ZIMMER BIOMET

Synovasure® Microbial ID *P. acnes* 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) *P. acnes* Test is a qualitative *in vitro* diagnostic test intended for the early detection of *P. acnes* antigen in synovial fluid of patients experiencing joint pain and/or inflammation. 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 *P. acnes* Test is intended for laboratory use only.**

Synovasure Microbial ID *P. acnes* Ref# CD2004 must be run in conjunction with Synovasure Microbial ID Ref# CD2002 to interpret test results.

PRINCIPLES OF THE TEST

The Synovasure Microbial Identification (MID) *P. acnes* Test is an immunometric assay and involves the reaction of the microbial antigen present in the sample with a species-specific anti-microbial antibody conjugated to MagPlex® microspheres (beads). The bead contains two (2) internal classification dyes of similar excitation requirements, but with unique emission profiles. Synovial fluid samples are incubated with an extraction buffer (PBS, 200 µg/mL hyaluronidase, 500 µg/mL lysozyme, 1% Tween 20), boiled, and centrifuged. The resultant supernatants are added to microplate wells that contain microspheres coupled to an antibody generated against *P. acnes*. After a wash step, a biotinylated antibody conjugate (anti-*P. acnes* antibody) is added and 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 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 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 then divided by a specific cut-off factor for the *P. acnes* assay 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.
- **Assay must be run with Microbial ID Ref# CD2002 to be able to interpret results**.

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 state requirements as if they contain infectious agents.

DEFINITIONS

P. acnes assay (PAC)

MATERIALS PROVIDED & STORAGE CONDITIONS

Synovasure Microbial ID *P. acnes* Test Kit: REF CD2004 **Kit Configurations**

CD2004L Recommended for low volume; running partial plates on a routine basis (20 runs up to 48 test samples/run)
CD2004H Recommended for high volume usage; running full plates on a routine basis (20 runs up to 88 test samples/run)

Kit Component	REF #	CD2004L Quantity	CD2004H Quantity
MID *P. acnes* Biotin-Ab	P50069	6	10
QC1, MID (Negative Control)	P50046	20	20
QC9, MID (*P. acnes* Panel)	P50060	20	20
MID *P. acnes* Beads	P50064	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	M40023	1	1

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 containing 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 *P. acnes* Biotin-Ab –200 µL 60X concentrated detection antibody, Screw cap vial.	P50069	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.
QC9, MID - 250 µL aliquots of boiled antigens in assay diluent. Screw cap vials.	P50060	Frozen, Dry Ice	Frozen ≤ -65°C	Thaw before each use. One (1) time use only.
MID *P. acnes* Beads –200 µL of 60X concentrated. Screw cap vial.	P50064	Cold, Ice Packs	Refrigerated 2 to 8°C Protect from Light	Dilute before each use
Streptavidin-PE Conjugate – 200 µL 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	M40023			

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

- Lysozyme (Sigma L6876 or L4919 or Creative Enzymes NATE-4728 or equivalent)
- Hyaluronidase (Sigma H4272 or equivalent)

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 spinning at 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.
- **Lysozyme**
  Reconstitute a determined amount of lysozyme with de-ionized or distilled water to produce a 50 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. Solution is stable in the freezer for up to one (1) year.
- **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:
  - 0.97 mL of 1X Sample Preparation Buffer
  - 0.01 mL of Lysozyme (50 mg/mL)
  - 0.02 mL of Hyaluronidase (10 mg/mL)
- **QC Handling**
  The QCs (QC1 – negative control, QC9 – *P. acnes* positive control) are supplied on dry ice and stored frozen at less than ≤ -65°C. Thaw for 30 minutes at RT and mix 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 diluted 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 with four (4) parts of distilled or de-ionized water. Store at 2 to 8°C.
- **MID *P. acnes* Beads**
  The *P. acnes* specific antibodies coupled to beads are supplied concentrated (60X) at 2 to 8°C. Vortex beads for 30 seconds, then dilute beads 1X with 1X assay diluent prior to use.
- **MID *P. acnes* 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 *P. ACNES* TEST KIT PROCEDURE

The Synovasure Microbial ID *P. acnes* 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 P. acnes to yield 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 diluent. **Change tips between samples.** Balance plate by adding 400 µL water to the empty wells of the plate.

1.4. Cover 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 time 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 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 *P. acnes* 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 *P. acnes* panel protocol.

Luminex Plate Reading Specifications:

- Select the following bead region:
  P. acnes assay 042

- 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 the positive control (QC9) 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	QC9											
F												
G												
H												

Name	QC
Negative Control	QC1
P. acnes Positive Control	QC9

An assay will be considered valid if:

The calculated signal (S) to cut-off (C/O) value for the positive control (QC9) 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 diagnostic tests.

Abbreviations

P. acnes (PAC)
*Staphylococcu*s Panel A (SPA)

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

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

The results from this test should only be used in conjunction with results from **Synovasure Microbial ID Ref# CD2002** and information available from clinical evaluations and other tests.

Rules for Assay Interpretation

Reported results: positive (+), negative (-), indeterminate (I)

+	Positive for organism
-	Negative for organism
I	Indeterminate result
PAC	*P. acnes* MID Assay
SPA	SPA result from MID Ref#CD2002 assay results

Rule	Assay Results (Signal: cut-off)	P. acnes	Notes
1	If PAC < 1.0	-	
2A	If SPA < 10.0, PAC ≥ 1.0	+	
2B	If SPA ≥ 10.0, PAC ≥ 1.0	I	At high levels, Protein A found on cell surface of *S. aureus* can result in cross-reactivity

PERFORMANCE CHARACTERISTICS

Diagnostic Accuracy and Method Comparison

The clinical performance of the MID *P. acnes* test was established by testing two sample sets: 99 clinical sample cohort consisting of 50 α-defensin negative and 49 α-defensin positive samples and 118 *P. acnes* contrived clinical samples. The MID *P. acnes* test sensitivity (percent agreement with

expected result) was determined on the basis of the contrived clinical synovial fluid sample set spiked with *P. acnes* while specificity was determined on the 99 clinical sample cohort that were *P. acnes* negative for culture. The calculated sensitivity was 93.2 % (110/118) (95% CI: 87.2 - 96.5 %) while the specificity was estimated at 99.0 % (98/99) (95 % CI: 94.5 - 99.8 %) (Table 2).

A comparison between MID *P. acnes* and PCR System (Curetis Unyvero ITI G2) showed a negative percent agreement (NPA) of 99.0 % (97/98) (95 % CI: 94.5 - 99.8 %) (Table 2). Positive percent agreement (PPA) could not be calculated as *P. acnes* positive clinical samples are infrequently encountered.

Table 1: Data tables for calculating sensitivity (percent agreement with the expected result) and specificity of Synovasure MID *P. acnes* test using 118 *P. acnes* contrived and 99 negative clinical samples (A). Method comparison between MID *P. acnes* and Curetis Unyvero ITI G2 for the 99 negative clinical samples (B).

A		Contrived Sample Results	Clinical Sample Results
		Positive	Negative
MID *P. acnes*	Positive	110	1
	Negative	8	98

B		Curetis Unyvero ITI G2	
		Positive	Negative
MID *P. acnes*	Positive	0	1
	Negative	1	97

Table 2: Sensitivity (percent agreement with expected result) and specificity of the Synovasure MID *P. acnes* test determined with contrived and negative clinical synovial fluid samples, respectively (A). Negative percent agreement (NPA) between Synovasure MID *P. acnes* and Curetis Unyvero ITI G2 (B).

A	Sensitivity (Contrived Clinical Results)			Specificity (Clinical Results)		
	TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI
	110/118	93.2	87.2 – 96.5	98/99	99.0	94.5 – 99.8

B	NPA (Compared to Curetis Unyvero ITI G2)		
	TN/(TN+FP)	%	95% CI
	97/98	99.0	94.5 – 99.8

Contrived clinical samples were used in lieu of *P. acnes* infected clinical samples due to the unavailability of infected samples. These surrogate specimens were prepared by spiking two bacterial isolates into 16 negative clinical synovial fluid pools at four different concentrations – 5 x 10^6 cfu/mL, 1 x 10^6 cfu/mL, 5 x 10^5 cfu/mL and 1 X 10^5 cfu/mL. On a concentration basis, MID *P. acnes* test accuracy ranged from 75% at the lowest spike (1 x 10^5 cfu/mL) to 100 % thereafter. The analytical assay sensitivity of MID *P. acnes* is near ~1 x 10^5 cfu/mL (Table 3).

Table 3: Accuracy of Synovasure MID *P. acnes* calculated for contrived clinical synovial fluid samples prepared by spiking heat-inactivated *P. acnes* cell lysates at various levels. Accuracy is defined as the number of positive samples observed divided by the total number of positive samples expected.

Bacterial Conc. (cfu/mL)	Positive Samples / Total	Accuracy (%)	Average (%)	95% CI
5X10^6	32/32	100	93.2	87.2 – 96.5
1X10^6	22/22	100		
5x10^5	32/32	100		
1x10^5	24/32	75		

Precision

Four (4) precision pools (negative positive pools) were tested in quadruplicate two (2) times/day over five (5) days to estimate the precision of the assay. The following table summarizes the overall precision performance for five (5) days of testing:

Overall Precision		
Pool		*P. acnes*
1	Mean (S:C/O)	0.12
	% CV	16%
2	Mean (S:C/O)	2.19
	% CV	20%
3	Mean (S:C/O)	2.91
	% CV	20%
4	Mean (S:C/O)	3.13
	% CV	21%

Reproducibility

A two-laboratory reproducibility study was performed on the MID *P. acnes* test with positive *P. acnes* quality control spiked into assay diluent. The experimental design consisted of five (5) days of testing two (2) runs per day each by a different operator. Negative percent agreement (NPA) and Positive percent agreement (PPA) are both 100% with a 95% confidence interval of 83.9 – 100%.

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%)
PAC	100	100	100	83.9, 100.0	83.9, 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. None of the interferents had a clinically-relevant effect in 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 were established for the assay using boiled microbial antigen. The values for the assay were determined from 16 different concentrations derived from 44 separate titration curves. The results are summarized below:

	CFU/mL		
Test	**C5**	**C50**	**C95**
P. acnes assay	4.00×10^4	6.50×10^4	9.00×10^4

Hook Effect

High concentrations of boiled microorganisms (1.0×10^7 - 1.0×10^9 CFU/mL) 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 tested.

Analytical Specificity (Exclusivity)

Unless noted, at least one of the following bacterial and fungal isolates were tested for cross-reactivity in the MID *P. acnes* Test: *Staphylococcus spp.* (*epidermidis (4), aureus (4), caprae, capitis, lugdunensis,* and *hominis)*, *Candida spp.* (*glabrata, albicans,* and *tropicalis*), *Streptococcus spp.* (*agalactiae (3)* and *mitis/oralis*), *Enterococcus faecalis (3)*, *Pseudomonas aeruginosa (7)*, *Escherichia coli (8), Corynebacterium striatum (2),* and *Serratia marcescens (3)*. This panel represents 80-90% of organisms cultured from patient synovial fluid obtained from PJI. The microorganisms were tested at $1x\ 10^4$ - $1x\ 10^6$ CFU/mL. There was no cross-reactivity observed in the MID *P. acnes* assay with any of the microbial isolates tested up to 1×10^6 cfu/mL.

REFERENCES

1. NCCLS. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. NCCLS document EP5-A2 [ISBN 1-56238-542-9]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2004.
2. 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.
3. CLSI. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition (Interim Revision)*. CLSI document EP09-A2-IR (ISBN 1-56238-731-6). Clinical Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2010.
4. CLSI EP12-A2 *User protocol for Evaluation of Qualitative Test Performance, Approved Guideline, Second Edition*. Clinical Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2010

SYMBOL KEY

	Instructions for Use (Read)		Lot Number
	Item Number		Manufacturer
	Storage Temperature Limit		Authorized Representative
	Expiration Date		Do Not Reuse

CD Diagnostics, Inc
650 Naamans Rd, Suite 100
Claymont, DE 19703 USA