510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number: k05 2485

B. Purpose for Submission: New 510k

C. Measurand: Fatty acid methylated esters extracted from bacterial cell membranes

D. Type of Test: Gas Chromatographic, qualitative identification of *B. anthracis*

E. Applicant: MIDI, Inc.

F. Proprietary and Established Names: MIDI Sherlock Microbial Identification System BioDef™ and Gas chromatography, *Bacillus anthracis* membrane fatty acids

G. Regulatory Information:

1. Regulation section: Unclassified
2. Classification: Unclassified
3. Product code: NWZ, Gas chromatography, Bacillus anthracis membrane fatty acids
4. Panel: 83

H. Intended Use:

1. Intended use(s): aid in the identification of B. anthracis
2. Indication(s) for use:

The MIDI Sherlock Microbial Identification System for BioDef™ is intended to aid in the identification of *Bacillus anthracis* through the analysis of membrane fatty acids derived from cultured bacterial samples, using gas chromatography and pattern recognition software.

Warnings:

- The Sherlock BioDef system is not intended for use with spore preparations or materials other than colonies from trypticase soy agar (with 5% defibrinated sheep blood) that have been presumptively identified as *Bacillus* spp. BioDef cannot assess presence or absence of virulence factors.
- Accurate identification of *B. anthracis* is dependent on purity of isolates, length of incubation (24 h) at 35º C, and media type, along with well-trained and experienced technicians. Only

BBL™ 5% sheep blood agar has been validated for identifying *B. anthracis* with the MIDI Sherlock Microbial Identification System for BioDef™.

- The definitive identification of *B. anthracis* from colony growth requires additional testing and confirmation procedures in consultation with public health or other authorities to whom reporting is required.
- Identification of organisms other than *B. anthracis* in the Sherlock BioDef's system's BTR library database has not been evaluated.

3. Special conditions for use statement(s):

Prescription Use

4. Special instrument requirements: Gas chromatograph equipped with a 25 m capillary column having dimensions of 0.2 mm internal diameter and 0.33 film thickness of 5% phenol methyl siloxane as the liquid phase. Requires hydrogen as carrier gas and a flame ionization detector using air and nitrogen. Oven temperature must increase linearly from 170 to 270° during analysis and an autoinjector to inject 2μL.

I. Device Description:

MIDI Sherlock Microbial Identification System for BioDef™ is based on gas chromatorgraphy (GC) with a 25 x 0.2 mm capillary column (0.33-film thickness of 5% diphenyl-95%-dimethyl siloxane), an oven with 170-270°C increase range during analysis, an autoinjector, and MIDI Sherlock Microbial Identification System software that includes the BTR library [installed on a PC, HP or Dell, with 8 gb HD, 128 mb RAM, 450MHz, Pentium III or higher, CD ROM drive and LAN interface (Lan card or GPIB card); GPIB communication, NT 4.0, SP 5 or higher, 2000 or XP]. H_2 carrier gas and N_2 makeup gas (both 99.999% pure) is needed along with industrial grade air. *B. cereus* (ATCC 14579) is run as a control. Reagents must be prepared using ACS NaOH and NaCl with HPLC grade methanol, hexane, and methyl-tert-butyl ether.
The BTR library is used with the standard clinical (CLIN) database. The program is written in Microsoft Visual Basic vrs 6.0.

A calibration standard (made and sold by Microbial ID (a sister company to MIDI) is also needed to perform the testing procedure. The standard is a mixture of fatty acids used to characterize peaks based on retention time.

Fatty acid methyl ester (FAME) composition of bacterial cell membranes are distinctive. For *B. anthracis*, 13:0 ISO and 17:0 ANTEISO are unique and distinguishing from other Bacillus spp. The BTR library contains approximately 150 Bacillus spp. and is used to match similarity of an unknown to the library profiles. A Similarity Index (SI) is calculated that expresses the relative distance of an unknown's profile from the population mean for a particular species. The SI reflects both distance and variance from the mean (4.69 standard deviations is maximum for a 4.0 SI).

J. Substantial Equivalence Information:

1. Predicate device name(s): Tetracore RedLine Alert™

2. Predicate 510(k) number(s): k030370

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Aid in the identification of *B. anthracis* grown on solid culture medium	Presumptive identification of *B. anthracis* from non-hemolytic Bacillus colonies cultured on sheep blood agar plates
Samples	Solid media colony isolates	Solid media colony isolates
Media growth	22-26 h 5% sheep blood agar growth	12-24 h 5% sheep blood agar growth

Differences		
Item	Device	Predicate
Reagents	Calibration Standard	Buffer and test cassette
Technology	Gas chromatographic characterization of fatty acid methyl esters	Lateral flow immunoassay detecting a cell surface protein
Instrumentation	Gas chromatograph with automated peak naming and library comparison using pattern recognition software	None – visual recognition
Sample Preparation	Saponification, extraction, and derivitization of membrane fatty acids	Suspend colony in buffer
Biosafety indications	BSL-2, BSL-3 practices	BS level-2
Quality control recommended	*Bacillus cereus* culture *B. anthracis* Sterne culture	Lyophilized antigen from *B. anthracis* Sterne

K. Standard/Guidance Document Referenced (if applicable): NA

L. Test Principle:

Growth from 24 h 5% SBA is treated to saponify and methylate fatty acids, followed by extraction into an organic solvent. Fatty acid methyl esters are characterized by gas chromatography, using retention times to calculate ECLs (equivalent chain length) based on comparison to a calibration standard containing a mixture of saturated fatty acids (9-20 carbons length). Software compares ECL values of straight chain and branched chain acids to those of known isolates. Capillary columns are calibrated for variations and long-term drift to center ECLs of polar hydroxy fatty acids with expected ECLs in the library.

Temperatures and gas pressures may be adjusted to calibrate drift. Samples must be prepared from sufficient growth to allow detection of smaller fatty acid peaks. Contaminating peaks can occur from a faulty injection port liner or septum. Testing is designed to be run on the Agilent 6850 or 5890 gas chromatographic systems.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

B. anthracis and non-B. anthracis samples were tested at 11 different laboratories. The % correct identifications overall in these labs for the B. anthracis samples was 96.3% and for non-B. anthracis was 99.7%.

	Number of Samples	**Positive for B. anthracis[@]**	**Negative for B. anthracis[@]**	**% Correct**
Bacillus-anthracis	383	369	14	96.3%
Near Neighbors*	381	1	380	99.7%

b. Linearity/assay reportable range: NA

c. Traceability, Stability, Expected values (controls, calibrators, or methods): Calibrator standard is a mixture of fatty acid methy esters (n-hexane, normal hexane, hexyl hydride).

d. Detection limit: NA, a sufficient amount of growth must be used (40 mg or one heaping 4 mm loopful).

e. Analytical specificity: 169 samples of 8 non-anthracis *Bacillus* species most closely similar phenotypically to *B. anthracis* were correctly separated

f. Assay cut-off: A BA similarity index must be >0.400 to correctly identify as *B. anthracis*

2. Comparison studies:

a. Method comparison with predicate device: Not done

b. Matrix comparison: NA

3. Clinical studies: NA – the MIDI test uses solid culture media growth and is dependent on culture for recovery of growth from clinical specimens or other sample types. Evaluations were performed with B. anthracis and near-neighbor isolates, some of which were obtained originally from clinical specimens

a. Clinical Sensitivity:

b. Clinical specificity:

c. Other clinical supportive data (when a. and b. are not applicable):

The *B. anthracis* entry of the Sherlock ***BioDef***™ library contains 116 analyses of 66 strains of the pathogen and 169 analyses of 8 near neighbor *Bacillus* species. It was earlier determined that only *B. cereus, B.* thuringiensis and *B. mycoides* are sufficiently close in FAME profile to potentially give rise to problems of discrimination. The comparison species, and numbers of strains and analyses are as follows.

B. cereus	(38 analyses)	*B. megaterium*	(11 analyses)
B. circulans	(37 analyses)	*B. mycoides*	(10 analyses)
B. coagulans	(31 analyses)	*B. subtilis*	(16 analyses)
B. licheniformis	(8 analyses)	*B. thuringiensis*	(18 analyses)

By comparison to more than 400 species entries in the clinical (Sherlock CLIN version 5.0) library, no other species was likely to give FAME profiles similar to *B. anthracis*.

	# Species	**# Samples**	**Positive for *B. anthracis*@**	**Negative for *B. anthracis*@**	**Percent Correct**
Bacillus-anthracis	1	116	116	0	100%
Non-anthracis *Bacillus* Species*	8	169	0	169	100%
Other Bacterial Species**	12	303	0	303	100%

4. Clinical cut-off: Discriminating *B. anthracis* from other *Bacillus* spp. using a >0.400 Similarity Index based on a maximum distance of 4.69 standard deviations from the mean for the species.

5. Expected values/Reference range:

Average Percent of Cellular Fatty Acid (CFA) for Bacillus entries in BTR20 library database

	anthracis A		**anthracis B**		**anthracis C**		**cereus A**		**cereus B**	
Fatty Acid Peak	Mean %	SD*	Mean %	SD	Mean %	SD	Mean %	SD	Mean %	SD
13:0 ISO	2.82	0.56	0.39	0.16	4.02	2.98	7.02	0.51	8.2	1.23
14:0 ISO	1.75	0.48	1.26	0.34	2.76	1.42	2.63	0.58	3.06	1.27
14:0	2.29	0.45	2.2	0.47	2.14	0.96	2.89	0.26	2.7	0.16
15:0 ISO	44.85	3.32	47.55	2.83	26.21	5.94	40.05	1.35	41.33	5.65
15:0 ANTE	4.02	0.97	3.66	0.74	3.94	1.34	3.03	0.57	4.39	0.46
16:1 w7c alcohol	0	0	1.79	0.23	0	0	0	0	0	0

16:0 ISO	5.98	1.29	8.73	1.28	8.93	2.87	3.92	0.79	4.99	1.54
16: w11c	0	0	1.26	0.09	0	0	0	0	0	0
16:0	3.91	0.62	4.94	0.59	6.22	2.05	3.17	0.36	5.51	0.61
ISO 17:1 w7c	0	0	4.11	0.88	0	0	2.31	0.61	1.98	1.32
ISO 17:1 w5c	5.06	1.54	1.58	0.61	6.01	1.12	7.18	0.65	2.12	0.81
17:1 ANTE A	1.6	0.41	0.67	0.26	1.96	0.37	0	0	0	0
17:0 ISO	11.57	2.02	9.05	1.84	18.06	5.91	8.48	0.72	9.83	1.47
17:0 ANTE	2.48	0.76	3.49	0.75	3.54	0.72	0	0	1.77	0.54
18:1 w9c	1.16	0.46	1.52	0.21	0.69	0.59	3.35	0.68	4.57	2.37
18:0	1.04	0.44	0.7	0.27	1.72	0.63	1.48	0.62	1.41	0.73
Summed 2	2.27	0.68	1.02	0.14	2.58	1.39	2.26	0.44	0.86	0.98
Summed 3	7.99	1.06	4.53	0.53	9.74	4.13	9.42	0.54	5.5	2.07
Summed 5	0.91	0.42	1.29	0.51	0.57	0.52	1.64	1.08	0.77	0.6

	circulans		**coagulans A**		**coagulans B**		**coagulans C**		**licheniformis**	
Fatty Acid Peak	Mean %	SD	Mean %	SD	Mean %	SD	Mean %	SD	Mean %	SD
13:0 ISO	0	0	0	0	0	0	0	0	0	0
14:0 ISO	3.62	1.02	0	0	6.88	0.66	0	0	0	0
14:0	2.24	0.79	1.44	0.08	2.94	0.24	0.63	0.6	0	0
15:0 ISO	11.33	2.69	6.79	0.11	14.36	0.62	8.15	5.03	39.1	4.46
15:0 ANTE	53.32	4.17	58.02	0.6	32.04	0.64	45.45	7.24	24.82	2.98
16:1 w7c alcohol	0	0	0	0	0	0	0	0	0	0
16:0 ISO	7.68	3.04	2.06	0.12	10.96	0.34	2.61	1.83	3.33	0.53
16: w11c	0	0	0	0	0	0	0	0	0.41	0.38
16:0	7.29	1.78	13.81	0.64	14.96	0.39	9.38	1.02	2.97	0.35
ISO 17:1 w7c	0	0	0	0	0	0	0	0	1.24	0.87
ISO 17:1 w5c	0	0	0	0	0	0	0	0	0	0
17:1 ANTE A	0	0	0	0	0	0	0	0	0	0
17:0 ISO	1.3	0.99	0	0	3.4	0.32	1.88	1.74	14.82	2.09
17:0 ANTE	9.37	1.46	14.73	0.38	12.8	0.59	30.74	2.82	11.14	2.04
18:1 w9c	0.73	0.95	0	0	0	0	0	0	0	0
18:0	2.2	1.5	3.15	0.31	0.92	0.86	0.82	0.76	0.79	0.78
Summed 2	0	0	0	0	0	0	0	0	0	0
Summed 3	0	0	0	0	0	0	0	0	0	0
Summed 5	0	0	0	0	0	0	0	0	0	0

	megaterium		**mycoides**		**subtilis**		**thuringiensis A**		**thuringiensis B**	
Fatty Acid Peak	Mean %	SD	Mean %	SD	Mean %	SD	Mean %	SD	Mean %	SD
13:0 ISO	0	0	9.61	1.13	0	0	10.12	0.4	8.22	1.35
14:0 ISO	7.5	0.46	2.18	0.33	0.88	0.23	4.57	0.33	5.12	1.27
14:0	1.72	0.41	2.97	0.59	0	0	4.21	0.24	3.56	0.46
15:0 ISO	29.27	6.24	35.44	2.35	24.7	3.94	34.95	1.7	34.76	3
15:0 ANTE	46.36	6.37	2.43	1.14	36.23	3.82	4.62	0.12	2.74	0.78
16:1 w7c alcohol	0	0	0.58	0.5	0	0	1.13	0.05	0.55	0.17
16:0 ISO	1.73	0.62	5.33	0.73	2.68	0.72	5.43	0.24	7.64	1.52

16: w11c	1.63	0.74	1.13	0.98	0.71	0.22	0	0	0	0
16:0	5.21	0.46	7.82	0.96	3.51	1.31	3.77	0.51	4.85	0.95
ISO 17:1 w7c	0	0	2.41	2.08	1.6	0.52	3.08	0.14	1.76	0.42
ISO 17:1 w5c	0	0	1.96	1.34	0	0	3.78	0.21	3.73	0.91
17:1 ANTE A	0	0	0	0	0	0	0.94	0.04	0.59	0.13
17:0 ISO	1.35	0.68	11.23	3.72	15.56	2.68	4.58	0.21	9.65	1.52
17:0 ANTE	2.47	0.48	1.31	0.49	11.85	2.15	0.66	0.33	0.99	0.3
18:1 w9c	1.55	0.34	2.5	0.6	0	0	0	0	2.54	0.92
18:0	0.92	0.44	1.62	0.4	0.79	0.53	0	0	0.79	0.2
Summed 2	0	0	0	0	0	0	2.94	0.11	2.33	0.57
Summed 3	0	0	6.38	1.65	0	0	12.32	0.27	7.7	0.81
Summed 5	0	0	1.98	0.71	0	0	0	0	0.84	0.27

* SD = standard deviation

N. Instrument Name: Agilent 6850 or 5890 gas chromatographic systems with 25 mm capillary columns (0.2mm internal diameter, 0.33 film thickness of 5% phenyl methyl siloxane in liquid phase).

O. System Descriptions:

1. Modes of Operation: 5% phenyl methyl silicone fused silica capillary column with H_2 carrier gas, along with air and N_2 for flame ionization. Injector temperature = 250°C; detector = 300°C

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___x___ or No _____

3. Specimen Identification:

   NA

4. Specimen Sampling and Handling:

   NA

5. Calibration:

   An external calibration standard is used for each batch

6. Quality Control:

For 48 calibration standard determinations, the average SI was 0.998 with a range of 0.989-1.00. The manufacturer recommends a *B. cereus* and *B. anthracis* Sterne as control organisms for adequate saponification and methylation of fatty acids, and also for correct identification.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.