TABLE 1. Synovial cut-off values and associated test characteristics

Variable/Statistical Test	Acute Hip PJI [2]	Chronic Hip PJI [3]	Acute Knee PJI [4]	Chronic Knee PJI [5]
Cut-off Values WBC count (cells/μL); %PMNs	>12,800; >89%	>3,966; >80%	>10,700; >89%	>3000; >80%
Sensitivity (WBC count; %PMNs)	89%; 81%	89.5%; 92.1%	95%; 84%	80.6%; 83.9%
Specificity (WBC count; %PMNs)	100%; 90%	91.2%; 85.8%	91%; 69%	91.2%; 94.9%
Positive Predictive Value (WBC count; %PMNs)	100%; 91%	76.4%; 59.3%	62%; 29%	67.5%; 78.8%
Negative Predictive Value (WBC count; %PMNs)	88%; 79%	97.5%; 98.0%	99%; 97%	95.4%; 96.3%

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QUESTION 5: What is the role of alpha-defensin in the diagnosis of periprosthetic joint infections (PJIs)?

RECOMMENDATION: Measurement of alpha-defensin in synovial fluid is a complement to existing diagnostic tests for PJIs.

LEVEL OF EVIDENCE: Moderate

DELEGATE VOTE: Agree: 82%, Disagree: 14%, Abstain: 4% (Super Majority, Strong Consensus)

RATIONALE

Alpha-defensins are antimicrobial peptides released by neutrophils in response to pathogens. They can be measured in synovial fluid and have been proposed as an indicator for PJI. Alpha-defensin use as a PJI diagnostic marker was introduced first by Deirmengian et al. in 2014 [1].

There are two commercially available methods for measuring alpha-defensin in synovial fluid: (1) the enzyme-linked immunosorbent assay-based alpha-defensin immunoassay (Zimmer Biomet, Warsaw, IN, USA), which gives a numeric readout within 24 hours and (2) the alpha-defensin lateral flow test (Zimmer Biomet, Warsaw, IN, USA), which gives a binary readout within minutes. Both assays were developed with the intention of matching the MusculoSkeletal Infection Society (MSIS) criteria as the gold standard for diagnosis of PJI.

The Alpha-defensin Laboratory Test

The alpha-defensin laboratory-based immunoassay measures the alpha-defensin concentration in synovial fluid, providing results relative to a signal/cutoff ratio of one. This form of the assay has been studied at numerous institutions, including The Rothman Institute [1], The Mayo Clinic in Arizona [2], The Cleveland Clinic (Cleveland) [3], the Cleveland Clinic (Florida) [4] and the HELIOS ENDO-Klinik [5]. The following table demonstrates the results of these studies. Both the sensitivity and specificity of the alpha-defensin laboratory test exceed 95% when using the MSIS consensus criteria for PJI as a gold standard.

In addition to individual studies, there have been meta-analyses of the alpha-defensin laboratory test. Lee et al. [6] performed a meta-analysis of the performance of the synovial fluid leukocyte count, polymorphonuclear (PMN) %, C-reactive protein (CRP), alpha-defensin, leukocyte esterase, Interleukin-6 (IL-6), IL-8 and culture in diagnosing PJI. They found the alpha-defensin laboratory test to demonstrate the highest sensitivity (97%) of any individual test for PJI. No other test in this meta-analysis had a sensitivity >90%. In this same study, the alpha-defensin test was found to demonstrate the highest specificity (96%) of any individual test for PJI. A meta-

TABLE 1. Institutions studying the alpha-defensin laboratory-based immunoassay

Institution	N	Gold Standard	Sensitivity	Specificity
Rothman Institute	149	MSIS Criteria	97% (3637)	96% (107/112)
Mayo Clinic Arizona	61	MSIS Criteria	100% (33/33)	95% (83/87)
Cleveland Clinic	111	MSIS Criteria	100% (24/24)	98% (53/54)
HELIOS ENDO-Klinik	156	MSIS Criteria	97% (28/29)	97% (123/127)
Cleveland Clinic Florida	70	MSIS Criteria	97% (34/35)	97% (34/35)
Combined	**547**		**98.1% (95%CI: 95-100%)**	**96.4% (95%CI:94-98%)**

analysis by Yuan et al. [7] found that the alpha-defensin test had a sensitivity of 96% and a specificity of 95%. Similarly, a meta-analysis by Li et al. [8] demonstrated a sensitivity of 98% and a specificity of 97%.

The Alpha-defensin Lateral Flow Test

The alpha-defensin lateral-flow test is a rapid test that can be performed in the operating room. The user must follow the device directions and apply synovial fluid, followed by a waiting period which demonstrated the presence or absence of a line. The presence of a line is indicative of a positive test. Obviously, the results of this device not only depend on the inherent diagnostic characteristics of the test, but also compliance with the directions of use. The literature reporting on the performance of the alpha-defensin lateral flow test is not as consistent or controlled as the literature on the laboratory test. For example, whereas all the major studies reporting on the laboratory test are relatively large and utilize the MSIS criteria as a gold standard, the studies reporting on the lateral flow assay are greatly varied in the number of patients and do not all strictly utilize the MSIS or International Consensus Meeting (ICM) criteria.

Four small studies, each with very few PJIs and very large confidence intervals (CIs), reported on their initial experience with the alpha-defensin lateral flow test. Below is a table summarizing their results. It is important to note that the report by Sigmund et al. [9] was methodologically limited by an absence of availability of the synovial fluid white blood cell (WBC) and PMN % for diagnosis, and also by the inclusion of a very large number of spacer block aspirates. Both Kasparek et al. [10] and Sigmund et al. [9] suggested that the alpha-defensin lateral flow test could be used in place of frozen section histology intraoperatively, given the apparent equivalence between the methods in their studies. However, given the very small numbers and very large confidence intervals in these four studies, it is difficult to draw any significant conclusions.

TABLE 2. Smaller studies reporting on the alpha-defensin laterial flow test

Author	N	PJIs	Gold Standard	Sensitivity (95%CI)	Specificity (95%CI)
Kasparek et al.[10]	40	12	ICM	67% (35-89)	93% (75-99)
Sigmund et al.[9]	50	13	Modified MSIS	69% (46-92)	94% (84-100)
Suda et al.[11]	30	13	MSIS	77% (no range)	82% (no range)
Balato et al.[12]	51	16	ICM	88% (75-95)	97% (87-100)

There are also three large studies of the alpha-defensin lateral flow test that utilize the MSIS criteria as a gold standard. Below are the summarized results of their results in a table format. The report by Renz et al. [13] did include alternative results when compared to other diagnostic criteria, but for the purposes of remaining consistent, only MSIS criteria-based results are included in this Table 3.

TABLE 3. Larger studies reporting on the alpha-defensin laterial flow test

Author	N	PJIs	Gold Standard	Sensitivity (95%CI)	Specificity (95%CI)
Berger et al.[14]	121	34	MSIS	97% (85-100)	97% (90-99)
Gehrke et al.[15]	223	76	MSIS	92% (84-97)	100% (97-100)
Renz et al.[13]	212	45	MSIS	84% (71-94) 94% excluding sinuses	96% (92-99)

There are two studies attempting to use meta-analysis techniques to evaluate the lateral-flow test. One, by Suen et al. [16], does not include the recent large studies by Gehrke et al. [15], Berger et al. [14] or Renz et al. [13]. Furthermore, they included the report by Sigmund et al. [9] which is problematic due to the lack of diagnostic data and inclusion of a very large population of spacer block aspirates. A second study by Eriksson et al. [17], is similarly limited in that recent large studies are not included but includes the potentially limited study by Sigmund et al. [9].

Special Considerations

The alpha-defensin immunoassay test seems not to be influenced by prior administration of antibiotics and covers a wide spectrum of potential pathogens causing PJI [18,19]. Additionally, its results do not appear to be affected by patient-related factors such

the presence of inflammatory diseases [White Paper Synovasure alpha-defensin; CD Diagnostics, Claymont, DE, USA].

Given that the alpha-defensin tests are protein immunoassays, it is critically important that the fluid tested is actually synovial fluid. Aspirates resulting from a saline lavage are not appropriate for any biomarker testing. Furthermore, while blood contamination does not appear to alter the results of the alpha-defensin test, it is critical that the aspirate is actually synovial fluid, and not pure blood from a postoperative hematoma. The following are general precautions when utilizing the alpha-defensin test.

1. Do not request the test when the aspirated sample is from a saline lavage.
2. Pure blood aspirates (e.g., postoperative hematomas) should not be sent for biomarker testing. However, simple blood contamination does not appear to affect the test.
3. Aspirates from prosthetic joints with metallosis demonstrate approximately a 30% false positive alpha-defensin rate.
4. False-negative alpha-defensin results may be observed in the setting of a sinus tract (similar to that observed for the leukocyte count). Fortunately, a joint arthroplasty with a sinus tract is accepted by all criteria for PJI to be deterministic of the diagnosis of PJI. Therefore, a false-negative alpha-defensin result in the setting of a sinus tract should not cause a false diagnosis or be detrimental to patient care.
5. Immediate postoperative aspirates rarely demonstrate mature synovial fluid but are more likely to consist of hematoma. Biomarker assays should not be utilized in the first four to six weeks after surgery.
6. The alpha-defensin test has not been validated for use in the setting of a spacer block.

Summary

Appropriate use of the alpha-defensin test should be exercised. It is not intended to be utilized from aspirates from a saline lavage, gross postoperative hematoma, spacer block or a joint with a sinus tract. Furthermore, the test should be used with proper expectations in the setting of metallosis, as false positive testing appears to be demonstrated at a rate of 30%.

The alpha-defensin laboratory test appears to be the most sensitive and specific single test for PJI and therefore appears suitable to be included in the armamentarium of tests routinely used. Given its combination of a high sensitivity and high specificity as demonstrated in multiple institutions and meta-analysis, it serves well as both a good rule-in and rule-out test and could be given significant weight compared to other individual tests.

The alpha-defensin lateral flow test demonstrates results which appear at least equivalent to frozen section histology, providing for a more rapid and convenient intraoperative solution. Although several smaller studies suggest that the lateral flow test is substantially less sensitive than the laboratory assay, larger studies suggest that the sensitivity is only marginally less sensitive, but remains above 90%. The big advantages of the lateral flow test are that it can be utilized perioperatively and that it gives results within minutes. These features make the lateral flow test useful in ruling-in infection. These results must be carefully interpreted when they show negative results. Although further studies are needed to define the exact sensitivity of the lateral flow test, it appears to be the most accurate rapid test for PJI.

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