WHITE PAPER

A New Paradigm for the Diagnosis of Periprosthetic Joint Infection

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CD Diagnostics®
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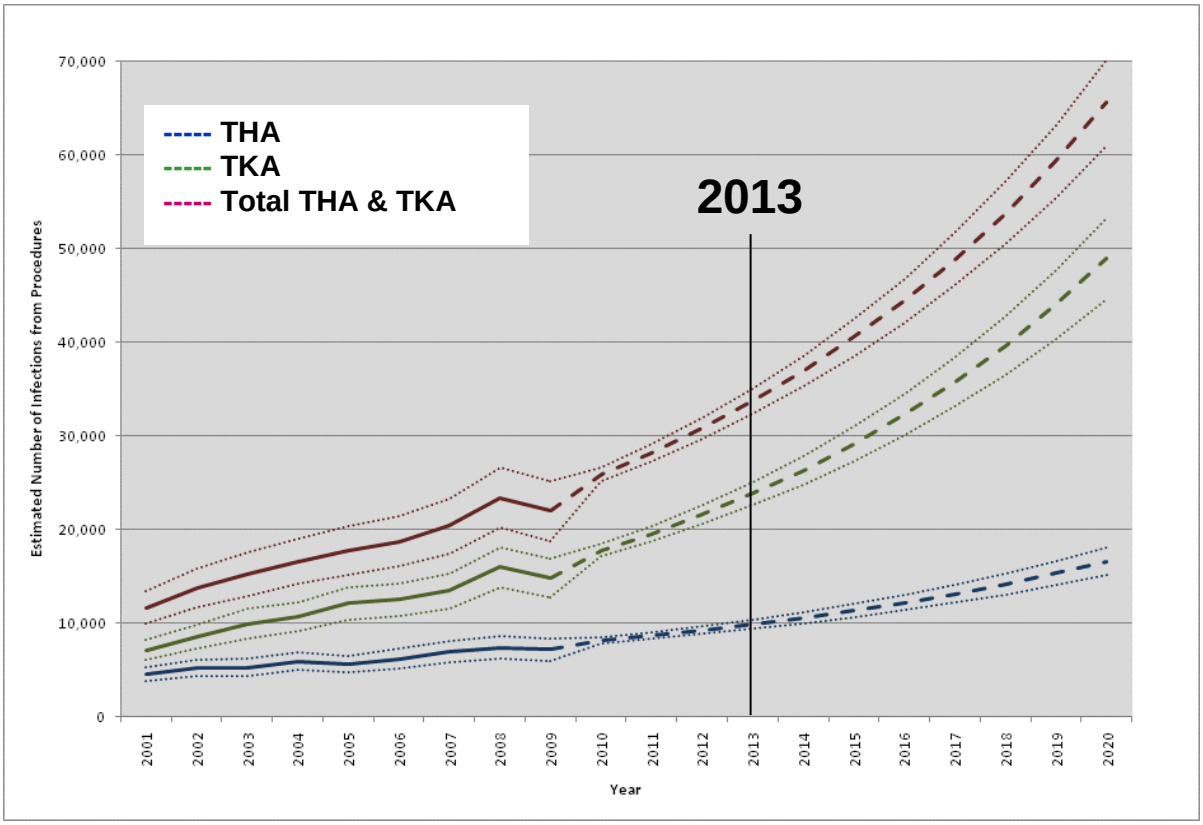
The Incidence and Burden of PJI

Total joint arthroplasty continues to gain acceptance as the standard of care for the treatment of severe degenerative joint disease (1), and is considered one of the most successful surgical interventions in the history of medicine. However, infection of these implants, called periprosthetic joint infection (PJI), remains one of the biggest challenges facing orthopaedics today. PJI can lead to additional surgeries, revision, fusion, amputation, and possibly even death (2, 3).

Also concerning is the fact that despite significant technological advancements in implants and techniques, the incidence of PJI is increasing. The annual number of PJIs in the US is currently estimated to be 33,000 and is expected to increase to almost 70,000 by 2020 (4). PJI now accounts for 25% of failed knee replacements (5) and 15% of failed hip replacements (6), and extrapolation of data suggests that by 2030 over 60% of all revision total joint procedures will be due to PJI. (7).



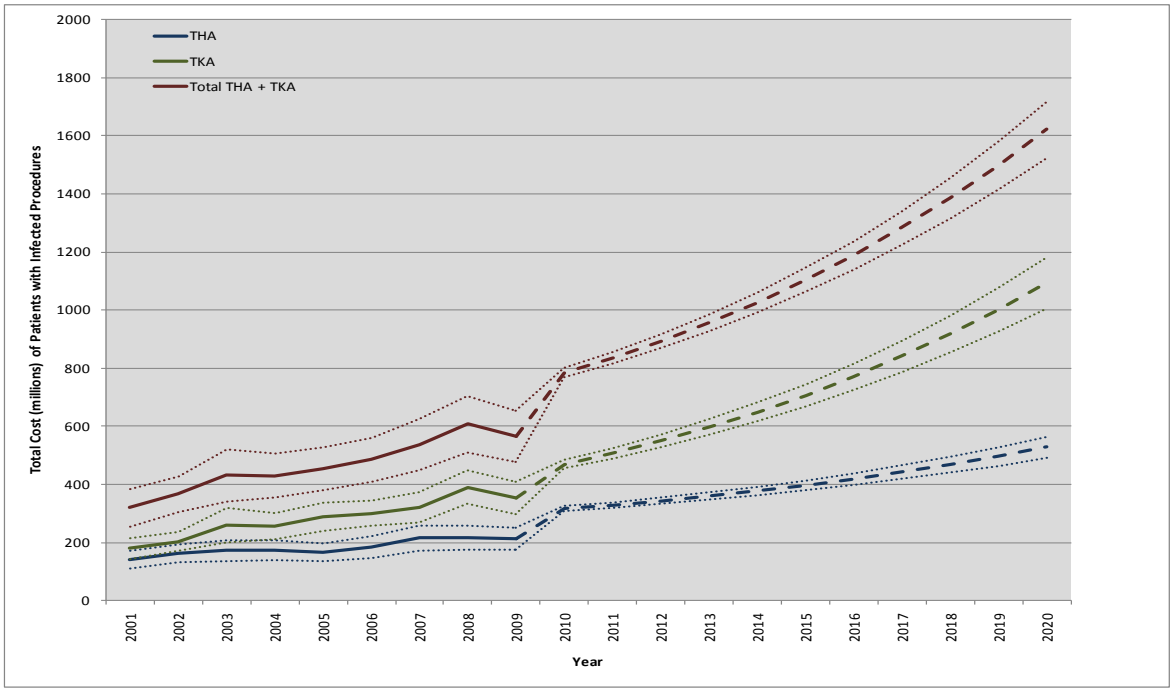
The rise in PJI is multifactorial. First, the number of total joint arthroplasties being performed on an annual basis has increased dramatically and will continue to do so for the foreseeable future (7). Second, patients undergoing total joint arthroplasty have an increasing number of comorbidities, with higher rates of obesity, diabetes, and cardiovascular disease contributing to a greater risk of infection (8). Third, the microorganisms responsible for PJI are becoming more resistant to treatment. Staphylococcal species account for 50-65% of

all PJI cases throughout the world and are the most common causative pathogens, and in some institutions, MRSA is the causative pathogen in more than half of PJI cases (9). Finally, it is likely that the orthopaedic community better understands how to identify and diagnose patients that may have a PJI, leading to a larger population of appropriately diagnosed infections than previously measured.

The Cost of PJI

Revision surgeries performed for PJI are associated with much higher costs than procedures performed for aseptic loosening or mechanical failure (10). Revision arthroplasty for PJI is a complex and technically demanding procedure, and the length of hospital stay for infected revisions has been increasing in recent years. The direct medical cost of treating PJI is $31,000 for the hip and $24,000 for the knee (10). This is 2.8 times greater than the direct cost of treating a case of aseptic loosening, and 4.8 times greater than the cost of an uncomplicated primary total joint arthroplasty (11). The average cost of a two-stage revision for infection is $93,000 for a hip and $75,000 for a knee (10). These costs do not include the associated financial burdens resulting from a decreased quality of life, such as lost productivity and functionality at work. Furthermore, they do not include the long-term postoperative morbidity that is difficult to quantify but likely far greater than healthcare costs (11).

Currently, it is estimated that the US healthcare system spends almost $1 billion per year treating PJI. That number is expected to rise to $1.6 billion by the end of this decade (10).



The Pathogenesis of PJI

The pathogenesis of PJI involves a complex series of interactions between the implant, the patient's immune system and the offending microorganisms. Only a small number of microorganisms are needed to seed the implant. Such organisms adhere to the implant and form a biofilm, which protects the organisms from detection, conventional antimicrobial agents and the host's immune system (12). Biofilm bacteria behave differently than unicellular or planktonic bacteria, allowing bacterial survival during suboptimal periods of growth and in the presence of an environmental stress such as antimicrobial therapy. (13)

Biofilm development follows several stages that include initial attachment, formation, maturation, and shedding:

1. Development is stimulated by bacterial adherence to a surface. Biofilm infection occurs when bacteria win the "race to the surface" and attach to an implant prior to osseointegration.

2. Microcolony formation occurs as bacteria sense and communicate with each other via "quorum sensing". A high microbial density, with increasing cell-to-cell signaling, leads to the activation of genes responsible for biofilm production. The microcolonies become surrounded by a protective matrix of polysaccharide expressed by the bacteria.

3. Microorganisms form complex communities resembling multicellular organisms. At this stage, the positively charged biofilm is relatively impenetrable by positively-charged, hydrophilic antibiotics.

4. The final phase of biofilm formation is shedding of the bacteria from mature biofilm, representing a dispersal of viable organisms capable of causing metastatic infection. This is particularly troubling in patients with an isolated periprosthetic joint infection who have other, well-functioning joint replacements.

Once established, biofilm infections cannot be eradicated by the host's immune mechanism. Even antimicrobial therapy is unlikely to be successful. Therefore, additional measures, including revision surgery are necessary (12).

Diagnosis of PJI

It is important to accurately diagnose PJI because its management differs from that of other causes of arthroplasty failure. The most common symptom of PJI is pain. In acute infection, the local signs and symptoms (e.g., severe pain, swelling, erythema, and warmth at the infected joint) of inflammation are generally present. On the other hand, chronic infection usually has a more subtle presentation, with pain

alone, and is often accompanied by loosening of the prosthesis at the bone-implant interface . The diagnosis of PJI has proven quite challenging, as both acute and chronic infections can be difficult to differentiate from other forms of inflammation (12).

The use of costly radiographic techniques, such as MRI, PET scans, bone scans, and indium-labeled WBC scans, is quite common in the orthopaedic community for diagnosing PJI. Unfortunately, the literature describing the utility of these techniques has failed to provide definitive direction and consistent methods to assure a high sensitivity and specificity of testing. Experts within the field of musculoskeletal infection believe that imaging other than x-rays does not play a direct role in the diagnosis of PJI.

Thus far, the reported literature on the diagnosis of PJI has focused on and evaluated laboratory tests that were never developed specifically for the diagnosis of PJI. These include the erythrocyte sedimentation rate (ESR), the serum C-reactive protein (CRP), the synovial fluid white blood cell count and the leukocyte differential. Because these tests were not made for the purpose of diagnosing PJI, it has been the responsibility of the orthopaedic community to evaluate and recommend their interpretation. This has resulted in significant confusion regarding the appropriate thresholds and optimal combination of tests. Therefore, in addition to providing suboptimal diagnostic performance, the current strategies for the diagnosis of PJI are susceptible to misinterpretation by those not familiar with the literature.

Appropriate thresholds are still unclear, despite a considerable volume of literature. Thresholds of 12 to 40 mm/hour for ESR and 3 to 20.5 mg/L for CRP have been proposed. Adding even more complexity, some studies have demonstrated that the optimal threshold magnitudes for various tests may vary not only between hips and knees, but also between acute-postoperative and chronic PJIs. These clinical details should also be taken into consideration when using the current test battery as a diagnostic criterion for PJI (17, 18).

The current tests, thresholds, sensitivity, specificity along with potential shortcomings are listed below. Conventional and evaluated cutoffs, averages and ranges for sensitivity and specificity are based on values obtained from cited references. (17-35)

Serum CRP

Shortcoming: A positive result is non-specific to joint infection. False positives may result from multiple conditions that elevate CRP. The units of reporting for CRP vary between institutions and CRP assays, and may be a source of result misinterpretation.

Year	Author	Journal	Cutoff	Sensitivity	Specificity
2013	Alijianipour et al.	CORR	Knee 10 mg/L Hip 10 mg/L	97% 88%	70% 77%
2010	Piper at al.	Plos One	Knee 14.5 mg/L	79%	88%
2010	Piper at al.	Plos One	Hip 10.3 mg/L	74%	79%
2009	Ghanem et al.	Int J Inf Diseases	20.5 mg/L	94%	81%
2007	Nilsdotter-Augustinsson et al.	Acta Ortho	10 mg/L	82%	71%
2012	Costa et al.	American Journal of Ortho	10 mg/L	93%	40%
1999	Spangehl et al.	JBJS	10 mg/L	96%	92%
2007	Greidanus et al.	JBJS	13.5 mg/L	91%	86%
		Average		**87%**	**77%**

Synovial Fluid Culture

Shortcoming: A low sensitivity is due to limitations in culture technique and low bacterial cell counts in fluid and tissues. Can be adversely affected by concurrent patient treatment with antibiotics.

Year	Author	Journal	Sensitivity	Specificity
2006	Bare et al.	CORR	53%	94%
2008	Gallo et al.	New Microbiol	44%	94%
1999	Spangehl et al.	JBJS	71%*	97%
2012	Gomez et al.	J Clin Micro	64%	97%
		Average	**59%**	**95%**

*When including patients on preoperative antibiotics

Synovial Fluid WBC Count

Shortcoming: Results may vary significantly between institutions. May be adversely affected by inflammatory conditions or immunocompromise. Can be elevated above threshold levels by other causes of synovial inflammation.

Year	Author	Journal	Cutoff	Sensitivity	Specificity
2013	Dinneen et al.	JBJS (BR)	1590 cells/mm3	90%	91%
2010	Shukla et al.	JOA	3528 cells/mm3	78%	96%
2008	Ghanem et al.	JBJS	1100 cells/mm3	91%	88%
2007	Nilsdotter-Augustinsson et al.	Acta Ortho	1700 cells/mm3	86%	92%
2012	Zimistowski et al.	JOA	3000 cells/mm3	93%	94%
2004	Trampuz et al.	Am J med	1700 cells/mm3	94%	88%
		Average		**89%**	**92%**

Erythrocyte Sedimentation Rate - ESR

Shortcoming: A positive result is non-specific to joint infection. False positives may be a result of multiple conditions that elevate the ESR.

Year	Author	Journal	Cutoff	Sensitivity	Specificity
2013	Alijianipour et al.	CORR	Knee 30mm/h Hip 30 mm/h	95% 94%	71% 68%
2012	Costa et al.	American Journal of Ortho	30 mm/h	89%	69%
1999	Spangehl et al.	JBJS	30 mm/h	82%	85%
2007	Nilsdotter-Augustinsson et al.	Acta Ortho	30 mm/h	64%	87%
2007	Greidanus et al.	JBJS	22.5 mm/h	93%	83%
2009	Ghanem et al.	Int J Inf Diseases	31 mm/h	95%	72%
		Average		**87%**	**76%**

Leukocyte Esterase Test Strip

Shortcoming: Up to 30% of samples cannot be interpreted due to blood and debris, and require centrifugation. Differentiating between various results involves subjective decision-making.

Year	Author	Journal	Sensitivity	Specificity
2012	Wetters et al.	JOA	93%	89%
2011	Parvizi et al.	JBJS	81%	100%
		Average	**87%**	**95%**

PCR

Shortcoming: A research tool that has had difficulty transitioning to clinical use. PCR has a quite poor sensitivity in the published literature. Adjusting PCR technique to have a higher sensitivity causes unexpectedly high rates of bacterial detection.

Year	Author	Journal	Sensitivity	Specificity
2008	Gallo et al.	New Microbiol	71%	97%
2007	Fihman et al.	Journal of Infection	54%	86%
2011	Bonilla et al.*	1.Diagnostic Micro and Infec Dis	63%	100%
2011	Bonilla et al.*	2.Diagnostic Micro and Infec Dis	63%	98%
2011	Bonilla et al.*	3. Diagnostic Micro and Infec Dis	44%	100%
		Average	**59%**	**96%**

**Assessed three different types of PCR*

The Musculoskeletal Infection Society (MSIS) recently published a consensus statement in response to the inconsistencies regarding the diagnosis of PJI, aiming to provide a unified definition of PJI for both clinical practice and research publication. The MSIS definition requires two clinical features (sinus and purulence), three synovial fluid tests (white blood cell count, neutrophil percentage, and culture), two blood tests (ESR and CRP), and one histologic tissue analysis (frozen section). Some of these are subjective criteria that can have significant variability between physicians and institutions, including the observation of purulence, the WBC count, and interpretation of the frozen section histology. The reported accuracy of the frozen section histology is driven significantly by the physician and pathologist performing the test. Additionally, the WBC

count and differential have been shown to vary significantly between institutions (36). The MSIS consensus definition has been a tremendous accomplishment which allows clinicians and researchers to speak the same language when discussing PJI. While this definition provides a "gold standard" for definitive retrospective diagnosis and research, its inherent complexity and required understanding of the literature may limit its wide-spread clinical adoption by the orthopaedic community in general.

The full MSIS criteria are listed below (15):

Based on the proposed criteria, a definite diagnosis of PJI can be made when the following conditions are met:

1. A sinus tract communicating with the prosthesis; or
2. A pathogen is isolated by culture from two separate tissue or fluid samples obtained from the affected prosthetic joint; or
3. Four of the following six criteria exist:
    a. Elevated serum erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) concentration
    b. Elevated synovial white blood cell (WBC) count
    c. Elevated synovial neutrophil percentage (PMN%)
    d. Presence of purulence in the affected joint
    e. Isolation of a microorganism in one culture of periprosthetic tissue or fluid
    f. Greater than five neutrophils per high-power field in five high-power fields observed from histologic analysis of periprosthetic tissue at 400 times magnification

However, it should be noted that PJI may be present even if fewer than four of these criteria are met.

The Value of a Better Test

Given the complexity in utilizing and interpreting the armamentarium of currently recommended tests, and considering the increasing incidence of PJI, it is clear that the orthopaedic community could benefit from a better diagnostic test. This ideal test would provide an objective laboratory result that performed at a high accuracy with no interpretation needed. Additionally, it would provide a rapid standardized result so that all clinicians, regardless of experience, would achieve similar diagnostic outcomes. An improved sensitivity and

specificity would allow clinicians to identify the infections that they would normally miss and prevent the misdiagnosis of patients with highly inflamed but non-infected joints.

The currently utilized diagnostic tests such as ESR, serum CRP, cultures, and WBC count generally have a sensitivity or specificity for PJI that is less than 90%. And most of the studies demonstrating these diagnostic values excluded patients with systemic inflammatory diseases and patients on antibiotics, which may comprise 10-30% of the population tested. The true sensitivity and specificity of these tests in daily practice, when including all patient groups, is likely lower than suggested by the literature.

Correctly diagnosing the group of patients with PJI requires a test with a high sensitivity. All of the currently utilized tests for infection listed above have a sensitivity lower than 90%, which means that reliance on any given test will lead to 10 missed PJIs out of every 100 patients with a PJI. A missed diagnosis of PJI is quite devastating for the patient, as his or her revision will likely fail due to a persistent infection.

Correctly identifying a patient who is not infected is equally important, requiring a test with a high specificity. Inflammatory diseases can affect both the systemic tests and local synovial fluid tests, resulting in falsely elevated values. The ESR, CRP, and WBC count have specificities in the low 90%, as reported mostly by studies that have excluded patients with inflammatory diseases. This means that reliance on any of these tests would result in at least 10 patients being falsely diagnosed as having an infection out of every 100 patients who is tested. The treatment for infection requires a lengthy two-stage revision procedure which has been shown to have poor functional outcomes when compared to an aseptic revision (16). Furthermore, six weeks of intravenous (IV) antibiotics are necessary, which can have significant and sometimes life-threatening consequences. Unfortunately, many aseptic diagnoses can mimic an infection in clinical appearance, resulting in a decision to proceed with a two-stage revision and antibiotic therapy.

The analysis above is highly dependent on the surgeon interpreting the testing results. Unfortunately, much of the existing analysis is subjective and dependent on an individual's personal experience. Each surgeon has a tendency to put more weight on their preferred test and their interpretation rather than an equal weighting of numerous tests. One advantage of an accurate laboratory test specifically intended for PJI is that all surgeons could utilize it with equal success, regardless of their bias. This would result in an improvement in PJI diagnosis. If the rates of false positive and false negative diagnoses for PJI can be significantly reduced, the impact on patients' outcomes would be dramatic, as would the reduction in healthcare spending devoted to the treatment of PJI.

A New Paradigm – The Synovasure™ Test for Periprosthetic Joint Infection (PJI)

The promising diagnostic capabilities of synovial fluid biomarkers for PJI have already been reported in the literature (37, 38). These biomarkers include inflammatory proteins, cytokines, and antimicrobial peptides that are known to be involved in the host response to infection. The optimal combination of biomarkers for the diagnosis of PJI has not yet been described.

CD Diagnostics has embarked on a very comprehensive synovial fluid biomarker evaluation program that studied synovial fluid biomarkers at both the genetic and proteomic level. Over 50 biomarkers of interest were initially tested from a population of revision arthroplasty patients to identify the most accurate biomarkers for PJI. The high-priority biomarkers that were identified in this study were then tested in a larger population of arthroplasty patients.

Identifying the Best Synovial Fluid Biomarker for PJI

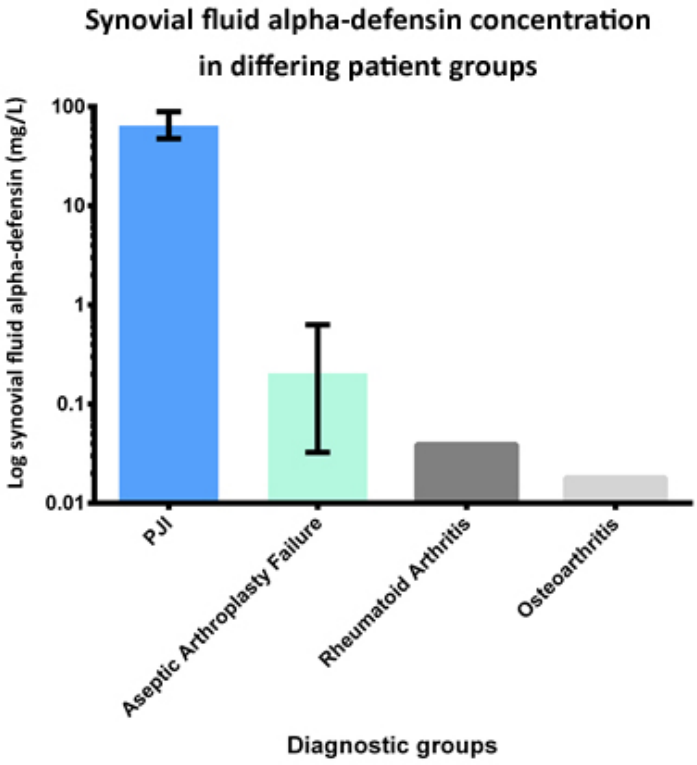
In an effort to identify the best diagnostic biomarker for PJI, the 16 most promising biomarkers were tested in the synovial fluid of 95 patients who were being evaluated for revision arthroplasty. The MSIS consensus definition for PJI was utilized to classify all patients in the study, which included 29 PJIs and 66 aseptic joints. The sensitivity and specificity of each biomarker was then calculated when comparing patients with PJI or an aseptic diagnosis. Additionally, this study included a separate cohort of 9 patients having a revision hip arthroplasty for metallosis. This group was specifically analyzed to understand the effect of metal products on the performance of the biomarkers tested, but were excluded from the calculation of sensitivity and specificity.

This study identified 5 synovial fluid biomarkers that had an area under the curve (AUC) of 1.0, with a specificity of 100% and sensitivity of 100%. The proteins with antimicrobial function outperformed the cytokine biomarkers. The alpha-defensin protein, human neutrophil elastase 2, the bactericidal/permeability increasing protein, the neutrophil gelatinase-associated lipocalin, and lactoferrin were able to predict the MSIS diagnosis in all study patients.

Diagnostic Characteristics of Synovial Fluid Biomarkers						
Biomarker	**AUC**	**Cut-off**	**Specificity (%)**	**95% CI - Specificity**	**Sensitivity (%)**	**95% CI - Sensitivity**
alpha-defensin	N/A*	5.2 ug/mL*	100.0	94.6 to 100.0	100.0	88.1 to 100.0
ELA2	1.000	2.0 ug/mL	100.0	94.6 to 100.0	100.0	88.1 to 100.0
BPI	1.000	2.2 ug/mL	100.0	94.6 to 100.0	100.0	88.1 to 100.0
NGAL	1.000	2.2 ug/mL	100.0	94.6 to 100.0	100.0	88.1 to 100.0
LF	1.000	7.5 ug/mL	100.0	94.6 to 100.0	100.0	88.1 to 100.0
IL-8	0.992	6.5 ng/mL	95.4	87.1 to 99.0	100.0	87.2 to 100.0
SF CRP	0.987	12.2 mg/L	97.0	89.5 to 99.6	89.7	72.7 to 97.8
RSTN	0.983	340 ng/mL	100.0	94.6 to 100.0	96.6	82.2 to 99.1
TSP	0.974	1061 ng/mL	97.0	89.5 to 99.6	89.7	72.7 to 97.8
IL-1b	0.966	3.1 pg/mL	95.4	87.1 to 99.0	96.4	81.7 to 99.9
IL-6	0.950	2.3 ng/mL	96.9	89.3 to 99.6	88.9	70.8 to 97.7
IL-10	0.930	32.0 pg/mL	89.2	79.1 to 95.6	89.3	71.8 to 97.7
IL-1a	0.922	4.0 pg/mL	90.6	80.7 to 96.5	82.1	63.1 to 93.9
IL-17a	0.892	3.1 pg/mL	98.5	91.7 to 100.0	82.1	63.1 to 93.9
G-CSF	0.859	15.4 pg/mL	92.1	82.4 to 97.4	81.5	61.9 to 93.7
VEGF	0.850	2.3 ng/mL	76.9	64.8 to 86.5	75.0	55.1 to 89.3

**The alpha-defensin protein had a cutoff determined before the study. AUC cannot be calculated.*

The alpha-defensin protein provided the best overall diagnostic characteristics in this study, when considering the needs of developing a diagnostic test. In the setting of PJI, alpha-defensin reaches concentrations above 5.2 mg/L, with a mean concentration of 65 mg/L. These concentrations are ideal for development of an immunoassay diagnostic test. In comparison, patients with an aseptic arthroplasty have a mean concentration of 0.41 mg/L, patients with osteoarthritis have a mean alpha-defensin concentration of 0.018 mg/L, and patients with rheumatoid arthritis have a mean concentration of 0.039 mg/L (39). Additionally, the alpha-defensin protein concentration demonstrated a tremendous separation between patients with an aseptic diagnosis and PJI, resulting in a bimodal distribution. The bar graph below demonstrates these concentrations on a log scale, showing the median and interquartile range for arthroplasty groups.



It was also observed that metallosis could trigger the pathogen response and cause elevation of many of the biomarkers. In the subgroup of 9 patients, most of the synovial fluid biomarkers demonstrated a false positive rate of 33%. However the synovial fluid CRP was not elevated above a threshold of 3 mg/L in the synovial fluid of any patient with metallosis.

The Diagnostic Performance of Alpha-Defensin and CRP

In an effort to further define the diagnostic performance of synovial fluid alpha-defensin and CRP, a follow-up study was completed assessing 158 synovial fluid samples from patients being evaluated for a revision.

Synovial Fluid Repository and Patient Inclusion

A synovial fluid repository was initiated jointly by CD Diagnostics and The Rothman Institute at Thomas Jefferson University, to generate a library of prospectively annotated synovial fluid samples from patients with an arthroplasty. Synovial fluid samples from this repository that met the inclusion criteria below were utilized to characterize the diagnostic performance of the Synovasure™ test for PJI.

Inclusion criteria:

(1) A total hip or knee arthroplasty/spacer, having an evaluation for revision hip or knee arthroplasty***.***
(2) Sufficient clinical information for use of the MSIS criteria for PJI.
(3) Sufficient synovial fluid for study methods.

Patients receiving antibiotics before aspirations, patients having the diagnosis of a systemic inflammatory disease, and patients with an infection remote from the joint **were included** in this study. Patients having a revision were classified as having metallosis if this diagnosis was demonstrated by laboratory results and operative findings.

Consecutive patients meeting these criteria were prospectively evaluated and classified as infected or aseptic as defined by the "gold standard" MSIS definition of PJI. Additionally, gender, age, joint, laterality, comorbidities, surgical findings and isolated organism were recorded.

Biomarker Measurements

Synovial fluid was delivered to the laboratory immediately after aspiration. Centrifugation was used to separate all particulate and cellular material from each synovial fluid sample, and the resulting supernatant was aliquoted and frozen at -80°C. Various conditions and preparations of synovial fluid were tested for optimal stability and accurate assessment of alpha-defensin concentrations.

The immunoassays for synovial fluid C-reactive protein (CRP) and human alpha-defensin were standard enzyme-linked immunosorbent assays (ELISA). The assays were optimized specifically for performance in synovial fluid. The assay for alpha-defensin was optimized to operate at a cutoff value of 5.2 ug/ml, providing a semi-quantitative signal to cutoff ratio of 1.0. The assay for CRP was optimized to operate at a cutoff of 3 mg/L.

Hemoglobin Adjustment

A spike of fresh blood into a synovial fluid sample does not usually result in the significant elevation of the alpha-defensin since the majority of the intact cells are centrifuged from the sample prior to performing the assay. To account for any cellular lysis that may occur during sample transport, the alpha-defensin cutoff is raised proportionately, based on using the lysis of the red blood cells as an indicator of cell lysis. The adjustment was determined utilizing high alpha-defensin blood samples which were fully lysed, and assessed for alpha-defensin levels. This analysis provided a quantification of the highest potential alpha-defensin

contamination that could be introduced by a bloody or hemolyzed synovial aspirate. This data is utilized to make an adjustment to the assay cutoff based on hemoglobin measurement in the synovial fluid. Therefore, the concentration of hemoglobin is assessed in all synovial fluid samples to appropriately adjust the synovial fluid alpha-defensin measurement. The adjustment expands the indeterminant range for the assay and increases the value required to report a positive sample.

Statistical Analysis of Data

The Synovasure™ test for infection was compared between PJIs and aseptic joints based on the MSIS definition. The PJI threshold for alpha-defensin was established before the study at 5.2 mg /L. The synovial fluid CRP threshold was optimized to identify potential false positive alpha-defensin results. These diagnostic measures were calculated for the entire cohort, and also calculated for the subgroup of patients having a revision for metal corrosion. (14)

Clinical Findings

158 consecutive patients met the criteria of the study, including 120 arthroplasties diagnosed with an aseptic etiology and 38 arthroplasties diagnosed with PJI. Of the aseptic diagnoses, 19 samples had a secondary diagnosis of metallosis as determined by serum metal levels and the observation of metal corrosion products at the time of revision. Patient demographics appear in the table below:

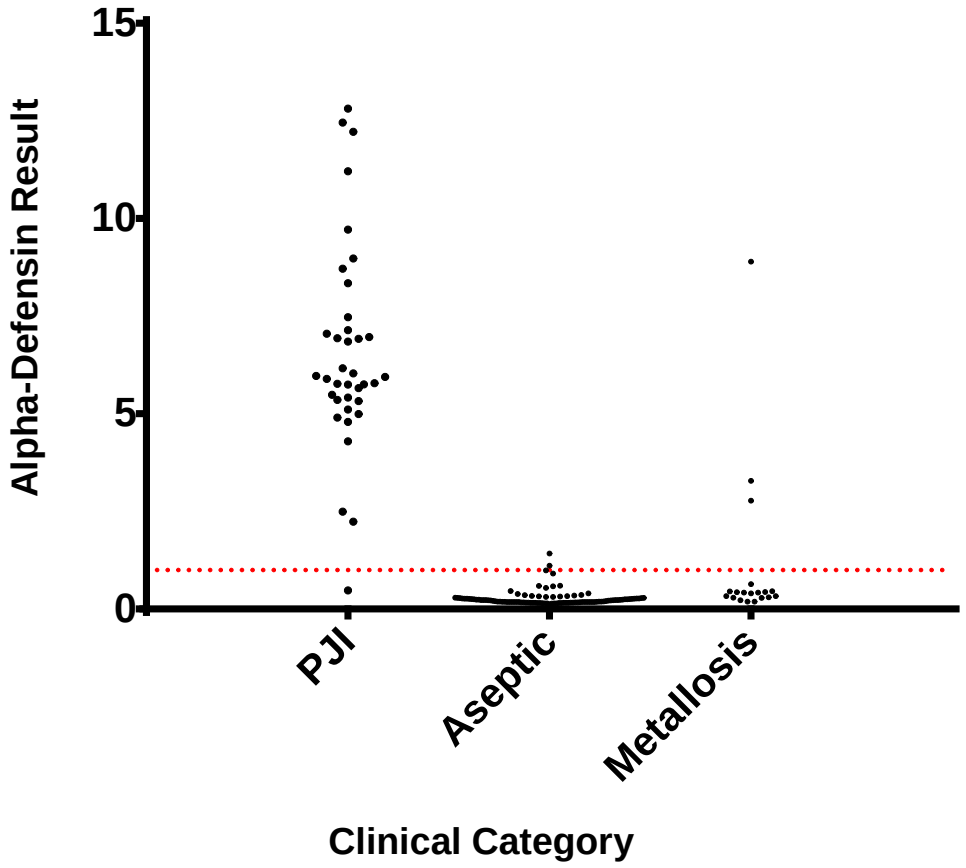
	Aseptic (101) without Metallosis	Metallosis (19)	Summary Aseptic Aseptic + Metallosis (120)	Infected PJI (38)
Classification	52 males 49 females	11 males 8 females	63 males 57 females	15 males 23 females
Patient Age	Ave. age 65 years (41 -86)	Ave. 67 years (55-78)	Ave. 66 (41-86)	Ave. 65 years(48-89)
Surgical History	14 THA 79 TKA 8 knee cement spacers	17 THA 2 TKA	31 THA 81 TKA 8 knee cement spacers	2 THA 35 TKA 1 knee cement spacer
Diagnosis **Included:**	59 – aseptic loosening 4 – instability 6 – bearing surface wear 23 – pain but no mechanical diagnosis 1 chronic quadriceps tendon rupture 8 spacer blocks	All hips had elevated preoperative serum cobalt and/or chromium levels. 7 patients had a pseudotumor. Both knees had metal staining of synovium	(see aseptic and metal)	24 Culture positive 14 Culture negative

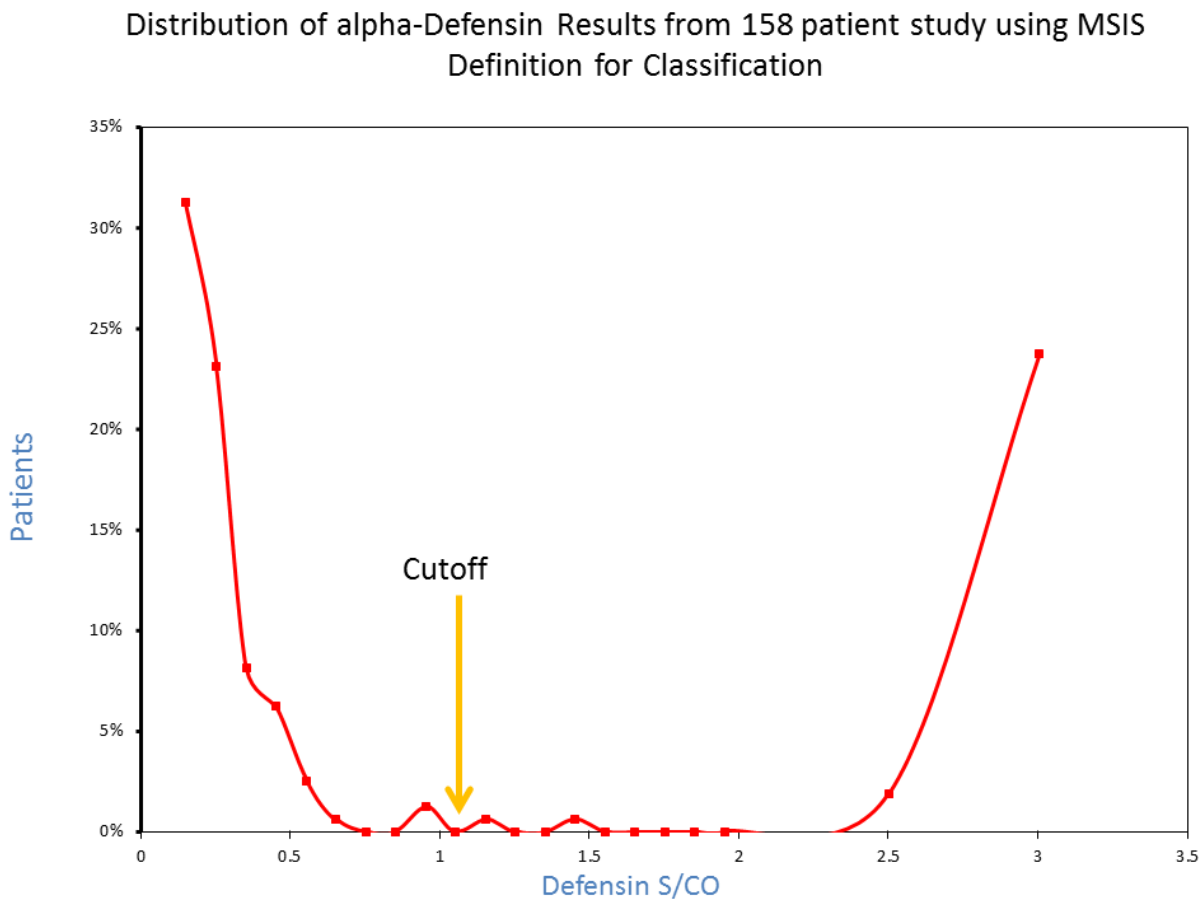
Alpha-Defensin Diagnostic Characteristics

Alpha-defensin correctly diagnosed 152 of all 158 arthroplasties, with an overall specificity of 96% (95% CI: 90.5-98.6%) and a sensitivity of 97% (95% CI: 86.1-99.6%). The specificity of alpha-defensin was 97.1% when excluding patients with metallosis. In the entire study, three of the misdiagnoses were false positive results among the 19 patients with metal corrosion, two were false positive results in the aseptic group, and one was a false negative result in the infected group. The false negative alpha-defensin result was from a synovial fluid sample that was also negative for neutrophil elastase and CRP, with 4 negative cultures. Nevertheless, there were three of five positive MSIS minor criteria and the subject was classified as PJI.

MSIS Classification and Defensin Result					
			MSIS Classification Summary		
			Aseptic (Total)	Infected	
	Aseptic	Metal	Aseptic + Metal	PJI	
Defensin Result					TOTAL
Negative	99	16	115	1	116
Positive	2	3	5	37	42
Total	101	19	120	38	158

The scatter plot below demonstrates the alpha-defensin separation of patients with PJI and aseptic diagnoses. Each dot represents a different synovial fluid sample. The alpha-defensin concentration is graphed as a signal to cutoff ratio, with 1.0 set as the threshold for PJI.





**All samples with values >3 were set to 3.*

Synovial Fluid alpha-Defensin		95% Confidence Interval
Sensitivity	97.4%	86.1% - 99.6%
Specificity (excluding cases of metallosis)	97.1%	93.0 % - 99.7%
Specificity (including cases of metallosis)	95.8%	90.5% - 98.6%

158 Patient Study Culture Results

Of 120 patients diagnosed by the MSIS consensus definition as being aseptic, 5 patients had a culture positive result from either synovial fluid (2) or tissues (3) at the time of surgery. Of 38 patients meeting the MSIS definition of infection, 24 had either synovial fluid (18 subjects) or tissues (24 subjects) that resulted in a positive culture. Therefore, 14 patients were diagnosed as having a culture-negative infection.

Confounding Conditions and Treatments

Alpha-defensin measurement also demonstrated a relative resistance to systemic influences such as inflammatory diseases and antibiotic treatment. In the current study, 23% of patients had a documented history of a systemic inflammatory disease such as rheumatoid arthritis, lupus, multiple sclerosis, psoriasis, Crohn's disease, gout, or hepatitis C. Of these patients, 40% were on an immunomodulating drug or steroid at the time of aspiration. This population of patients did not affect the Synovasure™ test, nor were the alpha-defensin levels significantly different in this population.

Furthermore, 27% of aspirates from patients with PJI were performed after the patient was already treated with antibiotics. While the mean serum CRP was 45% lower in this population on antibiotics ($p = 0.039$), the alpha-defensin level was unchanged, revealing consistent performance even in the setting of antibiotic treatment (see table).

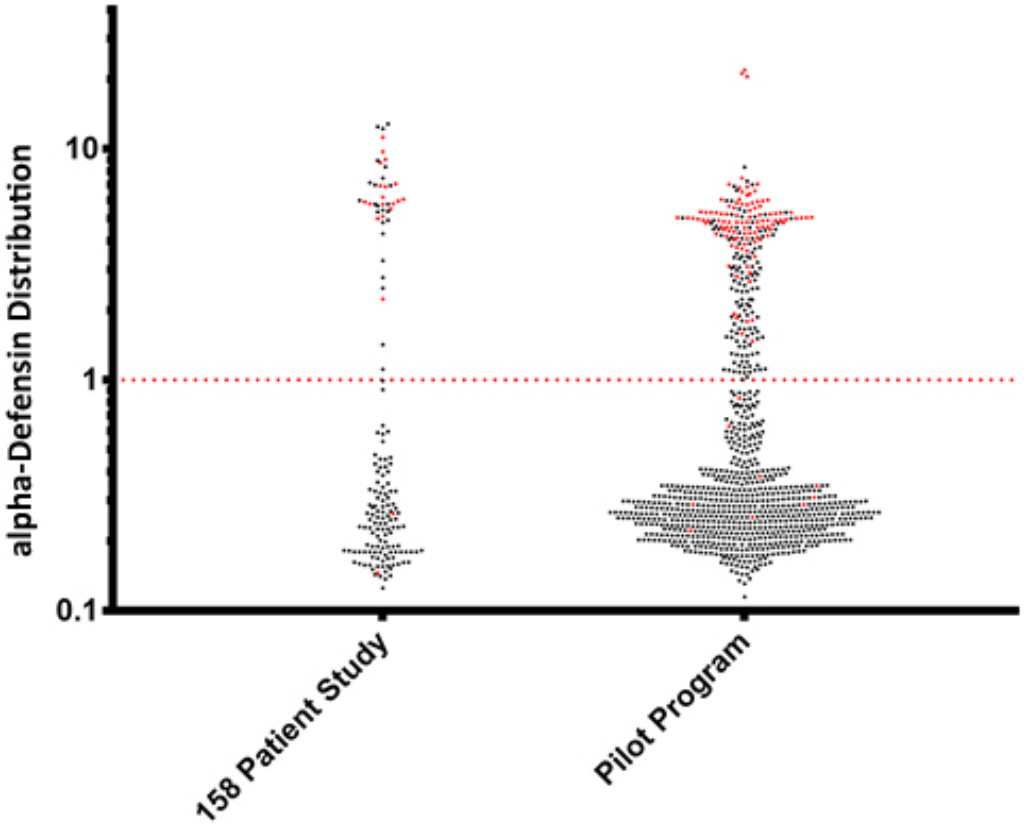
The Effect of Antibiotics Treatment on Tests for PJI						
Condition	Alpha-defensin	Serum CRP	ESR	Synovial fluid cell count	% Neutrophils	Culture Positive rate
PJI Group						
Patients receiving antibiotics	7.6	101.1	74.2	26,128	85.7	50%
Patients not receiving antibiotics	6.4	182.9	86.5	50,031	88.7	70%

Alpha-Defensin Testing in a Larger Population

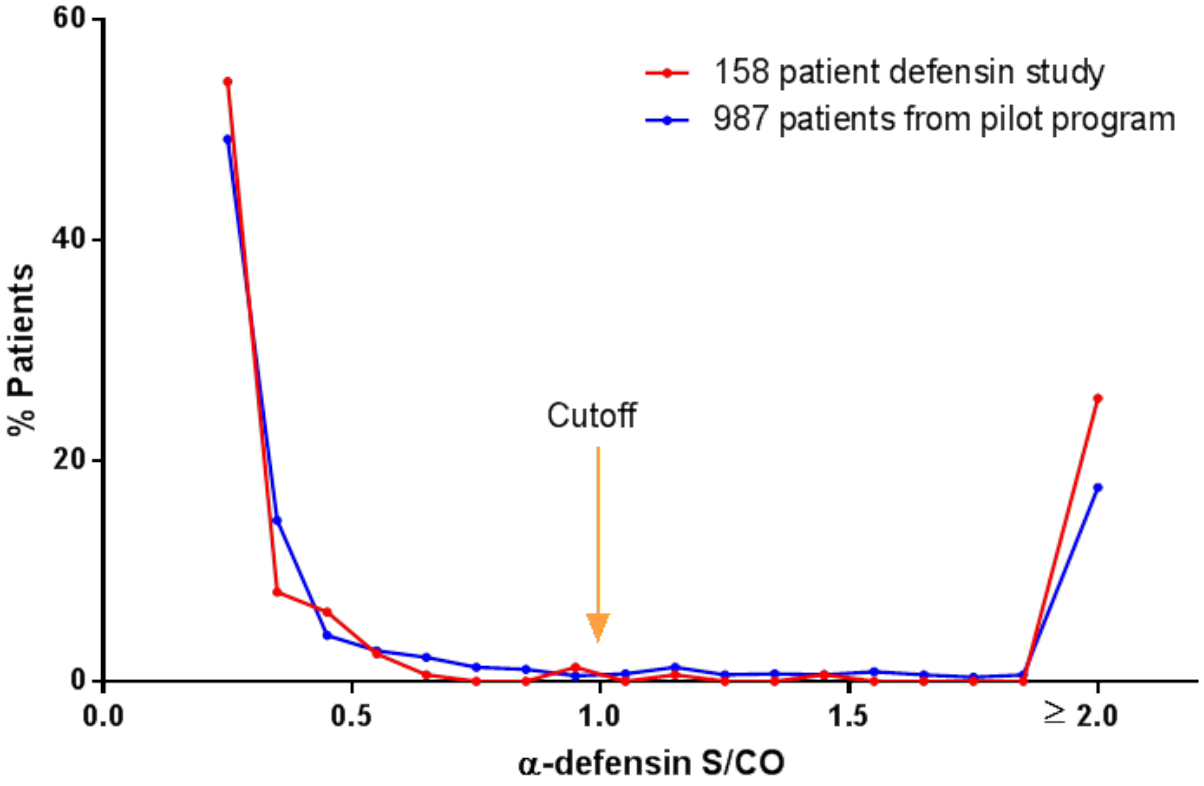
From 2012-2013, CD Diagnostics conducted a pilot program offering synovial fluid alpha-defensin testing and culture for surgeons in the United States. During this program, 1007 synovial fluid samples were received from 260 surgeons in 39 states. The synovial fluid alpha-defensin concentration was successfully measured and reported in 987 of the submitted samples. Of these, 883 had sufficient fluid for culture. There were 20 samples that could not be tested for synovial fluid alpha-defensin for the following reasons:

Cause	**# of Specimens**
Indeterminant	6
Invalid	2
Quantity Not Sufficient	12
Total Specimens	**20**

The scatter plot below demonstrates the distribution of alpha-defensin results in this pilot program compared to the 158 patient study described above. This data is also shown in a line graph, adjusted to report the percentage of patients with various alpha-defensin concentrations. It is evident that the CD Diagnostics pilot program and the 158 patient study exhibit an almost identical bimodal distribution of alpha-defensin concentrations. In the scatter plot, patients with a positive synovial fluid culture are colored red.



Distribution of alpha-Defensin Results from 158 patient study overlaying pilot program samples classified using established MSIS criteria



The pilot program included 649 samples that tested negative for alpha-defensin. The culture-positive rate among these negative patients was 1.4%, which is consistent with the expected false positive culture rate in a population of synovial fluid samples. The culture-positive rate among alpha-defensin-positive samples was 44%, which is consistent with reports of the culture-positive rate from synovial fluid aspirates of PJI. These data are comparable to the 158 patient MSIS defined study group, which had a 1.7% rate of false positive synovial fluid cultures among aseptic patients and a 47% rate of culture positive synovial fluid among samples with PJI. The distribution of organisms identified in samples that cultured positive is listed below:

Organism	Number	Percentage
S.epidermidis(+)	41	33%
E.faecalis(+)	11	9%
S.agalactiae(Gp B) (+)	7	6%
S.aureus(+)	7	6%
S.lugdunensis(+)	6	5%
S.aureus (MRSA) (+)	5	4%
S.mitis/oralis (+)	5	4%
S.capitis(+)	4	3%
C.striatum(+)	3	2%
P.aeruginosa(-)	3	2%
E.cloacae complex (-)	3	2%
B.fragilis(-)	2	2%
C.albicans(Yeast)	2	2%
E.coli (-)	2	2%
P.mirabilis(-)	2	2%
S.caprae(+)	2	2%

Organism	Number	Percentage
S.gordonii(+)	2	2%
S.warneri(+)	2	2%
A.defectiva(+)	1	1%
C.jeikeium(+)	1	1%
C.koseri(-)	1	1%
C.parapsilosis (yeast)	1	1%
G.adiacens(+)	1	1%
K.ohmeri(yeast)	1	1%
P.aeru(-)., E.faecalis(+)	1	1%
S. lugdunensis(+)	1	1%
S.cristatus(+)	1	1%
S.marcescens(-)	1	1%
S.parasanguinis(+)	1	1%
Streptococcus species(+)	1	1%

In summary, alpha-defensin distinguishes between PJI and aseptic infection with a level of sensitivity and specificity that exceeds that of currently used tests for infection. In a 158 patient, MSIS-classified study, alpha-defensin demonstrates a sensitivity of 97% and a specificity of 96% for infection, even when including patients with metallosis, systemic inflammatory diseases, and patients on antibiotics at the time of aspiration. The specificity when excluding patients with metallosis was 97.1%. Both the distribution of alpha-defensin levels and the culture positivity rates were preserved when alpha-defensin and culture were tested on a larger population of 883 synovial fluid samples.

Alpha-Defensin vs. the Leukocyte Esterase Test Strip

A study was conducted to compare the diagnostic characteristics of the synovial fluid alpha-defensin test to that of the leukocyte esterase (LE) colorimetric test strip. The MSIS criteria for PJI were used to diagnose 23 cases of aseptic arthroplasty failure and 23 cases of PJI. The synovial fluid was tested for both alpha-defensin levels and also the LE test strip. A "++" reading was considered positive for the LE test strip as previously described, while the alpha-defensin threshold used was an assay signal to cutoff ratio of 1.0.

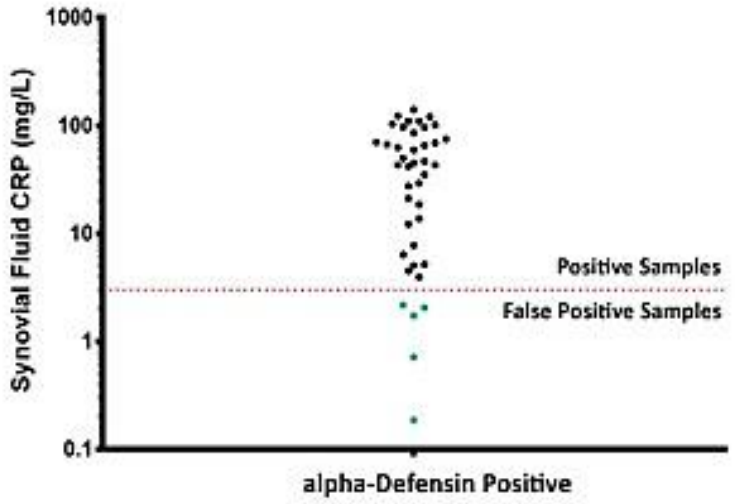
The synovial fluid alpha-defensin test correctly diagnosed all patients in this study, with a sensitivity and specificity of 100%. Bloody contamination did not prevent the alpha-defensin test from being interpreted in

any case. The LE test strip could not be interpreted due to bloody contamination in 8 of 46 samples (17%). In the remaining interpretable samples, the LE strip demonstrated a sensitivity of 67% and a specificity of 100%. The synovial fluid alpha-defensin test diagnostically outperforms the LE test strip, and is not subject to the high rate of invalid results due to bloody contamination.

Improving the Diagnosis of PJI by Adding Synovial Fluid CRP

Alpha-defensin alone has an excellent diagnostic profile that can provide critically useful information for arthroplasty surgeons. In the previously described 95-patient study evaluating 16 biomarkers, it was observed that the synovial fluid CRP was one of the few biomarkers that were not falsely triggered by some cases of metallosis. Therefore, the utility of adding synovial fluid CRP to the panel was studied.

The CRP concentration was assessed in 158 synovial fluid samples that were MSIS classified aseptic or PJI. Of these samples, there were 42 alpha-defensin-positive synovial fluid samples, including 37 true positives and 5 false positives by the MSIS definition. The scatter plot below demonstrates this data, with false positive alpha-defensin samples (3 metallosis and 2 aseptic) colored in green. As shown in the graph below, when a sample has a low (<3.0 mg/L) synovial fluid concentration of CRP and an elevated alpha-defensin, there exists a high probability that the elevated alpha-defensin result is a false positive.



CRP Distribution for Sample Positive for Alpha-Defensin

The Synovasure™ Test for PJI

Based on the preceding data on alpha-defensin and CRP, a diagnostic laboratory test was developed that provides clinicians with a new method of diagnosing PJI. This panel capitalizes on the diagnostic

characteristics of alpha-defensin, and also includes adjustment for samples with significant amounts of bloody contamination or hemolysis. Furthermore, the synovial fluid CRP test provides for the identification of samples that are most likely to represent false positive results due to confounding conditions such as metallosis. Synovasure™ maintained its diagnostic performance even among patients with metallosis, patients with systemic inflammatory diseases, and patients on antibiotics. Results are determined and returned to the clinician within 24 hours of receipt.

Synovasure™ Test		**95% Confidence Interval**
Sensitivity	97.4%	86.1% - 99.6%
Specificity (excluding cases of metallosis)	97.1%	93.0 % - 99.7%
Specificity (including cases of metallosis)	95.8%	90.5% - 98.6%

Synovasure™ results are reported as positive or negative based on the alpha-defensin measurement in the synovial fluid.

1. A negative Synovasure™ test result is highly suggestive that the aspirated arthroplasty is aseptic.
2. A positive Synovasure™ test result is highly suggestive that the aspirated arthroplasty has a PJI.
3. An indeterminate result will be reported for samples with an alpha-defensin level between 0.9 and the adjusted cutoff value.
4. A positive Synovasure™ test result including a low CRP concentration is potentially a false positive result, although CRP negative infections have been reported (40).

Summary

The Synovasure™ test was developed specifically for diagnosing PJI.

Alpha-defensin is an antibacterial peptide that is released into the synovial fluid in the presence of infection. Its levels are dramatically elevated in synovial fluid during PJI, signaling the presence of infection. The Synovasure™ test for PJI measures the concentration of synovial fluid alpha-defensin, providing a high sensitivity (97%) and specificity (96%) for the diagnosis of infection. The synovial fluid CRP level is also measured by Synovasure™, providing the clinician with an indication of which samples have the highest likelihood of a false positive test, which may occur in certain settings such as metallosis.

Note: *Some of the data and patient populations in this white paper were included in manuscripts submitted to the Journal of Bone and Joint Surgery and Clinical Orthopaedics and Related Research.*

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