510(k) SUMMARY

Date of Summary: October 28, 2013

Product Name MBT-CA System

Sponsor: Bruker Daltonics, Inc
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Device Identification

Trade or Proprietary Name: MALDI Biotyper CA System
Common or Usual Name: Mass spectrometer for clinical multiplex test systems
Product Code: PEX
Regulation Section: 21 CFR 862.3361 Instrumentation for clinical multiplex test systems
Device Class: Class II (special controls)
Panel: Microbiology

Substantial Equivalency

The Bruker Daltonics, Inc MBT-CA System is substantially equivalent to the Vitek® MS MALDI-TOF mass spectrometer system (K124067). Table 1 compares the characteristics of the MBT-CA System (New Device) and the Vitek® MS (predicate device).

Comparison of New Device with Predicate Device

TABLE 1: Substantial Equivalency Table

Similarities		
Characteristic	**NEW DEVICE** **Bruker Daltonics, Inc MBT-CA System** **(K130831)**	**PREDICATE DEVICE** **Vitek® MS** **(K124067)**
Product Codes	PEX	PEX
Intended use	The Bruker Daltonics, Inc MALDI Biotyper CA System is a qualitative *in vitro* diagnostic mass spectrometer system for the identification of Gram-negative bacterial colonies cultured from human specimens using matrix-assisted laser desorption/ ionization - time of flight (MALDI-TOF) mass spectrometry technology. The MALDI Biotyper CA System is indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of Gram negative bacterial infections.	The Vitek® MS is a mass spectrometer system using matrix-assisted laser desorption/ionization-time to flight (MALDI-TOF) for the identification of microorganisms cultured from human specimen. The VITEK MS is a qualitative in vitro diagnostic device indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial and yeast infections.
Sample type	Isolated colony from any patient sample source. Acceptable media: • Columbia blood agar with 5% sheep blood • Trypticase soy agar with 5% sheep Blood • Chocolate agar • MacConkey Agar	Isolated colony from any patient sample source. Acceptable media: • Columbia blood agar with 5% sheep blood • Trypitcase soy agar with 5% sheep Blood • Chocolate polyvitex agar • Campylosel agar • MacConkey Agar • Modified Sabouraud dextrose Agar • ChromID CPS
Type of Test	Automated Mass Spectrometry System	Automated Mass Spectrometry System
Matrix	α-Cyano-4-hydroxycinnamic acid	α-Cyano-4-hydroxycinnamic acid
Method of Testing	Bacteria: Direct testing If after initial analysis the log(score) is reported at < 2.00, organisms are processed using the extraction procedure.	Bacteria: Direct testing

Similarities		
Characteristic	**NEW DEVICE** **Bruker Daltonics, Inc MBT-CA System** **(K130831)**	**PREDICATE DEVICE** **Vitek® MS** **(K124067)**
Result Reporting	Organism identification is reported with high confidence if the log(score) is ≥ 2.00. An organism identification is reported with low confidence if the log(score) is between 1.70 and <2.00.	A single identification is displayed, with a confidence value from 60.0 to 99.9, when one significant organism or organism group is retained. "Low-discrimination" identifications are displayed when more than one but not more than four significant organisms or organism groups are retained. When more than four organisms or organism groups are found, or when no match is found, the organism is considered unidentified.
Matching Algoritm	Calculates matches by comparing a new spectrum against each single reference entry of a reference database.	Uses a proprietary process called "mass binning." In this process, the spectrum between 3,000 and 17,000 Daltons are divided into 1300 pre-defined intervals called "bins". Next, an algorithm based on supervised machine learning known as the "Advanced Spectrum Classifier", is used to determine how informative each bin was in differentiating that species from all other species in the database.
Recorded mass range	2,000 - 20,000 m/z	2,000 - 20,000 m/z

Differences		
Characteristic	**NEW DEVICE Bruker Daltonics, Inc MBT-CA System (K130831)**	**PREDICATE DEVICE Vitek® MS (K124067)**
Culture Age	Bacteria growth should be between 18h to 36h	Bacteria and yeast growth should be between 24 to 72 hours.
Calibration	Bruker US IVD Bacterial Test Standard (BTS)	E. coli ATCC 8739
MALDI Target Plate	US IVD 48 Spot Target • 48 positions reusable steel targets	VITEK MS-DS Target Slides • 48 positions disposable plastic targets
MALDI-TOF MS instruments	Bruker microflex (benchtop)	Shimadzu AXIMA® Assurance MS (floor standing)
Database	MALDI Biotyper for Clinical Applications (MBT-CA)	VITEK® MS V2.0 Knowledge Base

These differences do not affect substantial equivalence of the MBT-CA System and Vitek® MS system. Both systems are mass spectrometer systems using matrix-assisted laser desorption/ionization-time to flight (MALDI-TOF) for the identification of microorganisms cultured from human specimens. The differences noted above do not impact the intended use and do not raise questions as to the safety and effectiveness of the test (new) device.

Intended Use

The Bruker Daltonics, Inc MALDI Biotyper CA System is a qualitative *in vitro* diagnostic mass spectrometer system for the identification of Gram-negative bacterial colonies cultured from human specimens using matrix-assisted laser desorption/ ionization - time of flight (MALDI-TOF) mass spectrometry technology.

The MALDI Biotyper CA System is indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of Gram negative bacterial infections.

Methodology

Biochemical methods are currently the most commonly used methods for the identification of microorganisms. Organisms are tested against a range of reagents and organism identification is based on a microorganism's reaction to these reagents.

The MBT-CA System uses a different methodology for organism identification based on unique protein patterns of the microorganisms obtained from mass spectrometry. The test organism's spectrum (a pattern of mass peaks) is compared with a reference spectra library (database). Using biostatistical analysis, a probability ranking of the organism identification is generated. The probability ranking is represented as a log(score) between 0.00 and 3.00. Organism identification is reported with high confidence if the log(score) is ≥ 2.00. An organism identification is reported with low confidence if the log(score) is between 1.70 and <2.00.

Organisms to be identified with the MBT-CA System should be isolated for purity on appropriate isolation media.

Direct Transfer (DT): An individual colony from an overnight subculture plate is transferred to a selected position on an US IVD 48 Spot Target plate (target). The target is air dried and US IVD HCCA portioned (matrix) is added. The standard solvent (50% acetonitrile / 47.5% H_2O / 2.5% trifluoroacetic acid) in the matrix solution extracts proteins (mainly ribosomal proteins, which are present in high concentration) from the microorganisms. When dried matrix crystallizes, the inoculated target is ready to be analyzed on the MBT-CA System. If after initial analysis the log(score) is reported at < 2.00, organisms can be processed using the extraction procedure and analysis repeated.

Extraction Procedure (Ext): If after initial analysis the log(score) is reported at < 2.00, organisms are processed using the extraction procedure and analysis repeated. For this purpose, isolated colonies from the overnight subculture plate are extracted using ethanol / formic acid procedure. Afterwards they are transferred to the target and treated as described above.

MALDI-TOF Analysis:

Samples are analyzed using MALDI (matrix-assisted laser desorption/ionization) TOF (time-of-flight) mass spectrometry. The matrix transfers protons onto the extracted proteins and absorbs UV light. A laser in the MALDI- TOF mass spectrometer irradiates the matrix sample composite, causing evaporation and release of positively charged intact proteins and peptides ("soft" ionization technique). These ions are electrostatically accelerated over a short distance and arrive in the flight tube at a mass-dependent speed. As different proteins/peptides have different masses, ions arrive at the detector at different times (time of flight). The system measures the time (in the nanosecond range) between pulsed acceleration and the corresponding detector signal, the speed is converted into an exact molecular mass. The mass-to-charge ratio of an ion is proportional to the square of its drift time.

Highly abundant microbial proteins (mainly ribosomal proteins) result in a mass spectrum with characteristic mass and intensity distribution. It is species-specific for many bacteria and is interpreted as a molecular fingerprint to identify the test organism.

Data acquisition is controlled with MBT-CA Software. The spectrum of the unknown organism is first transformed into a peak list. Using a biostatistical algorithm, this peak list is compared to the reference peak lists of organisms in the reference library (database) and a log(score) is generated. A higher log(score) indicates a higher degree of similarity to the organism in the reference library. Organism identification is reported with high confidence if the log(score) is ≥ 2.00. An organism identification is reported with low confidence if the log(score) is between 1.70 and <2.00.

The log(score) ranges, defined in the MBT-CA System, are indicative of the probability of organism identification. Results should be reviewed by a trained microbiologist and final organism identification should be based on all relevant information available. This information includes but is not limited to: Gram staining, colony morphology, growth characteristics, sample matrix, etc.

Performance Data

Precision/Repeatability:

Validation of the whole MALDI Biotyper CA System was performed on twelve (12) working days with two (2) runs/day following manufacturer's instructions for use. Ten (10) test organisms were tested in triplicate in each run. The study also tested multiple sources of system variability including three (3) test operators, three (3) microflex LT/SH instruments, three (3) target lots, three (3) BTS lots and three (3) Matrix lots. As triplicates of each test organism were prepared and tested in each run, a total of 72 MALDI Biotyper CA System identifications were carried out for each test organism. Overall results from the precision/repeatability study are presented below.

Table 2: Overall Precision per Test Organism

Test Organism	# samples measured	# samples passed (DT)	# samples passed (DT+Ext)
Acinetobacter baumannii	72	62 (86.1%)	72 (100%)
Pseudomonas aeruginosa	72	70 (97.2%)	72 (100%)
Stenotrophomonas maltophilia	72	70 (97.2%)	72 (100%)
Enterobacter cloacae	72	70 (97.2%)	72 (100%)
Escherichia coli	72	71 (98.6%)	72 (100%)
Hafnia alvei	72	72 (100%)	72 (100%)
Proteus mirabilis	72	72 (100%)	72 (100%)
*Brevundimonas diminuta**	72	72 (100%)	72 (100%)
Haemophilus influenzae	72	72 (100%)	72 (100%)
Moraxella catarrhalis	72	72 (100%)	72 (100%)

** Brevundimonas diminuta was tested but is not included in the claim.*

Table 3: Overall Precision per Test Organism Average log(score)

Test Organism	# samples measured	Average log(score) [DT+Ext]
Acinetobacter baumannii	72	2.172 ± 0.113
Pseudomonas aeruginosa	72	2.368 ± 0.121
Stenotrophomonas maltophilia	72	2.364 ± 0.078
Enterobacter cloacae	72	2.138 ± 0.064
Escherichia coli	72	2.385 ± 0.097
Hafnia alvei	72	2.452 ± 0.095
Proteus mirabilis	72	2.587 ± 0.079
*Brevundimonas diminuta**	72	2.489 ± 0.045
Haemophilus influenzae	72	2.344 ± 0.118
Moraxella catarrhalis	72	2.523 ± 0.080

**Brevundimonas dimiuta was tested but is not included in the claim.*

Based upon the data presented, the study confirms repeatability and precision of the MALDI Biotyper CA System independent from:

- System Operators
- microflex LT/SH instruments
- Target Production Lots
- Matrix Lots
- BTS Lots

Limit of Detection/Dynamic Range:

The Limit of Detection study was designed to establish the estimated dynamic range of sample size for both the Direct Transfer (DT) and Extraction method (Ext) procedure. Seven (7) frequently occurring clinically relevant test organisms were chosen for this study. Cell density and cell concentration were estimated by measuring the optical density of the suspension at a wavelength of 600nm. Approximately $3x10^8$ cells/mL were reported to correspond to an optical density of $OD_{600} = 1$ according to the commonly used McFarland Standard. All suspensions were tested in duplicate. Each cell stock was diluted to a minimum five (5) concentrations and tested in duplicate. A cell concentration was considered within the dynamic range if the MBT-CA correctly identified the organism for both replicates with a log(score) of ≥2.00.

Study results concluded that the estimated dynamic range for the direct and extracted method is as follows:

Technique	Lower limit [cells/µL]	Upper limit [cells/µL]
Direct Transfer	$6.3x10^3$–$1.4x10^4$	$1.4x10^6$–≥$6.5x10^7$
Extraction	$9.0x10^3$–$1.3x10^5$	$1.1x10^7$ –≥$6.9x10^7$

Specificity:

The goal of the specificity study was to validate the performance of the proposed MALDI Biotyper CA System reference library by ensuring that organisms not included in the reference library would not yield an incorrect identification and would be reported as "No Identification." Additionally, the study was designed to further demonstrate that the MALDI Biotyper CA System identification is not impacted when closely related species not included in the reference library are run on the system.

The study was conducted in two phases. In phase one, organisms currently not included in the initial system reference library were tested via Direct Transfer and extraction method to ensure that the organisms would not be falsely identified by the MALDI Biotpyer CA system. Organisms tested fell into the following five groupings:

- Anaerobe bacteria
- Mycobacteria
- Gram-Negative bacteria (not currently claimed within the library)
- Gram-Positive bacteria
- Yeast species

Results from this phase are presented below:

Table 4: Phase 1: Summary Results

Organism	Strain	# of "No Identification"		# of false identification
		DT	Ext	
Bacteroides fragilis	DSM 2151	2 / 2	2 / 2	0
Bacteroides fragilis	DSM 9669	2 / 2	2 / 2	0
Prevotella copri	DSM 18205^T	2 / 2	2 / 2	0
Prevotella buccae	DSM 19025^T	2 / 2	2 / 2	0
Mycobacterium fortuitum ssp. *fortuitum*	DSM 43477	2 / 2	2 / 2	0

Organism	Strain	# of "No Identification"		# of false identification
		DT	Ext	
Mycobacterium fortuitum ssp. *fortuitum*	DSM 46621^T	2 / 2	2 / 2	0
Neisseria gonorrhoeae	DSM 9188^T	2 / 2	2 / 2	0
Neisseria gonorrhoeae	DSM 15130	2 / 2	2 / 2	0
Erwinia tasmaniensis	DSM 17949	2 / 2	2 / 2	0
Erwinia tasmaniensis	DSM 17950	2 / 2	2 / 2	0
Vagococcus fluvialis	DSM 5731^T	2 / 2	2 / 2	0
Vagococcus fluvialis	DSM 21402	2 / 2	2 / 2	0
Facklamia hominis	CCUG 59179	2 / 2	2 / 2	0
Facklamia hominis	CCUG 49614	2 / 2	2 / 2	0
Guehomyces pullulans	CBS 2532^T	2 / 2	2 / 2	0
Guehomyces pullulans	CBS 2542	2 / 2	2 / 2	0
Cyberlindnera mississippiensis	CBS 7023^T	2 / 2	2 / 2	0
Cyberlindnera mississippiensis	CBS 7027	2 / 2	2 / 2	0

In phase two of the study testing, *Burkholderia cepacia/multivorans/gladioli* were investigated via Direct transfer and extraction method to ensure that closely related organisms can be differentiated when tested on the MALDI Biotyper CA System. Results from this phase of testing are reported below:

Table 5: Phase 2: Summary Results

Organism	Strain	# of Correct Identifications		# of false identification
		DT	Ext	
Burkholderia cepacia	DSM 9241	2 / 2	2 / 2	0
Burkholderia cepacia	DSM 50181	2 / 2	2 / 2	0
Burkholderia multivorans	1A11237234_4v MVD	2 / 2	2 / 2	0
Burkholderia multivorans	H480 MCRF	2 / 2	2 / 2	0
Burkholderia gladioli	DSM 8361	2 / 2	2 / 2	0
Burkholderia gladioli	LMG 6956	2 / 2	2 / 2	0

Phase 1 data demonstrates with high confidence that Anaerobes, Mycobacteria, Gram-negative, Gram-positive and Yeast organisms not included in the MALDI Biotyper CA database are not identified confirming the specificity of the MALDI Biotyper CA reference library when following product instructions for use for both DT and extraction method. Phase 2 data confirms that closely related species can be unambiguously identified by the MALDI Biotyper CA System.

Mixed Culture:

Although MALDI Biotyper CA System users will be instructed to select only a single isolated colony for identification on the MALDI Biotyper CA System this study was conducted to assess the effect of testing a mixed culture on MALDI Biotyper CA identification. *Pseudomonas aeruginosa,* a frequently occurring Gram negative bacterium was chosen as the target organism for this study. Four (4) non-target organisms consisting of gram-negative and gram-positive bacteria where introduced with the target organism at varying concentrations to determine the affect a mixed culture would have on

MBT-CA identification.

Table 6: Summary of Mixed Culture Study

Condition	Target Organism Amount	Non-Target Organism Amount	# of MALDI Biotyper CA False Identifications
A	100%	0%	0/32
B	75%	25%	0/32
C	50%	50%	0/32
D	25%	75%	0/32

Although system users will be instructed to test a single isolated colony on the MALDI Biotyper CA System, it is important to note that when a mixed culture is analyzed on the system, no false results are obtained and the impact on final test results is greatly reduced when compared to the issues observed with alternative biochemical methods.

Media and Colony Stability

In accordance with product instructions for use, customers are advised that primary or secondary isolation plates of recommended media [Trypticase Soy Agar with 5% sheep blood (TSA), Columbia Blood Agar with 5% sheep blood (CBA), MacConkey Agar (MAC), and Chocolate Agar (Choc)] may be held for up to 12 hours at room temperature prior to testing on the MALDI Biotyper CA System. This study was conducted to confirm the acceptability of the recommended agar/media and stability of the colony for up to 12 hours prior to analysis.

Testing was conducted using seven (7) gram-negative organisms at two different incubation time points (18h, 24h). After initial incubation, isolates were further tested at two (2) temperatures (18°C, 25°C) for 12 hours post-incubation.

Table 7: Summary of Media and Colony Stability Study

Media	≥2.0 Identification (DT)	False Identification (DT)	≥2.0 Identification (Ext)	False Identification (Ext)
TSA	288/288	0/288	288/288	0/288
CBA	284/288	0/288	288/288	0/288
MAC	263/288	0/288	288/288	0/288
CHOC	288/288	0/288	288/288	0/288

The study results confirm that the following culture media can be used on the MALDI Biotyper CA System:

- Trypticase Soy Agar with 5% sheep blood (TSA)
- Columbia Blood Agar with 5% sheep blood (CBA)
- MacConkey Agar (MAC)
- Chocolate Agar (CHOC)

Study results conclude that sample colony is stable for up to 12 hours post-incubation.

Influence of Agar Media

This study was completed in order to demonstrate that impurities such as salts, peptides or carbohydrates introduced from culture media do not interfere with MALDI Biotyper CA identification. In

addition, the study set-out to prove that isolation media alone would not generate mass spectra leading to false identification on the MBT-CA system. TSA, CBA, MAC and CHOC agars were tested by the following methods:

- Each agar media was inoculated using the Direct Transfer (DT) and Extraction (Ext) method alone (6) six times each.
- Three (3) frequently occurring Enterobacteriacea and non-fermenting Gram Negative Bacteria were transferred to the target plate in duplicate via DT and Ext method to serve as a control.
- Each target organism was then inoculated in duplicate via DT and Ext method such that a sample agar media was included with the isolate.

A summary of results obtained is provided below:

Table 8: Summary of Influence of Agar Media Study

Media	Agar Alone		Target Organism Alone		Target Organism + Agar	
	# replicates	% No ID	# replicates	% False ID	# replicates	% False ID
TSA	12/12	100	12/12	0	10/12	0
CBA	12/12	100	12/12	0	12/12	0
MAC	12/12	100	12/12	0	12/12	0
CHOC	12/12	100	12/12	0	12/12	0

The study confirms that the media recommended for use on the MALDI Biotyper CA System do not interfere with MBT-CA performance or organism identification.

Organism Stability prior to MALDI Biotyper CA System Analysis

This study was conducted to assess isolate stability on the target plate prior to matrix overlay via Direct Transfer (DT) and Extraction (Ext) method. In addition, the study set out to confirm the stability of extracted material prior to target plate inoculation.

To test for isolate stability on the target plate prior to matrix overlay via DT, three (3) common gram negative bacteria were inoculated eight times and overlaid with matrix at five (5) different time points. After matrix overlay, isolates were tested in accordance with product instructions. For the Ext method, colonies were prepared following the extraction technique per product instructions for use. Extracts were overlaid with matrix at five (5) different time points and tested per product instructions. For the third phase of testing, the three (3) gram negative isolates were extracted twice. The extracts were stored at controlled room temperature for up to 24 hours and tested at five (5) time points in replicates of eight.

A summary of results obtained is provided below:

Table 9: Summary of Organism Stability Prior to MBT-CA Analysis Study

Test Phase	Testing Condition	Measurands	Correct Identification	False Identification
Direct Transfer (DT)	0 min	24	24/24	0/24
	15 min	24	24/24	0/24
	30 min	24	24/24	0/24
	60 min	24	24/24	0/24
	120 min	24	24/24	0/24

Test Phase	Testing Condition	Measurands	Correct Identification	False Identification
Extraction Method (Ext)	0 min	6	6/6	0/6
	10 min	6	6/6	0/6
	20 min	6	6/6	0/6
	30 min	6	6/6	0/6
	60 min	6	6/6	0/6
Extract #1	0 hour	24	24/24	0/24
	1 hour	24	24/24	0/24
	4 hours	24	24/24	0/24
	8 hours	24	24/24	0/24
	24 hours	24	24/24	0/24
Extract #2	0 hour	24	24/24	0/24
	1 hour	24	24/24	0/24
	4 hours	24	24/24	0/24
	8 hours	24	24/24	0/24
	24 hours	24	24/24	0/24

Study results confirm that samples are stable on the target plate when tested via DT or Ext method for up to sixty (60) minutes prior to analysis. In addition, extracts are stable for up to 24 hours when stored at room temperature.

Sample Stability overlaid with Matrix

This study was conducted to prove the stability of test organisms on the spotted target plate following matrix addition at various temperature and relative humidity conditions. In addition, the study served to prove that matrix alone will not influence MBT-CA identification. Three (3) gram negative target organisms were cultured on Columbia Blood Agar (CBA) and aging experiments were done at two (2) different temperature and relative humidity testing conditions. For each condition, two (2) target plates were inoculated and each contained four target spots of directly transferred test organism, four spots of extracted test organism and eight spots of matrix solution alone. All spots containing test organism were then overlaid with matrix in accordance with product instructions for use and tested immediately and then stored at one of the testing conditions and retested at 4±1 hour, 8±1 hour and 24±1 hour.

The results of the testing are summarized below:

Table 10: Summary of Sample Stability overlaid with Matrix

Test Condition	Test Age	Test Organism Correct Identification	Matrix "No Peaks Found"
DT **20 ± 1°C, 40 ± 5%**	0 hour	24/24	8/8
	4 hours	24/24	8/8
	8 hours	24/24	8/8
	24 hours	24/24	8/8
Ext **20 ± 1°C, 40 ± 5%**	0 hour	24/24	8/8
	4 hours	24/24	8/8
	8 hours	24/24	8/8
	24 hours	24/24	8/8

Test Condition	Test Age	Test Organism Correct Identification	Matrix "No Peaks Found"
DT **20 ± 1°C, 70 ± 5%**	0 hour	24/24	8/8
	4 hours	24/24	8/8
	8 hours	23/24	8/8
	24 hours	24/24	8/8
Ext **20 ± 1°C, 70 ± 5%**	0 hour	24/24	8/8
	4 hours	24/24	8/8
	8 hours	24/24	8/8
	24 hours	24/24	8/8
DT **25 ± 1°C, 30 ± 5%**	0 hour	24/24	8/8
	4 hours	24/24	8/8
	8 hours	24/24	8/8
	24 hours	24/24	8/8
Ext **25 ± 1°C, 30 ± 5%**	0 hour	24/24	8/8
	4 hours	24/24	8/8
	8 hours	24/24	8/8
	24 hours	24/24	8/8
DT **25 ± 1°C, 70 ± 5%**	0 hour	24/24	8/8
	4 hours	24/24	8/8
	8 hours	18/24	8/8
	24 hours	24/24	8/8
Ext **25 ± 1°C, 70 ± 5%**	0 hour	24/24	8/8
	4 hours	24/24	8/8
	8 hours	23/24	8/8
	24 hours	24/24	8/8

All study results confirmed that inoculated test organisms once overlaid with matrix are stable for up to 24 hours at room temperature. In addition, matrix alone does not interfere or influence MBT-CA identification.

Bacterial Test Standard (BTS) Stability

The Bruker Bacterial Test Standard is an *in vitro* diagnostic product for mass spectrum calibration and optimization as well as a performance control for the identification of microorganisms with the MBT-CA System. The testing summarized below was done to determine the stability of unreconstituted and reconstituted BTS material.

In one study, accelerated and shipping stability of BTS was assessed using three (3) lots of BTS material. Shipping and accelerated stability conditions were simulated by storing eight (8) vials of each BTS lot in a climate controlled chamber at 37±2°C for one (1), two (2) and three (3) weeks. At each time interval, two (2) vials of each BTS lot were removed, allowed to acclimate to room temperature, reconstituted and spotted on sixteen (16) target plate positions (each vial) and three (3) cross-joint positions. Target plates were then analyzed in accordance with product instructions for use.

In the second study, real-time stability of BTS was assessed using three (3) lots of BTS material. The three lot of material were maintained at recommended storage conditions per product instructions (≤-18°C) and tested at 3, 6, 9, 12 and 18 months following the same process as described above.

In the third and final study, in-use (reconstituted) stability of BTS was assessed using four vials of a single lot of BTS reagent. All four vials were reconstituted in accordance with product instructions for use. Two (2) vials each were then pooled. Testing was carried out in replicates of eight (8) immediately following reconstitution then frozen and retested at 1, 2, 3, 4, 5 and 6 months.

The summary of the results of the three (3) studies are provided below:

Table 11: Summary of BTS Stability

Study	Test Age	av. log(score) Lot# 1, Vial 1	av. log(score) Lot# 1, Vial 2	av. log(score) Lot# 2, Vial 1	av. log(score) Lot# 2, Vial 2	av. log(score) Lot# 3, Vial 1	av. log(score) Lot# 3, Vial 2
Accelerated/Shipping Stability	No Aging	2.334 ± 0.027	2.353 ± 0.023	2.344 ± 0.024	2.351 ± 0.019	2.370 ± 0.031	2.345 ± 0.023
	1 week	2.385 ± 0.030	2.362 ± 0.042	2.337 ± 0.034	2.364 ± 0.039	2.372 ± 0.034	2.353 ± 0.023
	2 weeks	2.384 ± 0.021	2.365 ± 0.043	2.344 ± 0.054	2.381 ± 0.034	2.372 ± 0.021	2.349 ± 0.026
	3 weeks	2.391 ± 0.022	2.372 ± 0.027	2.369 ± 0.031	2.347 ± 0.037	2.332 ± 0.031	2.349 ± 0.033
Real-Time Stability	No Aging	2.293 ± 0.030	2.276 ± 0.038	2.333 ± 0.057	2.320 ± 0.031	2.274 ± 0.041	2.250 ± 0.031
	3 months	2.391 ± 0.049	2.387 ± 0.042	2.358 ± 0.050	2.380 ± 0.036	2.296 ± 0.027	2.303 ± 0.019
	6 months	2.318 ± 0.035	2.340 ± 0.036	2.349 ± 0.035	2.338 ± 0.024	2.298 ± 0.029	2.296 ± 0.027
	9 months	2.361 ± 0.037	2.346 ± 0.027	2.356 ± 0.029	2.348 ± 0.028	2.348 ± 0.028	2.320 ± 0.023
	12 months	2.310 ± 0.024	2.327 ± 0.020	2.292 ± 0.033	2.292 ± 0.022	2.262 ± 0.028	2.284 ± 0.026
	18 months	2.414 ± 0.026	2.410 ± 0.043	2.338 ± 0.027	2.346 ± 0.014	2.340 ± 0.034	2.344 ± 0.026

Study	Test Age	av. log(score) Lot# 1, Vial 1	av. log(score) Lot# 1, Vial 2
In-Use Stability	No Aging	2.413 ± 0.038	2.392 ± 0.022
	1 month	2.396 ± 0.030	2.353 ± 0.054
	2 months	2.331 ± 0.020	2.360 ± 0.020
	3 months	2.365 ± 0.022	2.371 ± 0.034
	4 months	2.359 ± 0.017	2.362 ± 0.025
	5 months	2.332 ± 0.024	2.292 ± 0.044
	6 months	2.293 ± 0.023	2.240 ± 0.044

The shipping/accelerated stability study confirmed that BTS is stable for three (3) weeks at temperatures up to 37±2°C. Two of these weeks account for the shipping and one for the long-term storage. Real-time stability testing confirms that BTS is stable for up to 18 months when stored in accordance with product claims. (Bruker only claims stability for 12 months, though.) Lastly, in-use stability confirms that reconstituted BTS is stable for five (5) months when stored in accordance with product claims.

HCCA portioned (Matrix) Stability

HCCA portioned (Matrix) is used when processing test organisms for identification on the MBT-CA system. Matrix must be reconstituted prior to use. These studies were conducted to determine the stability of unreconstituted matrix as well as in-use (reconstituted) matrix.

In one study, accelerated/shipping stability was assessed using a single lot of matrix. Shipping conditions were simulated by storing the matrix at 37±2°C for two (2) weeks while accelerated stability studies conducted subsequently at the same temperature for 14 weeks. All testing was done using a common gram negative organism. At each time interval, matrix was removed, allowed to acclimate to room temperature, reconstituted and testing was done via direct transfer (DT) and extraction (Ext) method using two (2) targets in replicates of eight (8) in accordance with product instructions for use.

A second study assessed the real time stability of HCCA matrix using three (3) lots of material. All test lots were stored at the recommended storage condition per product instructions 2-8°C. All three lots were tested on two (2) target plates in replicates of eight (8) and tested at 3, 6, 9, 12 and 18 months in keeping with the process described for accelerated/shipping stability.

A third study assessed the recommended stability of reconstituted matrix for one (1) week at controlled room temperature (20-25°C). Three (3) lots of matrix material were reconstituted in accordance with product instructions for use and stored in a climate controlled chamber at 20±1°C and 25±1°C for seven (7) days. Testing was conducted at day one (1), three (3) and seven (7) using three (3) common gram negative bacteria on two (2) target plates, in replicates of eight. In addition, matrix alone was inoculated onto eight (8) positions at each time point.

A fourth study was conducted to assess the stability of reconstituted matrix at stressed temperatures for up to twelve (12) hours. A single lot of matrix material was reconstituted in accordance with product instructions for use and stored in a climate controlled chamber at 15±1°C and 30±1°C for twelve (12) hours. Testing was conducted at 6±1 hours and 12±1 hours using three (3) common gram negative bacteria on two (2) target plates, in replicates of eight. In addition, matrix alone was inoculated onto eight (8) positions at each time point.

The results of the four (4) studies are summarized below:

Table 12: Summary of Matrix Stability

Study	Test Condition	Test Age	# MBT-CA ID ≥2.0, (Target 1)	# "no ID", (Target 1)	# "false ID" (Target 1)	# MBT-CA ID ≥2.0, (Target 2)	# "no ID", (Target 2)	# "false ID", (Target 2)
Accelerated/ Shipping Stability	Matrix Lot #1	No Aging	16/16	0/16	0/16	16/16	0/16	0/16
		1 week	16/16	0/16	0/16	16/16	0/16	0/16
		2 weeks	16/16	0/16	0/16	16/16	0/16	0/16
		4 weeks	16/16	0/16	0/16	16/16	0/16	0/16
		7 weeks	16/16	0/16	0/16	16/16	0/16	0/16
		16 weeks	16/16	0/16	0/16	16/16	0/16	0/16
Real-Time Stability	Matrix Lot #1	No Aging	16/16	0/16	0/16	16/16	0/16	0/16
		3 months	16/16	0/16	0/16	16/16	0/16	0/16
		6 months	16/16	0/16	0/16	16/16	0/16	0/16
		9 months	16/16	0/16	0/16	16/16	0/16	0/16
		12 months	16/16	0/16	0/16	16/16	0/16	0/16
		18 months	16/16	0/16	0/16	16/16	0/16	0/16
	Matrix Lot #2	No Aging	16/16	0/16	0/16	16/16	0/16	0/16
		3 months	16/16	0/16	0/16	16/16	0/16	0/16
		6 months	16/16	0/16	0/16	16/16	0/16	0/16
		9 months	16/16	0/16	0/16	16/16	0/16	0/16
		12 months	16/16	0/16	0/16	16/16	0/16	0/16

Study	Test Condition	Test Age	# MBT-CA ID ≥2.0, (Target 1)	# "no ID", (Target 1)	# "false ID" (Target 1)	# MBT-CA ID ≥2.0, (Target 2)	# "no ID", (Target 2)	# "false ID", (Target 2)
		18 months	16/16	0/16	0/16	16/16	0/16	0/16
	Matrix Lot #3	No Aging	16/16	0/16	0/16	16/16	0/16	0/16
		3 months	16/16	0/16	0/16	16/16	0/16	0/16
		6 months	16/16	0/16	0/16	16/16	0/16	0/16
		9 months	16/16	0/16	0/16	16/16	0/16	0/16
		12 months	16/16	0/16	0/16	16/16	0/16	0/16
		18 months	16/16	0/16	0/16	16/16	0/16	0/16
In-Use Stability at Controlled Room Temperature	Matrix Lot #1; 20±1°C	No Aging	24/24	0/24	0/24	24/24	0/24	0/24
		1 day	24/24	0/24	0/24	24/24	0/24	0/24
		3 days	24/24	0/24	0/24	24/24	0/24	0/24
		7 days	24/24	0/24	0/24	24/24	0/24	0/24
	Matrix Lot #1; 25±1°C	No Aging	24/24	0/24	0/24	24/24	0/24	0/24
		1 day	24/24	0/24	0/24	24/24	0/24	0/24
		3 days	24/24	0/24	0/24	24/24	0/24	0/24
		7 days	24/24	0/24	0/24	24/24	0/24	0/24
	Matrix Lot #2; 20±1°C	No Aging	24/24	0/24	0/24	24/24	0/24	0/24
		1 day	24/24	0/24	0/24	24/24	0/24	0/24
		3 days	24/24	0/24	0/24	24/24	0/24	0/24
		7 days	24/24	0/24	0/24	24/24	0/24	0/24
	Matrix Lot #2; 25±1°C	No Aging	24/24	0/24	0/24	24/24	0/24	0/24
		1 day	24/24	0/24	0/24	24/24	0/24	0/24
		3 days	24/24	0/24	0/24	24/24	0/24	0/24
		7 days	24/24	0/24	0/24	24/24	0/24	0/24
	Matrix Lot #3; 20±1°C	No Aging	24/24	0/24	0/24	24/24	0/24	0/24
		1 day	24/24	0/24	0/24	24/24	0/24	0/24
		3 days	24/24	0/24	0/24	24/24	0/24	0/24
		7 days	24/24	0/24	0/24	24/24	0/24	0/24
	Matrix Lot #3; 25±1°C	No Aging	24/24	0/24	0/24	24/24	0/24	0/24
		1 day	24/24	0/24	0/24	24/24	0/24	0/24
		3 days	24/24	0/24	0/24	24/24	0/24	0/24
		7 days	24/24	0/24	0/24	24/24	0/24	0/24

	Test Condition	Test Age	# "no peaks found ID", (Target 1); T=20°C	# "no peaks found ID", (Target 2); T=20°C	# "no peaks found ID", (Target 1); T=25°C	# "no peaks found ID", (Target 2); T=25°C,
In-Use Stability at Controlled Room Temperature	Matrix Lot #1; Matrix Only	No Aging	8/8	8/8	8/8	8/8
		1 day	8/8	8/8	8/8	8/8
		3 days	8/8	8/8	8/8	8/8
		7 days	8/8	8/8	8/8	8/8
	Matrix Lot #2; Matrix Only	No Aging	8/8	8/8	8/8	8/8
		1 day	8/8	8/8	8/8	8/8
		3 days	8/8	8/8	8/8	8/8
		7 days	8/8	8/8	8/8	8/8
	Matrix Lot #3; Matrix Only	No Aging	8/8	8/8	8/8	8/8
		1 day	8/8	8/8	8/8	8/8
		3 days	8/8	8/8	8/8	8/8
		7 days	8/8	8/8	8/8	8/8

Study	Test Condition	Test Age	# MBT-CA ID ≥2.0, (Target 1)	# "no ID", (Target 1)	# "false ID" (Target 1)	# MBT-CA ID ≥2.0, (Target 2)	# "no ID", (Target 2)	# "false ID", (Target 2)

In-Use Stability at Stressed Temperatures	Matrix Lot #1; 15±1°C	No Aging	24/24	0/24	0/24	24/24	0/24	0/24
		6 hours	24/24	0/24	0/24	24/24	0/24	0/24
		12 hours	24/24	0/24	0/24	24/24	0/24	0/24
	Matrix Lot #1; 30±1°C	No Aging	24/24	0/24	0/24	24/24	0/24	0/24
		6 hours	24/24	0/24	0/24	24/24	0/24	0/24
		12 hours	24/24	0/24	0/24	24/24	0/24	0/24
	Test Condition	**Test Age**	**# "no peaks found ID", (Target 1) T=15°C**	**# "no peaks found ID", (Target 2) T=15°C**		**# "no peaks found ID", (Target 1) T=30°C**	**# "no peaks found ID", (Target 2) T=30°C,**	
	Matrix Lot #1; Matrix Only	No Aging	8/8	8/8		8/8	8/8	
		1 day	8/8	8/8		8/8	8/8	
		3 days	8/8	8/8		8/8	8/8	
		7 days	8/8	8/8		8/8	8/8	

Shipping/accelerated stability studies confirm that matrix is stable for sixteen (16) weeks at temperatures up to 37±2°C. Real-time stability studies confirm that matrix is stable for up to 18 months when stored in accordance with product instructions for use. In addition, in-use (reconstituted) stability testing confirmed that reconstituted matrix is stable for one (1) week when stored at controlled room temperature and for 12 hours when stressed by temperatures up to 15°C or 30°C.

Carry-Over and Cross Contamination:

This study was conducted to determine the effect of cross-contamination, defined as microbial sample convergence between adjacent target spots and carry-over defined as target contamination due to insufficient target cleaning after MALDI Biotyper CA organism identification. Two targets and two (2) frequently occurring Gram negative bacteria were chosen for this testing. Each target was inoculated with test organism four (4) times via Direct Transfer and extraction method in an alternating pattern. All sample positions were overlaid with matrix solution including the remaining unused target positions to serve as blank measurements. Testing then proceeded in accordance with product instructions for use. Targets were then cleaned in accordance with the Target Cleaning procedure and organism prepared in a similar fashion but in the reverse pattern. The test cycle described above was repeated four (4) times on each target plate. Results of this study are presented below.

Table 13: Target #1 (SN 00004) Summary Data

Test procedure		**Matrix only**	**Test Organism**		
		"no peaks found"	*# of MALDI Biotyper CA ID ≥2.0*	*# of "no ID"*	*# of "false ID"*
1st cycle	1st run	26 / 26 (100%)	16/16	0/16	0/16 (0%)
	2nd run	27 / 27 (100%)	16/16	0/16	0/16 (0%)
2nd cycle	1st run	26 / 26 (100%)	16/16	0/16	0/16 (0%)
	2nd run	27 / 27 (100%)	16/16	0/16	0/16 (0%)
3rd cycle	1st run	26 / 26 (100%)	16/16	0/16	0/16 (0%)
	2nd run	27 / 27 (100%)	16/16	0/16	0/16 (0%)
4th cycle	1st run	26 / 26 (100%)	16/16	0/16	0/16 (0%)
	2nd run	27 / 27 (100%)	16/16	0/16	0/16 (0%)
5th cycle	1st run	26 / 26 (100%)	16/16	0/16	0/16 (0%)
	2nd run	27 / 27 (100%)	16/16	0/16	0/16 (0%)

Table 14: Target #2 (SN 00012) Summary Data

Test procedure		Matrix only	Test Organism		
		"no peaks found"	*# of MBT-CA ID ≥2.0*	*# of "no ID"*	*# of "false ID"*
1st cycle	1st run	26 / 26 (100%)	16/16	0/16	0/16 (0%)
	2nd run	27 / 27 (100%)	16/16	0/16	0/16 (0%)
2nd cycle	1st run	26 / 26 (100%)	16/16	0/16	0/16 (0%)
	2nd run	27 / 27 (100%)	16/16	0/16	0/16 (0%)
3rd cycle	1st run	26 / 26 (100%)	16/16	0/16	0/16 (0%)
	2nd run	27 / 27 (100%)	16/16	0/16	0/16 (0%)
4th cycle	1st run	26 / 26 (100%)	16/16	0/16	0/16 (0%)
	2nd run	27 / 27 (100%)	16/16	0/16	0/16 (0%)
5th cycle	1st run	26 / 26 (100%)	16/16	0/16	0/16 (0%)
	2nd run	27 / 27 (100%)	16/16	0/16	0/16 (0%)

The study confirmed that there are neither cross-contamination nor carry-over effects in the automated MALDI Biotyper CA identification process.

Proficiency/Familiarity

Prior to method comparison study initiation, each intended study operator from the four (4) US Study sites underwent a proficiency/familiarity period to ensure that each operator was familiar with all aspects of instrument operation. Each intended operator was asked to test five (5) QC organisms using both the Direct Transfer (DT) and Extraction (Ext) method following product instructions for use.

Table 15: Proficiency/Reproducibility Study Summary

Site	Operator	Test Organism [Correct Identifications ≥2.0]	
		# Samples Passed (DT)	*# Samples Passed (Ext)*
#1	#1	25/25	25/25
	#2	30/31	30/30
	#3	32/32	30/35
#2	#1	25/25	25/25
	#2	29/30	30/30
#3	#1	25/26	25/25
	#2	25/25	25/25
	#3	25/25	25/25
	#4	25/25	25/25
	#5	30/32	30/30
	#6	25/25	25/25
#4	#1	25/25	25/25
	#2	25/25	25/25
	#3	25/26	25/25
	#4	29/30	30/30
	#5	25/25	25/25

All testing confirmed that, though the MBT-CA technology is of a higher complexity, intended operators are able to illustrate reproducible results using both testing methods.

Reproducibility:

The reproducibility study was conducted to confirm day-to-day reproducibility and precision of the MALDI Biotyper CA System on different clinical study sites. The study was conducted for five (5) days with two (2) runs each day/each clinical site. The sources of variability tested were:

* Two (2) operators/each clinical study site
* Four (4) clinical study sites
* Four (4) Target plates each clinical study sites
* Four (4) microflex LT/SH instruments

Ten (10) well-characterized organisms were chosen for this study and tested in duplicate via direct transfer method in accordance with product instructions. When the DT log(score) was <2.0, per product instructions, the test organism was tested following extraction method.

Table 16: Reproducibility Study Summary

Blinded Test Organism	Reproducibility Panel	# samples passed (DT)	# samples passed (DT+Ext)
Stenotrophomonas maltophilia	REPRO-1	77/80 (96%)	**80/80 (100%)**
Citrobacter koseri	REPRO-2	80/80 (100%)	**80/80 (100%)**
Enterobacter aerogenes	REPRO-3	77/80 (96%)	**80/80 (100%)**
Escherichia coli	REPRO-4	78/80 (98%)	**80/80 (100%)**
Klebsiella pneumoniae	REPRO-5	76/80 (95%)	**80/80 (100%)**
Morganella morganii	REPRO-6	80/80 (100%)	**80/80 (100%)**
Pasteurella multocida	REPRO-7	79/80 (99%)	**80/80 (100%)**
Proteus mirabilis	REPRO-8	80/80 (100%)	**80/80 (100%)**
Pseudomonas aeruginosa	REPRO-9	78/80 (98%)	**80/80 (100%)**
Salmonella sp	REPRO-10	78/80 (98%)	**80/80 (100%)**

100% of all blinded test organisms were correctly identified on the species level at each clinical test site. Thus, data confirm reproducibility and precision of the whole MALDI Biotyper CA System independent from:

- Clinical Site
- System operators
- microflex LT/SH instruments
- Target plates

Challenge Panel:

A panel of 100 organisms was tested at five (5) study sites. Eighty (80) of the Organisms included in the panel were selected from stored organisms tested during the clinical study. Twenty (20) were selected from strain collections. The study reference laboratory, prepared the panel. Organism identifications were blinded to test sites. Each site tested the challenge panel member via direct transfer method in accordance with product instructions. If DT result yielded a log(score) <2.00, the organism was retested using the extraction method.

Table 17: Challenge Panel Study Summary

Test procedure	Site A	Site B	Site C	*Site D	**Site E
Direct Transfer	99/100 (99%)	98/100 (98%)	99/100 (99%)	96/100 (96%)	86/87 (99%)
Extraction Transfer only organisms with log(Score) <2.0.	99/100 (99%)	99/100 (99%)	99/100 (99%)	97/100 (97%)	86/87 (99%)

* One sample was incorrectly identified due to a site error, while another sample was not exclude from the study because of mixed culture.

** The number of test organisms was reduced as 13 samples were not received.

Testing of the challenge panel confirms intra laboratory performance of the MALDI Biotyper CA System.

Method Comparison:

To demonstrate performance of the MALDI Biotyper CA (MBT-CA) System, a method comparison study was performed at four (4) clinical test sites and Bruker (Bremen, Germany). Fresh and frozen organisms were tested on the MALDI Biotyper CA System in accordance with manufacturer's instructions for use. All organisms included in the study were subcultured for purity. Testing on the MBT-CA system was done from a fresh isolated colony.

At the time of testing on the MBT-CA, the test organism was subcultured onto a TSA Agar slant and shipped to the study reference laboratory. The reference laboratory, transferred the isolate to a sequencing reference laboratory for sequencing in accordance with MM-18 A guidelines.

Performance of the MBT-CA was compared to sequencing and when necessary to biochemical identification (i.e: Vitek 2) and protein sequencing.

In total, 2263 fresh and stored isolates were tested to support the initial reference library claim. An overall performance table is presented below:

Table 18: Method Comparison Summary Table (Fresh & Stored Isolates)

All Isolates - ALL SITES	REFERENCE ALGORITHM		
	Positive	*Negative*	***Total***
Positive Organism ID; (High Confidence); log(score) ≥2.0	2174	16	2190
Positive Organism ID; (Low Confidence); log(score) ≥1.7; <2.0	48	23	71
Negative	2	n/a	2
Total	2224	39	2263

Gram negatives	Correct: Genus ID Correct: Species ID or Group ID or Complex ID		Correct: Genus ID Wrong: Species ID or Group ID or Complex ID		Wrong: Genus ID		no ID
ALL SITES	high confidence log(score) ≥2.0	low confidence log(score) ≥1.7 ... <2.0	high confidence log(score) ≥2.0	low confidence log(score) ≥1.7 ... <2.0	high confidence log(score) ≥2.0	low confidence log(score) ≥1.7 ... <2.0	log(score) <2.0
IDs	2174 / 2263 96.07%	48 / 2263 2.12%	14[1)] / 2263 0.62%	22[2)] / 2263 0.97%	2[3)] / 2263 0.09%	1[4)] / 2263 0.04%	2[5)] / 2263 0.09%
Combined IDs	2222 / 2263 98.19%		36 / 2263 1.59%		3 / 2263 0.13%		n/a

Of the 2263 isolates included in the initial reference library claim, one hundred fifty-seven (157) samples required extraction for an overall sample extraction rate of 6.9% (157/2263). Of the 193 Quality Control runs conducted during the course of the method comparison study, there were five (5) instances where

a Quality Control organism failed to yield an expected result. As a result, all isolates included in that plate run were repeated using a fresh QC organism transfer. The overall plate repeat rate was 2.6% (5/193).

In addition, it is important to note that of the 2263 isolates, 498 were "fresh" meaning that the isolates were never frozen. The performance of these isolates is provided below:

Table 19: Method Comparison Summary Table (Fresh Isolates)

All Isolates	**REFERENCE ALGORITHM**		
	Positive	*Negative*	***Total***
Positive Organism ID ≥ 2.0	488	0	488
Positive Organism (Low Discrimination)	9	1	10
Negative	0	0	0
Total	497	1	498

Positive		**Negative**
high discrimination	*high & low discrimination*	
98.0%	99.8%	0.00%

Statement of Safety and Efficacy

The data presented clearly demonstrate the safety and efficacy of the Bruker Daltonic, Inc MBT-CA System as compared to the reference method, 16s bi-directional sequencing, when the instructions for use are followed.