5. Articles with only limited data available such that one cannot calculate the sensitivity, specificity or predictive value of histology.
6. Studies which analyze different aspects of inflammation and therefore have no focus on the diagnostic quantification of granulocytes.

For each, it was attempted to define the results of histology and the influence of special or immunohistochemical stains with respect to true positives, false positives, true negatives and false negatives to calculate sensitivity, specificity, predictive value and accuracy. If that data was unavailable, the values reported by the authors were recorded. The threshold used for interpreting histology as favoring infection, the reference standard and other clinical metrics were also recorded.

Results

The initial search yielded 287 articles, 41 of which were automatically excluded as duplicates. The titles and abstracts of the remaining 246 articles were reviewed and 233 excluded. The remaining 13 articles, reviewed in their entirety, and 9 publications for excluded for the following reasons: 3 were not in English, 3 related to aseptic loosening (not infection), 1 did not involve the use of special stains and 2 had an inappropriate study design. The remaining three [5–7] studies were included in our review:

1. Kashima TG, Inagaki Y, Grammatopoulos G, Athanasou NA. Use of chloroacetate esterase staining for the histological diagnosis of prosthetic joint infection. Virchows Arch. 2015;466:595–601. doi:10.1007/s00428-015-1722-y.
2. Krenn VT, Liebisch M, Kölbel B, Renz N, Gehrke T, Huber M, et al. CD15 focus score: Infection diagnosis and stratification into low-virulence and high-virulence microbial pathogens in periprosthetic joint infection. Pathol Res Pract. 2017;213:541–547. doi:10.1016/j.prp.2017.01.002.
3. Munemoto M, Inagaki Y, Tanaka Y, Grammatopoulos G, Athanasou NA. Quantification of neutrophil polymorphs in infected and noninfected second-stage revision hip arthroplasties. Hip Int. 2016;26:327–330. doi:10.5301/hipint.5000365.

Based on the review of the literature, it is recommended that neutrophil counting methods be included when diagnosis is uncertain. In general, we recommend that 5 or more PMNs per field in each of 5 high power (400 X objective) magnification fields be used as the threshold to support the diagnosis of infection. Additional studies are needed to determine the optimum use of special stains. Although the literature supports the use of special stains for neutrophils to increase sensitivity, the stains reported to date can only be performed on sections of formalin-fixed, paraffin embedded tissue. Therefore, these stains are not available for use on frozen sections obtained during an operation. There is some evidence that findings derived from special stains can also correlate with the virulence of the pathogens involved in the infection.

The above recommendations are based on the review of three studies, one of which is high quality. Based on the range of sensitivity and specificity, the strength of the 5 PMNs threshold is strong, while the advocacy of special stains on permanent sections is moderate.

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2.4. DIAGNOSIS: PATHOGEN ISOLATION, CUTURE RELATED

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QUESTION 1: Should intraoperative cultures be taken during every revision total joint arthroplasty (RTJA)? If so, how many?

RECOMMENDATION: Yes, routine cultures should be taken during every RTJA. At least three intraoperative culture samples should be obtained.

LEVEL OF EVIDENCE: Moderate

DELEGATE VOTE: Agree: 87%, Disagree: 12%, Abstain: 1% (Super Majority, Strong Consensus)

RATIONALE

Using the following search terms and words (revision and joint infection; joint arthroplasty; aseptic loosening and culture), a total of 1,772 results were generated from PubMed, Ovid and Google Scholar. Sixty-five studies were found to have met the inclusion criteria. Publications that did not relate to the topic, case reports and those describing technical details of revision arthroplasty were all excluded. Furthermore, registry studies, articles with inadequate description of tissue sample methodology and studies with few patient numbers were

TABLE 1. Statistical analysis by minimum number of cultures sent per revision TJA (RTJA)

Minimum Number Cultures Sent (Mean)	Total Number of RTJA, n (# of Studies)	Sensitivity, % (Lower-Upper CI)	Specificity, % (Lower-Upper CI)	PPV, % (Lower-Upper CI)	NPV, % (Lower-Upper CI)
<3	2,038 (9)	72 (63-81)	94 (90-98)	80 (58-102)	79 (69-89)
≥3	2,283 (14)	62 (50-74)	93 (88-98)	78 (66-90)	85 (78-92)
Overall	4,321 (23)	66 (58-75)	94 (90-97)	78 (67-89)	83 (77-89)

also excluded. To ensure an acceptable strong to moderate strength body of literature evidence – only prospective, comparative and large retrospective studies were included. The literature search did not yield any randomized controlled trials. Across the studies which met the criteria, two that stated multiple tissue samples were taken and were recorded as at least two samples (due to lack of clarity on the number). In order to determine the optimal number of culture samples to be obtained intraoperatively, we included only studies with revision hip and knee arthroplasty that documented the total number of cultures taken at time of surgery and the corresponding diagnostic accuracy (sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)). The studies were then stratified according to the number of samples sent. Exclusion criteria were studies that did not include all four values of sensitivity, specificity, PPV and NPV. The number of cultures obtained and sent were reported as the mean of the minimum number of cultures sent, as reported in the studies. A meta-analysis was performed to obtain pooled estimates for specificity, sensitivity, PPV and NPV using exact likelihood methods normal-binomial model with empirical ("sandwich") variance estimator. Separate estimates were obtained for studies reporting < 3 cultures and those reporting ≥ 3 cultures.

The reviewed literature revealed that the mean number of culture samples taken across cohorts included in the studies was four (minimum two, maximum eight). There were 23 studies with a total of 4,321 patients undergoing revision hip and knee arthroplasty that documented the total number of cultures taken at time of surgery and the corresponding diagnostic accuracy (sensitivity, specificity, PPV and NPV). The analysis indicated that taking three or more intraoperative samples yielded higher negative predictive value to rule out infection without limiting the positive predictive value to confirm infection (Table 1). It is a known fact that periprosthetic joint infection (PJI) may be present in patients undergoing revision hip and knee surgery for aseptic etiologies, even when preoperative workup suggests that this might be the case. A varying degree of clinically relevant PJI has been associated with presumed aseptic loosening [1,2]. These cases were diagnosed from intraoperative cultures. It is for this reason that we suggest that intraoperative samples be sent for all revision hip and knee arthroplasties, irrespective of preoperative diagnosis.

Up to 12% of cases of total knee and hip arthroplasty (TKA and THA) are revised within ten years. Cases are revised for a variety of reasons, and making a preoperative diagnosis may be challenging [1]. PJI is one of the most morbid complications after total hip and knee arthroplasty. According to the Swedish Hip Arthroplasty Register between 2000 and 2013 the risk of PJI increased from 7.5-13.5%. In patients undergoing revision for an aseptic diagnosis after TKA and THA, 7.9 and 12.1%, respectively, had PJIs [2]. As no gold standard exits for the diagnosis of PJI, clinicians often must rely on a combination of tests to confirm or rule out a diagnosis [3]. There is also a paucity of available standards on how many intraoperative cultures should be taken. Attempts to standardize these practices have been published in the form of treatment guidelines, yet the approach still varies between practitioners and locations. This is in part owing to a paucity of strong evidence to support specific guidelines [4].

Atkins et al. had recommended that five or six intraoperative specimens be sent and that the cutoff for a definite diagnosis of PJI be three or more operative specimens positive for an indistinguishable organism due to the low sensitivity of cultures [5]. Some studies reported on their results when taking five to six intraoperative tissue samples from multiple areas of the infected prosthesis and hip joint including the capsule, pericapsular tissue and membrane around prosthesis. However, some other studies were carried out using a protocol where two to three tissue samples were taken intraoperatively for microbiology culture analysis [2,6–8]. Our present review of the literature shows an average of four tissue samples being taken across the studies which we examined. This is consistent with 25% of the cohort of studies assessed in this review.

There are obvious discrepancies and variations in the protocols and guidelines being adhered to which may vary according to institution. If patients with PJI can be accurately identified preoperatively or intraoperatively, a better outcome might be achieved from revision surgery. Although a combination of preoperative investigations can point towards infection, no test has yet proved to be completely accurate as a stand-alone test [9]. Therefore due to low sensitivity of intraoperative cultures [10], it is only imperative that definite guidelines on how many samples to be taken should be anchored on evidence based literature. In the current body of published studies, there are no randomized controlled studies answering this specific question.

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QUESTION 2: Are there significant differences in the yield of culture between preoperative aspiration and intraoperative culture samples? If so, which result should be utilized?

RECOMMENDATION: There may be differences in the yield of culture between preoperative aspiration and intraoperative culture samples, particularly in the case of polymicrobial infections or low-virulence organisms. The collection of multiple intraoperative tissue samples is considered by many experts to provide the highest yield in isolating organisms from a joint.

LEVEL OF EVIDENCE: Limited

DELEGATE VOTE: Agree: 98%, Disagree: 1%, Abstain: 1% (Unanimous, Strongest Consensus)

RATIONALE

When interpreting culture results in general, one should be aware that the literature demonstrates a lack of reproducibility, whether from the synovial fluid or from the tissue.

Due to inherent methodologic difficulties and limitations in the existing literature and variation in culture techniques between institutions, it is not possible to make a general statement regarding the relative yields of synovial fluid and tissue culture. In general, we recommend that synovial fluid and tissue samples both be sent for culture, as the growth of an organism from either source is highly informative. However, clinicians should be aware that in general, culture techniques have a relatively poor sensitivity for periprosthetic joint infections (PJIs) (40 to 85%), and that negative culture results do not rule out PJI. The current literature does not provide evidence-based guidance on how to interpret contradictory synovial fluid versus tissue culture results. Considerable research is needed to optimize and standardize culture techniques to provide improved yield for isolation of infective organisms.

There are inherent methodologic difficulties in studying the comparative yield between synovial fluid and tissue culture results. First is the fact that while synovial fluid is usually sent to the lab for a single culture, intraoperative tissue samples are usually sent in multiples. Whenever a diagnostic test is completed multiple times and the results are interpreted in combination, the sensitivity increases and the specificity decreases by definition. Therefore, even if the sensitivity and specificity of synovial fluid and tissue culture were identical, the multiplicity of testing associated with tissue culture sampling would result in the observation that intraoperative culture has a higher yield. Tissue samples have a greater opportunity to yield a positive result, whether real or due to contamination.

Second, is the fact that there are no universal standards in arthroplasty culture technique. The collection, transport, sample preparation, culture media and culture times vary greatly between institutions [1-18]. The techniques may even vary based on whether the sample is a fluid or a tissue sample at the same institution. Therefore, the results published at one institution regarding the yield of synovial fluid culture or tissue culture cannot be assumed to apply to all institutions.

Third, is the fact that the definition of PJI has varied over time and had great variability before the MusculoSkeletal Infection Society (MSIS) definition. Many historical studies considered positive tissue cultures to be the gold standard for infection, eliminating the possibility of properly assessing the diagnostic characteristics of tissue culture. Furthermore, different centers have different definitions of what qualifies as a positive tissue culture, with variation in the number of positive samples requirements, the virulence of the organisms yielded and the assessment of broth-only results.

Microorganisms involved in infection of orthopaedic devices are highly adapted on the implant or in the bone-cement interphase, adhering to the environment within the in vivo biofilm, but are only to a minor part in a planktonic state in the synovial fluid [19]. This fact can explain the high rates of preoperative aspiration with false negative bacteriology [11]. Moreover, other factors such as bacterial load or the type of germ may affect synovial culture, which may explain the higher sensitivity of aspiration fluid culture observed in acute versus chronic infections [20, 21]. Although a recent study from Shanmugasundaram et al. could not show any influence of microbial virulence on organism isolation from preoperative aspiration versus intraoperative culture [14], some studies showed insufficient accuracy of synovial fluid culture in isolating low virulent pathogens in chronic PJI compared to intraoperative tissue culture [11, 21].

For the aforementioned reasons, a comparison of the yield of synovial fluid versus tissue cultures cannot be made with any confidence. There are exceedingly few studies comparing the culture sensitivity of synovial fluid versus tissue [1-18]. Of these reports in the literature, there are very significant limitations which prevent the appropriate comparison of synovial fluid versus tissue culture yield. Many of these studies have fewer than 10 patients with PJI. The diagnosis of PJI varies greatly in these studies. And many of these studies fail to provide the proper data in evaluating their analysis and conclusions. Studies seeking to compare synovial aspiration and intraoperative tissue culture results have shown a wide range of concordance (57-92%) [1-18] in the sense of false-negative, false-positive, true-negative and true-positive results. Among these 18 studies, nine were retrospective and nine collected their data prospectively.