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RESEARCH ARTICLE

# Improved Diagnosis of Prosthetic Joint Infection by Culturing Periprosthetic Tissue Specimens in Blood Culture Bottles

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**ABSTRACT** **Despite known low sensitivity, culture of periprosthetic tissue specimens on agars and in broths is routine. Culture of periprosthetic tissue samples in blood culture bottles (BCBs) is potentially more convenient, but it has been evaluated in a limited way and has not been widely adopted. The aim of this study was to compare the sensitivity and specificity of inoculation of periprosthetic tissue specimens into blood culture bottles with standard agar and thioglycolate broth culture, applying Bayesian latent class modeling (LCM) in addition to applying the Infectious Diseases Society of America (IDSA) criteria for prosthetic joint infection. This prospective cohort study was conducted over a 9-month period (August 2013 to April 2014) at the Mayo Clinic, Rochester, MN, and included all consecutive patients undergoing revision arthroplasty. Overall, 369 subjects were studied; 117 (32%) met IDSA criteria for prosthetic joint infection, and 82% had late chronic infection. Applying LCM, inoculation of tissues into BCBs was associated with a 47% improvement in sensitivity compared to the sensitivity of conventional agar and broth cultures (92.1 versus 62.6%, respectively); this magnitude of change was similar when IDSA criteria were applied (60.7 versus 44.4%, respectively; $P = 0.003$). The time to microorganism detection was shorter with BCBs than with standard media ($P < 0.0001$), with aerobic and anaerobic BCBs yielding positive results within a median of 21 and 23 h, respectively. Results of our study demonstrate that the semiautomated method of periprosthetic tissue culture in blood culture bottles is more sensitive than and as specific as agar and thioglycolate broth cultures and yields results faster.**

**IMPORTANCE** **Prosthetic joint infections are a devastating complication of arthroplasty surgery. Despite this, current microbiological techniques to detect and diagnose infections are imperfect. This study examined a new approach to diagnosing infections, through the inoculation of tissue samples from around the prosthetic joint into blood culture bottles. This study demonstrated that, compared to current laboratory practices, this new technique increased the detection of infection. These findings are important for patient care to allow timely and accurate diagnosis of infection.**

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Prosthetic joint infections (PJIs) place an increasing burden on patients and health care resources (1–4). This diagnosis may be challenging, owing to imperfect definitions, alongside inadequate diagnostic techniques; the reported sensitivity for current periprosthetic tissue specimen (PPT) culture techniques ranges from 39 to 70% and is influenced by chronicity of infection, with low sensitivity reported for chronic infection (4–7). While the Infectious Diseases Society of America (IDSA) and the Musculoskeletal Infection Society (MSIS) criteria aim for a unified approach to define and report PJIs, misclassification remains, in part because both sets of criteria are somewhat reliant on microbiological findings (8, 9). Accurate detection of PJI pathogens aids diagnosis, ensures judicious, directed antimicrobial therapy, optimizes patient outcome, and reduces cost (4, 10). Although implant sonication increases culture sensitivity compared to that of PPT culture, slow adoption of sonication in clinical laboratories and increasingly used debridement and implant retention strategies obviate this approach, rendering PPTs the predominant specimens submitted for culture (11). PPT culture methods are nonstandardized; sensitivity using agar plates is low, though it is improved with broth culture (12, 13). We previously demonstrated that synovial fluid was ideally cultured in blood culture bottles (BCBs) (14); this has become a standard method.

In 2011, Hughes and colleagues (12) published a small study evaluating 23 histologically classified PJI cases cultured in BCBs on a Bactec 960 platform (BD Diagnostic Systems, Oxford, United Kingdom) for 5 days; this approach was as sensitive as traditional broth and more sensitive than agar plate culture (12, 15). The same research group more recently demonstrated that PPT culture in BCBs for 14 days yielded positive results in 84% of patients

with PJIs (15). However, no comparison with other methods was performed. In these studies, PJI was defined using histopathology alone, and details regarding anatomic location and chronicity of infection were not provided (7). Culture in BCBs provides a semiautomated method for culturing PPTs; however, before widespread adoption into clinical practice, a larger study comparing this new strategy to standard agar and broth cultures is needed.

There are also considerations regarding study design. The prior studies used paired-design methodologies. Given the unknown prevalence of PJI and limitations of conventional microbiological techniques, application of traditional statistical methods may lead to miscalculation of the true sensitivities of new tests and true disease prevalence (16–18). Bayesian latent class modeling (LCM) is a statistical method that overcomes potential flaws of traditional analysis. It is based on the assumption that no gold standard exists and that the true disease prevalence requires estimation, both germane to PJI. Bayesian LCM has been applied to diagnostic tests for infectious diseases, such as latent tuberculosis, but not to PJI (16–18). Further, although it is conventional to compare one new diagnostic method to the standard test, in the clinical setting, a number of tests may be applied to the same specimen to optimize the diagnostic yield. Research and clinical applications are therefore disconnected.

Herein, we report the results of a large prospective cohort study comparing inoculation of PPTs into BCBs to conventional agar and thioglycolate broth culture for the diagnosis of PJI, applying Bayesian LCM. We compare the sensitivity and specificity of different PPT culture techniques alone and in combination and assess time to positivity.

## RESULTS

During the study period, 421 consecutive patients underwent revision arthroplasty; 52 were excluded as zero or one PPT was inoculated into BCBs. Therefore, the study cohort consisted of 369 subjects (Table 1). A total of 117 study subjects met IDSA criteria for PJI; 82% of PJI cases were late chronic infections, 7% were early postoperative infections, and 11% were hematogenous infections. A total of 104 (28%) patients met MSIS criteria for PJI; all of these patients also met IDSA criteria for PJI. Of the 13 patients with discordant MSIS and IDSA criteria, 6 had intraoperative purulence, 6 had acute inflammation on histopathology, and 1 had both intraoperative purulence and acute inflammation on histopathology. Overall, 1,154 PPTs were studied (median [and interquartile range {IQR}], 3 PPTs per subject). Of note, 60 PJI subjects (51%) had received antibiotics in the 4 weeks prior to definitive surgery, compared to 17 (7%) of subjects without PJI ($P < 0.001$).

**Sensitivity and specificity.** Applying Bayesian LCM, with the assumption of no gold standard diagnostic test, anaerobic and aerobic BCBs were the most sensitive media for PPT culture (sensitivities of 90.2 and 82.0%, respectively), with aerobic and anaerobic agars and thioglycolate broth having sensitivities of 59.4, 32.2, and 74.8%, respectively. The specificities of anaerobic and aerobic BCBs, aerobic and anaerobic agars, and broth were 97.1, 96.3, 99.5, 99.5, and 99.4%, respectively. Using IDSA criteria as the gold standard, the sensitivities of all media were reduced; however, the sensitivities of anaerobic and aerobic BCBs remained the highest of all media studied (Table 2).

When combinations of different culture media were analyzed using Bayesian LCM, the sensitivity for paired aerobic and anaerobic BCBs was 92.1%, compared to 62.6% for the conventional combination of agar and thioglycolate broth culture media. The specificities using paired aerobic and anaerobic BCBs together and the conventional combination of culture methods were 99.7 and 98.1%, respectively. Using IDSA criteria for classification, the sensitivity using paired aerobic and anaerobic BCBs was 60.7%, compared to 44.4% for the conventional combination of culture methods ($P = 0.0003$). The specificities using paired aerobic and anaerobic BCBs and the conventional combination of culture methods were both 98.8%. Using all culture media combined marginally increased sensitivity to 99.1 and 67.5% using Bayesian LCM and IDSA classification, respectively. The alteration of the protocol with the extension of BCB cultures from 7 to 14 days did not demonstrate major changes in sensitivity and specificity (see Tables S1a and S1b in the supplemental material). When analyzed according to upper limb subgroup, the sensitivities of aerobic and anaerobic agar and aerobic BCBs were reduced, whereas the sensitivities of thioglycolate broth and anaerobic BCBs were similar compared to the overall cohort and to lower limb arthroplasties (Tables S2a and S2b).

**Time to detection.** Aerobic and anaerobic BCBs yielded positive results within the first day of incubation. Aerobic BCBs detected pathogen growth more rapidly than did any other medium studied (anaerobic BCBs, $P = 0.03$; thioglycolate broth and all agars, $P < 0.0001$). Anaerobic BCBs also more rapidly detected pathogen growth than did any other medium, except aerobic BCBs ($P < 0.0001$). The aerobic agar, thioglycolate broth, and anaerobic BCB cultures from PJI subjects were positive earlier than those from subjects not meeting criteria for PJI. In contrast, there was no difference in time to positivity in subjects meeting or not meeting criteria for PJI using aerobic BCBs or anaerobic agar (Table 3 and Fig. 1).

Microorganisms were isolated from PPTs after 7 days of incubation in 25 subjects. No aerobic BCBs gave positive results after 7 days. There were seven positive anaerobic agar cultures beyond 7 days, none of which yielded additional PJI diagnoses. Extending thioglycolate broth culture incubation beyond 7 days yielded 16 positive cultures from 15 subjects, including three additional diagnoses of PJI (all *Propionibacterium acnes*). In seven subjects, the same organism isolated in thioglycolate broth had been isolated in another medium by 7 days. In five patients not meeting criteria for PJI, a microorganism was isolated in thioglycolate broth beyond 7 days. Extending anaerobic BCB incubation beyond 7 days yielded 11 additional positive culture results for eight subjects, including five subjects with PJIs and three subjects not meeting IDSA criteria for PJI. Overall, extending anaerobic BCB incubation yielded three additional PJI diagnoses (one *Propionibacterium granulosum* case and two *P. acnes* cases; both *P. acnes* cases were also detected using extended thioglycolate broth culture). In an additional PJI case caused by *P. acnes*, two anaerobic BCBs yielded positive results prior to day 7, with the third BCB giving a positive result on day 12. In the fifth PJI subject, *P. acnes* was isolated from a single anaerobic BCB after 9 days, with *Staphylococcus epidermidis* isolated from multiple BCBs prior to 7 days. Extending anaerobic BCB incubation beyond 7 days also yielded three contaminants, all *P. acnes*.

**Microbiology.** A pathogen was isolated from two or more PPTs in 83 subjects (71%) (Table 4). In 10 PJI subjects, a microorganism was isolated from a single PPT specimen only. Twenty-two PJI subjects had a microorganism isolated from a single PPT specimen as well as a second different microorganism detected in

**TABLE 1** Demographic, perioperative biochemical, microbiological, and histopathological characteristics of study subjects

| Characteristic | No. of subjects meeting IDSA criteria for PJI or parameter value (%, unless IQR is specified)[a] | | |
|---|---|---|---|
| | No ($n = 252$) | Yes ($n = 117$) | *P* value |
| Age, yr [median (IQR)] | 64 (56, 74) | 62 (56, 70) | 0.2 |
| Female sex | 148 (59) | 45 (38) | <0.001 |
| Prosthetic joint type | | | 0.01 |
| Hip | 106 (42) | 32 (28) | |
| Knee | 104 (41) | 56 (48) | |
| Shoulder | 32 (13) | 17 (15) | |
| Elbow | 10 (4) | 12 (10) | |
| Indication for primary arthroplasty | | | 0.5 |
| Osteoarthritis | 188 (75) | 87 (74) | |
| Rheumatoid arthritis | 12 (5) | 8 (7) | |
| Fracture/trauma | 28 (11) | 16 (14) | |
| Avascular necrosis | 9 (4) | 2 (2) | |
| Tumor | 4 (2) | 2 (2) | |
| Native septic arthritis | 2 (1) | 0 | |
| Developmental dysplasia | 7 (3) | 1 (1) | |
| Other | 0 | 1 (1) | |
| Prior revision arthroplasty performed on index joint | 101 (41) | 73 (63) | <0.001 |
| Documented history of PJI | 35 (14) | 63 (54) | <0.001 |
| No. of days of symptoms prior to surgery [median (IQR)] | 288 (123, 633) | 114 (39, 324) | 0.001 |
| Implant age, days [median (IQR)] | 1,497 (520, 3,865) | 479 (166, 1,391) | <0.001 |
| Presenting symptoms | | | |
| Pain | 244 (97) | 97 (83) | <0.001 |
| Erythema along incision | 2 (1) | 23 (20) | <0.001 |
| Swelling | 18 (7) | 34 (29) | <0.001 |
| Drainage from the incision | 1 (0.4) | 42 (36) | <0.001 |
| Sinus tract | 0 | 36 (31) | |
| Fever | 5 (2) | 16 (14) | <0.001 |
| Antibiotics in the 4 wks prior to surgery | 17 (7) | 60 (51) | <0.001 |
| Surgery performed | | | <0.001 |
| Revision arthroplasty (including one-stage exchange) | 218 (87) | 14 (12) | |
| Resection arthroplasty (with or without arthrodesis) | 0 | 1 (1) | |
| Resection with insertion of a spacer | 24 (4) | 73 (62) | |
| Debridement and implant retention | 10 (4) | 27 (23) | |
| Amputation | 0 | 2 (2) | |
| Preoperative C-reactive protein concn, mg/liter [median (IQR)] | 3 (3, 6) | 25 (9, 56) | <0.001 |
| Preoperative erythrocyte sedimentation rate, mm/h [median (IQR)] | 9 (4, 19) | 29 (17, 52) | <0.001 |
| Preoperative synovial fluid aspirate performed | 100 (40) | 60 (51) | 0.04 |
| No. of leukocytes/$\mu$l in synovial fluid [median (IQR)] | 872 (365, 2,000) | 34048 (13355, 71502) | <0.001 |
| % neutrophils in synovial fluid [median (IQR)] | 14 (6, 51) | 91 (84, 96) | <0.001 |
| Positive synovial fluid culture | 7 (7) | 44 (75) | <0.001 |
| Pre- and/or intraoperative synovial fluid culture performed | 121 (48) | 65 (56) | 0.2 |
| Positive synovial fluid culture | 7 (6) | 42 (65) | <0.001 |
| Sonicate culture performed | 41 (16) | 39 (33) | <0.001 |
| Positive sonicate culture | 1 (2) | 23 (59) | <0.001 |
| Histopathology specimen obtained | 207 (82) | 68 (58) | <0.001 |
| No. of microbiological specimens obtained [median (IQR)] | 3 (3, 4) | 5 (4, 6) | <0.001 |
| No. of tissue cultures performed [median (IQR)] | 3 (2, 3) | 3 (3, 4) | <0.001 |

[a] The number of subjects meeting Infectious Diseases Society of America (IDSA) criteria for prosthetic joint infection (PJI) infection or parameter value (shown as a percentage, unless interquartile range [IQR] is specified) is shown.

**TABLE 2** Sensitivity and specificity of periprosthetic tissue culture techniques using Bayesian latent class modeling and Infectious Diseases Society of America criteria for prosthetic joint infection diagnosis as gold standards[a]

| Culture medium[b] | No gold standard (Bayesian LCM) | | IDSA PJI criteria as gold standard | |
|---|---|---|---|---|
| | Sensitivity (95% credible interval) | Specificity (95% credible interval) | Sensitivity (95% confidence interval) | Specificity (95% confidence interval) |
| Individual culture media | | | | |
| Aerobic agar | 59.4 (45.3, 72.5) | 99.5 (98.3, 100.0) | 26.5 (18.8, 35.5) | 100.0 (98.6, 100.0) |
| Anaerobic agar | 32.2 (20.8, 45.7) | 99.5 (98.3, 100.0) | 14.5 (8.7, 22.2) | 100.0 (98.6, 100.0) |
| Thioglycolate | 74.8 (61.5, 85.8) | 99.4 (98.1, 99.9) | 33.3 (24.9, 42.6) | 100.0 (98.6, 100.0) |
| Aerobic blood culture bottle | 82.0 (69.5, 91.1) | 97.1 (94.8, 98.6) | 42.7 (33.6, 52.2) | 100.0 (98.6, 100.0) |
| Anaerobic blood culture bottle | 90.2 (79.4, 96.5) | 96.3 (93.7, 98.1) | 47.9 (38.5, 57.3) | 99.6 (97.8, 100.0) |
| Combinations of culture media | | | | |
| Aerobic and anaerobic agars | 48.9 (38.3, 59.7) | 99.7 (98.7, 100.0) | 33.3 (24.9, 42.6) | 100.0 (98.6, 100.0) |
| Aerobic and anaerobic agars and thioglycolate | 62.6 (51.7, 72.5) | 98.1 (96.1, 99.3) | 44.4 (35.3, 53.9) | 98.8 (96.6, 99.8) |
| Aerobic and anaerobic BCBs | 92.1 (84.9, 97.0) | 99.7 (98.7, 100.0) | 60.7 (51.2, 69.6) | 98.8 (96.6, 99.8) |
| Aerobic and anaerobic BCBs and thioglycolate | 92.1 (84.9, 97.0) | 98.8 (97.0, 99.6) | 63.3 (53.8, 72.0) | 98.8 (96.6, 99.8) |
| Aerobic and anaerobic BCBs and aerobic agar | 94.6 (88.1, 98.6) | 99.7 (98.7, 100.0) | 62.4 (53.0, 71.2) | 98.8 (96.6, 99.8) |
| Aerobic and anaerobic BCBs and anaerobic agar | 96.8 (91.3, 99.3) | 99.8 (98.7, 100.0) | 62.4 (53.0, 71.2) | 98.0 (95.4, 99.4) |
| Aerobic and anaerobic BCBs and aerobic and anaerobic agars | 99.1 (95.7, 100.0) | 99.7 (98.7, 100.0) | 64.1 (54.7, 72.8) | 98.0 (95.4, 99.4) |
| All media combined | 99.1 (95.7, 100.0) | 97.3 (94.8, 98.7) | 67.5 (58.2, 75.9) | 96.8 (93.8, 98.6) |

[a] Using individual culture media in periprosthetic tissue culture techniques, the prevalence of prosthetic joint infection (PJI) was 13.7% (95% credible interval of 10.4% to 17.6%) with no gold standard (Bayesian latent class modeling [LCM]), and the prevalence of PJI was 31.7% (95% confidence interval of 27.0% to 36.7%) with Infectious Diseases Society of America (IDSA) PJI criteria as the gold standard. Using combinations of culture media in periprosthetic tissue culture techniques, the prevalence of PJI was 21.7% (95% credible interval of 17.7% to 26.1%) with no gold standard (Bayesian LCM), and the prevalence of PJI was 31.7% (95% confidence interval of 27.0% to 36.7%) with IDSA PJI criteria as the gold standard.
[b] BCBs, blood culture bottles.

two or more PPT specimens. PPTs gave culture-negative results for 24 PJI subjects (21%). Two PJI subjects with negative PPT cultures had an organism isolated from multiple synovial fluid specimens (*Staphylococcus aureus* in a shoulder PJI case and *S. epidermidis* in a hip PJI case), one subject with negative PPT cultures had >100 CFU/10 ml *Corynebacterium jeikeium* isolated from sonication culture of his/her knee implant, and one subject had a single synovial aspirate from which *Staphylococcus caprae* was isolated. Receipt of antibiotic therapy in the preceding month was not associated with negative microbiological cultures (57% subjects with culture-negative PJI received antibiotics prior to definitive surgery, compared to 50% of subjects with positive microbiological cultures; $P = 0.6$). Of note, 9 subjects (out of the 13) with discordant MSIS and IDSA criteria had no organism detected from any specimen, and four had a single positive culture for coagulase-negative *Staphylococcus* species.

For 13 PJI subjects, inoculation of PPTs into BCBs detected microorganisms not found from PPTs using any other culture media. Of these, the same microorganism was isolated from two or more BCB specimens in five subjects, including three cases with *S. aureus* and two cases with *Granulicatella adiacens*. In three additional PJI cases, a single BCB specimen yielded the same microorganism isolated in either sonication or synovial fluid culture (isolating *Enterococcus faecalis*, group G *Streptococcus* species, and *Staphylococcus epidermidis*), therefore confirming the diagnosis of PJI according to the IDSA criteria with the isolation of the same microorganism in ≥2 specimens. In addition, BCBs yielded a microorganism from single specimens alone in five PJI cases (two with single bottles detecting *S. aureus*, one each with single bottles detecting *S. epidermidis* and *Staphylococcus warneri*, and one with separate single bottles detecting *Staphylococcus hominis* and a Gram-positive bacillus).

In addition to the PPT culture-negative cases, inoculation of PPTs into BCBs failed to identify the pathogen in nine PJI subjects (8%). In five cases, the pathogen was detected from two or more PPT cultures using another culture medium including two cases of *P. acnes* PJI (both detected in thioglycolate broth culture; one case occurred in the initial study period before BCB incubation

**TABLE 3** Time to detection of microorganisms with different culture media[a]

| Culture medium | Median time (h) to detection (IQR) | | | |
|---|---|---|---|---|
| | Complete cohort ($n = 369$) | Met IDSA criteria for PJI | | |
| | | No ($n = 252$) | Yes ($n = 117.0$) | *P* value[b] |
| Aerobic agar | 41 (21, 63) | 49 (45, 95) | 33 (21, 62) | 0.003 |
| Anaerobic agar | 62 (43, 144) | 144 (50, 163) | 52 (43, 140) | 0.3 |
| Thioglycolate | 65 (43, 92) | 160 (65, 195) | 64 (43, 90) | 0.01 |
| Aerobic blood culture bottle | 21 (14, 45) | 27 (21, 42) | 21 (13, 45) | 0.8 |
| Anaerobic blood culture bottle | 23 (16, 47) | 52 (24, 147) | 22 (14.5, 38.5) | 0.02 |

[a] Time to detection was measured from the time the specimen was received in the laboratory until the time a microorganism was detected. In the case of thioglycolate broth and blood culture bottles, this was the time the thioglycolate broth was recorded as cloudy or the bottle gave a positive result with an organism detected by Gram staining, respectively.
[b] P value comparing time to positivity for each medium according to whether cases met IDSA criteria for PJI.

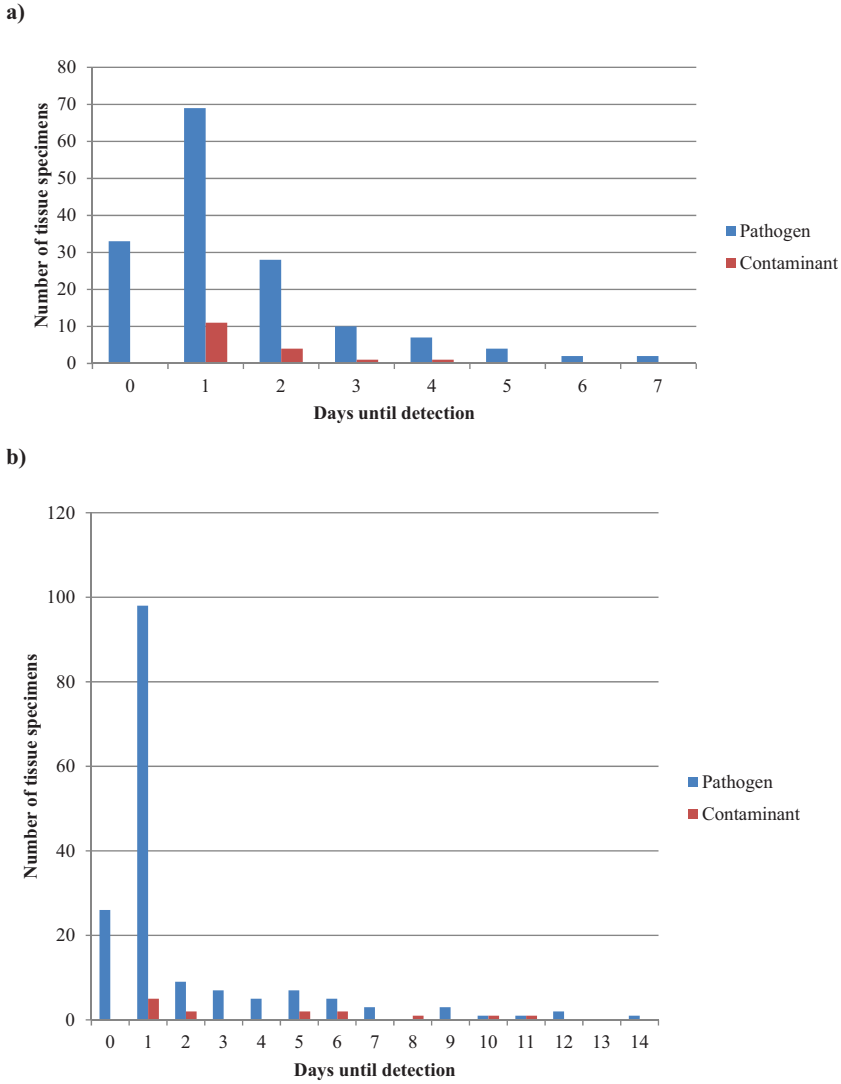


**FIG 1** Time to detection of pathogens compared to contaminants in aerobic (a) and anaerobic (b) blood culture bottles.

was extended to 14 days), one case of *S. hominis* PJI (detected on aerobic agar cultures only), and two cases of *Parvimonas micra* PJI (bilateral knees from the same subject detected on anaerobic agar cultures only). In another subject, *S. epidermidis* was isolated from the same PPT specimen inoculated on aerobic agar and thioglycolate broth in addition to synovial fluid culture, therefore confirming the diagnosis of PJI. There were three additional PJI subjects with indeterminate tissue culture results and negative results for BCBs, including one case isolating *C. jeikeium*, one isolating *Corynebacterium striatum*, and one isolating *P. acnes*. In 26 patients not meeting IDSA criteria for PJI, microorganisms were isolated from single PPTs inoculated into BCBs (presumed contaminants).

## DISCUSSION

Results of this study demonstrate that the use of BCBs for PPT culture increases the sensitivity of PJI diagnosis compared to the standard method of agar plate and thioglycolate broth culture; the magnitude of improved sensitivity was similar by Bayesian LCM and traditional methods. Of note, this improved diagnostic sensitivity was observed in a cohort of patients with primarily late chronic infection in whom sensitivity of culture-based diagnostics has been previously noted to be low compared to those with acute infection (7). Improved sensitivity was not at the detriment of specificity.

Overall, PPT culture in BCBs resulted in eight additional microbiological diagnoses of PJI and failed to identify six pathogens detected using other tissue culture media. The sensitivity for BCB cultures found herein is similar to that reported in the small study by Hughes et al. in which inoculation into BCBs yielded a sensitivity of 87% (12). The 74.8% sensitivity for thioglycolate broth culture (using Bayesian LCM) was slightly lower in the current study compared to the sensitivity found by Hughes and colleagues, a difference that may reflect differences in statistical methodologies, patient populations, or media studied (Robertson's cooked meat versus thioglycolate broth) (12). In addition, in the study by Hughes and colleagues, specimens were ground with glass beads prior to inoculation into BCBs; the optimal method of

**TABLE 4** Periprosthetic tissue culture results in subjects meeting Infectious Diseases Society of America criteria for prosthetic joint infection for different culture media

| Microorganism | Total no. (%) of culture-positive PJI ($n = 93$) | No. of culture-positive PJI with the indicated result in periprosthetic tissue culture medium | | | | | |
|---|---|---|---|---|---|---|---|
| | | Blood culture bottle | | Agar | | Thioglycolate broth | |
| | | ≥1 specimen positive | ≥2 specimens positive | ≥1 specimen positive | ≥2 specimens positive | ≥1 specimen positive | ≥2 specimens positive |
| *Staphylococcus* species | 46 (49) | 44 | 41 | 38 | 25 | 36 | 22 |
| *S. aureus* | 22 (24) | 22 | 22 | 18 | 14 | 18 | 13 |
| *S. epidermidis* | 19 (20) | 18 | 15 | 17 | 9 | 14 | 9 |
| *S. lugdunensis* | 2 (2) | 2 | 2 | 1 | 1 | 2 | 0 |
| *S. capitis* | 1 (1) | 1 | 1 | 0 | 0 | 1 | 0 |
| *S. hominis* | 1 (1) | 0 | 0 | 1 | 1 | 0 | 0 |
| *S. saccharolyticus* | 1 (1) | 1 | 1 | 1 | 0 | 1 | 0 |
| *Streptococcus* species | 4 (4) | 4 | 3 | 3 | 1 | 2 | 0 |
| *S. agalactiae* | 1 (1) | 1 | 1 | 1 | 1 | 0 | 0 |
| *S. bovis* | 1 (1) | 1 | 1 | 1 | 0 | 1 | 0 |
| *S. gordonii* | 1 (1) | 1 | 1 | 1 | 0 | 1 | 0 |
| *Streptococcus* group G | 1 (1) | 1 | 0 | 0 | 0 | 0 | 0 |
| *Enterococcus faecalis* | 2 (2) | 2 | 1 | 0 | 0 | 1 | 1 |
| Other Gram-positive cocci | 6 (6) | 4 | 4 | 4 | 2 | 3 | 1 |
| *Facklamia hominis* | 1 (1) | 1 | 1 | 1 | 0 | 1 | 1 |
| *Finegoldia magna* | 1 (1) | 1 | 1 | 1 | 0 | 1 | 0 |
| *Granulicatella adiacens* | 2 (2) | 2 | 2 | 0 | 0 | 0 | 0 |
| *Parvimonas micra* | 2 (2) | 0 | 0 | 2 | 2 | 1 | 0 |
| Gram-positive bacilli | 12 (13) | 10 | 10 | 8 | 4 | 11 | 8 |
| *Propionibacterium acnes* | 10 (11) | 8 | 8 | 6 | 3 | 10 | 7 |
| *Propionibacterium granulosum* | 1 (1) | 1 | 1 | 1 | 0 | 0 | 0 |
| *Corynebacterium amycolatum* | 1 (1) | 1 | 1 | 1 | 1 | 1 | 1 |
| Gram-negative bacilli | 7 (8) | 7 | 7 | 7 | 5 | 6 | 4 |
| *Escherichia coli* | 1 (1) | 1 | 1 | 1 | 1 | 1 | 1 |
| *Proteus mirabilis* | 1 (1) | 1 | 1 | 1 | 0 | 0 | 0 |
| *Enterobacter cloacae* | 1 (1) | 1 | 1 | 1 | 1 | 1 | 1 |
| *Serratia marcescens* | 1 (1) | 1 | 1 | 1 | 1 | 1 | 1 |
| *Pseudomonas aeruginosa* | 2 (2) | 2 | 2 | 2 | 1 | 2 | 0 |
| *Stenotrophomonas maltophila* | 1 (1) | 1 | 1 | 1 | 1 | 1 | 1 |
| *Candida albicans* | 1 (1) | 1 | 1 | 1 | 1 | 1 | 1 |
| Polymicrobial | 5 (5) | 5 | 4 | 4 | 4 | 5 | 4 |
| *S. aureus* + *P. acnes* | 1 (1) | 1 | 0 | 1 | 1 | 1 | 1 |
| *S. aureus*, *S. agalactiae*, *Enterobacter aerogenes*, and Gram-positive bacillus | 1 (1) | 1 | 1 | 1 | 1 | 1 | 1 |
| *S. epidermidis* + *P. acnes* | 1 (1) | 1 | 1 | 0 | 0 | 1 | 0 |
| *S. epidermidis* + *E. faecalis* | 1 (1) | 1 | 1 | 1 | 1 | 1 | 1 |
| *Streptococcus sanguis*, *Haemophilusparainfluenzae*, and *Veillonella* species | 1 (1) | 1 | 1 | 1 | 1 | 1 | 1 |
| Indeterminate (single positive periprosthetic tissue culture)[a] | 10 (11) | 7 | 3 | 2 | 0 | 0 | 0 |
| *S. aureus* | 2 (2) | 2 | 0 | 0 | 0 | 0 | 0 |
| *S. epidermidis* | 1 (1) | 1 | 0 | 0 | 0 | 0 | 0 |
| *S. epidermidis* + *S. capitis* | 1 (1) | 1 | 0 | 1 | 0 | 0 | 0 |
| *Staphylococcus warneri* | 1 (1) | 1 | 0 | 0 | 0 | 0 | 0 |
| *S. hominis* + Gram-positive bacillus | 1 (1) | 1 | 0 | 0 | 0 | 0 | 0 |
| *Corynebacterium jeikeium* | 1 (1) | 0 | 1 | 0 | 0 | 0 | 0 |
| *Corynebacterium striatum* | 1 (1) | 0 | 1 | 0 | 0 | 0 | 0 |
| *C. amycolatum* + *Cellulosimicrobium cellulans* | 1 (1) | 1 | 0 | 1 | 0 | 0 | 0 |
| *P. acnes* | 1 (1) | 0 | 1 | 0 | 0 | 0 | 0 |

[a] Excludes cases with indeterminate culture results in addition to the isolation of a different microorganism isolated from two or more periprosthetic tissue specimens.

PPT preparation (e.g., Stomacher, glass beads) for BCB inoculation has not been ascertained (12, 15).

This study is the first to apply Bayesian LCM in the PJI setting. This form of statistical analysis is ideally suited to this clinical scenario. We have highlighted challenges regarding classification of PJI using current criteria, illustrated by the discrepancy between classification using MSIS and IDSA criteria. A higher number of subjects met IDSA criteria for PJI compared to MSIS criteria

for PJI. These subjects with discrepant PJI classification were overrepresented in the culture-negative PJI cases or indeterminate cases with single positive cultures isolating coagulase-negative staphylococcal species; this raises the possibility that these cases classified as PJI according to IDSA criteria but not MSIS criteria may not be true PJIs. In addition, we have illustrated the disparity between sensitivity and specificity using the Bayesian LCM approach compared to applying the IDSA criteria for PJI. While a uniform definition is important, misclassification remains an issue when assessing new diagnostic techniques, compromising definition of performance characteristics of tests.

In addition to improved sensitivity, time to microorganism detection was faster using the automated BCB system, facilitating the diagnosis of PJI within the first 24 h of surgery. Similar findings were reported by Minassian et al., with 95% of organisms detected within the first 3 days of aerobic BCB incubation and 96% within the first 5 days of anaerobic BCB incubation (15). Using rapid diagnostics, such as matrix-assisted laser desorption ionization−time of flight (MALDI-TOF MS) mass spectrometry or rapid nucleic acid amplification tests, direct species identification and limited antimicrobial susceptibility testing from BCBs are theoretically possible and may inform rapid selection of antimicrobial therapy (19). In addition to minimizing the use of unnecessary antibiotic therapy, which is linked to the emergence of antimicrobial resistance, prior epidemiological studies have demonstrated an association between the time to optimal antimicrobial therapy and successful treatment outcomes for PJI (10, 20, 21). Inoculation of homogenized PPTs into BCBs provides a strategy for partial automation of tissue culture work-up, overcoming a current limitation of total laboratory automation, the inability to handle anaerobic cultures.

Several studies have focused on the duration of culture incubation for PJI diagnosis (15, 22, 23). In our study, no organism was isolated in aerobic BCBs after 7 days of incubation. Extending anaerobic BCB incubation to 14 days resulted in three additional PJI diagnoses and detection of three additional contaminants, all *P. acnes*. Our findings are in keeping with those reported by Minassian et al. (15). Incubating aerobic BCBs for 7 days and anaerobic BCBs for 14 days would be a reasonable approach. Although the benefit of extending culture incubation beyond 14 days was not examined, studies using less-sensitive approaches suggest that this should be unnecessary (22).

A limitation of our study is that we did not perform terminal subcultures on the blood culture bottles. However, Minassian et al. performed terminal subcultures after 14 days on 1,000 Bactec blood culture bottles inoculated with PPTs that had not yielded positive results and recovered only one additional organism, *Propionibacterium* species (15). In addition, the inoculum for the BCB and thioglycolate broth techniques was 10 times higher compared to the agar culture which theoretically could account for the improved sensitivity observed with these techniques. Furthermore, in this pragmatic study, half of all PJI cases had been pretreated with antibiotic therapy, which may account for the overall reduced sensitivity of all diagnostic tests. There were a small number of polymicrobial infections in our study, precluding formal conclusions regarding the optimal method(s) for their detection; it is likely that not all organisms present in polymicrobial infections will be detected using BCBs. Finally, in keeping with established laboratory practice, anaerobic agars and thioglycolate broths were not routinely examined until day 2 of culture, potentially impacting the time to detection using these media.

**Conclusions.** In summary, results of our study demonstrate that PPT culture in BCBs improves sensitivity for diagnosis of PJI compared to agar and thioglycolate broth culture. The use of automated blood culture systems also yields faster results with the potential for pathogen identification within 24 h of surgery.

## MATERIALS AND METHODS

**Study design.** The study population included all consecutive patients undergoing revision arthroplasty surgery, including patients undergoing revision for (i) presumed aseptic failure and (ii) suspected or proven septic failure, at Mayo Clinic, Rochester, MN, between August 2013 and April 2014. According to the Mayo Clinic Institutional Review Board (IRB 13-005302) approval, the inoculation of BCBs was performed after inoculation of agar plates and thioglycolate broth using the residual tissue in brain heart infusion broth (see "Microbiological methods" below). In the event that <1 ml of brain heart infusion broth remained, BCBs were not inoculated. Patients were excluded if zero or one PPT was inoculated into BCBs. In patients undergoing two-stage exchange for management of PJI, only samples at the initial prosthesis resection were included.

**Microbiological methods.** Fluid and tissue specimens were collected as previously described (11, 14, 25). Intraoperative tissue specimens were sampled based on macroscopic appearance, aiming to sample obviously inflamed or abnormal tissue at the judgment of the treating surgeon. PPT specimens were individually placed into sterile 30-ml screw-top vials filled with $CO_2$ for transportation to the Clinical Microbiology Laboratory (11, 14, 25). Tissue specimens were homogenized using a Seward Stomacher 80 Biomaster (Seward Inc., Port St. Lucie, FL) operated on high in 5 ml brain heart infusion broth for 1 min and inoculated as follows; 0.1 ml was inoculated onto sheep blood and chocolate agar and incubated aerobically at 35°C in 5% $CO_2$ for 5 days, 0.1 ml was inoculated onto CDC anaerobic blood agar and incubated anaerobically for 14 days, and 1 ml was inoculated into anaerobically prereduced thioglycolate broth (BD Diagnostic Systems, Sparks, MD), incubated anaerobically at 35°C for 14 days, and subcultured if cloudy. In addition, 1 ml was inoculated into a BCB containing Bactec Plus Aerobic/F medium and a BCB containing Bactec Lytic/10 Anaerobic/F medium, and the bottles were placed in a Bactec 9240 instrument (BD Diagnostic Systems). In keeping with prior published methods, initially, BCBs were incubated for 7 days (12, 14). On the basis of the work of Butler-Wu et al. (22), the incubation period for both aerobic and anaerobic BCBs was extended to 14 days. Bottles were subcultured only if the instrument gave a positive result. Methods for culture of synovial fluid samples and removed implants have been described previously (11, 14, 25). In brief, synovial fluid specimens were inoculated onto CDC anaerobic blood agar and into thioglycolate broth and incubated for 14 days in addition to inoculation into a Bactec Plus Aerobic/F BCB with incubation for 5 days (14). The removed implants were sonicated with sonicate fluid plated onto aerobic and anaerobic sheep blood agar and incubated aerobically and anaerobically for 5 and 7 days, respectively (25).

**Statistical analysis.** Descriptive statistics were based on percentages and frequencies for categorical variables and for continuous variables, means and standard deviations (SDs) or medians and interquartile ranges (IQRs). Proportions were compared using Fisher's exact test or chi-squared test for categorical data and for continuous data, Student's *t* test or Mann-Whitney U test. The times to positivity in different media were compared using a log rank test.

Performance of the study tests was assessed individually and in combinations using Bayesian LCM to estimate the prevalence of the disease and the sensitivity and specificity of PPT culture techniques with 95% credible intervals. Since all tests measured the same latent variable (that is, true disease status), conditional independence among tests was assumed.

The analysis was performed using Bayesian LCM software (version 1.9.2, June 2013) which uses the described statistical methods (26, 27). The prevalence of disease and sensitivity and specificity for each test or test combination were estimated using uniform (noninformative) prior. Five hundred burn-in iterations, which were discarded, were used to minimize the effect of initial values on the posterior inference. An additional 10,000 iterations of the Gibbs sampler were used for final estimates. The software generates a trace plot of each parameter (each parameter versus the iteration number of the Gibbs sampler) for an assessment of convergence of the Gibbs sampler algorithm. Convergence of the Gibbs sampler suggested that estimates were valid. Prevalence, sensitivity, and specificity with 95% confidence intervals were also estimated using IDSA criteria. Sensitivity estimates based on IDSA criteria for BCB media were compared to conventional culture media using McNemar's test of paired proportion. SAS version 9.3 (SAS Inc., Cary, NC) was used.

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.01776-15/-/DCSupplemental.

Table S1ab, DOCX file, 0.02 MB.
Table S2ab, DOCX file, 0.02 MB.
Table S3, DOCX file, 0.02 MB.

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