ZIMMER BIOMET

# Synovasure® Alpha Defensin Lateral Flow Test Kit

*For additional product information, visit www.cddiagnostics.com*

**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.

## NAME AND INTENDED USE

The Synovasure® Alpha Defensin Lateral Flow Test Kit is a visual immunochromatographic assay intended as an adjunct for the detection of periprosthetic joint infection (PJI) in the synovial fluid of patients experiencing pain and/or inflammation in a replacement joint. The Synovasure® Alpha Defensin Lateral Flow Test Kit detects human alpha defensins 1-3 in the synovial fluid of persons with a total joint replacement. Alpha defensins are antimicrobial peptides released by activated neutrophils in response to infection. Synovasure® Alpha Defensin Lateral Flow Test Kit results are intended to be used in conjunction with other clinical and diagnostic findings to aid in a patient's diagnosis of infection.

The Synovasure® Alpha Defensin Lateral Flow Test Kit is intended to determine whether there is an infection present in synovial fluid. It is not intended to identify a specific type of infection or establish the origin or severity of an infection. It is intended to provide a physician with a specific positive or negative result for the presence of alpha defensins 1-3 in the synovial fluid as a result of the infection.

For laboratory use only.

For *in vitro* diagnostic use only.

## 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. 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 synovial fluid with the disposable Microsafe® tube and adding the specimen 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-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 are read in ten (10) minutes.

## WARNINGS AND PRECAUTIONS

- 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 kit has been developed for use with 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 RBCs greater than 1 million/µL in the synovial fluid sample may lead to false negatives. This represents dilution of the synovial fluid sample 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 we do not recommend use of this test under those circumstances.
- False positives have been reported in the presence of metallosis.
- 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.
- 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.
- Wear appropriate personnel protective equipment when handling and testing patient specimens.
- 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.

## REAGENTS AND MATERIALS SUPPLIED

| REF 00-8888-125-05 | Synovasure® Alpha Defensin Lateral Flow Test Kit | Quantity |
|---|---|---|
| Instructions for Use | | 1 |
| REF P50023 | Test Device<br>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 | Sample Preparation Assembly<br>Sealed pouch containing:<br>(1) REF P50025 Sample Dilution Bottle (Pre-filled, phosphate buffer, dropper bottle)<br>(2) Disposable Microsafe® tube<br>(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)***

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

## MATERIALS REQUIRED BUT NOT PROVIDED

- Timer

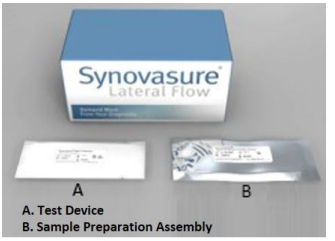
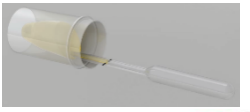
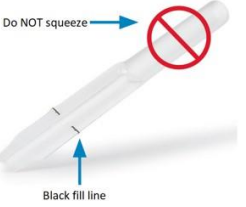
## ADDITIONAL MATERIALS

- **Synovasure® Alpha Defensin Control Kit (2pk)** REF 00-8888-125-02
  *Available as an accessory to the Synovasure® Alpha Defensin Lateral Flow Test Kit*

## SYNOVASURE® ALPHA DEFENSIN LATERAL FLOW TEST KIT PROCEDURE

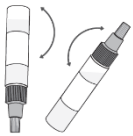
Review the following checklist before performing the test

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 1 white pouch and 1 silver pouch from the Synovasure box and allow to come to room temperature.<br>• **White pouch –** Test device<br>  ○ 1 x Synovasure device<br>• **Silver pouch –** Sample preparation assembly<br>  ○ 1 x Sample cup<br>  ○ 2 x Microsafe® tubes<br>  ○ 1 x Dilution bottle |  <br>**A. Test Device**<br>**B. Sample Preparation Assembly** |
| **2** | Open 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 if needed. |  |
| **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 sample. Capillary action will automatically draw the sample to the black fill line and stop. **Do not squeeze tube while sampling**.<br>*NOTE 1: A positive displacement pipette set at 15 µL can be used to obtain sample, if available.*<br>*NOTE 2: Do not squeeze Microsafe® tube while sampling. 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. Highly viscous synovial fluid samples may take longer to collect. If first tube is unable to obtain synovial fluid, discard and use the second tube provided.* |  <br>  |

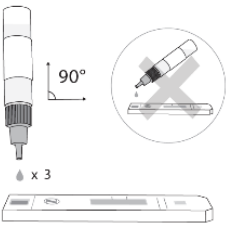
**4** After synovial fluid has been obtained, dispense the fluid into the prefilled dropper bottle. Rinse Microsafe® tube in prefilled dropper bottle by squeezing and releasing the bulb on the end of the Microsafe® tube five (5) times to expel viscous synovial fluid. Recap dropper bottle and mix by gentle inversion.

*NOTE: At this time, it is appropriate to squeeze the bulb to ensure contents are fully transferred.*



**5** Let device equilibrate to room temperature before use. Remove the test device from the foil pouch just prior to use. Do not use if the foil 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 red 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 4 hours.

**7** Allow the test to develop undisturbed for ten (10) minutes. Interpret the test results according to the description in section "Interpretation of test results". *Do not read the results after twenty (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/or 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 reddish-pink line, without regard to intensity, or size, is a line**. Do not interpret the test after twenty (20) minutes.

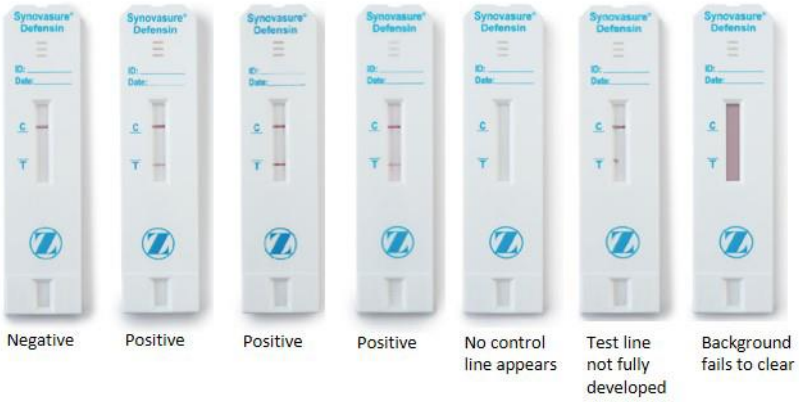
***Negative:*** A NEGATIVE result for alpha defensin is the presence of the reddish-pink device control line (C-line) ONLY, with no (T-line) test result line after ten (10) minutes.

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

***Test Valid:*** Before reading the test result lines, verify that the device control line, labelled "C", has formed. If the device control 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 4 hours.

**Examples of Results**

**Test valid** **Test invalid**




**PERFORMANCE CHARACTERISTICS OF ALPHA DEFENSINS**

The clinical performance of the alpha defensin biomarker for the diagnosis of periprosthetic joint infection was determined utilizing an alpha defensin ELISA in a 158 patient study utilizing the following inclusion criteria:

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

Patients receiving antibiotics before aspirations, patients having the diagnosis of a systemic inflammatory disease, and patients with an infection remote from the joint were included in this study.

Alpha defensin correctly diagnosed 152 of all 158 arthroplasties, with an overall specificity of 95.8% (95% CI: 90.5-98.6%) and a sensitivity of 97.4% (95% CI: 86.1-99.6%).

Further, alpha defensin correctly identified culture negative infections, which represented roughly 36% of patients with PJI in this study.

**Performance Characteristics of Lateral Flow**

The Lateral Flow performance was evaluated verses the laboratory test. The positive agreement was 100% (59/59) and the negative agreement was 95.6% (175/183).

**Precision**

Precision pools (4 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. Percent (%) correct result was 100, 97.5, 95.0, 100; respectively.

**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[3]. For each substance, the highest concentration, which was considered not to impact the clinical interpretation of results, is as follows: Hemoglobin 0.5 g/dL; Triglycerides 600 mg/dL; Bilirubin, conjugates 5 mg/dL and Bilirubin, unconjugated 15 mg/dL.

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**REFERENCES**


1. Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J. Diagnosing Periprosthetic Joint Infection: Has the Era of the Biomarker Arrived? Clin Orthop Relat Res. 2014 Nov; 472(11):3254-62.
2. Parvizi, J., Zmistowski, B., Della Valle, C., Bauer, T. W., Malizos, K. N., Alavi, A., Bedair, H., et al. (2013). Diagnosis of Periprosthetic Joint Infection. The Journal of Arthroplasty p200-220.
3. CLSI. Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition. CLSI document EP7-A2 (ISBN 1-56238-584-4). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2005.


**For additional product information, visit [www.cddiagnostics.com](http://www.cddiagnostics.com)**

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