EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR SYNOVASURE ALPHA DEFENSIN LATERAL FLOW TEST KIT DECISION SUMMARY

A. De Novo Number:

DEN180032

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the Synovasure Alpha Defensin Lateral Flow Test Kit.

C. Measurand:

Human Alpha Defensins 1-3

D. Type of Test:

Lateral flow immunoassay

E. Applicant:

CD Diagnostics, a Zimmer Biomet Subsidiary

F. Proprietary and Established Names:

Synovasure Alpha Defensin Lateral Flow Test Kit;
Synovasure Alpha Defensin Lateral Flow Test Kit (5 Test);
Synovasure Alpha Defensin Lateral Flow Test Kit (10 Test);
Synovasure Alpha Defensin Lateral Flow Test Kit (30 Test);
Synovasure Alpha Defensin Control Kit;

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3230

2. Classification:

   Class II

3. Product code(s):

   QGN

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Synovasure Alpha Defensin Lateral Flow Test Kit is a qualitative visually read immunochromatographic assay for the detection of human host response proteins, Alpha Defensins 1-3, in the synovial fluid of adults with a total joint replacement who are being evaluated for revision surgery. The Synovasure Alpha Defensin Lateral Flow Test Kit results are intended to be used in conjunction with other clinical and diagnostic findings as an aid in the diagnosis of periprosthetic joint infection (PJI). The Synovasure Alpha Defensin Lateral Flow Test Kit is not intended to identify the etiology or severity of a PJI.

   The Synovasure Alpha Defensin Control Kit is used in the Synovasure Alpha Defensin Lateral Flow Test Kit as assayed quality control samples to monitor performance and reliability of the Synovasure Alpha Defensin Lateral Flow Test Kit.

2. Indication(s) for use:

   Same as intended use.

3. Special conditions for use statement(s):

   For prescription use only.

   For *in vitro* diagnostic use only.

4. Special instrument requirements:

   Not applicable.

I. Device Description:

The Synovasure Alpha Defensin Lateral Flow Test Kit (Synovasure LFT) is an immunoassay for the detection of alpha defensin levels in the synovial fluid of patients with a potential PJI. Antibodies specific to alpha defensin bind host alpha defensin in the synovial fluid, become immobilized on the lateral flow test strip, and are detected as a colored line due to the use of a colloidal gold reporter.

Synovasure Alpha Defensin Lateral Flow Test Kit contains two sub components:

1. Synovasure Alpha Defensin Lateral Flow Test Device

2. Synovasure Lateral Flow Sample Prep Assembly

The Synovasure Lateral Flow Sample Prep Assembly further contains

1. One Synovasure Dilution Buffer Bottle

2. One Sample Cup

3. Two Microsafe Tubes

The Synovasure Alpha Defensin Lateral Flow Test Device is a cassette that includes a reagent strip. Each cassette contains a reagent strip with all the critical components for the assay.

The Synovasure Alpha Defensin Lateral Flow Test Kit is accompanied by the Synovasure Alpha Defensin Control Kit. The Synovasure Alpha Defensin Control Kit further contains

1. Synovasure Alpha Defensin Positive Control

2. Synovasure Alpha Defensin Negative Control

3. Synovasure Control Reconstitution Bottle

The positive control contains 0.25 mL of 16 µg/mL alpha defensin in synthetic synovial fluid and the negative control contains 0.25 mL of synthetic synovial fluid.

Additional materials required but not provided include

1. Timer

J. Standard/Guidance Document Referenced (if applicable):

EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition

EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition

EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition

EP14-A3, Evaluation of Commutability of Processed Samples; Approved Guideline—Third Edition

EP25-A , Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition

K. Test Principle:

Alpha defensins are antimicrobial peptides secreted by neutrophils in response to infection. The Synovasure Alpha Defensin Lateral Flow Test detects the presence of alpha defensin in synovial fluid specimens utilizing lateral flow technology. A sample of the synovial fluid is diluted and added to the lateral flow test device. The first pad in the device filters out the cellular material and the filtered sample contacts the buffering pad that contains the components for blocking and pH control. The sample then migrates to the pad containing the anti-alpha defensin antibody which is labeled with a colloidal gold conjugate. Finally, the sample mixture migrates to the test line which has immobilized anti-alpha defensin antibody and then to the control line that captures unbound antibody and verifies that the device flowed properly. A line present in the test zone indicates a positive result and the absence of a line in the test zone indicates a negative result.

L. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   The precision study was performed at three external laboratories over a minimum of 5 days with 3 operators per site, 3 runs per day, 3 reagent lots, and 18 blinded samples per run consisting of 2-4 blinded replicates of each sample. Combined results are shown in **Table 1**.

Table 1: Synovasure LFT Precision of Alpha Defensin Spiked into Synthetic Synovial Fluid

Panel Member	% Positive	95% Confidence Interval	% Negative	95% Confidence Interval
Negative	1.0% (4/403)	0.3-2.5%	99.0% (399/403)	97.5-99.7%
High Negative	9.9% (40/404)	7.2-13.2%	90.1% (364/404)	86.8-92.8%
Cutoff	49.9% (202/405)	44.9-54.9%	50.1% (203/405)	45.1-55.1%
Low Positive	79.9% (321/403)	75.4-83.5%	20.3% (82/403)	16.5-24.6%
Positive	96.0% (388/404)	93.6-97.7%	4.0% (16/404)	2.3-6.4%
High Positive	98.5% (396/402)	96.8-99.5%	1.5% (6/402)	0.5-3.2%

The low positive sample results failed to meet an acceptance criterion of 90% reactivity. To identify a root cause for this discrepancy, a sub-analysis was performed on data stratified form each site and obtained from each operator. The sponsor noted that data obtained from Operators 2 and 3 from Site 1 exhibited significantly lower reactivities in the low positive samples (**Table 2**). Furthermore, it was noted that the the actual concentration of some panel members was lower than expected due to an error in the preparation of the panel.

Table 2: Precision Performance Stratified by Site

Panel Member	% Positive Site 1	95% Confidence Interval	% Positive Site 2	95% Confidence Interval	% Positive Site 3	95% Confidence Interval
Negative	0.7% (1/134)	0-4.1%	1.5% (2/135)	0.2-5.2%	0.7% (1/134)	0.0-4.1%
High Negative	0.0% (0/135)	0-2.7%	3.7% (5/134)	1.2-8.5%	25.9% (35/135)	18.8-34.2%
Cutoff	25.2% (34/135)	18.1-33.4%	51.9% (70/135)	43.1-60.5%	72.6% (98/135)	64.3-79.9%
Low Positive	66.4% (89/134)	57.8-74.3%	80.0% (108/135)	72.3-86.4%	92.5% (124/134)	86.7-96.4%
Positive	93.3% (126/135)	87.7-96.9%	97.0% (131/135)	92.6-99.2%	97.8% (131/134)	93.6-99.5%
High Positive	97.8% (132/135)	93.6-99.5%	99.2% (132/133)	95.9-100%	98.5% (132/134)	94.7-99.8%

An additional analysis was performed excluding the low positive data from Operators 2 and 3 at Site 1 and the low positive sample reactivity becomes 87% (271/315). According to the probit regression obtained in the C5-C95 study, this is in close agreement with the expected reactivity (88.14%) for the actual alpha defensin concentration of the low positive panel member (10.3 µg/mL). Furthermore, the

positive and negative control kit includes an alpha defensin concentration near the LoD and could provide an adequate control for user test interpretation.

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Stability

External Controls

To monitor the assay performance, reagent performance, and procedural errors, positive and negative external controls must be run in accordance with the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. The positive and negative controls are packaged lyophilized. Prior to use, the end user rehydrates the lyophilized pellets using the reconstitution fluid provided in the Synovasure Control Reconstitution Bottle following the instructions in the Synovasure Alpha Defensin Control Kit package insert.

Reagent Stability:

Reagent Integral Stability:

The sponsor evaluated the effect of freeze-thaw cycles on Synovasure LFT devices over time. While not reflective of the recommended storage temperatures (2-30°C) for assay components, the product may experience freezing and thawing during transport. A total of 30 Synovasure LFT kits (including the device and buffer bottle with sample cup and Microsafe tube) were stored at -20°C for one week and thawed at room temperature to evaluate performance of spiked synthetic synovial fluid positive and negative controls (N = 10). Additionally, another set of 30 Synovasure LFT kits were stored at -20°C for 24 h, thawed, and returned to -20°C for at least another 24 h three times. Performance of the Synovasure LFT against three synthetic synovial fluid quality control (QC) samples is reported below in **Table 3**.

Table 3: Synovasure LFT Kit Freeze-Thaw Stability

3X Freeze-Thaw Percent Positive (N)		
QC1 (0 ug/mL)	**QC2 (4 ug/mL)**	**QC3 (12 ug/mL)**
0% (0/10)	0% (0/10)	90% (9/10)
RT Controls		
0% (0/3)	0% (0/3)	100% (3/3)
1X Freeze-Thaw		
Neg. Control	**Pos. Control**	
0% (0/10)	100% (10/10)	
RT Controls		
0% (0/3)	100% (3/3)	

Three lots of Synovasure LFT assay kits were subjected to real-time stability testing at both 2-8°C and 30°C over a period of either 1021, 990, or 772 days. Positive (16 µg/mL alpha defensin) and negative controls from the associated control kit were evaluated at each time point. Assay performance was evaluated by comparison to a color card unit scale with scores <1 being negative. While assay output does decrease at higher temperatures, all 3 lots met the acceptance criteria over the entire course of the study at both 2-8°C and 30°C. An additional lot was subjected to an accelerated stability study at both 37°C and 50°C. The assay kit stored at 37°C met acceptance criteria for 224 days. Extrapolating these results utilizing the Arrhenius equation did not support the desired 24-month assay stability claim. However, the assay kit stored at 50°C met acceptance criteria for 190 days. The Arrhenius equation extrapolation of these stability data to 30°C do support a 24-month stability claim.

Calibrator Stability:

To confirm stability of rehydrated control reagents, 6 vials each of positive and negative control were reconstituted and tested so that reactivity was assessed on day 0, 5, 7, 12, 17, and 20 after resuspension. All controls met predetermined acceptance criteria with positive specimens maintaining assay reactivity during the 20 day length of the study.

Three lots of Synovasure LFT Control Kits were subjected to real-time stability testing. Positive control specimens were stored at both 2-8°C and 30°C and evaluated on the Synovasure LFT over time to support a 36 month expiration date. The positive and negative controls, along with the Reconstitution Buffer, were shown to give correct test results with the lateral flow tests at 28 months for both storage temperatures.

Sample Stability:

Five negative synovial fluid specimens were each halved and divided into two independent sample groups with 1 group receiving a spike of alpha defensin at 23.2 µg/mL and the other group receiving a vehicle control (water). Each sample was then

aliquoted and stored at either 2-8°C, 13-16°C, 20-25°C, or 28-32°C and analyzed on days 0, 1, 3, 5, and 7. Aliquots were evaluated for correct identification by the Synovasure LFT. Results are listed below in **Table 4**.

Table 4: Specimen Stability Study Results

	% Correctly Identified at Indicated Storage Temperature			
Time (Days)	**4-8°C**	**13-16°C**	**20-25°C**	**28-32°C**
T_0	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)
T_1	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)
T_3	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)
T_5	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)
T_7	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)

All samples agreed with the expected results.

Freeze-Thaw Stability Study

All clinical synovial fluid specimens evaluated by the sponsor were tested fresh without freezing. The only specimens that were frozen prior to testing were the alpha defensin spiked synthetic synovial fluid panels prepared for the precision study. In order to determine the stability of alpha defensin spiked synthetic synovial fluid panel members, the sponsor evaluated the effects of multiple freeze-thaw cycles on samples containing 2, 4, 8, 12 µg/mL alpha defensin. Specifically, samples were assayed on an alpha defensin ELISA after 1 freeze-thaw cycle and after 3 separate freeze-thaw cycles. Generally, samples after a freeze-thaw cycle exhibited no loss of analyte signal due to the freezing process (**Table 5**).

Table 5: Freeze-Thaw Stability of C5-C95 Study Synthetic Synovial Fluid Specimens

Conc.:	**2 µg/mL**			**4 µg/mL**			**8 µg/mL**			**12 µg/mL**		
Repeat	**Wet**	**1X FT**	**3X FT**	**Wet**	**1X FT**	**3X FT**	**Wet**	**1X FT**	**3X FT**	**Wet**	**1X FT**	**3X FT**
1	0.26	0.24	0.21	0.44	0.48	0.58	0.90	1.24	0.86	1.04	1.14	1.06
2	0.26	0.24	0.25	0.47	0.47	0.55	0.90	0.95	0.87	1.18	1.18	1.11
3	0.26	0.24	0.25	0.47	0.59	0.57	0.96	0.99	1.00	1.19	1.33	1.10
4	0.25	0.24	0.25	0.46		0.50	0.88		1.08	1.30	1.19	1.31
5	0.25		0.23	0.50			0.82		1.24	1.13	1.21	1.07
Average:	**0.25**	**0.24**	**0.24**	**0.47**	**0.52**	**0.55**	**0.89**	**1.06**	**1.01**	**1.17**	**1.21**	**1.13**
Difference:		**-0.012**	**-0.014**		**0.05**	**0.081**		**0.17**	**0.12**		**0.041**	**-0.04**

Samples containing a range of alpha defensin concentrations were also compared to a standard curve of serially diluted alpha defensin to estimate any difference from target concentrations after a single freeze-thaw cycle (**Table 6**). No significant differences were observed.

Table 6: Freeze-Thaw Stability of Spiked Synthetic Synovial Fluid Samples

Target (µg/mL)	Fresh (µg/mL)	Difference (%)	1X FT (µg/mL)	Difference (%)	% Positive After 1X FT
0	0.1	-	0.4	-	0
2.0	1.7	15.0	1.8	9.0	0
4.0	3.8	5.0	4.3	7.0	0
4.8	4	16.7	4.8	0	0
5.6	5	10.7	5.5	1.0	0
6.4	6.1	4.7	6.2	3.0	0
8.0	7.9	1.3	8.9	12.0	33.3% (1/3)
9.6	8.7	9.4	10.7	11.0	100% (3/3)
10.4	10.2	1.9	11.8	13.0	100% (3/3)
11.2	10.5	6.2	12.1	8.0	100% (3/3)
12.0	11.1	7.5	12.9	8.0	100% (3/3)

d. Detection limit:

A study to establish the detection limit of the Synovasure LFT was conducted over 5 days utilizing 3 reagent lots. A panel of 88 samples was tested by 3 operators. This design results in a total of 120 replicates per sample. The results of this study were analyzed by probit regression to identify C5 and C95 concentrations. Results are listed below in **Table 7**.

Table 7: Establishment of C5/C95 Concentrations for Synovasure LFT

Concentration (µg/mL)	% Positive	95% Confidence Interval	% Negative	95% Confidence Interval
0	0% (0/118)	0-3.1%	100%	97.0-100%
2.2	0% (0/120)	0-3%	100%	97.0-100%
4.3	5% (6/120)	1.9-10.6%	95.0%	89.4-98.1%
4.8	8.3% (10/120)	4.1-14.8%	91.7%	85.2-95.9%
5.4	11.7% (14/120)	6.5-18.8%	88.3%	81.2-93.5%
6.1	49.2% (59/120)	39.9-58.5%	50.8%	41.6-60.1%
8.9	77.5% (93/120)	69.0-84.6%	22.5%	15.4-31.0%
10.7	93.3% (112/120)	87.3-97.1%	6.7%	2.9-12.7%
11.9	93.3% (112/120)	87.3-97.1%	6.7%	2.9-12.7%
12.2	92.5% (111/120)	86.2-96.5%	7.5%	3.5-13.8%
13	96.7% (116/120)	91.7-99.1%	3.3%	0.9-8.31%

The probit model identified C5, C50, and C95 alpha defensin concentrations of 4.17, 7.05, and 11.95 µg/mL, respectively.

The verification of the analytical sensitivity was performed over 1 day using 3 reagent lots. 20 replicates of 11 panel members that span the expected C5-C95 range were evaluated for a total of 220 samples per lot. Results are below in **Table 8**.

Table 8: Verification of C5/C95 Concentrations for Synovasure LFT

Concentration (µg/mL)	% Positive	95% Confidence Interval	% Negative	95% Confidence Interval
0	0% (0/60)	0-6.0%	100%	94.0-100%
1.385	0% (0/60)	0-6.0%	100%	94.0-100%
2.505	0% (0/60)	0-6.0%	100%	94.0-100%
4.675	21.7% (13/60)	12.1-34.2%	78.3%	65.8-87.9%
5.58	42.4% (25/59)	29.6-55.9%	57.6%	44.1-70.4%
6.875	53.3% (32/60)	40.0-66.3%	46.7%	33.7-60.0%
8.01	73.3% (44/60)	60.3-83.9%	26.7%	16.1-39.7%
10.46	93.3% (56/60)	83.8-98.2%	6.7%	1.9-16.2%
11.035	91.7% (55/60)	81.6-97.2%	8.3%	2.8-18.4%
11.87	98.3% (59/60)	91.1-100%	1.7%	0.04-8.9%
13.88	96.7% (58/60)	88.5-99.6%	3.3%	0.4-11.5%

The results of the verification study establish C5 and C95 alpha defensin concentrations of 3.44 and 11.66 µg/mL, respectively.

The sponsor evaluated Synovasure LFT performance at temperatures including room temperature (22-25°C), 12-18°C, and 27-32°C. Results included in **Table 9** demonstrate that assay sensitivity deteriorates at temperatures ≥27°C. The assay package insert specifically states that the assay should be performed at room temperature only.

Table 9: Lateral Flow Assay In-Use Temperature Effects

			% Correctly Classified According to QC Criteria			
Target Temp.	**Actual Temp.**	**N**	**0 µg/mL**	**4 µg/mL**	**12 µg/mL**	**16 µg/mL**
27-32	30.3-30.8	20	100	100	15	85
27	26.8	20	100	100	80	100
22-25	23.3-23.7	20	100	100	100	100
12-18	12.8-17.9	20	100	100	100	100

e. *Analytical specificity:*

Cross reactivity:

Not applicable

Interference:

Exogenous and endogenous interfering substances were evaluated for interference by spiking into negative clinical synovial fluid specimens. Half of samples tested contained 17 µg/mL alpha defensin and the other half a water vehicle control. Both alpha defensin spiked and unspiked specimens were further subdivided to included

interferent or carrier solvent controls. Since synthetic synovial fluid used by the sponsor primarily contains physiological levels of Hyaluronic acid (HA), the sponsor also wanted to evaluate the effects of differing HA concentrations on assay performance.

Table 10: Interfering Substances Percent Agreement with Expected Results

		Low Positive		Negative	
Substance	**Test Concentration**	**N**	**% Correct**	**N**	**% Correct**
Rheumatoid Factor	300 IU	25	100%	20	100%
Whole Blood Hemoglobin	12.1 g/dL	15	100%	20	100%
Lysed Blood Hemoglobin	8.7 g/dL	9	100%	3	100%
Bilirubin (unconjugated)	20 mg/dL	25	100%	20	100%
Bilirubin (conjugated)	29 mg/dL	25	100%	20	100%
Triglyceride	418 mg/dL	25	96%	20	100%
Hyaluronic Acid	0 mg/mL	25	100%	25	100%
Hyaluronic Acid	2 mg/mL	25	100%	25	100%
Hyaluronic Acid	4 mg/mL	25	100%	25	100%
Hyaluronic Acid	8 mg/mL	25	100%	25	100%
Metal Ion Cobalt	150 mg/L	25	96%	20	100%
Metal Ion Chromium	150 mg/L	25	100%	20	100%
Metal Ion Titanium	150 mg/L	25	100%	20	100%
Bone Cement	10 mg/mL	25	100%	20	100%
Ultra-high-molecular-weight polyethylene	10 mg/mL	25	92%	20	100%

An interference effect was observed when lyophilized hemoglobin was evaluated on the Synovasure LFT with an effect that disappeared at 25 mg/dL concentrations. Hemoglobin interference was further assessed by testing synovial fluid samples containing both lysed and unlysed whole blood. Since no noticeable interference was observed with these specimens, the interefence from lyophilized hemoglobin was likely an artifact. Importantly, while whole blood does not appear to negatively interfere with assay performance, too much whole blood in the synovial fluid sample could cause false negative results by diluting any alpha defensin present. This point is noted in a limitation described on the package insert and evidenced in the clinical studies.

f. High Dose Hook Effect Study

Performance of the Synovasure LFT was evaluated utilizing high concentrations of alpha defensin to demonstrate the absence of a negative hook effect. A single operator utilizing a single lot of reagents tested specimens corresponding to physiological

alpha defensin concentrations of 0, 50, 100, 500, 1,000, 5,000 and 10,000 µg/mL. There was no visible decline in band intensity at these higher analyte concentrations.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Some of the assay validation studies utilized synthetic synovial fluid (SSF) as a sample matrix while other testing utilized samples prepared from clinical synovial fluid specimens. Clinical matrix was specifically utilized for interference testing, sampling method comparison studies, stability studies, and in-use stability testing. A study performed to assess in-use temperature as well as the precision and C_5-C_{95} studies were conducted utilizing SSF as a sample matrix due to limited availability of clinical specimen volumes to meet experimental designs. Preliminary comparisons of these two distinct sample matrices utilized only an alpha defensin concentration of 16.4 µg/mL. An additional matrix comparison study was therefore performed to demonstrate equivalent performance of the device at analyte levels below the assay LoD for both matrices. Alpha defensin was spiked into negative clinical pool (NP) of synovial fluid and synthetic synovial fluid at concentrations of 0, 6.7, ad 8 µg/mL and both matrices were evaluated on the Synovasure LFT. No significant difference between synthetic synovial fluid and clinical synovial fluid matrix was observed.

Table 11 : Performance Comparison of Clinical Matrix and Synthetic Synovial Fluid

Matrix	Alpha defensin (µg/mL)	Percent Positive	95% CI
SSF	0	0% (0/40)	0 - 8.81%
NP	0	0% (0/40)	0 - 8.81%
SSF	6.7	5.0% (2/40)	0.6 – 16.9%
NP	6.7	5.0% (2/40)	0.6 – 16.9%
SSF	8.0	55.0% (22/40)	38.5 – 70.7%
NP	8.0	67.5% (27/40)	50.9 – 81.4%
SSF	16.4	100% (25/25)	86.3-100%
NP	16.4	100% (25/25)	86.3-100%

3. Clinical studies:

a. Clinical Sensitivity:

Prospective Study

Reference method

The reference method utilized by the sponsor entails the identification of Musculoskeletal Infection Society (MSIS) criteria as listed below. Patients are diagnosed with periprosthetic joint infection if they meet one major criterion or any 3 of the 5 minor criteria. Final status determination was adjudicated by a two-physician panel, with discrepant opinions being resolved by consultation of a third physician.

Table 12: MSIS PJI Diagnostic Criteria

Major Criteria
1. A sinus tract communicating with the prosthesis
2. A pathogen is isolated by culture from two separate tissue or fluid samples obtained from the affected prosthetic joint
Minor Criteria
1. Elevated Erythrocyte Sedimentation rate (≥30mm/hr) and C-reactive protein levels (≥10 mg/L)
2. Elevated synovial fluid WBC count (≥3000)
3. Elevated synovial fluid neutrophil percentage (≥80%)
4. Isolation of a microorganism in one periprosthetic tissue or fluid culture
5. ≥5 neutrophils per high-powered field in 5 high-power fields from histological analysis of periprosthetic tissue at 400X magnification

Acceptance Criteria

The sponsor employed a sensitivity criterion of ≥90% with lower confidence bound of ≥85% and a specificity criterion of >90% with a lower confidence bound of ≥90% for combined prospective and retrospective data.

Study Demographics

A total of 386 subjects were evaluated for prospective study eligibility. 81 subjects were excluded because there was insufficient information for an MSIS diagnosis, the subject did not have a total knee or hip arthroplasty, the subject did not have revision surgery, the subject had a joint injection or synovial fluid collection recently, or there was insufficient sample volume for testing. The clinical study therefore analyzed a total of 305 prospective synovial fluid samples and 65 retrospective fresh remnant samples. The sponsor provided the demographic breakdown for prospective clinical study samples as identified in **Table 13**.

Table 13: Prospective Clinical Study Demographics and Baseline Characteristics

Variable	Value
Age (years)	
Mean	64.8
STDEV	9.76
Min.	38
Max.	92
Gender (n, %)	
Male	131 (43.0%)
Female	174 (57.0%)
Race (n, %)	
White	266 (87.2%)
Asian	1 (0.3%)
African American	26 (8.5%)
Native American	2 (0.7%)
Other	10 (3.3%)
Infection History (n, %)	
Yes	87 (28.5%)
No	218 (71.5%)
Antibiotic History (n, %)	
Yes	40 (13.1%)
No	77 (25.2%)
Inflammatory Medications within the last month (n, %)	
Yes	43 (14.1%)
No	262 (85.9%)
Immunocompromised Conditions (n, %)	
Yes	19 (6.2%)
No	286 (93.8%)
Medical History (n, %)	
Yes	219 (71.8%)
No	86 (28.2%)

External Controls

During the conduct of the clinical trial protocol, external control testing was performed once per week per operator performing lateral flow testing. The tests were conducted per kit instructions and no control failures were observed over the 74 weeks of clinical testing across all sites.

Enrollment Inclusion/Exclusion Criteria

The prospective clinical study had the following subject inclusion criteria:

1. Subject had a total knee and/or hip joint arthroplasty
2. Subject was evaluated for revision surgery
   a. Operative samples were required for full MSIS classification
3. Subject was ≥22 years of age
4. Subject had no recent injections or surgeries of the joint (within past 6 weeks)
5. Subject had all the medical tests required to allow MSIS classification
6. Subject signed informed consent form.

The prospective clinical study had the following subject exclusion criteria:

1. Subject did not have a total knee and/or hip joint arthroplasty
2. Healthy subject without medical need for aspiration
3. Subject did not have a revision surgery
4. Subject had a diagnostic synovial fluid specimen collection within the past 14 days
5. Subject was <22 years of age
6. Subject had an injection, lavage, or surgery of the joint within the past 6 weeks
7. Subject did not have all the medical tests required for MSIS classification
8. Subject did not sign informed consent form

Analysis

The sponsor conducted a prospective clinical trial at 3 US medical centers with high volumes of revision surgery. Enough specimens were collected to ensure at least 50 positives were obtained as determined by Musculoskeletal Infection Society (MSIS) criteria. After adjudication, 57 prospective samples were identified as positive and the Synovasure LFT achieved a sensitivity of 89.5% (95% CI:78.5-96.1%). (**Table 14**).

Table 14: Clinical Performance of Synovasure LFT in Prospective Clinical Specimens

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	51	13	64
	N	6	235	241
	Total	57	248	305
Sensitivity (95% CI): 89.5% (78.5-96.0%)				
Specificity (95% CI): 94.8% (91.2-97.2%)				

Whole blood contamination potentially reduces the alpha defensin concentration in the synovial fluid sample. Therefore, the sponsor performed an additional analysis excluding those samples with higher levels of whole blood contamination (>20%

dilution by volume) (**Table 15**). Out of 17 samples with greater than 20% whole blood contamination, 1 was a true positive, 3 were false negatives, and 13 were true negatives. To address this risk of generating false results, a specific limitation noting the potential for false negative results in samples containing a significant amount of blood is listed in the package insert.

Table 15: Clinical Performance of Synovasure LFT in Prospective Clinical Specimens (RBC>1x10^6/µL excluded)

		MSIS Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	50	13	63
	N	3	222	225
	Total	53	235	288
Sensitivity (95% CI): 94.3% (84.3-98.8%)				
Specificity (95% CI): 94.5% (90.7-97.0%)				

Two false negative Synovasure LFT results from the clinical trial were identified in patients with a draining sinus tract connecting to the joint. One of these was also identified as contaminated with greater than 20% whole blood. A draining sinus should be readily observable and is also a major diagnostic criterion for PJI; therefore, the impact of this on the patient's diagnosis would be minimal.

Retrospective Remnant Clinical Samples

To supplement the testing of fresh prospectively collected specimens, the sponsor also obtained fresh retrospective synovial fluid collections to bring the total number of positive specimens tested to at least 100. The residual samples were anonymized, assigned new case ID numbers and tested. Synovasure LFT results were compared to a status determination based positive confirmation of three minor MSIS criteria (neutrophil %, positive culture, and WBC count). A total of 65 MSIS positive retrospective samples were evaluated interspersed with sufficient negative specimens to maintain relative disease prevalence consistent with what was observed in the prospective study population. The Synovasure LFT exhibited a 98.5% (95% CI: 91.7-100%) positive percent agreement with MSIS diagnosis for these retrospective specimens.

Synovasure LFT Analysis of Covariates

Performance of the Synovasure LFT in the prospective clinical study was further assessed among several covariates which could potentially impact device performance: age, gender, race, history of infection, history of inflammatory disease, use of anti-inflammatory medication, gram positive or gram negative culture isolates, type of prosthetic joint, and individual MSIS criteria. Insignificant differences in assay performance were observed based upon these variables (**Table 16 – Table 32)**.

Table 16: Prospective Study Performance of Synovasure LFT for White Subjects

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	45	13	58
	N	6	202	208
	Total	51	215	266
Sensitivity (95% CI): 88.2% (76.1-95.6%)				
Specificity (95% CI): 94.0% (89.9-96.7%)				

Table 17: Prospective Study Performance of Synovasure LFT for African American Subjects

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	5	0	5
	N	0	21	21
	Total	5	21	26
Sensitivity (95% CI): 100% (47.8-100%)				
Specificity (95% CI): 94.0% (83.9-100%)				

Table 18: Prospective Study Performance of Synovasure LFT for Other[1] Races

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	1	0	1
	N	0	12	12
	Total	1	12	13
Sensitivity (95% CI): 100% (2.5-100%)				
Specificity (95% CI): 100% (73.5-100%)				

1. Other races include Asian, American Indian or Alaskan Native, and races identified as "Other"

Table 19: Prospective Study Performance of Synovasure LFT for Male Subjects (excluding RBC>1 x10^6 /µL samples)

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	24	9	33
	N	1	90	91
	Total	25	99	124
Sensitivity (95% CI): 96.0% (79.6-99.9%)				
Specificity (95% CI): 90.9% (83.4-95.8%)				

Table 20: Prospective Study Performance of Synovasure LFT for Female Subjects (excluding RBC>1 x10^6/µL samples)

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow	P	26	4	30
	N	2	132	134

Test	**Total**	28	136	164
Sensitivity (95% CI): 92.9% (76.5-99.1%)				
Specificity (95% CI): 97.1% (92.6-99.2%)				

To evaluate whether prior infections impact device performance, assay results were tabulated among patients with any prior history of infection or infectious disease (e.g., sinusitis, pneumonia or other respiratory infections, urinary tract infections, the common cold, HIV, lyme disease, viral hepatitis, etc.) within the last 6 months (Table 21) and for for those without such history (Table 22).

Table 21: Prospective Study Performance of Synovasure LFT for Subjects with Infection History (excluding RBC>1 x10^6/µL samples)

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	**P**	30	6	36
	N	1	48	49
	Total	31	54	85
Sensitivity (95% CI): 96.8% (83.3-99.9%)				
Specificity (95% CI): 88.9% (77.4-95.8%)				

Table 22: Prospective Study Performance of Synovasure LFT for Subjects without Infection History (excluding RBC>1 x10^6 /µL samples)

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	**P**	20	7	27
	N	2	174	176
	Total	22	181	203
Sensitivity (95% CI): 90.9% (70.8-98.9%)				
Specificity (95% CI): 96.1% (92.2-98.4%)				

Ongoing use of antibiotics might reduce infection severity and affect the performance of the Synovasure Lateral Flow test. Assay sensitivity and specificity were evaluated among study subjects who were actively on an antibiotic regimen upon study enrollment (Table 23) and those not currently taking antibiotic medications (Table 24).

Table 23: Prospective Study Performance of Synovasure LFT for Subjects with Ongoing Antibiotic Use (excluding RBC>1 x10^6/µL samples)

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	25	1	26
	N	1	12	13
	Total	26	13	39
Sensitivity (95% CI): 96.2% (83.3-99.9%)				
Specificity (95% CI): 92.3% (64.0-99.8%)				

Table 24: Prospective Study Performance of Synovasure LFT for Subjects without Ongoing Antibiotic Use (excluding RBC>1 x10^6/µL Samples)

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	8	5	13
	N	1	60	61
	Total	9	65	74
Sensitivity (95% CI): 88.9% (51.8-99.7%)				
Specificity (95% CI): 92.3% (83.0-97.5%)				

The presence of an underlying inflammatory or autoimmune disease (e.g., rheumatoid arthritis, lupus, diabetes, multiple sclerosis, autoimmune hepatitis, psoriasis, scleroderma, sarcoidosis, etc.) was also evaluated for potential impact on Synovasure LFT performance. Study subjects with a history of any inflammatory disease at least 6 months before enrollment were analysed (Table 25) and compared to those with a medical history of no inflammatory or autoimmune disorders (Table 26).

Table 25: Prospective Study Performance of Synovasure LFT for Subjects with Inflammatory Disease History (excluding RBC>1 x10^6/µL samples)

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	12	2	14
	N	1	52	53
	Total	13	54	67
Sensitivity (95% CI): 92.3% (64.0-99.8%)				
Specificity (95% CI): 96.3% (87.3-99.5%)				

Table 26: Prospective Study Performance of Synovasure LFT for Subjects without Inflammatory Disease History (excluding RBC>1 x10^6/µL samples)

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	38	11	49
	N	2	170	172
	Total	40	181	221
Sensitivity (95% CI): 95.0% (83.1-99.4%)				

Specificity (95% CI): 93.9% (89.4-96.9%)

The performance of the Synovasure LFT was also evaluated in subjects who are currently on anti-inflammatory or autoimmune medications (e.g., steroids or immunosuppressants) or have taken such medications in the past 6 months (Table 27) and those subjects who have not taken such medications (Table 28).

Table 27: Prospective Study Performance of Synovasure LFT for Subjects Using Anti-Inflammatory Medication (excluding RBC>1 x10^6/µL samples)

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	10	0	10
	N	1	30	31
	Total	11	30	41
Sensitivity (95% CI): 90.9% (58.7-99.8%)				
Specificity (95% CI): 100% (88.4-100%)				

Table 28: Prospective Study Performance of Synovasure LFT for Subjects without Current Anti-Inflammatory Medications (excluding RBC>1 x10^6/µL samples)

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	40	13	53
	N	2	192	194
	Total	42	205	247
Sensitivity (95% CI): 95.2% (83.8-99.4%)				
Specificity (95% CI): 93.7% (89.4-96.6%)				

Since alpha defensins are host response proteins, it is possible that their expression level varies depending on the type of organism responsible for the joint infection. Performance of the Synovasure LFT was compared in study subjects who had a gram positive pathogen isolated by culture (Table 29) versus those study subjects with a gram negative pathogen isolated by culture (Table 30).

Table 29: Prospective Study Performance of Synovasure LFT for Subjects with Gram Positive Culture (excluding RBC>1 x10^6/µL samples)

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	29	2	31
	N	3	21	24
	Total	32	23	55
Sensitivity (95% CI): 90.6% (75.0-98.0%)				
Specificity (95% CI): 91.3% (72.0-98.9%)				

Table 30: Prospective Study Performance of Synovasure LFT for Subjects with Gram Negative Culture (excluding RBC>1 x10^6/µL samples)

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	6	0	6
	N	1	0	1
	Total	7	0	7
Sensitivity (95% CI): 85.7% (42.1-99.6%)				
Specificity (95% CI): Non-estimable				

All of the data from the prospective study (including specimens with greater than 1 x10^6/µL RBC contamination) were also analyzed to evaluate Synovasure LFT Performance in the presence of a positive culture (Table 31), a key component of MSIS diagnostic criteria, and in samples for which no culture was positive (Table 32).

Table 31: Prospective Study Performance of Synovasure LFT for Subjects with at least one Positive Culture

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	36	2	38
	N	5	23	28
	Total	41	25	66
Sensitivity (95% CI): 87.8% (73.8-95.9%)				
Specificity (95% CI): 92.0% (74.0-99.0%)				

Table 32: Prospective Study Performance of Synovasure LFT for Subjects with all Negative Cultures

		MSIS PJI Diagnosis		Total
		P	N	
Synovasure Lateral Flow Test	P	15	11	26
	N	1	210	211
	Total	16	221	237
Sensitivity (95% CI): 93.8% (69.8-99.8%)				
Specificity (95% CI): 92.0% (91.3-97.5%)				

Prevalence Analysis

A detailed description of the type of infections observed during the clinical study is provided in **Table 33**.

Table 33: PJI Prevalence Overall and by Type of Infection for All Prospective Subjects with a Synovial Fluid and/or Tissue Culture Performed

	Positive PJI Diagnosis (N, [%, 95% CI])	Negative PJI Diagnosis (N, [%, 95% CI])	Total (N, [%, 95% CI])
Subjects with Gram positive culture	35 (61%, 48-74%)	24 (10%, 6-14%)	59 (19%, 15-24)
Subjects with a Gram Negative Culture	7 (12%, 5-24%)	1 (0%, 0-2%)	8 (3%, 1-5%)
Subjects with a Fungal Culture	1 (2%, 0-9%)	1 (0%, 0-2%)	2 (1%, 0-2%)
Subjects with any positive culture	41 (72%, 58-83%)	25 (10%, 7-15%)	66 (22%, 17-27)
Subjects with all negative cultures	16 (28%, 17-42)	221 (90%, 85-93%)	237 (78%, 73-83%)
Total subjects with culture performed	57	246	303

b. Clinical specificity:

See section M.3a above.

c. Other clinical supportive data (when a. and b. are not applicable):

See section M.3a above.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

Not Applicable

M. Instrument Name:

Not applicable. The device does not utilize an instrument for result generation.

N. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes _____ or No ____X___

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ___X_____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes _____ or No ____X____

   The device does not contain any software or instrument components.

3. Specimen Identification:

   Not applicable.

4. Specimen Sampling and Handling:

   Not applicable.

5. Calibration:

   Not applicable.

6. Quality Control:

   Not applicable.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

None.

P. Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

Q. Identified Risks to Health and Identified Mitigations

Identified Risks to Health	Mitigation Measures
Risk of false test results	Certain device descriptions, performance characteristics, results interpretation information, limitations, and study details in labeling. Certain device description information, demographic analysis, validation procedures, risk mitigation strategies and end user trainings, and studies. Collection device specification.
Failure to correctly interpret test results	Certain device descriptions, performance characteristics, results interpretation information, limitations, and study details in labeling. Certain demographic analysis, validation procedures, risk mitigation strategies and end user trainings, and studies.
Failure to correctly operate the device	Certain device descriptions, performance characteristics, results interpretation information, limitations, and study details in labeling. Certain demographic analysis, validation procedures, risk mitigation strategies and end user trainings, and studies. Collection device specification.

R. Benefit/Risk Analysis:

Summary of the Assessment of Benefit

The benefit of the assay is aiding the accurate diagnosis of prosthetic joint infection (PJI). Accurate diagnosis of prosthetic joint infection can be helpful to initiate appropriate treatment for prosthetic joint infection, including, but not limited to, antibiotics and revision surgery. Appropriate treatment of PJI can lead to alleviation of symptoms associated with infection and restoration of function. Additionally, appropriate exclusion of a prosthetic joint infection will aid clinicians in deciding to retain existing hardware.

Summary of the Assessment of Risk

The risks associated with the device, when used as intended, are those related to the risk of false test results, failure to correctly interpret the test results, and failure to correctly operate the device.

The risk of a false positive test result is improper patient management, including inappropriate administration of prolonged courses of antibiotics and inappropriate

explanting of hardware. Inappropriate administration of prolonged courses of antibiotics is associated with toxicity, allergic reactions, and adverse outcomes, including secondary infections such as *C. difficile* colitis. Inappropriate explanting of hardware may further obscure the anatomy of a joint, decrease function, worsen symptoms, and complicate further surgical manipulation of the joint. The risk of a false positive test is mitigated by the intended use clearly stating that the assay is intended as an adjunct to aid in the diagnosis of infection, in conjunction with other clinical and diagnostic findings.

The risk of a false negative test result is improper patient management, including inappropriate discontinuation of antibiotics or failure to treat a prosthetic joint infection with antibiotics and explanting of infected hardware. Failure to treat a prosthetic joint infection could lead to decreased function of the joint and worsen symptoms of infection. If not treated, a prosthetic joint infection could develop into a more complicated or a systemic infection, including skin and soft tissue infection, acute or chronic osteomyelitis, bacteremia, and/or sepsis. A patient who is symptomatic from a prosthetic joint infection will likely return to care, most likely delaying treatment instead of resulting in a complete failure to diagnose and treat. The risk of a false negative test is mitigated by the fact that this assay is intended as an adjunct to aid in the diagnosis of infection in conjunction with other clinical and diagnostic findings.

Failure to correctly operate the device can lead to false test results. Failure to correctly interpret test results can lead to treatment of a clinically positive patient in the same manner as a false negative test result and a clinically negative patient in the same manner as a false positive test result with the corresponding implications discussed above.

Summary of the Assessment of Benefit-Risk

General controls are insufficient to mitigate the risks associated with the device. However, the probable clinical benefits outweigh the potential risks for the proposed assay, considering the mitigations of the risks provided for in the listed special controls established for this device as well as general controls. The required special controls will help ensure that errors will be uncommon and will facilitate accurate assay implementation and interpretation of results. The clinical performance observed in the clinical trial suggests that errors will be uncommon and that the assay will provide substantial benefits to patients in the diagnosis of prosthetic joint infections and when used in conjunction with other clinical and diagnostic findings.

Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

S. Conclusion

The information provided in this De Novo submission is sufficient to classify this device into class II under regulation 21 CFR 866.3230. FDA believes that the stated special controls, in combination with the applicable general controls, provide a reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code(s): QGN
Device Type: Device to detect and measure non-microbial analytes to aid in the detection and identification of localized human infections
Class: II (special controls)
Regulation: 21 CFR 866.3230

(a) Identification: A device to detect and measure non-microbial analytes to aid in the detection and identification of localized human infections is identified as an *in vitro* device intended for the detection and qualitative measurement, quantitative measurement, or both of one or more non-microbial analytes in human clinical specimens to aid in the assessment, identification, or both of a localized microbial infection when used in conjunction with clinical signs and symptoms and other clinical and laboratory findings.

(b) Classification: Class II (special controls). The special controls for this device are:

1. Any sample collection device used must be FDA-cleared, -approved, or -classified as 510(k) exempt (standalone or as part of a test system) for the collection of human specimens; alternatively, the sample collection device must be cleared in a premarket submission as a part of this device.

2. The labeling required under 21 CFR 809.10(b) must include:

   i. An intended use with a detailed description of what the device detects and measures, the type of results provided to the user, the sample type, whether the measure is qualitative and/or quantitative, the clinical indications for the test use, and the specific population(s) for which the device is intended.

   ii. A detailed description of the performance characteristics of the device for all intended specimen types from the analytical and clinical studies (as applicable) required under paragraphs 3(ii) and 3(iii).

   iii. A detailed explanation of the interpretation of results, including acceptance criteria for evaluating the validity of individual runs (e.g., assessment of internal and/or external quality controls, as applicable).

   iv. The following limiting statements:

      (A) A statement that a negative test result does not preclude the possibility of infection;

      (B) A statement that the test results should be interpreted in conjunction with other clinical and laboratory data available to the clinician;

      (C) A statement that consistent device performance is dependent on adequate specimen collection, transport, storage, and processing. Failure

to observe proper procedures in any one of these steps can lead to incorrect results; and

(D) A statement that details any limitations associated with the samples, as appropriate (e.g., collected on the day of admission to the ICU).

3. Design verification and validation must include the following:

i. A detailed device description, including as appropriate, all device parts; control elements incorporated into the test procedure; instrument requirements; reagents required but not provided; and the principle of device operation and test methodology, including all pre-analytical methods for the processing of specimens and the methodology from obtaining a sample to the result; design of primer/probe sequences; rationale for target analyte selection; and computational path from collected raw data to reported result (e.g., how collected raw signals are converted into a reported result).

ii. Detailed documentation of analytical studies including analytical sensitivity (Limit of Detection, Limit of Quantitation, and Limit of Blank), inclusivity, cross-reactivity, microbial interference, interfering substances, competitive inhibition, carryover/cross-contamination, specimen stability, within-lab precision, reproducibility, and linearity, as applicable.

iii. Detailed documentation and results either from: a clinical study, that includes prospective (sequentially collected) samples for each intended specimen type that are representative of the intended use populations and, when determined to be acceptable by FDA, additional characterized clinical samples; or, when determined to be acceptable by FDA, an equivalent sample set. The clinical study must compare the device performance to results obtained from an FDA accepted reference method and/or FDA accepted comparator method, as appropriate. Documentation from the clinical studies must include the clinical study protocol (e.g., the predefined statistical analysis plan), clinical study report, testing results, and results of all statistical analyses.

iv. An evaluation of the level of the non-microbial analyte in asymptomatic patients with demographic characteristics (e.g., age, racial, ethnic, and gender distribution) similar to the intended use population of the device.

v. Documentation of an appropriate end user device training program that will be offered as part of efforts to mitigate the risks of false results, failure to correctly operate the device, and failure to correctly interpret test results.

vi. An appropriate risk mitigation strategy to ensure that the device does not prevent any other device(s) with which it is indicated for use, including incorporated device(s), from achieving their intended use (e.g., safety and effectiveness of the functions of the indicated device(s) remain unaffected).

vii. A detailed description of the impact of any software, including software applications and hardware-based devices that incorporate software, on the device's functions.