ZIMMER BIOMET

Synovasure® Alpha Defensin Lateral Flow Test Kit

For *in vitro* diagnostics use
Caution: Federal law restricts this device to sale by or on the order of a physician.

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.

RESTRICTIONS
This assay has not been validated for use in patient populations without a total joint replacement. This assay is for prescription use only.

NAME AND INTENDED USE
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 to aid in 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 with the Synovasure Alpha Defensin Lateral Flow Test Kit as quality control samples to monitor performance and reliability of the Synovasure Alpha Defensin Lateral Flow Test Kit. The Synovasure Alpha Defensin Lateral Flow Test Kit and the Synovasure Alpha Defensin Control Kit are non-automated.

COUNTRY SPECIFIC REQUIREMENTS
This assay is categorized as CLIA moderately complex for prescription use only in the United States

The Synovasure Alpha Defensin Lateral Flow Test Kit and Control Kit is intended for laboratory use in Canada and Australia.

In the European Union, the Synovasure Alpha Defensin Lateral Flow Test Kit and Control Kit are intended for laboratory use in a clinical laboratory by laboratory professionals and also for near patient testing in orthopedic clinics by users in routine professional care environments and operating rooms by users in critical care environments.

This assay is for professional use only in all countries outside of the United States, European Union, Canada, and Australia.

PRINCIPLES OF THE TEST
The Synovasure Alpha Defensin Lateral Flow Test Kit is an immunoassay for the detection of alpha defensin levels in the synovial fluid of patients with a potential PJI. Alpha defensins are antimicrobial peptides released by activated neutrophils in response to infection. The test system is comprised of a single use device, a premeasured vial of dilution buffer, a disposable Microsafe® tube, and a sample cup.

Each device contains a reagent strip with all of the critical components for the assay. Dilution is performed by collecting a sample from an aspirated synovial fluid specimen using the disposable Microsafe tube and adding the sample to the premeasured dilution buffer. Three (3) full, free-falling drops of the diluted sample are then added to the test device to begin the testing process. Cellular material is removed by the first pad. The solution then migrates to the buffering pad and mixes with the gold conjugate that has been labelled with an anti-alpha defensin antibody. The test mixture then migrates across the test line and the control line. A test result line ("T") will form if the level of alpha defensin in the sample is greater than the cut-off concentration. A control line ("C") will form to confirm that the solution has properly flowed across the device. Results can be read between 10 to 20 minutes.

WARNINGS, PRECAUTIONS, AND LIMITATIONS 

- This test is for in vitro diagnostic use. Test results should be utilized in conjunction with other clinical and diagnostic findings to aid the diagnosis of PJI.
- Failure to follow the instructions provided may lead to inaccurate results.
- Use all test kit components only once and dispose of properly. ***Do NOT reuse any of the test components.***
- This product is non-sterile and should **NOT** be placed into sterile fields.

- This kit has been developed for use with freshly collected synovial fluid only. The use of this test kit with any other specimen type may lead to inaccurate test results. The use of synovial fluid diluted with saline, blood, contrast agent, or any substances injected into the joint may lead to false negatives. Presence of red blood cells (RBCs) greater than 1 million/µL in the synovial fluid specimen may lead to false negatives. This represents dilution of the synovial fluid specimen with greater than 20% blood.
- This test should only be used for patients with a total joint prosthesis.
- The performance of this test has only been validated for conditions evaluated by the Musculoskeletal Infection Society (MSIS) criteria.
- A decrease in sensitivity (an increased likelihood of false negative results) has been observed in the presence of a sinus tract communicating with the prosthesis. Since the presence of a sinus tract is definitive evidence of PJI, use of this test under those circumstances is **NOT** recommended.
- False positives have been reported in the presence of metallosis.
- A negative test result does **NOT** preclude the possibility of infection.
- Synovial fluid obtained after repeated aspirations within a short time period might lead to false negatives due to the lack of buildup of alpha defensin.
- This test is **NOT** intended to be used to determine timing for reimplantation in two-stage procedures.

SAFETY PRECAUTIONS ⚠

- Used Synovasure Alpha Defensin Lateral Flow Test Kit devices are considered a potential biohazard and should be disposed of according to local, state, and federal waste disposal requirements.
- Handle all specimens and waste materials as if they could transmit infectious agents. Observe safety guidelines such as those outlined in CDC/NIH Biosafety in Microbiological and Biomedical Laboratories [1] the CLSI Document M29-A4 Protection of Laboratory Workers from Occupationally Acquired Infections [2], or other appropriate guidelines.
- Wear appropriate Personal Protective Equipment (PPE) when handling and testing patient specimens, including (but not limited to) disposable powder free gloves and lab coats. Protect skin, eyes, and mucus membranes. Change gloves often when handling reagents or specimens.
- Follow your institution's safety procedures for handling biological specimens.
- The Dropper Bottle included in this kit contains a Dilution Buffer. The Dilution Buffer contains a preservative which may cause an allergic skin reaction. Avoid breathing mist or fumes. May cause skin, eye, and respiratory irritation. Complete Safety Data Sheet is available at www.cddiagnostics.com.

REAGENTS AND MATERIALS SUPPLIED

REF 00-8888-125-05 REF	**Synovasure Alpha Defensin Lateral Flow Test Kit**	**Quantity** QTY
• Instructions for Use	-	1
• Instructional Insert	-	1
• REF P50023 REF	Test Device Sealed pouch containing coated membrane with an anti-alpha defensin test line and an anti-mouse control line; anti-alpha defensin coated gold particle pad, sample buffering pad and a cellular material removing pad contained in a protective plastic housing sealed in a pouch with desiccant	5
• REF P50024 REF	Sample Preparation Assembly Sealed pouch containing: - 1 REF P50025 Sample Dilution Bottle (Pre-filled, phosphate buffer, dropper bottle) - 2 Disposable Microsafe® tubes - 1 Sample Cup	5

Also available as Quantity 1 (REF 00-8888-125-01); Quantity 10 (REF 00-8888-125-10); Quantity 30 (REF 00-8888-125-30) depending on country-specific regulatory registration approval.

*NOTE: Do **NOT** interchange test devices or components with other devices or components from kits with different lot numbers.*

REAGENT STORAGE, HANDLING, AND STABILITY

- This test should be performed at room temperature (12-25°C). Do **NOT** run outside this range.
- The recommended storage temperature for the test kit is 2-30°C, which is valid until the expiration date printed on the package label.

- Always check the expiration date prior to use and do **NOT** use reagents beyond the expiration date printed on the package label.
- Avoid storage of any materials near heating or cooling vents or in direct sunlight.
- Do **NOT** open the pouch packaging until a sample is ready to be tested. Once the pouch packaging has been opened, the device should be used as soon as possible.
- Performance of the Synovasure Alpha Defensin Lateral Flow Test Kit was established using synovial fluid specimens collected per standard of care procedures by aspirating synovial fluid into a polypropylene syringe and transporting it in a polyethylene terephthalate (PET) tube with no additives (Ex: Clear top, red stopper tube). If specimen shipment is required, same-day or overnight courier is recommended.
- Synovial fluid specimens are stable up to seven (7) days at 4-32°C. Inadequate specimen collection, transport, or storage could adversely affect test performance.

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer

ADDITIONAL MATERIALS

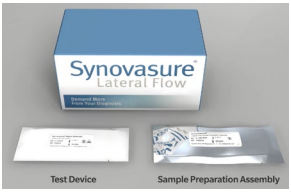
- **Synovasure Alpha Defensin Control Kit** REF 00-8888-125-02
  External positive and negative controls available for the Synovasure Alpha Defensin Lateral Flow Test Kit

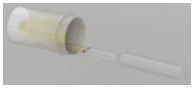
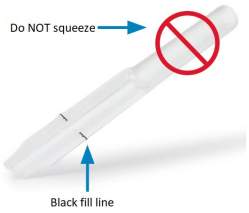
SYNOVASURE ALPHA DEFENSIN LATERAL FLOW TEST KIT PROCEDURE

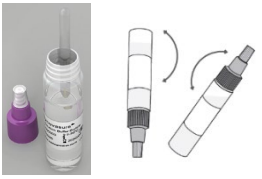
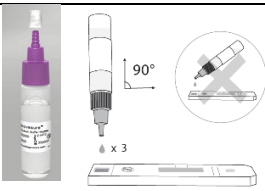
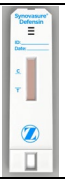
Review the following checklist before performing the test

1. Open the kit box and inspect components. Check expiration date before using. Do **NOT** use test kits beyond the expiration date printed on the box. If any of the components have been damaged, select a new component for testing.
2. Test must be performed on a flat, stable surface in an area with adequate lighting.
3. Conduct the test at room temperature, between 12°C – 25°C.
4. Ensure timer is available and set to 10 minutes.
5. Synovasure Alpha Defensin Control Kit (00-8888-125-02), should be used in accordance with local requirements.

Step	Instructions	Representation

1	Remove one (1) white pouch and one (1) silver pouch from the Synovasure box. If the kit was not stored at room temperature allow pouches to come to room temperature.
	• **White pouch –** Test device
	  ○ 1 x Synovasure device
	• **Silver pouch –** Sample preparation assembly
	  ○ 1 x Sample cup
	  ○ 2 x Microsafe® tubes
	  ○ 1 x Dilution bottle
		

2	Open the silver pouch packaging of the Sample Preparation Assembly. Aspirated synovial fluid must be in a vessel that allows sampling with a Microsafe® tube or positive displacement pipette. A sample cup is provided in the Sample Preparation Assembly.	
3	Dilution: Remove purple cap from the dropper bottle and set aside. Use Microsafe® tube to obtain synovial fluid for dilution. Hold the Microsafe® tube **horizontally** and touch the tip of the Microsafe® tube to the synovial fluid specimen. Capillary action will automatically draw the specimen to the black fill line and stop. **Do NOT squeeze tube while sampling**. *NOTE 1: A positive displacement pipette set at 15 µL can be used to obtain sample, if available.* *NOTE 2: Do **NOT** cover the vents at the black fill line. Allow fluid to reach fill line before proceeding. If synovial fluid cannot be obtained from the container holding the synovial fluid, transfer a small amount of the fluid to the sample cup provided. It may take longer to collect a sample from highly viscous synovial fluid specimens. If first tube is unable to obtain synovial fluid, discard and use the second tube provided.*	 

4	After synovial fluid has been obtained, dispense the fluid into the prefilled dropper bottle. Rinse Microsafe tube in prefilled dropper bottle by squeezing and releasing the bulb on the end of the Microsafe tube five (5) times to expel viscous synovial fluid. Recap dropper bottle and mix by gentle inversion. *NOTE: At this time, it is appropriate to squeeze the bulb to ensure contents are fully transferred*	
5	Open the white pouch packaging of the Test Device. Do **NOT** use if the pouch is not intact. Place device on a flat surface. Remove clear cap from dropper bottle. Hold dropper bottle in a vertical position (90°) while dispensing three (3) full, free-falling drops of diluted synovial fluid sample into the sample well of the test device. **The device must remain flat during processing.**	
6	Monitor the device to ensure that sample flows across the read window (background should turn reddish-pink and eventually clear). If no sample flows across the read window, it is most probable that insufficient sample volume was added to the device. Repeat testing using a new device. The previously prepared dilution bottle can be used. The repeat testing must be performed within four (4) hours.	
7	Allow the test to develop undisturbed for ten (10) minutes. Results can be read between 10 and 20 minutes after the sample is dispenses into the sample well. Interpret the test results according to the description in section "Interpretation of test results". *Do **NOT** read the results after 20 minutes.* A reddish-pink control line appears as the viewing window background clears. The control line [C] is the uppermost line in the test channel.	

SYNOVASURE ALPHA DEFENSIN CONTROL KIT PROCEDURE

External controls should be run in accordance with the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations regulations. It is recommended that controls be run by a new user to assess competency, when using a new kit lot or when a new shipment of kits is received, and if storage conditions of the material fluctuate outside of specified limits.

1. Prepare controls according to Instructions for Use provided in the Synovasure Alpha Defensin Control Kit.
2. Run controls as clinical samples, per instructions above for the Synovasure Alpha Defensin Lateral Flow Test Kit.

INTERNAL QUALITY CONTROL: A procedural control line ("C") is built into each test device, demonstrating the fluid is properly flowing through the device.

INTERPRETATION OF RESULTS

Note**: Alpha defensin is represented as "T" on the device.** The intensity of the device control and test result lines may vary. Any complete reddish-pink line, without regard to intensity, or size, is a line**. Do** NOT **interpret the test after twenty (20) minutes.**

Negative: A NEGATIVE result for alpha defensin is the presence of the reddish-pink device control line (C) ONLY, with no test result line (T) after ten (10) minutes. **The presence of the control line indicates the test is valid.**

Positive (Non-Negative): A POSITIVE result for alpha defensin is the presence of the reddish-pink device control line (C) AND a reddish-pink test result line (T). **The presence of the control line indicates the test is valid.**

Test Invalid: Before reading the test result line, verify that the device control line, labelled "C", has formed. If the device control line does not appear, the test line does not develop completely, or the background fails to clear, the test is invalid, and the test results must not be used. The test should be repeated using a new device. The previously prepared dilution bottle can be used. The repeat testing must be performed within four (4) hours.

Examples of Results

Test Valid

Test Invalid

PERFORMANCE CHARACTERISTICS OF SYNOVASURE ALPHA DEFENSIN LATERAL FLOW

Performance Characteristics of Lateral Flow

The clinical trial was a prospective trial of the Synovasure Alpha Defensin Lateral Flow Test with specimens collected at three (3) US medical centers with a high volume of revision surgery. Patients with a total knee and/or hip joint replacement who were being evaluated for revision surgery were recruited for the trial.

Specimens were collected from at least 300 total patients, until specimens were collected from at least 50 MSIS-defined PJI positive (infected) patients. Specimen processing, standard of care testing and investigational device testing occurred at a minimum of two (2) collection sites. There were additional sites that performed specimen collection and standard of care testing but transferred specimens to a central laboratory for investigational device testing. These specimens were transferred from the collection location to the testing location via a same-day or overnight courier.

Upon receipt into the laboratory, the specimens were first aliquoted for use in the physician-ordered diagnostic laboratory testing and the remainder of the specimen was used for clinical trial testing with the Synovasure Alpha Defensin Lateral Flow Test. The specimens were tested using the Synovasure Alpha Defensin Lateral Flow Test, following the testing and quality control procedures defined in the package insert. MSIS criteria-defined PJI diagnosis was determined by an independent three (3) physician adjudication panel with expertise in infection who have access to all the necessary patient data for clinical diagnosis (e.g., sufficient MSIS criteria and patient history). The adjudication panel was blinded to the results of the Synovasure Alpha Defensin Lateral Flow test results.

The results of the Synovasure Alpha Defensin Lateral Flow Test were compared separately to the clinical diagnosis for each patient based on MSIS criteria. The clinical performance of the test calculated against the MSIS criteria for all prospective samples is shown below (Table 1). This includes samples with >20% dilution with blood.

Table 1: Estimates of Clinical Performance for Synovasure Alpha Defensin Lateral Flow Test

		Positive	Negative
Clinical PJI Diagnosis	PJI Positive	51	6
	PJI Negative	13	235

Sensitivity	89.5% (51/57) (78.5% - 96.0%)
Specificity	94.8% (235/248) (91.2% - 97.2%)
PPV	79.7% (51/64) (67.8% - 88.7%)
NPV	97.5% (235/241) (94.7% - 99.1%)

The clinical performance of the Synovasure Alpha Defensin Lateral Flow Test calculated against the MSIS criteria with samples diluted with > 20% blood (RBC >1,000,000) excluded is shown below (Table 2).

Table 2: Estimates of Clinical Performance for Synovasure Alpha Defensin Lateral Flow Test excluding samples diluted with >20% blood (RBC >1.000,000)

		Positive	Negative
Clinical PJI Diagnosis	PJI Positive	50	3
	PJI Negative	13	222

Sensitivity	94.3% (50/53) (84.3% - 98.8%)
Specificity	94.5% (222/235) (90.7% - 97.0%)
PPV	79.4% (50/63) (67.3% - 88.5%)
NPV	98.7% (222/225) (96.2% - 99.7%)

Note: Presence of RBCs greater than 1 million/µL which represents dilution of the synovial fluid sample with greater than 20% blood in the synovial fluid sample may lead to false negatives.

The clinical performance estimated using prospective data was supplemented with retrospective positive specimens consecutively collected at CD Laboratories. From May 16, 2017 to August 31, 2017, 65 specimens designated as positive per a modified MSIS criteria (≥3000 WBCs, ≥80% neutrophils, and culture positive) were collected and tested using the Synovasure Alpha Defensin Lateral Flow Test. The Positive Percent Agreement (PPA) of the Synovasure Alpha Defensin Lateral Flow test with the modified MSIS criteria estimated from these retrospective positive samples is shown below (Table 3).

Table 3: Estimates of Clinical Performance for All Retrospective Positive Samples

	Positive	Negative	Total
PJI Positive	64	1	65

Positive Percent Agreement (PPA)	98.5% (64/65) (91.7% - 100.0%)

Using the prospective population of specimens where RBC < 1 million cells/µL, analyses of covariates were performed to determine the potential impact of ongoing antibiotic use (Table 4), inflammatory disease history (Table 5), and gram positive and negative culture (Table 6) on the clinical performance of the Synovasure Alpha Defensin Lateral Flow Test. The results of these analyses are shown below. Additional covariates evaluated included age, race, gender, infection history, anti-inflammatory medication use, and affected joint. No significant differences in test performance were observed.

Table 4. Covariate Analysis to Estimate Clinical Performance for Subjects with and without Ongoing Antibiotic History

Subjects with Ongoing Antibiotic History	
Sensitivity	96.2% (25/26) (80.4% - 99.9%)
Specificity	92.3% (12/13) (64.0% - 99.8%)
PPV	96.2% (25/26) (80.4% - 99.9%)
NPV	92.3% (12/13) (64.0% - 99.8%)
Subjects without Ongoing Antibiotic History	
Sensitivity	88.9% (8/9) (51.8% - 99.7%)
Specificity	92.3% (60/65) (83.0% - 97.5%)
PPV	61.5% (8/13) (31.6 - 86.1%)
NPV	98.4% (60/61) (91.2% - 100.0%)

Table 5. Covariate Analysis to Estimate Clinical Performance for Subjects with and without Inflammatory Disease History

Subjects with Inflammatory Disease History	
Sensitivity	92.3% (12/13) (64.0% - 99.8%)
Specificity	96.3% (52/54) (87.3% - 99.5%)
PPV	85.7% (12/14) (57.2% - 98.2%)
NPV	98.1% (52/53) (89.9% - 100.0%)
Subjects without Inflammatory Disease History	
Sensitivity	95.0% (38/40) (83.1% - 99.4%)
Specificity	93.9% (170/181) (89.4 - 96.9%)
PPV	77.6% (38/49) (63.4% - 88.2%)
NPV	98.8% (170/172) (95.9% - 99.9%)

Table 6. Covariate Analysis to Estimate Clinical Performance for Subjects with Gram Positive and Gram Negative Culture

Gram Positive Culture	
Sensitivity	90.6% (29/32) (75.0% - 98.0%)
Specificity	91.3% (21/23) (72.0% - 98.9%)
PPV	93.5% (29/31) (78.6% - 99.2%)
NPV	87.5% (21/24) (67.6% - 97.3%)
Gram Negative Culture	
Sensitivity	85.7 (6/7) (42.1% - 99.6%)
Specificity	Non-estimable
PPV	100.0% (6/6) (54.1% - 100.0%)
NPV	0.0% (0/1) (0.0% - 97.5%)

Precision

The Precision Study was performed at three (3) external laboratories over a minimum of five (5) days with three (3) operators per site, 3 runs per day, 18 blinded samples per run consisting of two to four (2-4) blinded replicates of each sample. All samples were tested in singlet. Each run included negative and positive controls in singlet. Results are provided below.

Table 7: Percent positive and percent negative results for all precision panel members

Sample	**% Positive**	**95% CI**	**% Negative**	**95% CI**
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	79.7% = 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%)

Interfering Substances

Endogenous interferences from naturally-occurring substances found in the patient specimens were tested. Exogenous interferences originating from materials found in a patient's specimen due to the presence of a prosthetic joint implant were also tested. Results are provided below.

Table 8: Interference Testing Results

Substance	**Concentration at which Device Exhibits No Interference**
Rheumatoid Factor	300 IU
Bilirubin (unconjugated)	20 mg/dL
Bilirubin (conjugated)	29 mg/dL
Triglyceride (TG)	418 mg/dL
Whole Blood Hemoglobin	12.1 g/dL
Lysed Blood Hemoglobin	8.7 g/dL
Hyaluronic Acid (HA)	8 mg/mL
Metal Ion Cobalt	150 mg/L
Metal Ion Chromium	150 mg/L
Metal Ion Titanium	150 mg/L
Bone Cement	10 mg/mL
UHMWPE	10 mg/mL

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

Please contact Zimmer Biomet at the following number if you have additional questions: +1-800-348-2759 or +1-800-253-6190.

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
8	Oct 2023	• Updated Intended use statement to meet EU IVDR 2017/746 requirements. • Updated country specific requirement to meet EU IVDR 2017/746 requirements. • Updated 'REAGENTS AND MATERIALS SUPPLIED' section to add Instructional Insert. • Updated symbols, symbol key as per EU IVDR 2017/746 requirements. Updated CE mark with CE number. • Added a Note to users with Zimmer Biomet contact information to report complaints/issues • Added Revision History Section
9	Aug 2024	• Revision to correct typographical error in the Performance Characteristics section of Synovasure Alpha Defensin Lateral Flow.

Symbol Key

	Instructions For Use		Lot Number
	Item Number		Manufacturer
	Storage Temperature		Authorized representative in the European Community/European Union
	Use-By Date		In Vitro Diagnostic Medical Device
	Contains sufficient for <n> tests		Precautions/Warnings
	Do not reuse		Quantity
	Near patient Testing (EU only)		Non-sterile

CE Mark on the package insert (IFU) is not valid unless there is a CE Mark on the product label.