ZIMMER BIOMET

Synovasure® Microbial Identification Test

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.

NAME AND INTENDED USE

The Synovasure Microbial Identification (MID) Test is a qualitative *in vitro* diagnostic test intended for the early detection of microbial antigen in synovial fluid of patients experiencing joint pain and/or inflammation. The Synovasure MID Test detects s antigen from *Staphylococcus* species*, Candida* species*,* and *Enterococcus* species in the synovial fluid. The test results are intended to be used as an additional test to microbial culture and to detect the presence of an organism in culture negative samples. **The Synovasure MID Test is intended for laboratory use only.**

PRINCIPLES OF THE TEST

The Synovasure Microbial Identification (MID) Test is an immunometric multiplexed assay and involves the reaction of the microbial antigen present in the sample with a genera-specific anti-microbial antibody conjugated to MagPlex® microspheres (beads). Each bead contains two (2) internal classification dyes of similar excitation requirements, but with unique emission profiles. The unique spectral characteristics of each bead allows them to be differentiated from all other beads in a multiplex cocktail. Synovial fluid samples are incubated with an extraction buffer (PBS, 200 µg/mL hyaluronidase, 40 µg/mL lysostaphin, 1% Tween 20), boiled and centrifuged. The resultant supernatants are added to microplate wells that contain multiple microspheres with antibodies against different microbial species or genera (*Staphylococcus spp., Candida spp.* and *Enterococcus spp.*). After a wash step, a cocktail of biotinylated antibody (anti-microbial antibodies) is added and this complexes with captured microbial antigen. Following another wash step, a streptavidin-phycoerythrin (SA-PE) conjugate is added, which binds to the biotinylated antibody conjugate. Unbound SA-PE is removed by an additional washing step. After resuspension of beads in sheath fluid, the beads are interrogated by the Luminex 200® instrument, which identifies the individual bead type by its fluorescent spectrum as well as any fluorescent conjugate (PE) intensity associated with the bead. The median fluorescent intensity (MFI) of PE bound to 50 beads is determined for each assay and each well. The MFI is directly proportional to the concentration of microbial antigen in the sample.

Results are calculated as a normalized signal, relative to a background (S/N) and divided by a specific cut-off factor to generate a cut-off value. A sample with a cut-off value ≥ 1 is considered positive. Cut-off values are calculated to two (2) significant figures.

WARNINGS AND PRECAUTIONS

For *in vitro* Diagnostic Use

- This kit should only be used by qualified laboratory personnel.
- This test should be performed at room temperature (RT) (20 to 27°C). Do NOT run outside this range.
- This kit is for use with synovial fluid only. The use of this kit with any other specimen type may lead to inaccurate results. The use of synovial fluid with saline, blood, contrast agent, or any substances injected into the joint may lead to false negatives.
- Do NOT mix lot numbers of conjugated beads, biotin conjugates, and/or controls. These are a matched set and tested as a unit. Mixing lot numbers of these materials without QC release data can give erroneous results.
- Do NOT use reagents beyond their labeled expiration date.
- Visual inspection of the reagents should be performed prior to use to check for color change, cloudiness, and precipitates. If a color change, cloudiness or precipitates is observed, reagent should be rejected. If crystals are observed in buffers, the buffer can be warmed to RT and stirred until crystals are solubilize.
- Distilled or de-ionized water must be used for wash buffer, sample preparation buffer, and assay diluent buffer preparation. Clinical laboratory reagent water Type I or Type II is acceptable. Store the water in nonmetallic containers.
- All pipetting equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.
- Ensure that the specimen is prepared appropriately before adding to the microplate. Failure to prepare a specimen properly may produce an erroneous result. Failure to add specimen may produce an erroneous aseptic result.
- Use new pipette tips for each specimen, QC, or reagent to be added. Cross contamination between reagents will invalidate the test results. Labeled, dedicated reservoirs for the appropriate reagents are recommended.
- Ensure that the microtiter plate is level during the test procedure.
- Strict adherence to the specified wash procedure is crucial to ensure optimum assay performance (*See Test Procedure*). A magnet must be used on the plate washer to avoid loss of microspheres.
- Control results that are invalid (*See Quality Control section*) may indicate a technique problem or product deterioration.
- Use new adhesive seals between assay steps to avoid cross contamination.

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.
- Handle all biological samples and testing materials using Universal Precautions as samples are potentially hazardous and capable of transmitting diseases.
- Dispose of all specimens and materials used to perform the test per local, state, and federal requirements as if they contain infectious agents.

MATERIALS PROVIDED & STORAGE CONDITIONS

Synovasure Microbial ID Test Kit: REF CD2002 **Kit Configurations**

CD2002L Recommended for low volume; running partial plates on a routine basis (20 runs up to 48 samples/run)
CD2002H Recommended for high volume usage; running full plates on a routine basis (20 runs up to 88 samples/run)

Kit Component	REF #	CD2002L Quantity	CD2002H Quantity
MID Biotin-Ab	P50055	6	10
QC1, MID (Negative Control)	P50046	20*	20*
QC2, MID *(Candida* Panel*)*	P50047	20*	20*
QC3, MID (*Enterococcus* Panel)	P50048	20*	20*
QC4, MID (*Staphylococcus* Panel A)	P50049	20*	20*
QC5, MID (*Staphylococcus* Panel B)	P50050	20*	20*
MID Beads	P50056	6	10
Streptavidin-PE Conjugate	P50054	1	1
Assay Diluent	P50052	1	2
Sample Preparation Buffer	P50051	1	2
Wash Buffer	P50010	1	1
Instructions for Use	M40022	1	1

** QC1-5 are packaged in 2 boxes with 10 in each box*

Shipping and Storage Information

Kits are delivered in three (3) boxes:

- One box containing dry ice for frozen materials
- Second box containing ice packs for refrigerated materials
- Third box for ambient materials

Upon arrival, the materials need to be unpacked and stored at the recommended Storage Conditions.

Do NOT use kit past expiration date. Expiration dates are stated on each component label. If outer packaging is damaged, be sure to check that immediate container packaging is intact. If immediate packaging is leaking, discard contents. Storage conditions of each component are stated on label and in the following chart:

Kit Component	REF #	Shipping Condition	Storage Condition	Storage Conditions and 'use by' dates of thawed and diluted materials
MID Biotin-Ab –200 µL 60X concentrate. Screw cap vial.	P50055	Frozen, Dry Ice	Frozen ≤ -65°C	Store prepared Biotin-Ab (1X) refrigerated (2 to 8°C) for no more than 28 days.
QC1, MID- 1000 µL aliquots of assay diluent. Screw cap vials.	P50046	Frozen, Dry Ice	Frozen ≤ -65°C	Thaw before each use. One (1) time use only.
QC2, MID - 250 µL aliquots of boiled antigens in assay diluent. Screw cap vials.	P50047	Frozen, Dry Ice	Frozen ≤ -65°C	Thaw before each use. One (1) time use only.
QC3, MID - 250 µL aliquots of boiled antigens in assay diluent. Screw cap vials.	P50048	Frozen, Dry Ice	Frozen ≤ -65°C	Thaw before each use. One (1) time use only.
QC4, MID - 250 µL aliquots of boiled antigens in assay diluent. Screw cap vials.	P50049	Frozen, Dry Ice	Frozen ≤ -65°C	Thaw before each use. One (1) time use only.
QC5, MID - 250 µL aliquots of boiled antigens in assay diluent. Screw cap vials.	P50050	Frozen, Dry Ice	Frozen ≤ -65°C	Thaw before each use. One (1) time use only.

MID Beads –200 µL of 60X concentrated mixture. Screw cap vial.	P50056	Cold, Ice Packs	Refrigerated 2 to 8°C Protect from Light	Dilute before each use.
Streptavidin-PE conjugate – 200 µL in a screw cap vial.	P50054	Cold, Ice Packs	Refrigerated 2 to 8°C Protect from Light	Dilute 1:2000 before each use.
Assay Diluent - 100 mL of a 5X concentrate, amber bottle (5X PBS, 0.25% Tween20, 2.5% BSA, 0.5% Proclin 950).	P50052	Cold, Ice Packs	Refrigerated 2 to 8°C	Store prepared diluent (1X) refrigerated (2 to 8°C) for no more than 28 days.
Sample Preparation Buffer- 25 mL of a 10X concentrate, opaque bottle (10X concentrate of PBST with 10.3% Tween 20)	P50051	Ambient	Ambient 15 to 30°C	Dilute before each use.
Wash Buffer - 1000 mL of 10X concentrate, opaque bottle (10X PBS, 0.5% Tween 20, 1.0% Proclin 950)	P50010	Ambient	Ambient 15 to 30°C	Store prepared buffer (1X) at ambient temperature (15 to 30°C) for no more than 28 days.
Instructions for Use	M40022			

OTHER MATERIALS REQUIRED BUT NOT PROVIDED

- Sheath Fluid (20 L Bio-Rad, 171-000055; 20 L Luminex 40-50035; or equivalent)
- Luminex 200 Calibrator (LX200-CAL-K25) and Performance Verification (LX200-CON-K25) kits
- 3 M sodium acetate, pH 4.5 (Bio world, 41920024-1 or equivalent)
- Lysostaphin (AMBI LSPN-50)
- Hyaluronidase (Sigma H4272)

EQUIPMENT

- Precision positive displacement pipettes for handling synovial fluid during sample preparation
- Pipettes to deliver 1-1000 µL
- Polypropylene tubes
- Plate sealers (adhesive film)
- Washing device suitable for microtiter plates
- Foil or foil-adhesive plate sealers
- Distilled or de-ionized water
- Plates 96-well deep well or equivalent (Eppendorf deep well plates, 951031801, Greiner Bio-One Cat# 786201)
- Plates 96-well black flat bottom medium binding or equivalent (Greiner Bio-One Plates, cat# 655096, Thermo Scientific Cat # 265301, Corning Cat# 3631)
- Water bath (95 to 100°C)
- Incubator (37°C+/-2)
- Microtiter plate shaker capable of 500 rpm
- Centrifuge capable of spinning plates at 3700g
- Luminex instrument
- Flat plate magnet (Biotek 96F, 7103016)
- Analytical balance, if necessary, for enzyme preparation

SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Specimen Requirement

Synovial fluid aspirated from the joint and transferred to a sterile tube without clot activator.

NOTE: Modified synovial fluid; such as saline wash or fluid drawn immediately post injection will negatively impact the performance of the test.

Specimen Collection and Preparation

Synovial fluid should be collected by approved medical techniques then transferred to clear top, red stopper tubes without clot activator.

Specimen Handling and Storage Conditions

Samples can be stored up to seven (7) days at 2 to 27°C before testing. **Do NOT centrifuge synovial fluid samples.** Avoid more than one (1) freeze/thaw cycle of clinical synovial fluid samples intended for Microbial ID determination.

PROCEDURE

This assay can be completed in one (1) business day. To ensure adequate time for sample preparation, assay completion, and results generation, advance verification of technician training, material and equipment availability and proper Luminex instrument setup is recommended.

- **Reagent Preparation**
  Allow all the reagents to equilibrate to RT (20 to 27°C) prior to use. Return to proper storage conditions immediately after use.
- **Sample Preparation Buffer**
  Prepare the 1X Sample Preparation Buffer by diluting one (1) part of the 10X sample preparation buffer with nine (9) parts of distilled or de-ionized water.
- **20 mM acetate, pH 4.5**
  Mix 0.333 mL of 3 M sodium acetate in a total of 50 mL of distilled or de-ionized water. Store at ambient temperature.
- **Lysostaphin**
  Reconstitute a determined amount of lysostaphin with 20 mM sodium acetate pH 4.5 to produce a 5 mg/ml stock. Mix well to ensure complete reconstitution and allow the stock to sit for at least 15 minutes with gentle agitation prior to use. Aliquot and store at -20°C in **single-use aliquots**. Avoid multiple freeze-thaw cycles.
- **Hyaluronidase**
  Reconstitute a determined amount of hyaluronidase with de-ionized or distilled water to produce a 10 mg/mL stock. Mix well to ensure complete reconstitution and allow the stock to sit for at least 15 minutes with gentle agitation prior to use. Aliquot and store at -20°C in **single-use aliquots**. Avoid multiple freeze-thaw cycles.
- **Sample Preparation Diluent (0.2 mL per sample, 21 mL per plate)**
  For 1 mL add and mix gently by inversion:
  - 0.972 mL of 1X Sample Diluent
  - 0.008 mL Lysostaphin (5 mg/mL)
  - 0.020 mL Hyaluronidase (10 mg/mL)
- **QC Handling**
  QC (QC1 is a negative control, and QC2, QC3, QC4 and QC5 are positive controls) are supplied on dry ice and stored frozen at less than ≤ -65°C. Thaw for 30 minutes at RT and mixed thoroughly using a vortex before use. Controls are to be discarded after a single use.
- **Wash Buffer**
  Wash buffer is supplied as a 10X concentrate and diluted to 1X prior to use. Preparation of 1X wash buffer: Mix 100 mL of 10X Wash Buffer Concentrate with 900 mL of distilled or de-ionized water. Store prepared buffer at RT. Discard Wash Buffer (1X) if visibly contaminated.
  NOTE: Wash Buffer Concentrate may contain crystals if temperature falls below 15°C. Bring concentrate to RT prior to dilution. Thoroughly mix the concentrate prior to use and ensure crystals dissolve prior to use.
- **Assay diluent**
  Assay diluent is supplied as a 5X concentrate and diluted to 1X prior to use. Prepare the desired volume of 1X assay diluent by diluting one (1) part of the 5X assay diluent buffer with four (4) parts of distilled or de-ionized water. Store at 2 to 8°C.
- **MID Beads**
  The bead cocktail with conjugated antibodies specific to each panel (*Candida* Panel, *Enterococcus* Panel*, Staphylococcus* Panel A, *Staphylococcus* Panel B) is supplied concentrated (60X) at 2 to 8°C. Vortex beads for 30 seconds, then dilute beads to 1X with 1X assay diluent prior to use.
- **MID Biotin-Ab**
  The biotin conjugate is supplied concentrated (60X) and frozen at ≤ -65°C. Thaw vial for 30 minutes at RT and dilute biotin conjugate to 1X with 1X assay diluent prior to use.
- **Streptavidin-Phycoerythrin (SA-PE) Conjugate**
  The streptavidin-phycoerythrin (SA-PE) conjugate is supplied concentrated at 2,000X. Dilute to 1X in 1X assay diluent prior to use.
- **Sheath fluid (not included)**
  Sheath fluid is ready to use if using Bio-Rad 171-000055 or Luminex 40-50035.

SYNOVASURE MICROBIAL ID TEST KIT PROCEDURE

The Synovasure Microbial ID panel assay will be performed per the following steps:

1. Sample Preparation Procedure:

1.1. Aliquot 200 µL of patient synovial fluid (SF) sample or QC control into a 96-well Eppendorf deep well plate (500 µL/well capacity) using a positive displacement pipette. **Change tips between samples.**
NOTE: QCs are assay diluent spiked with a concentration of the appropriate bacterium or fungus, which gives a low-to-mid positive response. These samples are pre-prepared and ready for the extraction procedure.

1.2. Add 200 µL of the prepared sample preparation diluent to the wells of the Eppendorf deep well plate.

1.3. Pipet up and down a minimum of five (5) times to thoroughly mix sample and sample preparation buffer. **Change tips between samples.** Balance plate by adding 400 µL of water to the empty wells of the plate.

1.4. Cover the plate with adhesive film.

1.5. Incubate plate for 30± 2 minutes in a 37± 2°C incubator. Record actual temperature, start and end times, and calculate the elapsed time that samples were incubated.

1.6. Boil plate for 5 to 5.5 minutes in 95-100°C water bath. Monitor plate during this step to avoid overheating. Record actual temperature, start and end times, and calculate the elapsed time that samples were boiled.
NOTE: Care should be taken when handling boiling water to prevent burning.

1.7. Centrifuge plate for 30 minutes at 3,700g.

2. Assay Procedure:

2.1. Add 250 µL of 1X assay diluent to the test wells of the flat bottom polystyrene black medium binding plate and incubate at RT for 10 to 60 minutes.

2.2. Remove assay diluent from plate by inversion and blot plate dry.

2.3. Vortex beads for 30 seconds, then dilute the conjugate beads 1:60 in 1X assay diluent. Vortex briefly, then add 50 µL per well.

2.4. Add 50 µL of processed patient SF sample or control sample per well and seal plate with adhesive film.

2.5. Incubate while shaking (~500 rpm) at RT for 60±2 minutes. **Use a NEW adhesive seal.** Protect the assay from light by keeping the microtiter plate in the dark by either sealing it with aluminum-adhesive film or sealing with a clear adhesive and covering it with aluminum foil.

2.6. Ensure that the plate washer is equipped with the appropriate magnet and that the customized wash program has been selected prior to executing the washing step.

2.7. Position the microtiter plate on the magnet and wait for 1 minute prior to starting the washing step. Wash three (3) times with 200 µL/well of prepared wash buffer with a 1-minute delay between washes to allow beads to adhere to magnet. **Do NOT invert or blot microtiter plate onto paper towels.**

2.8. Dilute the MID Biotin Ab 1:60 in 1X assay diluent and mix by inversion. Add 50 µL of the biotin conjugate per well and seal plate with a NEW adhesive seal. Protect from light as described in step 2.5.

2.9. Incubate while shaking at RT for 60 minutes, as described in step 2.5.

2.10. Wash the plate 3X as described in steps 2.6 and 2.7. **Do NOT invert or blot microtiter plate onto paper towels.**

2.11. Dilute the SA-PE 1:2000 in 1X assay diluent, mix by inversion, and add 50 µL per well. Seal plate with adhesive film. Incubate while shaking (~500 rpm) at RT for 30 minutes. **Use a NEW adhesive seal.** Protect from light as described in step 2.5.

2.12. Wash the plate 3X as described in steps 2.6 and 2.7. **Do NOT invert or blot microtiter plate onto paper towels.**

2.13. Add 100 µL sheath fluid per well. Seal plate with adhesive film. Use a NEW adhesive seal.

2.14. Incubate while shaking at RT for a minimum of five (5) minutes on speed 4.5. Plates can be read up to two (2) hours after adding the sheath fluid with plate shaking to keep the beads dispersed. **Use a NEW adhesive seal.** Protect from light as described in step 2.5.

2.15 Read plate on Luminex instrument using the Microbial ID panel protocol.

<u>Luminex Plate Reading Specifications</u>:

- Select the following bead regions:
  Staphylococcus Panel A: 026
  Staphylococcus Panel B: 034
  Candida Panel: 043
  Enterococcus Panel*:* 046
- Read 50 beads per region
- Sample size = 50 µL
- Default gate settings: 5,000 – 25,000

QUALITY CONTROL

The negative control (QC1) should be run in quadruplicate and each positive control (QC2-QC5) should be run in singlicate per the plate map below. A set of controls will be run on each plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**QC1**											
B	**QC1**											
C	**QC1**											
D	**QC1**											
E	**QC2**											
F	**QC3**											
G	**QC4**											
H	**QC5**											

Name	QC
Negative Control	QC1
Candida Panel	QC2
Enterococcus Panel	QC3
Staphylococcus Panel A	QC4
Staphylococcus Panel B	QC5

An assay will be considered valid if:
The calculated signal (S) to cut-off (C/O) value for each positive control (QC2 – QC5) must be ≥ 1.0.

INTERPRETATION OF RESULTS
The results from this test should only be used in conjunction with information available from clinical evaluations and other tests.

Abbreviations
Staphylococcus Panel (SP)
Staphylococcus Panel A (SPA)
Staphylococcus Panel B (SPB)
Candida Panel (CP)
Enterococcus Panel (EP)

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

- Cut-off (C/O) = 1
- Calculate Signal to Noise (S/N) = MFI (raw data) of sample ÷ mean MFI of QC1
- Calculate Signal to C/O ratio = S/N ÷ [Lot specific cutoff factor for each panel (*provided on Certificate of Analysis)*]

Rules for Assay Interpretation
Reported results: positive (+), negative (-), indeterminate (I)

+	Positive for organism
-	Negative for organism
I	Indeterminate result

1. If all assays < C/O, then all are reported as negative.
2. If only one assay is ≥ C/O, then only that assay is reported as positive. If both SPA and SPB are ≥ C/O, then only SP is reported as positive.
3. If CP and EP are ≥ C/O and SPA and SPB < C/O, then CP and EP are both reported as positive.
4. If SPA ≥ C/O and CP and/or EP ≥ C/O but ≤ SPA, then SP is reported as positive and CP and/or EP are reported as indeterminate.
5. If SPA ≥ C/O and CP and/or EP ≥ C/O and > SPA, then SP and CP and/or EP are reported as positive.
6. If SPA < C/O, SPB ≥ C/O and < 6, EP ≥ C/O and EP > SPB, then SP is reported as indeterminate and EP is reported as positive, but if EP ≤ SPB, then SP and EP are reported as positive.
7. If SPA < C/O, SPB ≥ 6 and CP and/or EP ≥ C/O, then SP is reported as positive and CP and/or EP are reported as positive.

Rule #	Assay results (Signal: cut-off)	SP	CP	EP
R1	If SPA and SPB < 1.0 and CP and EP < 1.0	-	-	-
R2	If SPA and/or SPB ≥ 1.0 and CP and EP < 1.0	+	-	-
R3	If SPA and SPB < 1.0, CP < 1.0 and EP ≥ 1.0	-	-	+
R4	If SPA and SPB < 1.0, CP ≥ 1.0 and EP < 1.0	-	+	-

R5	If SPA and SPB < 1.0, CP and EP ≥ 1.0 [E1]	-	+	+
R6	If SPA ≥ 1.0, CP and EP ≥ 1.0 and CP and EP ≤ SPA [E2]	+	I	I
R7	If SPA ≥ 1.0, CP and EP ≥ 1.0 and CP and EP > SPA [E3]	+	+	+
R8	If SPA ≥ 1.0, CP and EP ≥ 1.0 but CP ≤ SPA and EP > SPA	+	I	+
R9	If SPA ≥ 1.0, CP and EP ≥ 1.0 but CP > SPA and EP ≤ SPA	+	+	I
R10	If SPA ≥ 1.0, CP < 1.0 and EP ≥ 1.0 but EP ≤ SPA	+	-	I
R11	If SPA ≥ 1.0, EP < 1.0 and CP ≥ 1.0 but CP ≤ SPA	+	I	-
R12	If SPA ≥ 1.0, CP < 1.0 but EP ≥ 1.0 and EP > SPA	+	-	+
R13	If SPA ≥ 1.0, EP < 1.0 but CP ≥ 1.0 and CP > SPA	+	+	-
R14	If SPA < 1.0, SPB ≥ 1.0 but < 6.0, CP < 1.0, EP ≥ 1.0 and EP > SPB [E4]	I	-	+
R15	If SPA < 1.0, SPB ≥ 1.0 but < 6.0, CP < 1.0, EP ≥ 1.0 but EP ≤ SPB	+	-	+
R16	If SPA < 1.0, SPB ≥ 1.0 but < 6.0, CP ≥ 1.0, EP ≥ 1.0 and EP > SPB	I	+	+
R17	If SPA < 1.0, SPB ≥ 1.0 but < 6.0, CP ≥ 1.0, EP ≥ 1.0 but EP ≤ SPB	+	+	+
R18	If SPA < 1.0, SPB ≥ 6.0, CP < 1.0, EP ≥ 1.0	+	-	+
R19	If SPA < 1.0, SPB ≥ 6.0, CP ≥ 1.0, EP ≥ 1.0	+	+	+
R20	If SPA < 1.0, SPB ≥ 1.0, EP < 1.0, CP ≥ 1.0	+	+	-

In R6-R13, the SPB result is insignificant to the reported results

Evidence (E):

[E1] *Candida* and *Enterococcus* are common organisms associated together in poly-microbial infections

[E2] Protein A found on cell surface of *S. aureus* can result in low-level cross-reactivity in *Candida* and *Enterococcus spp.* assays

[E3] Cross-reactivity experiments do not indicate that high levels of *Candida* or *E. faecalis* result in positivity in *Staphylococcus* Panel A

[E4] High levels of *E. faecalis* are known to result in low-level cross-reactivity in the *Staphylococcus* Panel B assay

PERFORMANCE CHARACTERISTICS

Diagnostic Accuracy

The Microbial ID (MID) assay identifies organisms in synovial fluid (*Staphylococcus spp., Enterococcus spp.* and *Candida spp*.) The assay is composed of four (4) panels (*Staphylococcus Panel A and B* (SPA & SPB), *Candida Panel* (CP), and *Enterococcus Panel* (EP). The *Staphylococcus* results are combined into one (1) result (Positive in at least one panel is a positive result for the presence of a Staphylococcus antigen). The assay was validated using clinical patient synovial fluid (SF) known to be either infected with an organism which had been identified through standard culture techniques and alpha-defensin positivity, or aseptic samples which were negative for α-defensin, CRP, and culture (-/-/-). The *Staphylococcus* Panels detected antigen in 76% of all *Staphylococcus (S. aureus, S. epidermidis*, and *S. lugdunensis*)-culture positive SF samples tested. The *Candida* panel detected antigen in 80% of *Candida (C. albicans, C. tropicalis, C. glabrata, C. parapsilosis*, and *C. parapsilosis* species complex)-culture positive patient SF samples. *E. faecalis* was detected in 92% of *E. faecalis*-culture-positive SF samples in the *Enterococcus* panel. There was no antigen detected in > 98% of the -/-/- samples. *S. aureus* and *Streptococcus agalactiae* culture positive samples may react across two or more panels due to protein A and protein G respectively on the bacterial cell.

The assay was launched at CD Laboratories, Baltimore, MD. A prospective analysis of the results of the MID assay on results from February 2017 to December 14, 2017 was performed. The prospective analysis shown below demonstrates that MID has sensitivities against *Staphylococcus* (94%), *Enterococcus* (97.1%), and *Candida* (90%) and a total assay specificity of 98%.

α-defensin+/culture+ PJI and Native sample			
Organisms	**Samples (#)**	**MID Positive**	**Sensitivity**
Staphylococcus spp.	**418**	**393**	**94.0%**
S. aureus	146	143	97.9%
S. epidermidis	192	176	91.7%
S. lugdunensis	38	34	89.5%
Other *Staphylococcus spp.*	**42**	40	95.2%
Candida spp.	**20**	**18**	**90.0%**
Enterococcus spp.	**35**	**34**	**97.1%**

α-defensin-/culture- PJI and Native sample (N=4406)		
Organisms	**MID Positive (#)**	**Specificity**
Staphylococcus spp.	45	99.0%
Candida spp.	16	99.6%
Enterococcus spp.	23	99.5%
Polymicrobial (positive in more than one assay)	9	99.8%

Total	**93**	**97.9%**

In addition, 37% of α-defensin positive/culture negative samples were positive in the MID assay:

α-defensin+/culture- PJI and Native sample (N=506)		
Organisms	**MID Positive (#)**	**% positive**
Staphylococcus spp.	127	25.1%
Candida spp.	12	2.4%
Enterococcus spp.	22	4.4%
Polymicrobial (positive in more than one assay)	26	5.1%
Total	**187**	**37.0%**

Precision

Four (4) precision pools (low negative and three positive pools) were tested in quadruplicate two (2) times/day over five (5) days to estimate the precision of the assay. (Note: Pool 2 for *Candida* is a high negative pool)

The following table summarizes the overall precision performance for all five (5) days of testing:

	***Candida* Panel**		***Enterococcus* Panel**		***Staphylococcus* A**		***Staphylococcus* B**	
Pool	**Mean (S:C/O)**	**%CV**	**Mean (S:C/O)**	**%CV**	**Mean (S:C/O)**	**%CV**	**Mean (S:C/O)**	**%CV**
1	0.4	12	0.4	9	0.5	10	0.3	13
2	0.9	13	1.3	11	1.5	13	1.3	10
3	1.2	15	2.2	13	1.9	15	1.6	13
4	1.5	15	3.0	16	2.3	19	2.0	17

Reproducibility

A two-laboratory reproducibility study was performed on the MID1 test with contrived samples spiked with killed *Staphylococcus aureus, S. epidermidis, Candida albicans, or Enterococcus faecalis*. The experimental design consisted of five (5) days of testing, two (2) runs per day each (different operators) and two (2) locations.

The following table summarizes the overall reproducibility for all five (5) days of testing, both runs and both laboratories.
Negative percent agreement (NPA), Positive percent agreement (PPA), and total percent agreement are shown.

Assay	**Agreement**			**Confidence Intervals**	
	PPA (%)	**NPA (%)**	**Total (%)**	**PPA CI (95%)**	**NPA (95%)**
SPA	100	95	96	83.9, 100.0	87.8, 98.0
SPB	100	100	100	91.2, 100.0	94.0, 100.0
CA	97.5	100	99	87.1, 99.6	94.0, 100.0
EF	100	100	100	91.2, 100.0	94.0, 100.0

Interfering Substances

The effect of hemoglobin, triglyceride, bilirubin (conjugated and unconjugated) and rheumatoid factor (RF) were tested in negative synovial fluid spiked with low and high microbial antigen levels following the procedures described in CLSI Protocol EP7-A2 (Appendix D). None of the interferents affected the assay at the concentrations listed below:

Interferent	**High Test Level**
Hemoglobin	200 mg/dL
Triglyceride	418 mg/dL (37 mM)
Bilirubin, conjugated	20 mg/dL (0.237 mM)
Bilirubin, unconjugated	20 mg/dL (0.342 mM)
RF	300 IU/mL

Establishment of C5, C50, and C95

The C5, C50 and C95 was established for the assay using boiled microbial isolates as antigen in the assay. The values were determined from eight (8) separate concentrations from 22 separate titration curves. The results are summarized below:

	CFU/ml		
Test	**C5**	**C50**	**C95**

Staphylococcus Panel A	4.0 x 10^4	6.4 x 10^4	8.9 x 10^4
Staphylococcus Panel B	2.0 x 10^4	2.5 x 10^4	3.0 x 10^4
Candida Panel	9.0 x 10^2	1.4 x 10^3	2.0 x 10^3
Enterococcus Panel	3.0 x 10^2	4.8 x 10^2	6.7 x 10^2

Hook Effect

High concentrations of boiled microorganisms were tested to determine if the assay was subject to hook Effect. The concentrations tested were above the expected CFU/mL in the majority of patient SF samples. No hook effect was detected at the concentration ranges below:

Test	Range tested (CFU/mL)
Staphylococcus Panel A	1.0 x 10^7 - 1.0 x 10^9
Staphylococcus Panel B	1.0 x 10^7 - 4.0 x 10^8
Candida Panel	1.0 x 10^7 - 1.3 x 10^9
Enterococcus Panel	1.0 x 10^7 - 1.0 x 10^9

Cross reactivity

Many bacterial and fungal isolates were tested in the assays to identify potential cross reactants. Included in the panel were at least one of the following: *Staphylococcus spp.* (*epidermidis, aureus, caprae, capitis, lugdunensis* and *hominis)*, *Candida spp.* (*glabrata, albicans,* and *tropicalis*), *Streptococcus spp.* (*agalactiae* and *oralis*), *E. faecalis*, *P. aeruginosa*, and *E. coli*. The organisms were tested at 10^4 to 10^6 CFU/mL as this represents a mid-to-high concentration of what would be expected in a patient synovial fluid sample. The following were demonstrated to react in the assays:

	Organisms detected in the MID assays			
CFU/mL	***Staphylococcus* Panel A**	***Staphylococcus* Panel B**	***Candida* Panel**	***Enterococcus* Panel**
10^6	*Staphylococcus spp.* (*epidermidis, aureus, caprae, capitis,* and *hominis)*	*Staphylococcus spp.* (*epidermidis, aureus, caprae, capitis, lugdunensis* and *hominis) E. faecalis*	*Candida spp.* (*glabrata, albicans,* and *tropicalis*) *S. aureus**	*E. faecalis, S. aureus**
10^5	*Staphylococcus spp.* (*epidermidis, aureus, caprae, capitis, and hominis)*	*Staphylococcus spp.* (*epidermidis, aureus, caprae, capitis, and hominis) E. faecalis*	*Candida spp.* (*glabrata, albicans,* and *tropicalis*) *S. aureus**	*E. faecalis, S. aureus**
10^4	*Staphylococcus spp.* (*epidermidis, aureus)*	*Staphylococcus spp.* (*epidermidis, caprae, capitis, and hominis)*	*Candida spp.* (*albicans,* and *tropicalis*)	*E. faecalis*
**S. aureus* expresses protein A on cell surface which interacts nonspecifically with Fc portion of multiple antibody types				

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SYMBOL KEY