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

I Background Information:

A 510(k) Number

K193419

B Applicant

Bruker Daltonik GmbH

C Proprietary and Established Names

MBT Sepsityper

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QNJ	Class II	21 CFR 866.3378 - Clinical Mass Spectrometry Microorganism Identification and Differentiation System	MI

II Submission/Device Overview:

A Purpose for Submission:

1. Addition of MBT Sepsityper consisting of a MBT-CA (Sepsityper) software extension and a reagent kit (MBT Sepsityper Kit US IVD)
2. Update of MBT-CA to Windows 10
3. Addition of MBT Galaxy System – US IVD
4. Addition of MBT Pilot System – US IVD

B Measurand:

See Indications for Use (III.B below)

C Type of Test:

The MBT Sepsityper is an in vitro, qualitative device for species identification of Gram positive and negative bacteria and yeasts in positive blood cultures. The MBT Sepsityper device comprises a sample preparation kit (MBT Sepsityper Kit US IVD) and a MBT-CA (Sepsityper) software extension to be used in conjunction with the MBT-CA automated mass spectrometry system. The MBT Sepsityper uses the same MBT-CA Library as the MBT-CA colony identification. Methods of spotting include MBT Sepsityper “Rapid Sepsityper DT" (RS_DT), “Rapid Sepsityper eDT” (RS_eDT) and “Full Sepsityper“ (Ext); analogous to the MBT-CA (DT, eDT and Ext).

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use.

B Indication(s) for Use:

The MBT Sepsityper is a qualitative in vitro diagnostic device consisting of a MBT-CA (Sepsityper) software extension and a reagent kit (MBT Sepsityper Kit US IVD) for use in conjunction with other clinical and laboratory findings to aid in the early diagnosis of bacterial and yeast infections from positively flagged blood cultures using the MALDI Biotyper CA System.

The MBT Sepsityper Kit US IVD is a disposable blood culture processing device that includes associated reagents that are intended to concentrate and purify microbial cells from blood culture samples identified as positive by a continuous monitoring blood culture system and confirmed to demonstrate the presence of a single organism as determined by Gram stain. This sample preparation manual method is performed by laboratory health professionals in a clinical diagnostic setting.

Subculturing of positive blood cultures is necessary to recover organisms for identification of organisms not identified by the MBT-CA System, for susceptibility testing and for differentiation of mixed growth.

Positive MBT Sepsityper results do not rule out co-infection with organisms that may not be detected by the MBT-CA System. Results of the MBT Sepsityper should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Results of the MBT Sepsityper should be correlated with Gram stain results and used in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial and yeast bloodstream infections.

Organisms recovered from positive blood culture bottles that are suitable for identification using the MBT Sepsityper include:

Abiotrophia defectiva
Achromobacter xylosoxidans
Acinetobacter baumannii / nosocomialis group

Acinetobacter calcoaceticus
Acinetobacter haemolyticus
Acinetobacter johnsonii
Acinetobacter junii
Acinetobacter lwoffii
Acinetobacter pittii
Acinetobacter radioresistens
Acinetobacter ursingii
Actinomyces europaeus
Actinomyces funkei
Actinomyces graevenitzii
Actinomyces hyovaginalis
Actinomyces meyeri
Actinomyces neuii
Actinomyces odontolyticus
Actinomyces oris
Actinomyces radingae
Actinomyces turicensis
Actinomyces urogenitalis
Actinotignum schaalii group
Aerococcus sanguinicola
Aerococcus urinae
Aerococcus viridans
Aeromonas hydrophila / caviae group
Aeromonas salmonicida
Aggregatibacter actinomycetemcomitans
Aggregatibacter aphrophilus
Aggregatibacter segnis
Alcaligenes faecalis
Alloiococcus otitis
Alloscardovia omnicolens
Anaerococcus murdochii
Anaerococcus vaginalis
Arthrobacter cumminsii
Bacteroides caccae
Bacteroides fragilis
Bacteroides nordii
Bacteroides ovatus group
Bacteroides pyogenes
Bacteroides salyersiae
Bacteroides stercoris group
Bacteroides thetaiotaomicron group
Bacteroides uniformis
Bacteroides vulgatus group
Bifidobacterium breve
Bordetella hinzii
Bordetella pertussis / bronchiseptica / parapertussis
Brevibacterium casei
Brevundimonas diminuta group
Burkholderia cepacia complex
Burkholderia gladioli
Burkholderia multivorans
Campylobacter coli
Campylobacter jejuni
Campylobacter ureolyticus
Candida albicans
Candida auris
Candida boidinii
Candida dubliniensis
Candida duobushaemulonii
Candida famata
Candida glabrata
Candida guilliermondii
Candida haemulonis
Candida inconspicua
Candida intermedia
Candida kefyr
Candida krusei
Candida lambica
Candida lipolytica
Candida lusitaniae
Candida metapsilosis
Candida norvegensis
Candida orthopsilosis
Candida parapsilosis
Candida pararugosa
Candida pelliculosa
Candida tropicalis
Candida valida
Candida zeylanoides
Capnocytophaga ochracea
Capnocytophaga sputigena
Chryseobacterium gleum
Chryseobacterium indologenes
Citrobacter amalonaticus complex
Citrobacter freundii complex
Citrobacter koseri
Clostridium beijerinckii
Clostridium bifermentans
Clostridium butyricum
Clostridium clostridioforme group

Clostridium difficile
Clostridium innocuum
Clostridium paraputrificum
Clostridium perfringens
Clostridium ramosum
Clostridium septicum
Clostridium sordellii
Clostridium sporogenes / Clostridium botulinum (group I)
Clostridium tertium
Corynebacterium accolens
Corynebacterium afermentans group
Corynebacterium amycolatum
Corynebacterium aurimucosum group
Corynebacterium bovis
Corynebacterium coyleae
Corynebacterium diphtheriae
Corynebacterium freneyi
Corynebacterium glucuronolyticum
Corynebacterium glutamicum
Corynebacterium jeikeium
Corynebacterium kroppenstedtii
Corynebacterium macginleyi
Corynebacterium minutissimum
Corynebacterium mucifaciens / ureicelerivorans group
Corynebacterium propinquum
Corynebacterium pseudodiphtheriticum
Corynebacterium pseudotuberculosis
Corynebacterium resistens
Corynebacterium riegelii
Corynebacterium striatum group
Corynebacterium tuberculostearicum
Corynebacterium ulcerans
Corynebacterium urealyticum
Corynebacterium xerosis
Cronobacter sakazakii group
Cryptococcus gattii
Cryptococcus neoformans var grubii
Cryptococcus neoformans var neoformans
Cupriavidus pauculus group
Cyberlindnera jadinii
Delftia acidovorans group
Dermabacter hominis
Dermacoccus nishinomiyaensis
Edwardsiella tarda
Eikenella corrodens
Elizabethkingia meningoseptica group
Enterobacter aerogenes
Enterobacter amnigenus
Enterobacter cloacae complex
Enterococcus avium
Enterococcus casseliflavus
Enterococcus durans
Enterococcus faecalis
Enterococcus faecium
Enterococcus gallinarum
Enterococcus hirae
Enterococcus mundtii
Enterococcus raffinosus
Escherichia coli
Escherichia hermannii
Escherichia vulneris
Ewingella americana
Facklamia hominis
Finegoldia magna
Fluoribacter bozemanae
Fusobacterium canifelinum
Fusobacterium necrophorum
Fusobacterium nucleatum
Gardnerella vaginalis
Gemella haemolysans
Gemella morbillorum
Gemella sanguinis
Geotrichum candidum
Geotrichum capitatum
Granulicatella adiacens
Haemophilus haemolyticus
Haemophilus influenzae
Haemophilus parahaemolyticus group
Haemophilus parainfluenzae
Hafnia alvei
Helcococcus kunzii
Kingella denitrificans
Kingella kingae
Klebsiella oxytoca / Raoultella ornithinolytica
Klebsiella pneumoniae
Klebsiella variicola
Kloeckera apiculata
Kocuria kristinae
Kytococcus sedentarius

Lactobacillus gasseri
Lactobacillus jensenii
Lactobacillus rhamnosus
Lactococcus garvieae
Lactococcus lactis
Leclercia adecarboxylata
Legionella longbeachae
Legionella pneumophila
Leuconostoc citreum
Leuconostoc mesenteroides
Leuconostoc pseudomesenteroides
Listeria monocytogenes
Macrococcus caseolyticus
Malassezia furfur
Malassezia pachydermatis
Mannheimia haemolytica group
Micrococcus luteus
Micrococcus lylae
Mobiluncus curtisii
Moraxella sg Branhamella catarrhalis
Moraxella sg Moraxella nonliquefaciens
Moraxella sg Moraxella osloensis
Morganella morganii
Myroides odoratimimus
Myroides odoratus
Neisseria bacilliformis
Neisseria cinerea
Neisseria elongata
Neisseria flavescens / subflava group
Neisseria gonorrhoeae
Neisseria lactamica
Neisseria meningitidis
Neisseria sicca group
Neisseria weaveri
Nocardia brasiliensis
Nocardia cyriacigeorgica
Nocardia farcinica group
Nocardia nova
Nocardia otitidiscaviarum
Ochrobactrum anthropi
Oligella ureolytica
Oligella urethralis
Pantoea agglomerans
Parabacteroides distasonis
Parabacteroides goldsteinii
Parabacteroides johnsonii / merdae group
Parvimonas micra
Pasteurella multocida
Pediococcus acidilactici
Pediococcus pentosaceus
Peptoniphilus harei group
Peptostreptococcus anaerobius
Pichia ohmeri
Plesiomonas shigelloides
Pluralibacter gergoviae
Porphyromonas gingivalis
Porphyromonas somerae
Prevotella bivia
Prevotella buccae
Prevotella denticola
Prevotella intermedia
Prevotella melaninogenica
Propionibacterium acnes
Proteus mirabilis
Proteus vulgaris group
Providencia rettgeri
Providencia stuartii
Pseudomonas aeruginosa
Pseudomonas fluorescens group
Pseudomonas oryzihabitans
Pseudomonas putida group
Pseudomonas stutzeri
Ralstonia pickettii
Rhizobium radiobacter
Rhodotorula mucilaginosa
Rothia aeria
Rothia dentocariosa
Rothia mucilaginosa
Saccharomyces cerevisiae
Salmonella sp
Serratia fonticola
Serratia liquefaciens
Serratia marcescens
Serratia odorifera
Serratia plymuthica
Serratia rubidaea
Sphingobacterium multivorum
Sphingobacterium spiritivorum
Sphingomonas paucimobilis group
Staphylococcus aureus
Staphylococcus auricularis
Staphylococcus capitis

Staphylococcus caprae
Staphylococcus carnosus
Staphylococcus cohnii
Staphylococcus delphini
Staphylococcus epidermidis
Staphylococcus equorum
Staphylococcus felis
Staphylococcus haemolyticus
Staphylococcus hominis
Staphylococcus intermedius
Staphylococcus lentus
Staphylococcus lugdunensis
Staphylococcus pasteuri
Staphylococcus pettenkoferi
Staphylococcus pseudintermedius
Staphylococcus saccharolyticus
Staphylococcus saprophyticus
Staphylococcus schleiferi
Staphylococcus sciuri
Staphylococcus simulans
Staphylococcus vitulinus
Staphylococcus warneri
Staphylococcus xylosus
Stenotrophomonas maltophilia
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus canis
Streptococcus constellatus
Streptococcus dysgalactiae
Streptococcus equi
Streptococcus gallolyticus
Streptococcus gordonii
Streptococcus intermedius
Streptococcus lutetiensis
Streptococcus mitis / oralis group
Streptococcus mutans
Streptococcus parasanguinis
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarius / vestibularis group
Streptococcus sanguinis
Streptococcus sobrinus
Streptococcus thermophilus
Sutterella wadsworthensis
Trichosporon asahii
Trichosporon inkin
Trichosporon mucoides group
Trueperella bernardiae
Turicella otitidis
Vagococcus fluvialis
Veillonella parvula group
Vibrio parahaemolyticus
Vibrio vulnificus
Weeksella virosa
Yersinia enterocolitica
Yersinia frederiksenii
Yersinia intermedia
Yersinia kristensenii
Yersinia pseudotuberculosis

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

MBT-CA System including:

- MALDI Biotyper CA System
- MALDI Biotyper smart CA System
- MALDI Biotyper sirius CA System

Optional Instrument Requirements:

- MBT Galaxy System - US IVD
- MBT Pilot System - US IVD

IV Device/System Characteristics:

A Device Description:

The MBT Sepsityper is an in vitro, qualitative device for species identification of Gram positive and negative bacteria as well as yeasts by matrix-assisted laser desorption/ionization - time-of-flight mass spectrometry (MALDI-TOF MS) from positive blood cultures. The MBT Sepsityper device comprises a sample preparation kit (MBT Sepsityper Kit US IVD) and a MBT-CA (Sepsityper) software extension that is used with the MBT-CA automated mass spectrometry system (DEN170081). Methods of spotting include Rapid Sepsityper DT (RS_DT), Rapid Sepsityper eDT (RS_eDT) and Full Sepsityper (Ext); analogous to the MBT-CA spotting methods (DT, eDT and Ext).

The device differs from the predicate device in the use of the MBT Sepsityper Kit US IVD which contains lysis and wash buffers for the lysis of blood cells and enrichment of microorganisms from positive blood culture bottles and in the use of the MBT-CA (Sepsityper) software extension. The MBT-CA (Sepsityper) software extension is considered an extension of the claimed MBT-CA System software. The algorithm remains the same except for the mass range. Due to noise of red blood cells between 3,000-4,000 m/z, the peak picking mass range is limited to 4,000-15,000 m/z instead from 3,000-15,000 m/z for isolated colonies. The MBT Sepsityper performs a standard Main Spectrum reference Pattern (MSP) match of the peak list from the unidentified spectrum against the MBT-CA MSP Reference Library. The MSP Library is the same for the MBT-CA and MBT Sepsityper; however, a different confidence score range is used to denote high confidence (1.8 to 3), low confidence (1.6 to 1.79), and no identification (0 to 1.59). The MBT Sepsityper is for use with BACTEC (BD), BacT/ALERT without charcoal (bioMérieux), and VersaTREK (Thermo Scientific) positive blood culture bottles. The MBT Sepsityper also provides mixed culture hints (See IV.A.4).

MBT Sepsityper Device

The MBT Sepsityper is comprised of:

- MBT-CA (Sepsityper) software extension
- MBT Sepsityper Kit US IVD

Required Materials Supplied by Bruker:

- A Bruker MALDI-TOF Mass Spectrometer instrument connected to a computer with dedicated MBT-CA software installed:
  - Bruker microflex LT/SH (benchtop)
  - Bruker microflex LT/SH smart (benchtop)
  - Bruker MBT sirius (benchtop)
- US IVD 48 Spot Target [P/N: 8604532]
- MBT Biotarget 96 US IVD [P/N: 1840380]
- MSP Biotarget Adapter [P/N: 8267615]
- US IVD BTS (Bacterial Test Standard) [P/N: 8604530]
- US IVD HCCA portioned [P/N: 8604531]

Required Materials Not Supplied by Bruker:

- Standard solvent
- Acetonitrile

- HPLC-grade water
- Formic Acid (FA)
- Absolute Ethanol (EtOH)
- Trifluoroacetic acid (TFA)
- Other common laboratory supplies

B Principle of Operation:

MBT Sepsityper Kit US IVD Procedures:
Note: US IVD HCCA solution must be added within 30 minutes after sample spots have dried, otherwise the spotting must be performed again.

1. Harvesting blood culture fluid
    a. Disinfect the septum of the blood culture bottle with 70% ethanol or similar.
    b. Collect sufficient blood culture fluid.

    Preparing MBT Sepsityper samples using the MBT Sepsityper Kit US IVD:

    c. Transfer 1 mL blood culture fluid to a microcentrifuge tube.
    d. Add 200 µL Lysis Buffer and mix by vortexing (full speed) for 10 (± 5) seconds.
    e. Centrifuge the tube for 2 minutes at 13,000-15,000 rpm at room temperature.
    f. Remove the supernatant by pipetting and discard.
    g. Add 1 mL Washing Buffer and resuspend the pellet by pipetting up and down.
    h. Centrifuge the tube for 1 minute at 13,000-15,000 rpm at room temperature.
    i. Remove the supernatant from the pellet (in the following 'Sepsityper Pellet') by pipetting and discard.

2. Performing the Rapid Sepsityper Workflow using Direct Transfer (RS_DT) and extended Direct Transfer (RS_eDT) sample preparation procedures
    a. Using a transfer device such as a toothpick, transfer some Sepsityper Pellet material onto two (2) MALDI target plate positions.
    b. Overlay the 2nd spot with 1 µL 70% formic acid and dry at room temperature (RS-eDT).
    c. For validation of the IVD system, inoculate US IVD BTS onto two target plate positions following Instructions for Use of US IVD BTS.
    d. Overlay each sample spot and US IVD BTS spots with 1 µL US IVD HCCA solution.

Note: If one of the two Rapid Sepsityper samples (RS_DT or RS_eDT) show high confidence results the Sepsityper procedure ends up here. If low confidence results or no ID results were observed continue with Full Sepsityper.

3. Performing the Full Sepsityper Workflow using extraction (Ext) sample preparation procedures
    a. Add 300 µL HPLC-grade water and resuspend the Sepsityper Pellet by pipetting up and down.
    b. Add 900 µL ethanol and mix the suspension with a vortex mixer (full speed) for 10 (± 5) seconds.
    c. Centrifuge the tube for 2 minutes at 13,000-15,000 rpm at room temperature.
    d. Remove the supernatant by pipetting and discard.
    e. Centrifuge the tube for 2 minutes at 13,000-15,000 rpm at room temperature.
    f. Remove the residual ethanol by pipetting and discard.

g. Allow the pellet to dry for 5 (±1) minutes at room temperature.
h. Add 2-50 µL (depending on the pellet size) 70% formic acid and thoroughly resuspend the pellet by pipetting up and down.
i. Add an equal volume of acetonitrile and mix the suspension by pipetting up and down two to three times.
j. Centrifuge the tube for 2 minutes at 13,000-15,000 rpm at room temperature.
k. Pipette 1 µL of the supernatant onto an unoccupied MALDI target plate position and dry at room temperature.
l. For validation of the IVD system, inoculate US IVD BTS onto two target plate positions following Instructions for Use of US IVD BTS.
m. Overlay each sample spot and US IVD BTS spots with 1 µL US IVD HCCA solution.

C Instrument Description Information:

1. Instrument Name:

   MBT Sepsityper

2. Specimen Identification:

   The user manually enters the specimen identification information into the MALDI Biotyper CA System. The user first defines active sample positions and US IVD BTS control positions. The defined sample positions are required to have a user entered and valid sample identifier in the Id column along with an optional description. All inoculated positions must contain a valid ID before the run can proceed. After all sample positions have been input, at least two US IVD BTS control positions must be defined. It is recommended that the cross-joint positions on the US IVD 48 Spot Target are used as quality control positions.

   Alternatively, the user can use an optional Honeywell (Hyperion 1300g) Barcode Reader that is connected via a USB cable to the MALDI Biotyper CA System computer. The barcode reader scans the unique ten-digit target ID which appears in the Target ID box on the target plate. After the target ID has been entered, a new Run page opens, and the ten-digit target ID appears as the Plate Id and is appended to the Run name. Sample identifications are entered into the computer corresponding to the target plate position for that run.

   The user may also use the optional MBT Pilot System - US IVD. The MBT Pilot System - US IVD is a tool providing visual support for the manual preparation of biological samples on MALDI target plates in the MALDI Biotyper CA System workflow. The MBT Pilot System - US IVD indicates the correct MALDI target plate positions by means of projected cross hairs by providing guided MALDI target plate preparation, using patented micro-projection technology. Traceability is achieved with a barcode linking sample projects to specific MALDI target plates (i.e., US IVD 48 Spot Target (Bruker #8604532) and MBT Biotarget 96 US IVD (Bruker #1840380). A USB connection is utilized for data exchange. Usability studies were conducted and found acceptable.

3. Specimen Sampling and Handling:

   In addition to See IV.C.2 above, the MBT Galaxy System - US IVD may be utilized.

The MBT Galaxy System - US IVD device, as part of the MBT-CA System deposits matrix droplets (in DT, Ext workflows) or FA+matrix droplets (in eDT workflows) onto sample spot positions on MALDI targets. The MBT Galaxy System - US IVD reads the target barcode and loads the associated worklist from the MBT-CA server. Validation for the MBT Galaxy System - US IVD included droplet positioning test, carry over - cross-contamination studies, repeatability and reproducibility, sample stability of automatically overlaid with HCCA matrix solution or FA/HCCA matrix solution on test organism set at two temperatures [21(±2)°C (lower range) and at 25(±2)°C (upper range)], equivalence (manual vs automated sample prep) and spectra re-evaluation. All results were found acceptable and equivalent to manual methods. No carry over – cross-contamination was observed.

4. Calibration:

Calibration is achieved using the US IVD Bacterial Test Standard (BTS), an in-vitro-diagnostic product used for quality control and validation. US IVD BTS contains a manufactured extract of *Escherichia coli* DH5alpha that demonstrates a characteristic peptide and protein profile mass spectrum, when tested on the MALDI Biotyper CA System. US IVD BTS is spiked with two additional proteins that extend the upper boundary of the mass range of the US IVD BTS. The overall mass range covered by US IVD BTS is 3.6 to 17 kDa. If US IVD BTS does not meet all required performance specifications, the test run will be invalid.

5. Quality Control:

The user will follow local, state and/or federal regulations for Quality Control requirements.

V Substantial Equivalence Information:

A Predicate Device Name(s):
MALDI Biotyper CA System

B Predicate 510(k) Number(s):
DEN170081

C Comparison with Predicate(s):

Similarities		
Characteristic	**NEW DEVICE MBT-CA System with MBT Sepsityper (K193419)**	**PRIMARY PREDICATE DEVICE MBT-CA System (DEN170081)**
Product Codes	QNJ	QBN
Indications for Use	The MBT Sepsityper is a qualitative in vitro diagnostic device consisting of a MBT-CA (Sepsityper) software extension and a reagent kit (MBT Sepsityper Kit US IVD) for use in conjunction with other clinical and laboratory findings to aid in the early diagnosis of bacterial and yeast infections from positively flagged blood cultures using the MALDI Biotyper CA System. The MBT Sepsityper Kit US IVD is a disposable blood culture processing device that includes associated reagents that are intended to concentrate and purify microbial cells from blood culture samples identified as positive by a continuous monitoring blood culture system and confirmed to demonstrate the presence of a single organism as determined by Gram stain. This sample preparation manual method is performed by laboratory health professionals in a clinical diagnostic setting. Subculturing of positive blood cultures is necessary to recover organisms for identification of organisms not identified by the MBT-CA System, for susceptibility testing and for differentiation of mixed growth. Positive MBT Sepsityper results do not rule out co-infection with organisms that may not be detected by the MBT-CA System. Results of the MBT Sepsityper should not be used as the sole basis for diagnosis, treatment, or	The MALDI Biotyper CA System is a mass spectrometer system using matrix-assisted laser desorption/ionization – time-of-flight (MALDI-TOF) for the identification and differentiation of microorganisms cultured from human specimens. The MALDI Biotyper CA System 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 fungal infections. For measurand, see DEN170081

	other patient management decisions. Results of the MBT Sepsityper should be correlated with Gram stain results and used in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial and yeast bloodstream infections. Organisms recovered from positive blood culture bottles that are suitable for identification using the MBT Sepsityper include (see III.B above)	
Type of Test	Automated Mass Spectrometry System	Automated Mass Spectrometry System
Matrix	α-Cyano-4-hydroxycinnamic acid	α-Cyano-4-hydroxycinnamic acid
Matching Algorithm	Calculates matches by comparing a new spectrum against each single reference entry of a reference database.	Calculates matches by comparing a new spectrum against each single reference entry of a reference database.
Instrument Control Software	flexControl and AutoX	flexControl and AutoX
Calibration and Quality Control	Bruker US IVD Bacterial Test Standard (BTS)	Bruker US IVD Bacterial Test Standard (BTS)
MALDI Matrix	US IVD HCCA portioned	US IVD HCCA portioned
Library	MALDI Biotyper for Clinical Applications (MBT-CA)	MALDI Biotyper for Clinical Applications (MBT-CA)
MALDI Target Plate	US IVD 48 Spot Target (48 positions reusable steel targets)	US IVD 48 Spot Target (48 positions reusable steel targets)
	MBT Biotarget 96 US IVD (96 positions disposable targets)	MBT Biotarget 96 US IVD (96 positions disposable targets)
MALDI-TOF MS Instruments	Bruker microflex LT/SH (benchtop) Bruker microflex LT/SH smart (benchtop) Bruker MBT sirius (benchtop)	Bruker microflex LT/SH (benchtop) Bruker microflex LT/SH smart (benchtop) Bruker MBT sirius (benchtop)

Differences		
Characteristic	**NEW DEVICE MBT-CA System with MBT Sepsityper (K193419)**	**PRIMARY PREDICATE DEVICE MBT-CA System (DEN170081)**
Test Sample	**<u>Bottles of the BD BACTEC (Becton Dickinson) System:</u>** • BD BACTEC™ Standard - Aerobic / Anaerobic • BD BACTEC™ PLUS - Aerobic / Anaerobic • BD BACTEC™ - Lytic Anaerobic • BD BACTEC™ - BD Peds Plus™ • BD BACTEC™ - Myco/F Lytic • BD BACTEC™ - Mycosis IC **<u>Bottles without charcoal of the BacT/ALERT® (bioMérieux) System:</u>** • BacT/ALERT® SA Standard Aerobic • BacT/ALERT® SN Standard Anaerobic • BacT/ALERT® FA Plus • BacT/ALERT® FN Plus • BacT/ALERT® PF Plus **<u>Bottles of the VersaTREK® (Thermo Scientific) System:</u>** • VersaTREK® REDOX 1 • VersaTREK® REDOX 2	Isolated colony from any patient sample source. Acceptable media: • Columbia blood agar with 5% sheep blood (Gram negative bacteria) • Trypticase soy agar with 5% sheep blood (Gram negative bacteria, Gram positive bacteria, yeasts) • Chocolate agar (Gram negative bacteria, Gram positive bacteria) • MacConkey Agar (Gram negative bacteria) • Columbia CNA agar with 5% sheep blood (Gram positive bacteria) • Brucella Agar with 5% horse blood (Gram negative anaerobic bacteria, Gram positive anaerobic bacteria) • CDC anaerobe Agar with 5% sheep blood (Gram negative anaerobic bacteria, Gram positive anaerobic bacteria) • CDC anaerobe 5% sheep blood Agar with phenylethyl alcohol (Gram negative anaerobic bacteria, Gram positive anaerobic bacteria) • CDC anaerobe laked sheep blood Agar with kanamycin and vancomycin (Gram negative anaerobic bacteria) • Bacteroides bile esculin Agar with amikacin (*Bacteroides* species) • Clostridium difficile Agar with 7% sheep blood (*Clostridium difficile*) • Sabouraud-Dextrose Agar (Yeasts) • Brain Heart Infusion Agar (Yeasts) • Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood (*Campylobacter* species) • Bordet Gengou Agar with 15% sheep blood (*Bordetella* species) • Buffered Charcoal Yeast Extract Agar (*Legionella* species)

		• Buffered Charcoal Yeast Extract Selective Agar with polymyxin, anisomycin and vancomycin (*Nocardia* species) • Modified Thayer-Martin Agar (*Neisseria* species)
System Software	MBT-CA Software including the MBT-CA (Sepsityper) software extension	MBT-CA Software
Sample Type	Positive blood cultures	Isolated colonies
Sample Preparation Reagents	Lysis Buffer / Washing Buffer (MBT Sepsityper Kit US IVD)	No
Workflow	The approved MBT-CA Workflow plus the MALDI Sepsityper Workflows [Rapid Sepsityper DT (RS_DT), Rapid Sepsityper eDT (RS_eDT) and Full Sepsityper (Ext)]	MBT-CA Workflow (DT, eDT, Ext)
Analyzed Mass Range	4,000 – 15,000 m/z (Sepsityper samples)	3,000 – 15,000 m/z (Isolated colonies)
log(score) Values	MBT Sepsityper Samples: • High Confidence ID: 1.80 - 3.00; • Low Confidence ID: 1.60 - 1.79; • No Organism ID Possible: 0.00 - 1.59	Isolated colony from agar plate: • High Confidence ID: 2.00 - 3.00; • Low Confidence ID: 1.70 - 1.99; • No Organism ID Possible: 0.0 - 1.69
Mix Culture Hint	Yes	No

VI Standards/Guidance Documents Referenced:

CLSI documents EP07-Ed3 (Interference Testing in Clinical Chemistry)

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision: Repeatability and Reproducibility:

At each of the three sites (2 US; 1 Bruker) ten different samples were tested on five different days with two operators. Each site had two (2) operators test a panel of ten spiked samples (4x Gram positive, 4x Gram negative, 2x yeast) for five non-consecutive days representing two instrument runs a day. Each were tested in triplicate. No incorrect identifications were observed with any method sample preparation procedures (i.e., RS_DT, RS_eDT or Ext). A summary of combination of DT + eDT + Ext workflow is shown in Table 1 below.

Table 1: Repeatability and Reproducibility of the MBT Sepsityper

Site 1												
Sepsityper Result	Operator 1					Operator 2					Op 1	Op 2
	d1	d2	d3	d4	d5	d1	d2	d3	d4	d5	all days	
high confidence ID	100%	100%	100%	100%	100%	100%	90%	100%	100%	90%	100%	96%
low confidence ID	0%	0%	0%	0%	0%	0%	0%	0%	0%	10%	0%	2%
no ID	0%	0%	0%	0%	0%	0%	10%	0%	0%	0%	0%	2%
Site 2												
Sepsityper Result	Operator 1					Operator 2					Op 1	Op 2
	d1	d2	d3	d4	d5	d1	d2	d3	d4	d5	all days	
high confidence ID	100%	100%	90%	100%	100%	90%	90%	100%	90%	100%	98%	94%
low confidence ID	0%	0%	0%	0%	0%	0%	0%	0%	10%	0%	0%	2%
no ID	0%	0%	10%	0%	0%	10%	10%	0%	0%	0%	2%	4%
Site 3												
Sepsityper Result	Operator 1					Operator 2					Op 1	Op 2
	d1	d2	d3	d4	d5	d1	d2	d3	d4	d5	all days	
high confidence ID	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
low confidence ID	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
no ID	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%

2. Sample Stability:

Studies were performed to determine sample stability in various conditions:

- Organism stability in blood culture prior Sepsityper processing: Positive blood culture bottles can be stored at room temperature up to 24 hours prior to Sepsityper processing.
- Organism stability in extended incubation blood culture prior Sepsityper processing: Blood cultures which are flagged positive by the continuously monitoring blood culture instrument can remain on the instrument up to 12 hours prior to Sepsityper processing.
- Sample Stability of the Sepsityper Pellet: The concentrated Sepsityper Pellet can be maintained at room temperature for 1 hour prior to spotting the target plate.
- Sample Stability post matrix application: If HCCA matrix was applied to the ready prepared MBT Biotargets 96 or Steel Targets, the targets can be kept at room temperature and the measurement and identification can be performed within 24 h without reducing identification performance.

3. Analytical Specificity/Interference:

To assess the inhibitory effects of substances encountered in blood and blood culture media the substances listed in Table 2 were tested in the given concentration in blood cultures spiked with each one of the following species: *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Candida albicans*. Each interferent – species combination was prepared with the three Sepsityper workflows (RS_DT, RS_eDT, Ext) and MALDI target preparation was performed in quadruplicates. No interference was found.

Table 2: Substances and concentrations tested for interference in positive blood cultures

Routine Interferents		**Drug Interferents**	
Interferent	**Test Concentration**	**Interferent**	**Test Concentration**
Triglyceride-rich lipoproteins	10 g/L	Acetaminophen	1.324 mmol/L
Hemolysate (hemoglobin)	5 g/L	Acetylcysteine	10.2 mmol/L
Protein	120 g/L	Acetylsalicylic Acid	3.62 mmol/L
Conjugated Bilirubin	200 mg/L	Cefoxitin (Na)	1.55 mmol/L
Unconjugated Bilirubin	200 mg/L	Cyclosporine	5 mg/L
Further Interferents		Doxycycline (HCl)	0.0675 mmol/L
		Heparin	3,000 U/L
White blood cells	Five-fold increase (compared to usual concentration)	Ibuprofen	2.425 mmol/L
		Metronidazole	0.7 mmol/L
Sodium Polyanethole Sulfonate	0.5 mg/mL	Vancomycin	0.069 mmol/L

4. Interference (Polymicrobial Samples)

During the clinical studies polymicrobial samples were excluded via study design (Gram staining). However, polymicrobial samples may not always be detected by Gram stain and may be subsequently processed with the MBT Sepsityper. The mixed culture hint is a functionality of the MBT-CA (Sepsityper) software extension which is able to create a user warning about a possible polymicrobial infection.

The mixed culture warning is based on the ranking list of an identification. First, the algorithm scans the ranking list of a MBT Sepsityper identification until a defined log(score) is reached ("parameter #1"). The scanned ranking list is longer than the typical 10 positions. Within this ranking list ideally only one single species appears until the log(score) threshold is reached, however, if species are closely related the ranking list can contain both (or even more) related species until the defined log(score) is reached. Additionally, if a mixed culture is present, the dominant species will appear in higher ranking list positions with the second species also present within the listing. The distance (or the MALDI based similarity) between two species (between two single MSPs of a species) can be calculated.

The warning algorithm collects in the first step all unique species from the ranking list and checks in the second step the relation of all species in this list. For this second step a further log(score) ("parameter #2") is defined as a close relation between two species. If this log(score) is exceeded both species will be excluded from the mixed culture warning automatically. Only if the log(score) between two species is less than the threshold and a close relation is unlikely will both species be further processed in the warning generation. In these cases, if the parameter #2 went below the threshold both reference spectra (MSPs) of the two mentioned species are combined temporary and the match of the unknown spectrum will be compared against this temporary, artificial MSP. If the log(score) against this artificial MSP is higher than the highest log(score) in the normal ranking list the mixed culture warning is generated, and a second species will be reported.

For this study, the following combinations of polymicrobial samples, as listed in Table 3 below, were tested at different concentrations (i.e., 10:0, 10:1, 5:1, 1:1, 1:5, 1:10 and 0:10).

Table 3: Test organisms for polymicrobial validation

Organism Combination	Organisms Tested
Two taxonomically distant cleared species	*Escherichia coli : Pseudomonas aeruginosa* *Escherichia coli : Staphylococcus aureus* *Klebsiella pneumoniae : Staphylococcus aureus*
Two different cleared organisms (bacteria and yeast)	*Escherichia coli : Candida albicans* *Klebsiella pneumoniae : Candida albicans* *Staphylococcus aureus : Candida albicans*
Two taxonomically closely related cleared species	*Escherichia coli : Klebsiella pneumoniae* *Klebsiella pneumoniae : Klebsiella variicola*
Non-clinically validated species and cleared species (as part of the ncv MBT-CA Library)	*Escherichia coli : Corynebacterium argentoratense* *Staphylococcus aureus : Corynebacterium argentoratense*
Cleared species with contaminants (other cleared species)	*Escherichia coli : Corynebacterium glutamicum* *Escherichia coli : Propionibacterium acnes* *Klebsiella pneumoniae : Corynebacterium glutamicum* *Staphylococcus aureus : Corynebacterium glutamicum* *Staphylococcus aureus : Propionibacterium acnes* *Staphylococcus aureus : Staphylococcus epidermidis*
Cleared species with RUO Species	*Escherichia coli : Corynebacterium glaucum* *Staphylococcus aureus : Corynebacterium glaucum*

In all cases, clean ranking lists, mixed ranking lists and correct mixed culture warnings were observed. No incorrect identification was reported by mixing a "RUO species" to a blood culture bottle with a claimed organism. A mixed culture warning was not expected for all polymicrobial samples since different concentration of the mixed organisms leads to different output (i.e., a factor of LoD of testing).

The generation of a mixed culture hint is a strong indication, but not a confirmation, that a sample contains a mixture of organisms. Conversely, a sample containing a mixture of organisms might not generate a mixed culture hint. Therefore, as with all other MBT-CA analyses, final results from MBT Sepsityper samples must be assessed by a professional experienced in clinical microbiology and positive blood cultures should be subcultured for isolation and identification of organisms not identified by the MBT-CA System, for susceptibility testing, and differentiation of mixed growth.

5. Assay Reportable Range:

MBT Sepsityper: 4,000-15,000 m/z
MBT Sepsityper Samples:

- High Confidence ID: 1.80 - 3.00;
- Low Confidence ID: 1.60 - 1.79;
- No Organism ID Possible: 0.00 - 1.59

6. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

See IV.C.4 above

7. Detection Limit:

This study demonstrated the tolerance range (dynamic range) analogous to the limit of detection (LOD) to verify that the time of positivity call by the continuously monitoring blood culture instrument provided sufficient biological material for successful identification. Five organisms (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae* and *Candida albicans)* were tested (10 replicates each) at time of bottle positivity and at 1:10 and 1:100 dilutions. At "timepoint zero" (time of positivity call) of all positively flagged blood culture delivered sufficient biological material for correct MBT Sepsityper identifications. A few replicates of *Klebsiella pneumoniae,* were incorrectly identified as *K variicola* or *E.aerogenes* and a few replicates of *Streptococcus pneumoniae* were identified as *S.mitis/oralis* group. Diluted samples did not always reliably provide an identification; however, these dilutions demonstrated that lower concentration of microorganisms did not produce any incorrect identifications with the exception of the 1:100 dilution of *Klebsiella pneumoniae,* in which a few replicates were incorrectly identified as *K.variicola* or *E.aerogenes*. These are addressed as limitations in the instructions for use.

8. Assay Cut-Off:

The ability of the MBT Sepsityper to identify bacteria and yeast in positive blood cultures was evaluated for all 334 claimed species and compared to the isolated colony identification on the MBT-CA System. For all claimed species / species groups one representative strain of each species (group) was processed by each of the MBT Sepsityper workflows (i.e., RS_DT, RS_eDT and Ext). Some "low log(score) based" incorrect results were observed, however for organisms with low confidence scores, the user should perform further methods to confirm the MBT Sepsityper result.

A total of 14,247 analyzed log(scores) were determined in the assay cut-off study with the following results:

ID Level	# / % samples
High Confidence	7531 (52.1%)
Low Confidence	1760 (12.4%)
No ID	3807 (26.7%)
No Peaks	1249 (8.75%)
Incorrect Identification	207 (1.45%)

Discordant Results:

Altogether 207 samples (1.45%) of all analyzed 14,247 samples were incorrectly identified within the Assay Cut-Off study. "Contaminations": the majority (136 spectra) of incorrect results were resolved as contaminations during processing. After removing the samples suspected of contamination, sufficient test samples were measured for the mentioned species in the Assay Cut-Off Study. The remaining spectra are shown in brackets in the columns "# of Unique Spectra (minus discordant)" in Table 4 to Table 6. "Limitations": from the remaining 71 (0.5%) discordant

results 41 (0.29%) results represented minor errors since closely related species were incorrectly reported by the MBT-CA System. Where possible whenever cross identification occurred; a limitation was added to the matching hint table. “True Discordants”: the remaining 30 (0.21%) incorrect results were represented by 2 high confidence and 28 low confidence results. Some (9) of these incorrect results (all high confidence and 7 low confidence) were derived from a single “bad reference entry” in the library which was subsequently replaced due to its low quality. From the remaining 21 incorrect results 17 resulted from ncv reference entries and 4 incorrect results were derived from cleared species. Please see Table 4 to Table 6 for summary of discordant results.

Table 4: Assay Cut-Off Study Discordant Results “Contaminations”.

Claimed Species	# of Unique Spectra (minus discordant)	Discordant Results			
		confidence (# spectra)		species	Resolution
		high	low		
Acinetobacter johnsonii	36 (33)	3		Acinetobacter haemolyticus	contamination
Aerococcus urinae	45 (37)	7	1	Vagococcus fluvialis	contamination
Aerococcus viridans	45 (44)	1		Escherichia coli	contamination
Aggregatibacter actinomycetemcomitans	90 (87)		3	Yersinia enterocolitica	contamination
Aggregatibacter segnis	36 (29)	6	1	Strep. salivarius / vestibularis group	contamination
Bacteroides nordii	36 (33)		3	Propionibacterium acnes	contamination
Bifidobacterium breve	27 (23)	2	2	Staphylococcus hominis	contamination
Klebsiella pneumoniae	36 (34)	2		Escherichia coli	contamination
Legionella longbeachae	54 (52)	2		Escherichia coli	contamination
Neisseria elongata	36 (34)		2	Corynebacterium coyleae	contamination
Pediococcus pentosaceus	45 (36)	2	7	Staphylococcus haemolyticus	contamination
Plesiomonas shigelloides	36 (19)	16	1	Chryseobacterium indologenes	contamination
Porphyromonas gingivalis	27 (19)	5	3	Staphylococcus hominis	contamination
Rothia aeria	45 (40)	4	1	Staphylococcus epidermidis	contamination
Staphylococcus vitulinus	54 (49)	1	4	Staphylococcus capitis	contamination
Stenotrophomonas maltophilia	72 (69)	3		Yersinia kristensenii	contamination
Vagococcus fluvialis	36 (24)	8	4	Aerococcus urinae	contamination
Candida famata	36 (24)	9	3	Candida parapsilosis	contamination
Candida inconspicua	63 (57)	4	2	Pediococcus pentosaceus	contamination
Candida lambica	54 (49)	5		Pediococcus pentosaceus	contamination
Candida pararugosa	36 (24)	10	2	Pediococcus acidilactici	contamination
Candida tropicalis	54 (47)	6	1	Staphylococcus epidermidis	contamination
		96	40		
		136			

Table 5: Assay Cut-Off Study Discordant Results "Limitations", the species marked with (ncv) were from the clinically non validated library.

Claimed Species	# of Unique Spectra (minus discordant)	Discordant Results			
		confidence (# spectra)		species	Resolution
		high	low		
Corynebacterium pseudotuberculosis	63 (62)	1		Corynebacterium ulcerans	limitation
Enterococcus raffinosus	36 (35)		1	Enterococcus avium	limitation
Gemella haemolysans	54 (52)	1	1	Gemella sanguinis	limitation
Listeria monocytogenes	36 (35)	1		Listeria innocua	limitation (ncv)
Neisseria lactamica	36 (35)		1	Neisseria flavescens / subflava group	limitation
Neisseria weaveri	36 (35)	1		Neisseria zoodegmatis	limitation (ncv)
Ochrobactrum anthropi	36 (35)	1		Ochrobactrum intermedium	limitation (ncv)
Salmonella sp	45 (43)	1	1	Citrobacter freundii	limitation
Serratia plymuthica	36 (35)		1	Serratia liquefaciens	limitation
Staphylococcus delphini	54 (53)		1	Staphylococcus pseudintermedius	limitation
Staphylococcus pasteuri	54 (51)		3	Staphylococcus warneri	limitation
Staphylococcus pseudintermedius	45 (40)	4	1	Staphylococcus intermedius	limitation
Staphylococcus schleiferi	54 (53)		1	Staphylococcus pseudintermedius	limitation
Staphylococcus xylosus	72 (71)	1		Staphylococcus saprophyticus	limitation
Streptococcus dysgalactiae	54 (43)	11		Streptococcus canis	limitation
Streptococcus lutetiensis	36 (35)	1		Streptococcus gallolyticus	limitation
Streptococcus mitis / oralis group	36 (31)	3	2	Streptococcus pneumoniae	limitation
Yersinia intermedia	36 (35)	1		Yersinia enterocolitica	limitation
Candida dubliniensis	72 (71)		1	Candida albicans	limitation
		27	14		
		41			

Table 6: Assay Cut-Off Study Discordant Results "True Discordants", the species marked with (ncv) were from the clinically non validated library, the MSP marked with "bad MSP" was replaced in the library due to quality issues.

Claimed Species	# of Unique Spectra (minus discordant)	Discordant Results			
		confidence (# spectra)		species	Resolution
		high	low		
Acinetobacter ursingii	72 (71)		1	Actinomyces neuii (eDT, 1.61)	discordant
Actinomyces hyovaginalis	72 (71)		1	Actinomyces dentalis	discordant (ncv)
Actinomyces urogenitalis	54 (53)		1	Actinomyces graevenitzii (eDT, 1.60)	discordant
Anaerococcus vaginalis	36 (35)		1	Alloiococcus otitis	discordant (bad MSP)
Capnocytophaga sputigena	54 (51)		3	Actinomyces dentalis	discordant (ncv)
Clostridium paraputrificum	36 (35)		1	Alloiococcus otitis	discordant (bad MSP)
Corynebacterium amycolatum	54 (52)		2	Actinomyces bovis	discordant (ncv)
Corynebacterium aurimucosum group	36 (35)		1	Arthrobacter gandavensis	discordant (ncv)
Corynebacterium bovis	36 (35)		1	Lactobacillus plantarum	discordant (ncv)
Corynebacterium minutissimum	36 (35)		1	Lactobacillus paracasei	discordant (ncv)
Corynebacterium pseudodiphtheriticum	36 (35)		1	Alloiococcus otitis	discordant (bad MSP)
Dermacoccus nishinomiyaensis	36 (34)		2	Actinomyces oris (DT, 1.70; eDT, 1.63)	discordant
Finegoldia magna	36 (35)		1	Alloiococcus otitis	discordant (bad MSP)
Pseudomonas putida group	63 (62)		1	Pseudomonas alcaligenes	discordant (ncv)
Pseudomonas stutzeri	54 (53)		1	Alloiococcus otitis	discordant (bad MSP)
Rothia mucilaginosa	54 (50)	2	2	Alloiococcus otitis	discordant (bad MSP)
Staphylococcus sciuri	54 (53)		1	Corynebacterium cochlearium	discordant (ncv)
Streptococcus canis	36 (35)		1	Actinomyces dentalis	discordant (ncv)
Vibrio vulnificus	54 (52)		2	Actinomyces dentalis	discordant (ncv)
Yersinia kristensenii	54 (53)		1	Actinomyces dentalis	discordant (ncv)
Candida zeylanoides	36 (35)		1	Actinomyces dentalis	discordant (ncv)
Saccharomyces cerevisiae	81 (80)		1	Actinomyces dentalis	discordant (ncv)
		2	28		
		30			

9. Clearance of Further Blood Culture Bottles:

Clinical studies were performed across three clinical sites including two sites in the U.S. and one site in Germany. The following blood culture bottles were investigated:

- 187 bottles of the BD BACTEC™ (Becton Dickinson)
  - BD BACTEC™ Standard Aerobic / Anaerobic
  - BD BACTEC™ PLUS Aerobic / Anaerobic
- 145 bottles of the BacT/ALERT® (bioMérieux)
  - BacT/ALERT® FA Standard Aerobic
  - BacT/ALERT® FN Standard Anaerobic
- 47 bottles of the VersaTREK® (Thermo Scientific).
  - VersaTREK® REDOX 1
  - VersaTREK® REDOX 2

In addition to the clinical site method comparison which used bioMérieux, Becton Dickinson and Thermo Scientific blood culture bottles, additional bottle types were tested internally.

The following Blood Culture Media/Bottle Types were tested:

- BD BACTEC™ Pediatric Plus
- BD BACTEC™ Lytic/10 Anaerobic/F
- BD BACTEC™ Mycosis IC/F Medium Culture Vials
- BD BACTEC™ Myco/F Lytic Medium
- bioMérieux BacT/ALERT® SA Standard Aerobic
- bioMérieux BacT/ALERT® SN Standard Anaerobic
- bioMérieux BacT/ALERT® PF Plus

For bioMérieux BacT/ALERT bottles, three bottle types were assessed using ten different microorganism species (Gram positive, Gram negative and yeast). For BD BACTEC bottles, four bottle types were assessed using five different microorganisms

All sample preparations were performed (RS_DT, RS_eDT and Ext) and the measurement was executed using two MALDI instruments. Each sample preparation was prepared 12 times on the MALDI target and measured once. Altogether about 3,600 spectra were acquired and analyzed during this study. No misidentifications were seen.

NOTE:

- Reduced performance was seen with the BACTEC Lytic/10 Anaerobic/F.
- MBT Sepsityper should only be used with charcoal free bottles.

10. Carry-Over:

Five different organisms were cultivated in blood culture bottles. Additionally, five blood culture bottles were inoculated with sheep blood as negative samples. All bottles were placed on a blood culture instrument and pulled when positive (negative bottles were pulled at 19 hours) The bottles

were processed using the MBT Sepsityper Kit US IVD and with negative and positive samples spotted alternately on a MTB Biotarget 96 and a US IVD 48 Spot Target. No carry over or cross contamination on the target was observed using the Rapid Sepsityper workflow.

11. Stability of Blood Culture (Frozen, Cooled or Seeded)

Five blood culture bottles (BD BACTEC™ PLUS Aerobic) were inoculated with five different organisms (*Candida albicans* DSM 11943, *Klebsiella pneumoniae* DSM 16358^T, *Pseudomonas aeruginosa* DSM 50071^T, Staphylococcus aureus DSM 20231^T, and *Streptococcus pneumoniae* DSM 20566^T) with 10^2 cells/ml and cultivated in the blood culture instrument till positivity. Additionally, five blood culture bottles were inoculated with the same five organisms with 10^7 cells/ml (seeded) and were harvested immediately. (Seeded samples consisted of the medium from blood culture bottles, inoculated with the appropriate volume of blood, seeded with the desired number of microbial cells). Both types of prepared blood culture bottles were aliquoted in 1ml portions. The aliquots were stored at room temperature (RT), 4°C, and -18°C. Subsequently, the Rapid Sepsityper workflow was performed with one set of each fraction at different time points to show the equivalence between fresh (RT), cooled (4°C), frozen (-18°C) and seeded samples. Equivalency was demonstrated for the four types of positive blood cultures such that each could be independently from each other in the analytical studies performed.

B Comparison Studies:

1. Challenge Panel: Each site received a panel of 50 of the most common blood culture organisms (Gram positive, Gram negative and Yeast) in frozen blood culture aliquots. All three sample preparation workflows were applied in parallel. No incorrect identifications were obtained with Challenge Panel testing. Table 7 summarizes results below.

Table 7: Challenge Panel Summary Results

	site 1			site 2			site 3		
	high confidence	low confidence	no ID	high confidence	low confidence	no ID	high confidence	low confidence	no ID
DT	53%	17%	30%	62%	13%	25%	38%	26%	36%
eDT	75%	6%	19%	83%	9%	8%	58%	16%	26%
Ext	81%	13%	6%	85%	4%	11%	84%	6%	10%
DT → eDT → Ext	88%	6%	6%	94%	2%	4%	88%	6%	6%

2. Method Comparison with Predicate Device:

Samples for evaluation of the MBT Sepsityper performance were collected from three (3) clinical sites: Site 1 analyzed 187 samples, site 2 analyzed 145 samples and site 3 analyzed 47 samples. All samples were obtained from the sites daily routine samples without any pre-selection. During the clinical studies polymicrobial samples were excluded via study design (Gram staining). Overall 379 samples were evaluated. Sixty-seven different species were identified during the clinical tests. No misidentifications occurred. Organisms are summarized in Table 8 below.

Table 8: Breakdown of Clinical Study Organisms

2	Achromobacter xylosoxidans
1	Acinetobacter_baumannii_nosocomialis group
1	Actinomyces oris
1	Actinotignum_schaalii group
1	Aerococcus urinae
1	Bacteroides fragilis
1	Brevibacillus centrosporus (NCV)
1	Citrobacter_freundii complex
1	Clostridium perfringens
1	Corynebacterium_afermentans group
1	Corynebacterium_aurimucosum group
4	Corynebacterium_striatum group
1	Enterobacter aerogenes
6	Enterobacter cloacae_complex
1	Enterococcus casseliflavus
10	Enterococcus faecalis
7	Enterococcus faecium
1	Enterococcus gallinarum
63	Escherichia coli
1	Granulicatella adiacens
2	Haemophilus influenzae
1	Klebsiella_oxytoca Raoultella_ornithinolytica

10	Klebsiella pneumoniae
2	Klebsiella variicola
1	Lactobacillus rhamnosus
1	Lactobacillus salivarius
6	Micrococcus luteus
2	Morganella morganii
2	Neisseria gonorrhoeae
1	Neisseria meningitidis
1	Parvimonas micra
1	Pasteurella multocida
8	Propionibacterium acnes
6	Proteus mirabilis
13	Pseudomonas aeruginosa
1	Rhizobium radiobacter
2	Serratia marcescens
59	Staphylococcus aureus
8	Staphylococcus capitis
1	Staphylococcus caprae
2	Staphylococcus cohnii
75	Staphylococcus epidermidis
14	Staphylococcus hominis
1	Staphylococcus lugdunensis

1	Staphylococcus pasteuri
3	Staphylococcus pettenkoferi
1	Staphylococcus saccharolyticus
1	Stenotrophomonas maltophilia
4	Streptococcus agalactiae
1	Streptococcus anginosus
4	Streptococcus dysgalactiae
1	Streptococcus equi
1	Streptococcus gallolyticus
1	Streptococcus gordonii
1	Streptococcus intermedius
1	Streptococcus lutetiensis
8	Streptococcus_mitis_oralis group
1	Streptococcus parasanguinis
6	Streptococcus pneumoniae
3	Streptococcus pyogenes
2	Streptococcus sanguinis
1	Streptococcus thermophilus
5	Candida albicans
4	Candida glabrata
1	Candida lusitaniae
1	Candida parapsilosis
1	Candida tropicalis

Sixty-seven different species belonging to 31 genera were observed in the study. Fifty-two percent of all samples were identified as *Staphylococcus epidermidis, Escherichia coli* or *Staphylococcus aureus*. The two Gram positive species *Staphylococcus aureus* and *Staphylococcus epidermidis* appeared very frequently and were responsible for 134 out of 248 (54%) Gram positive samples.

All three sample preparations were performed for all clinical samples in this study, i.e., “Rapid Sepsityper DT" (RS_DT), “Rapid Sepsityper eDT (RS_eDT) and Full Sepsityper (Ext). Colonies were identified using the DT/eDT result. For result interpretation, spotting methods were analyzed separately as well as by MBT Sepsityper workflow. See Table 9 and Table 10 below. No discordant high & low confidence results were obtained.

The identification performance of the Rapid Sepsityper Workflows compared to the MBT Sepsityper Full extraction was lower for certain groups of organisms. The RS_DT sample preparation for Gram positive bacteria showed only 32% “high confidence ID” log(scores) compared to 55% scores for Gram negative organisms prepared with the same technique. The combination of RS_DT/RS_eDT showed nearly identical performance for both bacterial groups.

For Gram negative bacteria, the combination of RS_DT and RS_eDT led to 71 successful identifications compared to 89 identifications by using the extraction workflow alone. For Gram positive bacteria the combined rapid workflows identified 145 samples compared to 198 samples with extraction. This means that for both bacterial groups the rapid workflows delivered a final identification result in about 75% of cases compared to the “extraction only workflow”.

The general performance of Gram positive and Gram negative bacteria was found to be very similar. The rapid workflows combined identified about 60% of all samples of Gram positive as well as Gram negative bacteria. The entire workflow (DT → eDT → Ext) was slightly better for Gram positive bacteria (77% vs 84%).

Four percent of all positive blood culture samples were found to contain yeast and no false ID was observed. The identification performance for yeast was low with about 30% of isolates identified with high or low confidence after applying the entire workflow (RS_DT → RS_eDT → Ext) and the remainder (70%) reported as "no ID".

Clinical Study Polymicrobial Blood Cultures

If the positive blood culture Gram stain indicated a polymicrobial infection, the sample was excluded from the study. However, after the positive blood culture bottles were subcultured, plates were examined for purity and isolated colonies were identified using the MBT-CA System in accordance with instructions for use. If a polymicrobial sample (mixed culture) was observed, each organism was processed for identification on the MBT-CA System following instructions for use.

Thirteen samples were confirmed as "polymicrobial samples" after subculture and overnight incubation on agar plates. Of these, five polymicrobial samples were correctly identified within the identification ranking lists and eight were not identified within the identification ranking lists. In both cases, not a single completely false ID was observed in any MBT Sepsityper identification. In all cases of polymicrobial samples one part of the two species was identified correctly or a "no identification" was reported.

Table 9: Overall Identification Results (%) by Spotting Method.

Number of Identifications - Clinical Studies													
organisms	Number (%) of Samples	RS_DT				RS_eDT				Ext			
		confidence		no		confidence		no		confidence		no	
		high	low	ID	peak	high	low	ID	peak	high	low	ID	peak
Gram negative bacteria	119 (31.4)	65 (54.6)	8 (6.7)	22 (18.5)	24 (20.2)	64 (53.8)	8 (6.7)	20 (16.8)	27 (22.7)	89 (74.8)	4 (3.4)	19 (16)	7 (5.9)
Gram positive bacteria	248 (65.4)	80 (32.3)	38 (15.3)	87 (35.1)	43 (17.3)	132 (53.2)	37 (14.9)	44 (17.7)	35 (14.1)	198 (79.8)	25 (10.1)	23 (9.3)	2 (0.8)
Yeast	12 (3.2)	0 (0)	1 (8.3)	8 (66.7)	3 (25)	1 (8.3)	0 (0)	7 (58.3)	4 (33.3)	2 (16.7)	1 (8.3)	8 (66.7)	1 (8.3)
All samples	379 (100)	145 (38.3)	47 (12.4)	117 (30.9)	70 (18.5)	197 (52)	45 (11.9)	71 (18.7)	66 (17.4)	289 (76.3)	30 (7.9)	50 (13.2)	10 (2.6)

Table 10: Overall Results (%) by Spotting Workflow

Number of Identifications - Clinical Studies							
organisms	Number (%) of Samples	RS_DT PLUS RS_eDT			RS_DT ► RS_eDT ► Ext		
		High Confidence	High & Low Confidence	No ID	High Confidence	High & Low Confidence	No ID
Gram negative bacteria	119(31.4)	71(59.7)	82(68.9)	37(31.1)	92(77.3)	99(83.2)	20(16.8)
Gram positive bacteria	248(65.4)	145(58.5)	185(74.6)	63(25.4)	209(84.3)	228(91.9)	20(8.1)
Yeast	12(3.2)	1(8.3)	2(16.7)	10(83.3)	2(16.7)	4(33.3)	8(66.7)
All samples	379(100)	217(57.3)	269(71)	110(29)	303(79.9)	331(87.3)	48(12.7)

3. Matrix Comparison:

   N/A

C Clinical Studies:

1. Clinical Sensitivity:

   See B.1 Method Comparison with Predicate Device:

2. Clinical Specificity:

   See B.1 Method Comparison with Predicate Device:

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   N/A

D Clinical Cut-Off:

See Assay Reportable Range VII.A.5 above.

E Expected Values/Reference Range:

See Assay Reportable Range VII.A.5 above.

F Other Supportive Instrument Performance Characteristics Data:
N/A

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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

As all blood culture bottles are subcultured and growth will be assessed as compared to MBT Sepsityper identification, reduced performance for MBT Sepsityper blood culture identification is acceptable as compared to isolated colony identification. Users should follow the Instructions for Use (IFU) which indicates all results should be reviewed by a trained microbiologist and final organism identification should be based on all relevant information available.