84.8, 99.9)	96.6 (82.8, 99.9)

[a]PPV, positive predictive value; NPV, negative predictive value; *, statistically significant difference from other tests in the same comparison group, $P < 0.05$.
[b]Values are % (95% confidence interval) for IDSA PJI and % (95% credible interval) for Bayesian latent-class modeling.
[c]Combination includes tissue culture and sonicate fluid culture.

TABLE 4 Effect of preoperative antimicrobial therapy on culture results in 104 PJI subjects

Culture type	No. (%) of subjects with positive culture		
	Previous antimicrobial therapy ($n = 38$)	No previous antimicrobial therapy ($n = 66$)	P^a
Tissue culture	27 (71.1)	42 (63.6)	0.44
Sonicate fluid culture	29 (76.3)	47 (71.2)	0.57
P^b	0.41	0.10	

[a]*P* value for comparison of sensitivity of each culture method alone in subjects who received and those who did not receive antimicrobial therapy.
[b]*P* value for comparison of sensitivity between tissue culture and sonicate fluid culture in the subgroups of subjects who received or those who did not receive antimicrobial therapy.

been used to define diagnostic accuracy in infectious diseases, including typhoid fever, malaria, and pertussis (23–25), as well as, in our prior studies, PJI (8, 19). In both analyses, we found that tissue culture in BCBs reached sensitivity similar to that of sonicate fluid culture and that the combination of the two tests returned the best sensitivity. Notably, we have recently shown culture of periprosthetic tissue in BCBs to be cost saving compared to conventional (i.e., plate and broth) periprosthetic tissue culture methods (26).

Compared to conventional methods of tissue culture, culturing tissue in BCBs improves the performance of this method, reaching the performance of sonicate fluid culture. There are several possible reasons for this improvement. First, the resins in the Bactec Plus Aerobic/F bottle (BD Diagnostic Systems) may have bound antibiotics, neutralizing their effects and enhancing recovery of bacteria for subjects with previous antimicrobial therapy (27). Second, the inoculum volume into BCBs is large (1 ml into each bottle; 2 ml in total) compared to the volume inoculated onto agar plates (0.1 ml) and into thioglycolate broth (1 ml) using conventional tissue culture methods (8). The inoculum volume used for BCBs derived from a study by Hughes et al. (28) and has become our institutional standard. Third, culture conditions, including shaking, may increase sensitivity (29). Many studies have demonstrated that using BCBs can increase microbiological diagnostic sensitivity of PJI with various types of specimens, including synovial fluid, periprosthetic tissue, and even sonicate fluid (28–30). In addition, multiple tissue specimens cultured per patient (median, 4 for PJI subjects and 3 for aseptic failure subjects in this study) increase the chance of detecting microorganisms. Culturing tissue in BCBs also provides partial automation of the work flow and provides faster results than conventional tissue cultures (8, 22, 26), as addressed in our previous study (26). However, contamination of blood culture bottles is always a concern. It is not possible to enumerate the microorganisms grown in BCBs. Multiple tissue specimens are required; an identical microorganism isolated from two or more BCBs is defined as a positive result (19). Processing multiple tissue specimens increases the workload and takes a longer time than handling one prosthesis sonicate fluid culture (21). Interestingly, using Bayesian LCM analysis, tissue culture had the trend of lower sensitivity than sonicate fluid culture in the shoulder and elbow subgroup (68.4% versus 80.7%), with the same trend noted when using IDSA nonmicrobiological PJI criteria (72.2% versus 88.9%, $P = 0.179$).

In this study, we isolated multiple organisms from 11 of 85 (12.9%) subjects. This is similar to findings in previous studies (10, 13). In agreement with a previous study (8), *S. aureus*, followed by *S. epidermidis*, was the most common organism isolated from PJI subjects. *C. acnes* was the dominant pathogen in subjects with prosthetic shoulder infections, as previously reported (11), which also demonstrated that using anaerobic BCBs as well as aerobic ones for tissue culture is important, especially in shoulder PJIs.

Some studies suggest that preoperative antimicrobial therapy differentially affects the sensitivity of tissue and sonicate fluid culture (10, 31), while others have not corroborated this (32, 33). In this study, we found that preoperative antimicrobial therapy had no effect on culture sensitivities of tissues in BCBs or sonicate fluid. For periprosthetic tissue culture, the reason may be the resin in the Bactec Plus Aerobic/F

bottle. Additionally or alternatively, the liquid medium in the BCBs may dilute out any antibiotic present. For sonicate fluid culture, a possible explanation could be that the Ringer's solution added for sonication was removed after centrifugation, diluting out antibiotics. This is supported by results of a study demonstrating that concentration of orthopedic implant sonicate fluid through centrifugation yielded higher culture sensitivity than did membrane-filtered sonicate fluid (34). In our previous study, we concluded that antimicrobial use reduced the sensitivity of periprosthetic tissue and sonicate fluid cultures (10); however, periprosthetic tissue specimens were not placed into BCBs and sonicate fluid was not concentrated in that study. In a study by Portillo et al. in 2013, the authors reported that antimicrobial therapy reduced the sensitivity of unconcentrated sonicate fluid culture (31). In a later study by the same group, it was demonstrated that when inoculating sonicate fluid into BCBs, the sensitivity was not affected by antimicrobial treatment (35). Overall, these results suggest that either of the methods studied here have the potential to overcome some effects of prior antimicrobial therapy.

In this study, 23.1% (24/104) of PJI subjects (defined by nonmicrobiological IDSA diagnostic criteria) had culture-negative infections, despite having tissue cultures in BCBs and sonicate fluid cultures performed. A possible explanation could be that they were infected by unusual microorganisms (e.g., mycobacteria, fungi) that were not detected by the culture methods under evaluation and would need specialized culture methods (36, 37). One PJI subject in this study had *M. bovis* BCG detected through periprosthetic tissue mycobacterial culture, while the aerobic and anaerobic cultures of periprosthetic tissue and sonicate fluid were both negative. Notably, not all subjects had mycobacterial or fungal cultures performed. Tools other than culture, such as organism-specific PCR or targeted or shotgun metagenomic sequencing, may be needed to detect pathogens (e.g., *Ureaplasma* species, *Mycoplasma* species) undetected by conventional culture (38, 39). Another possible reason could be that some of these subjects were misclassified as having infection.

There are several limitations in our study. First, we have used nonmicrobiological IDSA criteria to classify PJI. This could lead to misclassifying PJI patients with positive culture results but not meeting any of the other two criteria into the aseptic failure group. However, using Bayesian latent class modeling allows us to estimate diagnostic performance in the absence of a "gold standard." Second, we defined tissue culture positivity as the identical microorganism detected in two tissue specimens and sonicate fluid culture positivity as growth of $\geq$20 CFU/10 ml (with some exceptions). The cutoff values chosen may underestimate the sensitivity of the evaluated tests. However, cutoff values are important for distinguishing pathogens from contaminants (8, 10). Third, this study did not include mycobacterial and fungal cultures. Finally, only the Bactec blood culture system and specific types of Bactec BCBs were evaluated.

In conclusion, periprosthetic tissue culture in BCBs reaches sensitivity similar to that of prosthesis sonicate fluid culture for diagnosis of PJI, but the combination of those two tests has the highest sensitivity without compromising the specificity. The combination of tissue culture in BCBs and sonicate fluid culture is recommended to achieve the highest level of microbiological diagnosis of PJI.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.00319-18.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

This work was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health (R01 AR056647). R.P. is also supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (R01 AR056647, R21 AI125870).

R.P. reports grants from CD Diagnostics, BioFire, Curetis, Merck, Hutchison Biofilm

Medical Solutions, Accelerate Diagnostics, Allergan, and The Medicines Company. R.P. is or has been a consultant to Curetis, Specific Technologies, Selux Dx, GenMark Diagnostics, PathoQuest, and Genentech; monies are paid to Mayo Clinic. In addition, R.P. has a patent on a *Bordetella pertussis*/*B. parapertussis* PCR assay issued, a patent on a device/method for sonication, with royalties paid by Samsung to Mayo Clinic, and a patent on an antibiofilm substance issued.

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