ZIMMER BIOMET

Synovasure® Alpha Defensin ELISA Test

For *in vitro* diagnostics use

- **Users must read this package insert in its entirety before using the product. Follow the instructions carefully when conducting the test. Failure to do so may cause inaccurate test results.**

CD2000-10 Synovasure® Alpha Defensin ELISA Test

RESTRICTIONS

- This assay has not been validated for use in patient populations without a total joint replacement.

NAME AND INTENDED USE

The Synovasure Alpha Defensin ELISA Test is a qualitative, immunometric assay intended 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 ELISA Test results are intended to be used in conjunction with other clinical and diagnostic findings to aid in diagnosis of periprosthetic joint infection (PJI). The Synovasure Alpha Defensin ELISA Test is not intended to identify the etiology or severity of a PJI. The device is non-automated.

The Synovasure Alpha Defensin ELISA Test is intended only for use within a clinical laboratory by qualified personnel.

PRINCIPLES OF THE TEST

The Synovasure Alpha Defensin ELISA Test is an immunometric assay and involves the reaction of the alpha defensins 1-3 peptides present in the sample with an anti-alpha defensin monoclonal antibody coated onto the wells. Prior to utilization in the assay, synovial fluid specimens are centrifuged for 10 minutes at 1000g to remove cellular material. The samples are then diluted 1:8,000 in assay diluent and added to a microwell that is coated with anti-defensin. After incubation, unbound materials are removed by washing. Subsequently a biotinylated antibody conjugate (mouse monoclonal anti-alpha defensin) is added and this complexes with bound alpha defensin.

Following another wash step a streptavidin-(horseradish) peroxidase (SA-HRP) conjugate is added and this complexes with bound biotinylated antibody conjugate. Unbound materials are removed by washing. The bound SA-HRP conjugate is measured by a colorimetric reaction. A substrate containing 3,3',5,5'-Tetramethylbenzidine (TMB) is added to the wells. The SA-HRP in the bound conjugate catalyzes the oxidation of TMB substrate, producing a colored product. The color formation is stopped after 30 minutes by the addition of maleic acid to the wells and measured by reading the optical density at 450 nm. The amount of SA-HRP conjugate bound is proportional to the concentration of alpha defensins 1-3 present.

Results are calculated as a normalized signal, relative to a cutoff value.

WARNINGS AND PRECAUTIONS ⚠

For *in vitro* Diagnostic Use

- **This kit should only be used by qualified laboratory staff.**
- **Distilled or deionized water must be used for wash buffer preparation, assay buffer preparation and rehydration of the calibrator and controls. Clinical laboratory reagent water Type I or Type II is acceptable. Store the water in nonmetallic containers.**
- **Do not mix lot numbers of coated microwell plates, assay diluent, conjugate reagents, calibrator or controls from kits with different lot numbers.**
- **The microwell strips are sealed in protective pouches with a desiccant. If the desiccant is missing the microwell strips should not be used. When resealing the foil bag ensure to include the desiccant.**
- **Cross-contamination between reagents will invalidate the test results. Labeled, dedicated reservoirs for**

the appropriate reagents are recommended.

- **Ensure that specimen is diluted appropriately and added to the microwell. Failure to dilute a specimen may produce an erroneous infected result. Failure to add specimen may produce an erroneous aseptic result.**
- **When using a single-channel micropipette for manual sample addition, use a new pipette tip for each specimen to be assayed. When using a multichannel micropipette, new tips are to be used for each reagent to be added.**
- **Strict adherence to the specified wash procedure is crucial to ensure optimum assay performance. (See Step 7 of Test Procedure.)**
- **Do not touch the bottom exterior surface of the microwells. Fingerprints or scratches may interfere with reading the microwells.**
- **Ensure that the microwell strips are level in the microwell strip holder during the test procedure. If necessary, wipe the bottom of the microwell strips carefully with a soft, lint-free, absorbent tissue to remove any moisture, dust or debris before reading.**
- **Control values which are not within the expected range (refer to Quality Control section) may indicate a technique problem or product deterioration.**
- **All pipetting equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.**
- **Delays in plate processing may affect absorbance values.**
- **This test should be performed at room temperature (15-30°C), do not run outside this range.**
- **Open glass vials carefully: vials are under vacuum.**
- **Visual inspections of the reagents should be performed prior to use to check for color change, cloudiness, and precipitates.**
- **Do not use the device if any components are damaged**

Safety Precautions ⚠

- Wear disposable gloves while handling kit reagents and specimens. Thoroughly wash hands afterwards.
- Do not ingest or inhale any of the kit components.
- The Synovasure Alpha Defensin ELISA Test kit contains MIT (ProClin 950 or StabilZyme), and maleic acid (the stop solution) . Direct contact with skin and eyes should be strictly avoided. If contact occurs, rinse immediately with plenty of water and seek medical advice. **EU Hazard Code: Xn; Sensitising Risk statement: 34. CLP regulations (EC) No 1272/2008 Hazard statement H317 May cause an allergic skin reaction.**
- Handle kit components and all biological samples and testing materials using Universal Precautions as they are potentially hazardous and potentially infectious.
- Dispose of all specimens and materials used to perform the test as if they contain infectious agents. Disposal of all specimens and materials should comply with all local, state and federal waste disposal requirements.

Limitations of The Procedure

- **Known Interferences**
  - Samples with significant hemolysis may give false positive results.
- **Other Limitations**
  The presence of alpha defensin in synovial fluid does not constitute a diagnosis of PJI but may be indicative of recent and/or past infection. An aseptic test result does not exclude the possibility of PJI. Levels of alpha defensin may be undetectable in early infection.
- The results from this device should only be used in conjunction with information available from clinical evaluations and other tests for the diagnosis of Peri-prosthetic Joint Infection.

MATERIALS PROVIDED & STORAGE CONDITIONS

Synovasure Alpha Defensin ELISA Test REF CD2000-10

Store the unopened kit refrigerated (2-8°C). Store Wash Buffer at ambient temperature (15-30°C). Do not use past kit expiration date. Expiration dates are stated on the kit box and vial labels. See Table 1 for more details on materials and storage conditions.

NOTE: Wash Buffer is shipped outside of the kit box for ease of storage.

Table 1 Materials Provided and Storage Conditions

Kit Component	REF REF	Quantity QTY	Storage and 'use by date' of Opened/ Reconstituted/Diluted Materials
Alpha Defensin coated Microwell plates - 12 strips per plate (total 96 microwells per plate) sealed in a re-sealable foil bag with desiccant	P50000	10 plates	Return unused wells to the re-sealable foil bag containing the desiccant pack. Re-seal along the entire edge of the zip seal. Store refrigerated. Can be used for up to four (4) weeks.
Quality Control 1 (QC1), Alpha Defensin – 0.25 mL of synthetic synovial fluid, glass vial	P50002	10 vials	Store refrigerated. Rehydrated: replace cap. Can be used for up to 2 weeks.
Quality Control 2 (QC2), Alpha Defensin – 0.25 mL at 4µg/mL in synthetic synovial fluid, glass vial	P50003	10 vials	
Quality Control 3 (QC3), Alpha Defensin – 0.25 mL at 12µg/mL in synthetic synovial fluid, glass vial	P50004	10 vials	
Calibrator, Alpha Defensin – 0.25 mL at in synthetic synovial fluid, glass vial	P50005	10 vials	
Biotin Conjugate, Lyophilized – 0.25 mL of 100-fold concentrate, glass vial	P50001	10 vials	Store refrigerated Rehydrated: Replace cap. Can be used for up to two (2) weeks. Diluted: Can be used for up to two(2) weeks.
Streptavidin-Peroxidase (SA-HRP) Conjugate - 60 mL, amber bottle	P50011	3 bottles	Store refrigerated Use by labeled expiration date.
Assay Diluent – 500 mL of 5x concentrate), opaque bottle	P50009	1 bottle	Store refrigerated Concentrate: Use by labeled expiry date. Diluted: Can be used for up to four (4) weeks.
Wash Buffer – 1000 mL of 10x concentrate, opaque bottle	P50010	1 bottle	Store ambient temperature Concentrate: Use by labeled expiry date. Diluted: Can be used for up to four (4) weeks.
TMB (color) Solution – 60 mL, amber bottle	P50012	3 bottles	Store refrigerated. Use by labeled expiration date.
Stop Solution – 250 mL, opaque bottle	P50013	1 bottle	Store refrigerated. Use by labeled expiration date.
Instructions for Use		1	

OTHER MATERIALS REQUIRED BUT NOT PROVIDED

- Precision positive displacement pipettes for sample preparation
- Pipettes to deliver 100-1000 µL.
- Polypropylene tubes.
- Plate sealers.
- Washing device suitable for microwell plates.
- Microplate reader capable of measuring absorbance at 450 nm.
- Distilled or deionized water.
- Polypropylene transfer plate
- Centrifuge
- Pipette basins
- Uncoated ELISA strips from Costar microplates
- Aluminum foil
- Delicate task wipes (Kimtech Science Kimwipes SKU 34155 or equivalent)

SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Specimens Recommended

Synovial fluid aspirated from joint.

Specimens Not Recommended
Blood, serum/plasma, or modified synovial fluid such as saline wash or fluid drawn immediately post injection will impact the performance of the test.

Specimen Collection and Preparation
Synovial fluid should be collected by approved medical techniques by aspirating synovial fluid using a polypropylene syringe and transferring it into a sterile polyethylene terephthalate (PET) tube with no additives (Ex: Clear top, red stopper tube). Samples must be centrifuged at 1000g for 10 minutes prior to processing in the assay. Samples should be tested as soon as possible following collection. Do not use heat-treated specimens.

Severe hemolysis in synovial fluid samples that are contaminated with blood during sample collection can elevate alpha defensin levels. Hemolysis should be noted and reported when providing a result.

Specimen Handling and Storage Conditions
Samples should be processed to remove the cellular material as soon as possible. Samples can be stored up to 3-4 days at 2-8°C.

TESTING PROCEDURE
The test duration is typically 3-4 hours

Reagent Preparation
Allow all the reagents to equilibrate to room temperature (15-30°C) prior to use.
Return to proper storage conditions immediately after use.

- **Calibrator Handling**
  The Calibrator is supplied lyophilized and should be rehydrated with 250 µL (0.25 mL) of deionized water mix thoroughly using a vortex. Let stand for 10 minutes. Make sure there are no bubbles in the calibrator prior to using.

- **Controls Handling**
  The Controls are supplied lyophilized and should be rehydrated with 250 µL (0.25 mL) of deionized water mix thoroughly using a vortex. Let stand for 10 minutes. Make sure there are no bubbles in the calibrator prior to using.

- **Wash Buffer**
  Wash buffer is supplied as a 10X concentrate, dilute to 1X prior to use. Preparation of wash buffer (1X): Mix 100 mL of 10X Wash Buffer Concentrate with 900 mL of distilled or deionized water. Record the date the Wash Buffer (1X) is prepared and the 'Use By' date on the container. Discard Wash Buffer (1X) if visibly contaminated.

  Note: Wash Buffer Concentrate may contain crystals if temperature falls below 15°C. Bring concentrate to room temperature prior to dilution. Mix the concentrate thoroughly prior to use. Ensure crystals dissolve prior to use.

- **Assay Diluent**
  Assay diluent is supplied as a 5X concentrate, dilute to 1X prior to use. Prepare assay diluent by mixing 40 mL of the 5x Assay Diluent with 160 mL of distilled or de-ionized water; which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of dilution buffer by diluting 1 part of the 5x dilution buffer with 4 parts of distilled or de-ionized water. Record the date the Assay Diluent (1X) is prepared and the 'Use By' date on the container.

- **Biotin Conjugate**
  The biotin conjugate is supplied as a 100X concentrate; lyophilized and sealed under vacuum. Dilute to 1X prior to use. Carefully remove the cap and reconstitute by pipetting 0.25 mL distilled or de-ionized water into the vial. Dilute the reconstituted conjugate 1:100 with 1X assay diluent. Record the date prepared and the 'Use By' date on the conjugate (1X) container. Record the date reconstituted and the 'Use By' date on the 100X conjugate vial.

- **Streptavidin-Peroxidase Conjugate**
  The streptavidin-peroxidase conjugate is supplied ready to use.

- **TMB/Stop Solution**
  The TMB substrate and stop solution are both provided ready to use.

SYNOVASURE ALPHA DEFENSIN ELISA TEST PROCEDURE

Calibrator, QC Controls and samples should be diluted to a final dilution of 1: 8,000 as follows:

Table 2 Sample preparation and Dilution Factor

Step	Sample prep	Dilution factor
1	50 µL of sample into 950 µL of Assay Diluent	1:20
2	50 µL of sample diluted 1:20 (step 1) into 950 µL of Assay Diluent	1:400
3	50 µL of sample diluted 1:400 (step 2) into 950 µL of Assay Diluent	1:8,000

Diluted samples should be mixed well at each step. It is recommended to use a positive displacement pipet when dispensing synovial fluid as it is usually a viscous material.

1. Prior to the beginning of the procedure, prepare all reagents as directed in the previous sections and bring all reagents and samples to room temperature (15-30°C). Gently mix diluted samples and reagents by gently inverting several times before use, but avoid vigorous agitation and foaming.

2. Determine the total number of wells needed for the assay. In addition to specimens, one calibrator and three controls will be included on each plate or partial plate. It is recommended that samples, calibrator and controls are assayed in duplicate. Record the date the pouch is opened and the 'Use By' date of the unused wells on the pouch.

   CAUTION: Handle microwell strips with care. Do not touch the bottom exterior surface of the wells.

3. Assemble the microwell strips into the microwell strip holder, if necessary. Microwell strips must be level in the microwell strip holder.

4. Prepare a record (plate map) identifying the placement of the controls and specimens in the microwells.

5. Verify that any manual dispensing equipment is set to deliver the specified volumes as stated in the procedure, following the equipment manufacturer's instructions. Add 100 µL of the diluted calibrator, controls and specimens to the microwells using a micropipette, capable of delivering 100 µL with at least ± 5% accuracy.

6. Cover the microwell strip holder with a plate sealer. Incubate at room temperature for 60 minutes ± 5 minutes.

7. With an aspirator-washer device, aspirate and wash all wells four times with prepared 1x Wash Buffer.
   CAUTION: Strict adherence to the specified wash procedure is crucial to ensure optimum assay performance.

   Follow the steps specified in order to ensure thorough washing:

   a. Aspirate the sample solutions from microwells and then completely fill wells with Wash Buffer. Do not allow the wells to overflow.
   b. Complete the aspirate/fill sequence three additional times.
   c. Completely aspirate wells. Invert the plate and firmly tap on a clean paper towel to remove excess Wash Buffer, if necessary.

8. Add 100 µL of Biotinylated Antibody Conjugate to all wells using an adjustable multichannel micropipette or equivalent reagent dispenser capable of delivering 100 µL with at least ± 5% accuracy.

9. Cover the microwell strip holder with a new unused plate sealer. Incubate at room temperature for 30 minutes ± 3 minutes.

10. After the second incubation, wash the wells as described in Step 7.

11. Add 100 µL of Streptavidin-peroxidase Conjugate to all wells using an adjustable multichannel micropipette or equivalent reagent dispenser capable of delivering 100 µL with at least ± 5% accuracy.

12. Cover the microwell strip holder with a new unused plate sealer. Incubate at room temperature for 30 minutes ± 3 minutes

13. After the third incubation, wash the wells as described in Step 7

14. Add 100 µL of TMB Solution to all wells using an adjustable multichannel micropipette or equivalent reagent dispenser capable of delivering 100 µL with at least ± 5% accuracy.

15. Incubate at room temperature in the dark for 30± 1 minutes.

16. Add 100 µL of Stop Solution to all wells using an adjustable multichannel micropipette or equivalent reagent dispenser capable of delivering 100 µL with at least ± 5% accuracy. If necessary, gently tap the plate or use a microwell plate shaker to mix the contents. Care should be taken to avoid splashing of the contents of the microwells.

17. If necessary, wipe moisture from the bottom of the microwell strips carefully with a soft, lint-free, absorbent tissue before reading. If necessary, level the strips in the microwell holder. Read the microwell strip plate at a wavelength of 450 nm.

NOTE: Microwell strip plates must be read within 30 minutes following the addition of Stop solution. Plates must be stored in the dark until read.

CALIBRATION

The assay cutoff of the Synovasure Alpha Defensin ELISA Test is based on comparing controls/specimen with in-house absolute calibrator which has been value-assigned an absolute calibrator factor to set the optimal cut-off for clinical sensitivity and specificity performance.

OD (Optical Density) results are measured and normalized relative to a cutoff value. Alot-specific parameter is used to determine a valid cutoff value for the Synovasure Alpha Defensin ELISA Test.

QUALITY CONTROL

Three (3) quality controls should be used as QC1, QC2 and QC3 for controlling the Synovasure Alpha Defensin ELISA Test. The low negative control (QC1) ensures that the assay is not giving elevated background results. The high negative control (QC2) is designed to be just below the clinical decision point and to ensure that specificity performance is in control. The low positive control (QC3) is designed to be just above the clinical decision point and ensure that sensitivity performance is maintained. Acceptable results are in the table below.

Table 3 Acceptance Criteria

Parameter	Acceptable Result (Classification)
QC1	≤ 0.30 (ASEPTIC)
QC2	≤ 0.89 (ASEPTIC)
QC3	≥ 1.00 (INFECTED)

Good laboratory practice requires that controls be processed to verify the performance of the test.

To ensure performance, analyze control materials in the same manner as patient specimens. If control results fall outside the acceptable range, investigate the cause before deciding whether to report patient results.

Interpretation of Results

Result Calculation

Results are calculated as a normalized signal, relative to a cutoff value.

Result = OD for test sample/OD at the Cutoff (Cutoff value)

Cut-off value = OD for Calibrator x [Lot specific Calibrator factor – *provided on Certificate of Analysis*].

A result of ≥1.00 indicates a positive sample and the possible presence of PJI, and can be defined as infected.
A result of <0.90 indicates a negative sample, and can be defined as aseptic.

A result of ≥0.90 and <1.00 indicates an indeterminate sample.

A sample found indeterminate in the Synovasure Alpha Defensin ELISA Test should be retested in duplicate to verify its status. If results on repeat testing are <0.90 for both replicates, the sample should be considered negative. If either

duplicate retest result is ≥0.90, the sample should be tested by supplemental tests to confirm the result. In the case of repeatedly indeterminate results, analysis of follow up samples is recommended.

Performance Characteristics

Clinical Diagnostic Accuracy:

A prospective and retrospective study of the Synovasure Alpha Defensin ELISA Test with subject specimens collected at 3 US investigational sites was conducted for 23 months. The study subjects were determined to be PJI positive or negative based on MSIS defined criteria by an independent three-physician adjudication panel with expertise in infection and who had access to all the necessary patient data for clinical diagnosis (e.g., all MSIS criteria and patient history). The adjudication panel was blinded to the results of the Synovasure PJI Tests. A total of 851 subjects were screened for study inclusion, including 386 prospective subjects, and 465 samples were evaluated retrospectively. From the total 386 prospective subjects studied, 304 subjects were included for ELISA study analysis, while the remaining were excluded from the study based on subject/sample inclusion and exclusion criteria. Of the 465 retrospective samples identified, 3 samples were excluded due to insufficient volume. The clinical sensitivity, clinical specificity, PPV, and NPV for the Synovasure Alpha Defensin ELISA Test were determined by comparison to the detection of PJI using the MSIS definition of PJI diagnosis. Point estimates and associated 95% confidence intervals (CIs) were calculated for clinical sensitivity, clinical specificity, PPV and NPV. Categorical variables were summarized by frequency counts and percentages. Continuous variables were summarized by number of observations (N), sensitivity, specificity, and 95% CIs. Result for the Synovasure Alpha Defensin ELISA Test was reported as "Positive", "Negative", or "Indeterminate". If an initial result was indeterminate, the sample was retested and the final result was reported.

Based on MSIS criteria, for 100 PJI positive samples, the acceptance criteria for this study was to have Clinical sensitivity point estimate ≥ 90% and Clinical specificity point estimate ≥90%. PPV and NPV were calculated with a consideration of 95% CI; however, pre-established success criteria were not set. The performance rate or values data reporting Synovasure Alpha Defensin ELISA test sensitivity and specificity is presented in Tables below

Table 4 Estimates of Clinical Performance for All Prospective Subjects and Retrospective Positive Subjects

Synovasure® Alpha Defensin ELISA Test				
		Positive	**Negative**	**Total**
Clinical PJI Diagnosis	PJI Positive	113	9	122
Clinical PJI Diagnosis	PJI Negative	6	241	247
Clinical PJI Diagnosis	Total	119	250	369
Sensitivity		92.6% (113/122) (86.5% - 96.6%)		
Specificity		97.6% (241/247) (94.8% - 99.1%)		
PPV		95.0% (113/119) (89.3% - 98.1%)		
NPV		96.4% (241/250) (93.3% - 98.3%)		

CI = confidence interval; NPV = negative predictive value; PJI = periprosthetic joint Infection; PPV = positive predictive value;

Table 5 Estimates of Clinical Performance for All Prospective Subjects and Retrospective Positive Subjects Excluding Subjects with Samples where RBC >1 Million Cells/µL

Synovasure® Alpha Defensin ELISA Test				
		Positive	**Negative**	**Total**
Clinical PJI Diagnosis	PJI Positive	112	6	118
Clinical PJI Diagnosis	PJI Negative	6	228	234
Clinical PJI Diagnosis	Total	118	234	352
Sensitivity		94.9% (112/118) (89.3% - 98.1%)		
Specificity		97.4% (228/234) (94.5% - 99.1%)		
PPV		94.9% (112/118) (89.3% - 98.1%)		
NPV		97.4% (228/234) (94.5% - 99.1%)		

CI = confidence interval; NPV = negative predictive value; PJI = periprosthetic joint Infection; PPV = positive predictive value;

Precision

Repeatability and Reproducibility: Four (4) precision pools (low negative, high negative, low positive, and high positive) were tested in duplicate 2 times/day over 5 days to estimate the precision of the assay.

- Pool 1 (P-1) represents a low negative sample with a result ≤ 0.35
- Pool 2 (P-2) represents a high negative sample with a result between 0.50-0.80
- Pool 3 (P-3) represents a low positive sample with a result between 1.20-1.60
- Pool 4 (P-4) represents a high positive with a result between 2.00-3.00

Synovasure Alpha Defensin ELISA Test met the precision performance acceptance criteria as total precision and within- run precision is ≤ 20% for the high negative, low positive and high positive pools. The following tables summarizes study results (mean, SD and %CV) from each run.

Table 6 : Total Precision covering 5 days, 2 runs, and 4 pools

Pool	**Sample**	**Mean Result**	**SD**	**%CV**
P-1	Low negative	0.16	0.019	12%
P-2	High negative	0.72	0.093	13%
P-3	Low positive	1.30	0.082	6%
P-4	High positive	2.48	0.217	9%

Table 6 Estimation of Repeatability Parameter

Pool	**Sample**	**Sr***	**Sr/mean result**
P-1	Low negative	0.008	5.2%
P-2	High negative	0.041	5.8%
P-3	Low positive	0.064	4.9%
P-4	High positive	0.124	5.0%

*Sr is repeatability parameter calculated based on CLSI EP5-A2

Reproducibility data is further supported by the C5, C95 study listed below.

Detection around the cutoff
C5- C95 establishment and verification study: A C5- C95 establishment and verification study was conducted at two different sites (CD Laboratory, Baltimore (CDL) and CD Diagnostics, Claymont (CDD)) with three different operators, with the study panel of total 11 members. The study was conducted at for 5 days in which three reagent and calibrator lot combinations (i.e., Reagent Lot #1/Calibrator Lot #1, Reagent Lot #2/Calibrator Lot #2, Reagent Lot #3/Calibrator Lot #3) were used. The testing was performed on a panel of 88 samples by three operators (one for each lot combination) including 2 at CDL and 1 at CDD. For each lot, each panel was tested by one operator within 1 run each day for a total of 120 establishment replicates per sample (5-11 replicates/run x 1 run/day x 5 days/site x 3 lots).

For the verification of C5-C95 range, study was conducted at CDL and CDD for one day, using three reagent and calibrator lot combinations. The testing site was provided with 3 sets of C5/C95. The study panels used in the testing consisted of 20 replicates of 11 panel members that span the assay's expected C5-C95 ranges for a total of 220 blinded samples per operator. With 3 operators, including 2 at CDL and 1 at CDD, a total of 60 determinations for each panel member were acquired for 660 total blinded samples amongst the 11 panel members. Each panel was labeled for testing on a specific test lot and run (e.g., L1 /R1; L2 /R2).

The S/CO data for C5, C50 and C95 (predicted 0.69 at 5%, 1.01 at 50%, and 1.48 at 95%) from the establishment study and verification study S/CO data for C5, C50 and C95 (predicted 0.73 at 5%, 1.01 at 50%, and 1.38 at 95%) are shown in Table 7

Table 8. Estimates of C5, C50 and C95 S/CO

% Positive Response	Test	Establishment S/CO	Verification S/CO
C_{05}	Synovasure Alpha Defensin ELISA	0.69	0.73
C_{50}	Synovasure Alpha Defensin ELISA	1.01	1.01
C_{95}	Synovasure Alpha Defensin ELISA	1.48	1.38

Point estimates with 95% CI were calculated for all the positive and negative tests results. It was found that the estimated proportion of negative tests for C5 was 95% for the 0.71 S/CO sample with a 95% CI interval of 86.06-98.96%. The estimated proportion of positive tests for C95 was 98.33 % for the 1.42 S/CO sample with a 95% CI interval of 91.06-99.96%. The cutoff was also verified with a 38.33% positive which met the acceptance criteria of 35-65% positive. The low negative and moderate positive were also found to meet the acceptance criteria.

Interfering substances
The effect of Hemoglobin, Triglyceride, and Bilirubin (conjugated and unconjugated) were tested in an alpha defensins 1-3 negative and an alpha defensins 1-3 positive sample following the procedures described in CLSI Protocol EP7-A2[5]. For each substance, the highest concentration, which was considered not to impact the clinical interpretation of results, are shown in the table 8 below:

Table 9. Interfering Substances

Type	Interferent	Concentration at which Device Exhibits No Interference
Exogenous	UHMWPE (10 mg/mL)	10 mg/ml
	Bone Cement (10 mg/mL)	10 mg/ml
	Cobalt (10 mg/L)	10 mg/ml
	Chromium (10 mg/L)	10 mg/ml
	Titanium (10 mg/L)	10 mg/ml
Endogenous	Rheumatoid Factor 300 IU/mL	300 IU/mL
	Hemoglobin 200 mg/dL	200 mg/dl
	Bilirubin (Unconjugated) 20 mg/dL	20 mg/dL
	Bilirubin (Conjugated) 29 mg/dL	29 mg/dL
	Triglyceride 418 mg/dL	418 mg/dL

Note: Any complaints should be reported to Zimmer Biomet using the Product Experience Report Form (Form number GBLF04001) to product.experience@zimmerbiomet.com. Serious adverse events shall be reported to the Competent Authority of the respective EU Member State in which the event occurred

Healthcare professionals, users and patients should report any suspected serious incident related to the device by informing the Manufacturer at www.zimmerbiomet.com, or the local Zimmer Biomet distributor, and the competent authority, ministry of health, or delegated agency in the country where the suspected serious incident occurred. For patients in Australia please visit the Therapeutic Goods Administration (TGA) website: https://www.tga.gov.au

Please contact Zimmer Biomet at the following number if you have additional questions. In the USA, call 1-800-348-2759. For calls outside the USA, call the local international access code +1-574-267-6131

Synovasure is a licensed trademark of Zimmer Biomet Holdings Inc., the successor of Zimmer Holdings Inc

Revision History

Version	Effective Date	Changes made from Previous version
4	September/2022	• Updated Intended use statement to meet EU IVDR 2017/746 requirements. • Updated symbols, symbol key as per EU IVDR 2017/746 requirements. Updated CE mark with CE number. • Updated "Performance Characteristics" section with Clinical diagnostic study, C5-C95, Precision and Interference study data. • Added a Note to users with Zimmer Biomet contact information to report complaints/issues • Added Revision History to meet EU IVDR 2017/746 requirements
5	August 2024	• Updated safety precautions section. • Updated symbols, symbol key as per EU IVDR 2017/746 and EC regulation 1272/2008 requirements.
6	July 2025	• Updated safety precautions section.

SYMBOL KEY

Instructions For Use

Item Number


Storage Temperature


Use-By Date

Non-sterile


Keep Dry


Warning: Skin Sensitizer

Lot Number


Manufacturer

Authorized representative in the European Community/European Union

IVD


Precautions/Warnings

Quantity

Unique device identifier

CD Diagnostics, Inc.
650 Naamans Road
Suite 100
Claymont, DE 19703

CE
2797