510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K162950

B. **Purpose for Submission:** To add the additional organism identification claims (moulds, mycobacteria and nocardia) to the already cleared device DEN130013 (K124067).

C. Measurand: See Intended Use

D. Type of Test: A mass spectrometer system for clinical use for the identification of microorganisms is a qualitative *in vitro* diagnostic device intended for the identification of microorganisms cultured from human specimens. The device is comprised of an ionization source, a mass analyzer and a spectral database. The device is indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial and fungal infections.

E. Applicant: bioMérieux, Inc.

F. Proprietary and Established Names:

VITEK MS

G. Regulatory Information:

1. Regulation section: 21 CFR 866. 3361
2. Classification: Class II (special controls)
3. Product code: PEX
4. Panel: Microbiology (83)

H. Intended Use:

1. Intended use(s):

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 specimens.

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, yeast and mould infections.

The following organisms are claimed:

Abiotrophia defectiva
Achromobacter denitrificans [1]
Achromobacter xylosoxidans [1]
Acinetobacter baumannii complex
Acinetobacter haemolyticus
Acinetobacter junii
Acinetobacter lwoffii
Actinomyces meyeri
Actinomyces neuii
Actinomyces odontolyticus
Aerococcus viridans
Aeromonas hydrophila/caviae [2]
Aeromonas sóbria [2,3]
Aggregatibacter actinomycetemcomitans
Aggregatibacter aphrophilus
Aggregatibacter segnis
Alcaligenes faecalis ssp faecalis
Bacteroides caccae
Bacteroides fragilis
Bacteroides ovatus [4]
Bacteroides thetaiotaomicron
Bacteroides uniformis
Bacteroides vulgatus
Bordetella parapertussis
Bordetella pertussis
Brevundimonas diminuta
Burkholderia multivorans
Campylobacter coli
Campylobacter jejuni
Candida albicans
Candida dubliniensis
Candida famata
Candida glabrata
Candida guilliermondii
Candida haemulonii
Candida inconspicua
Candida intermedia
Candida kefyr
Candida krusei
Candida lambica
Candida lipolytica
Candida lusitaniae
Candida norvegensis
Candida parapsilosis
Candida pelliculosa
Candida rugosa
Candida tropicalis
Candida utilis
Candida zeylanoides
Chryseobacterium indologenes
Citrobacter amalonaticus
Citrobacter braaki [5]
Citrobacter freundii [5]
Citrobacter koseri
Citrobacter youngae [5]
Clostridium clostridioforme
Clostridium difficile
Clostridium perfringens
Clostridium ramosum
Corynebacterium jeikeium
Cronobacter sakazakii
Cryptococcus neoformans
Edwardsiella hoshinae
Edwardsiella tarda
Eikenella corrodens
Elizabethkingia meningoseptica
Enterobacter aerogenes
Enterobacter asburiae [6]
Enterobacter cancerogenus
Enterobacter cloacae [6]
Enterococcus avium
Enterococcus casseliflavus
Enterococcus durans
Enterococcus faecalis
Enterococcus faecium
Enterococcus gallinarum
Escherichia coli [7]
Escherichia fergusonii
Escherichia hermannii
Ewingella americana
Finegoldia magna
Fusobacterium necrophorum
Fusobacterium nucleatum
Gardnerella vaginalis
Gemella haemolysans
Gemella morbillorum

Geotrichum capitatum
Granulicatella adiacens
Haemophilus influenzae
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Hafnia alvei
Kingella denitrificans
Kingella kingae
Klebsiella oxytoca
Klebsiella pneumoniae
Kodamaea ohmeri
Lactococcus garvieae
Lactococcus lactis ssp lactis [8]
Leclercia adecarboxylata
Legionella pneumophila
Leuconostoc mesenteroides
Leuconostoc pseudomesenteroides
Listeria monocytogenes
Malassezia furfur
Malassezia pachydermatis
Micrococcus luteus
Mobiluncus curtisii
Moraxella (Neisseria) ovis
Morganella morganii
Neisseria cinerea
Neisseria gonorrhoeae [9]
Neisseria meningitidis
Neisseria mucosa [10]
Ochrobactrum anthropi
Oligella ureolytica
Oligella urethralis
Pantoea agglomerans
Parvimonas micra
Pasteurella multocida
Pediococcus acidilactici
Peptoniphilus asaccharolyticus
Peptostreptococcus anaerobius
Pluralibacter gergoviae
Prevotella bivia
Prevotella buccae
Prevotella denticola
Prevotella intermedia
Prevotella melaninogenica
Propionibacterium acnes
Proteus mirabilis
Proteus penneri [11]
Proteus vulgaris [11]
Providencia rettgeri
Providencia stuartii
Pseudomonas aeruginosa
Pseudomonas fluorescens
Pseudomonas putida
Pseudomonas stutzeri
Ralstonia pickettii
Raoultella ornithinolytica
Raoultella planticola
Rhizobium radiobacter
Rhodotorula mucilaginosa
Rothia mucilaginosa
Saccharomyces cerevisiae
Salmonella group [9]
Saprochaete capitata
Serratia fonticola
Serratia liquefaciens
Serratia marcescens
Serratia odorifera
Sphingobacterium multivorum
Sphingobacterium spiritivorum
Sphingomonas paucimobilis
Staphylococcus aureus
Staphylococcus capitis
Staphylococcus cohnii ssp cohnii
Staphylococcus cohnii ssp urealyticus
Staphylococcus epidermidis
Staphylococcus haemolyticus
Staphylococcus hominis ssp hominis [12]
Staphylococcus lugdunensis
Staphylococcus saprophyticus
Staphylococcus schleiferi
Staphylococcus sciuri
Staphylococcus simulans
Staphylococcus warneri
Stenotrophomonas maltophilia
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus constellatus
Streptococcus dysgalactiae
Streptococcus gallolyticus ssp gallolyticus

Streptococcus infantarius ssp coli
Streptococcus infantarius ssp infantarius
Streptococcus intermedius
Streptococcus mitis/Streptococcus oralis
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarius ssp salivarius [13]
Streptococcus sanguinis
Trichosporon asahii
Trichosporon inkin
Trichosporon mucoides
Vibrio cholerae
Vibrio parahaemolyticus
Vibrio vulnificus
Yersinia enterocolitica
Yersinia frederiksenii
Yersinia intermedia
Yersinia kristensenii
Yersinia pseudotuberculosis [14]

MYCOBACTERIUM

Mycobacterium abscessus
Mycobacterium avium
Mycobacterium chelonae
Mycobacterium fortuitum group: [15]
- *Mycobacterium alvei*
- *Mycobacterium farcinogenes*
- *Mycobacterium fortuitum*
- *Mycobacterium houstonense*
- *Mycobacterium peregrinum*
- *Mycobacterium porcinum*
- *Mycobacterium senegalense*

Mycobacterium gordonae
Mycobacterium haemophilum
Mycobacterium immunogenum
Mycobacterium intracellulare
Mycobacterium kansasii
Mycobacterium lentiflavum
Mycobacterium malmoense
Mycobacterium marinum
Mycobacterium mucogenicum
Mycobacterium scrofulaceum
Mycobacterium simiae
Mycobacterium smegmatis
Mycobacterium szulgai
Mycobacterium tuberculosis complex: [16]
- *Mycobacterium africanum*
- *Mycobacterium bovis*
- *Mycobacterium canettii*
- *Mycobacterium microti*
- *Mycobacterium pinnipedii*
- *Mycobacterium tuberculosis*

Mycobacterium xenopi

NOCARDIA

Nocardia abscessus
Nocardia asteroides
Nocardia brasiliensis
Nocardia cyriacigeorgica
Nocardia farcinica
Nocardia nova [17]
Nocardia otitidiscaviarum
Nocardia paucivorans
Nocardia pseudobrasiliensis
Nocardia transvalensis
Nocardia veterana
Nocardia wallacei

MOULDS

Acremonium sclerotigenum
Alternaria alternata
Aspergillus brasiliensis
Aspergillus flavus/oryzae
Aspergillus fumigatus
Aspergillus lentulus
Aspergillus nidulans
Aspergillus niger complex
Aspergillus sydowii
Aspergillus terreus complex [18]
Aspergillus calidoustus
Aspergillus versicolor
Blastomyces dermatitidis
Cladophialophora bantiana
Coccidioides immitis/posadasii
Curvularia hawaiiensis
Curvularia spicifera
Epidermophyton floccosum
Exophiala dermatitidis

Exophiala xenobiotica [19]
Exserohilum rostratum [19]
Fusarium oxysporum complex
Fusarium proliferatum
Fusarium solani complex
Histoplasma capsulatum
Lecythophora hoffmannii
Lichtheimia corymbifera
Microsporum audouinii
Microsporum canis
Microsporum gypseum
Mucor racemosus complex [20]
Paecilomyces variotii complex
Penicillium chrysogenum
Pseudallescheria boydii
Purpureocillium lilacinum
Rasamsonia argillacea complex [21]
Rhizopus arrhizus complex
Rhizopus microsporus complex
Sarocladium kiliense
Scedosporium apiospermum
Scedosporium prolificans
Sporothrix schenckii complex
Trichophyton interdigitale
Trichophyton rubrum
Trichophyton tonsurans
Trichophyton verrucosum
Trichophyton violaceum [22]

1. *Achromobacter denitrificans* and *Achromobacter xylosoxidans* identifications should be considered as a slashline result, *Achromobacter denitrificans/ Achromobacter xylosoxidans*. The final identification should be confirmed before sending the final selection to the LIS or to the VITEK 2 for AST results
2. *Aeromonas hydrophila/caviae* and *Aeromonas sobria* should be considered as an *Aeromonas* species group identification.
3. In KB V3.0.0, *Aeromonas sobria* is displayed as a low discrimination result with *Aeromonas veronii* but only *Aeromonas sobria* has been clinically validated.
4. In KB V3.0.0, *Bacteroides ovatus* is grouped in a slashline with *Bacteroides xylanisolvens*. It is not possible to distinguish between the two species. *B. ovatus* is more commonly associated with human infection.
5. *Citrobacter freundii*, *Citrobacter braakii* and *Citrobacter youngae* should be considered as *Citrobacter freundii* complex.
6. *Enterobacter cloacae* and *Enterobacter asburiae* identifications should be considered as a slashline result, *Enterobacter cloacae/ Enterobacter asburiae*. The final identification should be confirmed before sending the final selection to the LIS or to the VITEK 2 for AST results.
7. *Shigella* species and *E. coli* O157 are identified as *Escherichia coli*. Confirmatory tests are required to differentiate *Escherichia coli* from *Shigella* species or *E. coli* O157.
8. In KB V3.0.0 *Lactococcus lactis ssp lactis* is integrated in a new group *Lactococcus lactis* (with two other subspecies *Lactococcus lactis ssp cremoris* and *Lactococcus lactis ssp hordniae*). In the *Lactococcus lactis* group only subspecies *Lactococcus lactis ssp lactis* has been clinically validated.
9. Confirmatory tests recommended for *Neisseria gonorrhea* and *Salmonella* species.
10. In KB V3.0.0, *Neisseria mucosa* is grouped in a slashline with *Neisseria sicca*. It is not possible to distinguish between the two species. Both species have been associated with human infection.

11. *Proteus penneri* and *Proteus vulgaris* identifications should be considered as a slashline result, *Proteus penneri/ Proteus vulgaris*.
12. In KB V3.0.0 *Staphylococcus hominis ssp hominis* is integrated in a new group *Staphylococcus hominis* (with another subspecies *S. hominis ssp novobiosepticus*). In the *Staphylococcus hominis* group only subspecies *Staphylococcus hominis ssp hominis* has been clinically validated.
13. In KB V3.0.0, *Streptococcus salivarius* ssp *salivarius* is displayed as a low discrimination result with *Streptococcus salivarius* ssp *thermophilus* and *Streptococcus vestibularis* but only *Streptococcus salivarius* ssp *salivarius* has been clinically validated.
14. There is a possibility of cross-identification between *Yersinia similis* and *Yersinia pseudotuberculosis*.
15. The *Mycobacterium fortuitum* complex or group displayed in VITEK MS includes the seven most prominent and closely related species. The VITEK MS group differs from the ones reported in the literature that may include up to 13 species.
16. The *Mycobacterium tuberculosis* complex displayed in VITEK MS includes the four most prevalent species based on different worldwide geographic regions. It does not include two additional species *Mycobacterium microti* and *Mycobacterium pinnipedii* as reported in the literature.
17. In KB V3.0.0, *Nocardia nova* is displayed as a low discrimination result with Nocardia africana but only *Nocardia nova* has been clinically validated.
18. Both *Aspergillus alabamensis* and *Aspergillus niveus* are not identified as *Aspergillus terreus* complex. No identification is expected with either species.
19. All of the no identifications for this organism were from multiple replicates of the same isolate.
20. *Mucor racemosus f. sphaerosporus* is not identified as *Mucor racemosus* complex. No identification is expected. The *Mucor racemosus* complex is comprised of *M. racemosus* f. *brunneus*, *M. racemosus* f. *chibinensis*, *M. racemosus* f. *racemosus*, and *M. racemosus* f. *sphaerosporus*.
21. Three out of the five no identifications for this organism were from multiple replicates of the same isolate.
22. All of the discordant identifications for this organism were from multiple replicates of the same isolate.

2. Indication(s) for use: Same as intended use.

3. Special conditions for use statement(s):

   The VITEK MS is for prescription use only in accordance with 21 CFR 801.109.

4. Special instrument requirements:
   - VITEK MS: Shimadzu AXIMA® Assurance mass spectrometer
   - VITEK MS Prep Station
   - VITEK MS-DS Target Slides

Reagents:

- VITEK MS-CHCA (Alpha-cyano-4-hydroxy-cinnamic acid) solution
- VITEK MS-FA (Formic acid) reagent
- VITEK MS MYCOBACTERIUM/NOCARDIA KIT
- VITEK MS LIQUID MYCO SUPPLEMENTAL KIT
- VITEK MS MOULD KIT

Database:

- VITEK MS V3.0 Knowledge Base (KB)

Software:

- VITEK MS Sample Prep Station software,
- VITEK MS V3 acquisition station 1.5.0.2
- VITEK MS Analysis Server / Software
- VITEK MS Computation Engine
- Myla® Middleware

I. Device Description:

The VITEK MS v3.0 system is a system consisting of kit reagents (VITEK MS-CHCA, VITEK MS-FA, VITEK MS Mycobacterium/Nocardia Kit, VITEK MS Mould Kit), VITEK MS-DS target slides, VITEK MS Prep Station, Knowledge Base v3.0, software, and the VITEK MS (original equipment manufacturer (OEM)-labeled Shimadzu AXIMA® Assurance mass spectrometer).

Reagent Description:

- VITEK MS-CHCA (Alpha-cyano-4-hydroxy-cinnamic acid) is the solution that serves as a matrix which will crystalize with the microbial sample on the target slide spot. 1.0 µl of the matrix is added to the spot with the sample and allowed to dry forming crystals.
- The VITEK MS-FA (Formic acid) reagent is used to pre-treat yeast in order to extract protein before the VITEK MS-CHCA matrix is added to the spot containing the sample.
- VITEK MS-DS target slides are single-use disposables which contain 3 acquisition groups of 16 sample spots. Each group includes 1 calibration spot. Target slides are for single use only.
- VITEK MS MYCOBACTERIUM/NOCARDIA KIT includes ethanol and vials with glass beads to inactivate mycobacteria and nocardia by disrupting the cells. The kit also includes formic acid and acetonitrile to complete the extraction of proteins.
- VITEK MS LIQUID MYCO SUPPLEMENTAL KIT includes the additional consumables (i.e., 5 mL conical bottom tubes and safety backed absorbent pads) needed to process samples for mycobacterium with liquid media.
- VITEK MS MOULD KIT provides ethanol, formic acid and acetonitrile to inactivate moulds and extract their proteins.

Knowledge Base:

The reference database for the VITEK MS system includes data representing 1046 taxa, including 882 bacteria and 164 fungi. Each species or species group is represented by an average of 17 isolates (range 2* - 712). In order to capture the degree of acceptable variation within spectra from the same species, each reference isolate was grown on multiple media types under several growth conditions. The raw spectra were then acquired by more than one technician using multiple instruments. This process resulted in an average of 36 reference spectra per species (or 53 spectra by species or group of species).

* NOTE: For two very rare species (*Prevotella baroniae* and *Prevotella ruminicola*), it was only possible to test a single strain for these organisms in the reference database build.

Additional laboratory tests as determined by Microbiology laboratory protocols for low discrimination results or non-clinically validated organisms are necessary for the completion of the organism identification. Non-clinically validated organisms are displayed as (N) in the report.

Testing of non-clinically validated species or species not found in the database may result in an unidentified result or a misidentification.

Note: Interpretation of results and use of the VITEK MS system require a competent laboratorian who should judiciously make use of experience, specimen information, and other pertinent procedures before reporting the identification of test organisms. Additional information known to the user, such as Gram stain reaction, colonial and cellular morphology, and growth aerobically or in CO2 should be considered when accepting VITEK MS results.

NOTE: *Candida auris* is not currently in the knowledge base; testing of this species will usually give no identification but may also result in a misidentification as either *Candida haemulonii* or *Candida lusitaniae*.

Software:

The VITEK MS system consists of a suite of applications that perform the overall system function. The system functions as a kiosk, not allowing the end-user to access any operating system functions. The end-user cannot access the native operating system or any system configuration panels. The software application contains several processes that include handling all user interactions, all network activity, communication, and synchronization with the all the components. The VITEK MS system software is comprised of four software components and MYLA middleware.

1. VITEK MS Sample Prep Station software: The VITEK MS Prep Station is used to prepare VITEK MS-DS target slides. It consists of a computer workstation equipped with a barcode reader, Touch Screen and Virtual Keyboard.

2. VITEK MS acquisition station: The Acquisition Station Software controls the VITEK MS to acquire spectral data from each sample in turn and displays the spectra for the operator to review. The Acquisition Station displays the spectra and peak lists and transfers the peak lists to the VITEK MS Analysis Server.
3. VITEK MS Analysis Server / Software: The VITEK MS Analysis Server is the software that manages the VITEK MS workflow and computes VITEK MS identification results. It is a software component that resides on the Myla Server (PC).
4. VITEK MS Computation Engine: The VITEK MS analysis server sends the acquired data to the computation engine that calculates the identification results. The algorithms and mapping files required for identification are contained within the computation engine.
5. Myla Middeware: Myla™ is a computer application ("Middleware"), based on Web technology, which allows data related to the laboratory workflow, laboratory instruments, Laboratory Information System (LIS), analysis results, etc. to be grouped together. Myla™ interfaces between the bioMérieux instruments connected to the application (e.g., VITEK MS) and the Laboratory Information System (LIS). Myla™ manages the VITEK MS workflow and computes the identification results with the use of a computation engine and organism knowledge bases.

VITEK MS

bioMérieux's VITEK MS, is the same instrument as the Shimadzu Axima Assurance MALDI-TOF spectrometer. The VITEK MS is manufactured for bioMérieux by Kratos Analytical (a Shimadzu subsidiary) in Manchester, UK. The VITEK MS contains a Class 1 laser product containing a Class 3b invisible-light laser. The laser is a 337 nm nitrogen laser, fixed focus. This speed depends on the mass of the ions with heavier molecules having a higher moment of inertia resulting in a lower velocity. The time of transit is measured precisely by the ions' arrival at a particle detector. Based on the time of flight, the m/z ratio of each particle can be determined, and a mass spectrum of the sample mixture is generated. The recorded signal is processed by the Acquisition Station software and presented as a spectrum of intensity versus mass in Daltons (Da).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   VITEK MS

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

   DEN130013 (K124067)

3. Comparison with predicate:

Similarities		
Characteristic	**New Device VITEK MS v3 / KB v3.0.0 (K162950)**	**Predicate Device VITEK MS (DEN130013 / K124067)**
Classification	21 CFR 866.3361 Class II System, mass spectrometry, MALDI-ToF, microorganism identification, cultured isolates	21 CFR 866.3361 Class II System, mass spectrometry, MALDI-ToF, microorganism identification, cultured isolates
Product Code	PEX	PEX
Intended Use	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, yeast and mould infections. **NOTE:** Addition of mould, mycobacteria and nocardia indications for use added in VITEK MS v3 / KB v3.0.0	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.
Type of Test	Automated Mass Spectrometry System	Automated Mass Spectrometry System
Calibration	*E. coli* ATCC 8739	*E. coli* ATCC 8739
Matrix	Alpha-cyano-4-hydroxy-cinnamic acid	Alpha-cyano-4-hydroxy-cinnamic acid
Target Slide	VITEK MS DS Target Slides 48 positions disposable plastic targets	VITEK MS DS Target Slides 48 positions disposable plastic targets
MALDI TOF MS Instrument	Shimadzu AXIMA® Assurance MS	Shimadzu AXIMA® Assurance MS
Method of Testing	(For bacteria and yeast) Direct testing from isolated colonies	(For bacteria and yeast) Direct testing from isolated colonies
Age of Culture	(For bacteria and yeast) Incubation of culture should be 18 – 72 hours	(For bacteria and yeast) Incubation of culture should be 18 – 72 hours
Sample / Media Type	Isolated colony from a patient sample source, from a media used during development of the VITEK MS Knowledge Base, including: • Columbia blood agar with 5% sheep blood • Trypticase soy agar with 5% sheep blood • Trypticase soy agar • Chocolate polyvitex agar • Campylosel agar • MacConkey agar • Modified Sabouraud dextrose agar (glucose: 20 g/l - pH: 6.1) • chromID CPS	Isolated colony from a patient sample source, from a media used during development of the VITEK MS Knowledge Base, including: • Columbia blood agar with 5% sheep blood • Trypticase soy agar with 5% sheep blood • Trypticase soy agar • Chocolate polyvitex agar • Campylosel agar • MacConkey Agar • Modified Sabouraud dextrose Agar • ChromID CPS
Algorithm	An algorithm process known as "mass binning" is used for the differentiation of the species included in the knowledge base.	An algorithm process known as "mass binning" is used for the differentiation of the species included the knowledge base.

Similarities		
Characteristic	**New Device VITEK MS v3 / KB v3.0.0 (K162950)**	**Predicate Device VITEK MS (DEN130013 / K124067)**
Result reporting	To detect microbial growth, the VITEK MS System uses a knowledge base developed from spectra of a number of microbial species. The resulting spectra from the VITEK MS / Acquisition Station are evaluated against this knowledge. Identification are displayed, with a confidence value when an organism or organism group is identified. Low-discrimination identifications are displayed when more than one, but not more than four identifications, are made. When more than four identifications are made, or when no match is found, the organism is considered unidentified. Data analysis is performed using software embedded on the MYLA® Server.	To detect microbial growth, the VITEK MS System uses a knowledge base developed from spectra of a number of microbial species. The resulting spectra from the VITEK MS / Acquisition Station are evaluated against this knowledge. Identification are displayed, with a confidence value when an organism or organism group is identified. Low-discrimination identifications are displayed when more than one, but not more than four identifications, are made. When more than four identifications are made, or when no match is found, the organism is considered unidentified. Data analysis is performed using software embedded on the MYLA® Server.
Recorded Mass Range	2,000 - 20,000 m/z	2,000 - 20,000 m/z
System Components, Software, Reagents	The VITEK MS system consists of a number of components (e.g. a Prep Station, the MS Instrument, an Acquisition Station), software/middleware solutions (e.g. Acquisition Station Software, an Analysis Server, a Computation Engine, and MYLA®), and the same disposables (e.g. the VITEK MS-DS Target Slides, CHCA Matrix, and Formic Acid as applicable).	The VITEK MS system consists of a number of components (e.g. a Prep Station, the MS Instrument, an Acquisition Station), software/middleware solutions (e.g. Acquisition Station Software, an Analysis Server, a Computation Engine, and MYLA®), and the same disposables (e.g. the VITEK MS-DS Target Slides, CHCA Matrix, and Formic Acid as applicable).
Laser	The laser used for the excitation of molecules on the VITEK MS v3 is the same as the laser used for the VITEK MS (v2) (operating power and repetition rate is unchanged).	A laser is used for the excitation of molecules (the laser operates on a set power and repetition rate).

Differences		
Characteristic	**New Device VITEK MS v3 / KB v3.0.0 (K162950)**	**Predicate Device VITEK MS (v2) (DEN130013 / K124067)**
Knowledge Base and Indications for Use	VITEK MS v3.0 Knowledge Base (includes the previous bacteria and yeast, as well as new mycobacterium, nocardia, and mould indications for use)	VITEK MS V2.0 Knowledge Base (included bacteria and yeast isolates)
Culture Media	The VITEK v3 / KB v3.0.0 includes new mycobacteria indications for use from both solid & liquid culture media, and nocardia and mould indications for use from solid culture media only.	VITEK MS (v2) only included indications for use for isolates from solid culture media.
Age of Culture	(Includes new mycobacterium, nocardia, and mould indication for use):	As mycobacterium, nocardia, and mould are new indications for use, there were no previous

Differences		
Characteristic	**New Device VITEK MS v3 / KB v3.0.0 (K162950)**	**Predicate Device VITEK MS (v2) (DEN130013 / K124067)**
	▪ Incubation of rapid growing mycobacteria from solid media should be 3 – 7 days ▪ Incubation of slow growing mycobacteria from solid media should be 7 – 28 days ▪ Incubation of mycobacteria from liquid media should be 24 – 72 hours post positivity ▪ Incubation of nocardia cultures should be 24 – 72 hours ▪ Incubation of rapid growing moulds should be 2 – 8 days ▪ Incubation of slow growing moulds should be 5 – 25 days	Age of Culture requirements for these organisms on the predicate device.
Rastering Pattern	The VITEK MS Acquisition Station was updated so that the rastering pattern exhausts raster points.	The VITEK MS Acquisition Station rastering pattern found sweet spots.
Media Type	With the introduction of the *Mycobacterium* indications for use, the following new media types were used during the development of the VITEK MS KB v3.0 for mycobacteria identifications. • BacT/ALERT® MP • Lowenstein-Jensen • MGIT™ • Middlebrook 7H10 agar • Middlebrook 7H11 agar • Coletsos With the introduction of the mould indications for use, the following new media types were used during the development of the VITEK MS KB v3.0 for mould identifications. • Potato dextrose agar • Sabouraud dextrose agar (glucose: 40 g/l - pH: 5.6) • Sabouraud dextrose agar with Gentamicin & Chloramphenicol • Tryticase soy agar with neutralizers With the introduction of the nocardia indications for use, the following new media types were used during the development of the VITEK MS KB v3.0 for nocardia identifications. • Sabouraud dextrose agar (glucose: 40 g/l - pH: 5.6) • Buffered Charcoal Yeast Extract	As *Mycobacterium* and mould, are a new indication for use, there were no previous media types or age of culture requirements for these organisms on the predicate device.

Differences		
Characteristic	**New Device VITEK MS v3 / KB v3.0.0 (K162950)**	**Predicate Device VITEK MS (v2) (DEN130013 / K124067)**
Sample Prep	With the introduction of the mycobacteria, nocardia and mould indications for use, an inactivation and extraction process is required for sample prep, prior to spotting the sample to the VITEK MS DS Slide.	As the predicate device only included indications for use with bacteria and yeast, sample prep consisted of direct spotting to VITEK MS DS Slide. Inactivation of the sample occurs during the addition of the CHCA Matrix.

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

Table 1: Clinical & Laboratory Standards Institute (CLSI) Standards

Standards No.	Standards Title	Date
C5O-A	Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline, 1st Edition	10/29/2007
EP09-A3	Measurement Procedure Comparison and Bias Estimate Using Patient Samples; Approved Guideline, 3rd Edition	08/30/2013
EP12-A	User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline, 2nd Edition	1/25/2008
MM09-A	Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline, 1st Edition	12/20/2004
MM18-A	Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline, 1st Edition	4/28/2008
M24-A2	Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard, 2nd Edition	03/31/2011
M48-A	Laboratory Detection and Identification of Mycobacterium; Approved Guideline, 1st Edition.	05/27/2008
M54-A	Principles and Procedures for Detection of Fungi in Clinical Specimens-Direct Examination; Approved Guideline, 1st Edition	10/31/2012

L. Test Principle:

The VITEK MS system is based on a matrix-assisted laser desorption ionization-time of flight mass spectrometer (MALDI-TOF MS). The colony is mixed with a saturated matrix solution and forms crystals. The ionization of this mixture by the laser induces the desorption and transfer of protons from photo-excited matrix to analyte to form a protonated molecule. During the analysis process, proteins are ionized without fragmentation by the coordinated

action of the laser and the small organic acids of the matrix and separated on the basis of their mass-to-charge ratios, a process which results in a characteristic mass spectral profile. Microbial identification is based on the comparison of the protein spectrum generated from intact whole bacterial cells to the knowledge database of species-specific reference protein profiles using a particular algorithm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A panel of 10 reproducibility strains consisting of five moulds, three mycobacteria and two Nocardia organisms were tested at each of three trial sites by two technologists in duplicate on each of five days with two runs per day for a total of 60 replicates per organisms. The reproducibility study was conducted at five external sites; three sites tested the mould reproducibility strains and three sites tested the mycobacteria and Nocardia reproducibility strains. Three different lots of the VITEK MS Mycobacterium/Nocardia kit and the VITEK MS Mould kit were used during reproducibility testing. On each day of testing, sequential positions on the target slide were assigned to the first run of the selected reproducibility samples, and randomized for the second run. Spot positions were alternated each day between the technologist on each day of testing. Three organisms were used for positive quality control, and reagents alone were used for the negative control. The quality control strains were *Aspergillus brasiliensis*, ATCC 16404, *Mycobacterium smegmatis*, ATCC 19420 and *Nocardia farcinica*, ATCC 3308. Results are shown in Table 2 below.

Table 2: Reproducibility Testing for Moulds, Mycobacteria and Nocardia –all sites combined

Sample	Organism	Day					Total (%)	95% CI
		1	2	3	4	5		
R1	*Mycobacterium abscessus*	12/12	12/12	12/12	12/12	12/12	60/60 (100.0%)	[94.0, 100.0]%
R2	*Mycobacterium chelonae*	12/12	12/12	12/12	12/12	12/12	60/60 (100.0%)	[94.0, 100.0]%
R3	*Mycobacterium smegmatis*	12/12	12/12	12/12	12/12	12/12	60/60 (100.0%)	[94.0, 100.0]%
R4	*Nocardia wallacei*	12/12	12/12	12/12	12/12	11/12	59/60 (98.3%)	[91.1, 100.0]%
R5	*Nocardia otitidiscaviarum*	12/12	12/12	12/12	12/12	12/12	60/60 (100.0%)	[94.0, 100.0]%
R6	*Aspergillus fumigatus*	12/12	12/12	12/12	12/12	12/12	60/60 (100.0%)	[94.0, 100.0]%
R7	*Fusarium proliferatum*	12/12	12/12	12/12	12/12	12/12	60/60 (100.0%)	[94.0, 100.0]%
R8	*Purpureocillium lilacinum*	12/12	12/12	12/12	11/12	11/12	58/60 (96.7%)	[88.5, 99.6]%
R9	*Lecythophora hoffmannii*	12/12	12/12	12/12	12/12	12/12	60/60 (100.0%)	[94.0, 100.0]%
R10	*Penicillium chrysogenum*	12/12	12/12	12/12	12/12	12/12	60/60 (100.0%)	[94.0, 100.0]%
All		120/120	120/120	120/120	119/120	118/120	597/600 (99.5%)	[98.5, 99.9]%

b. Linearity/assay reportable range:

Not applicable, qualitative assay.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability:

Calibrator: *E. coli* ATCC 8739 is used to as a calibrator. This organism is deposited with VITEK MS-CHCA matrix on positions: xA1, xB1, xC1, of the VITEK MS-DS target slides depending on the number of samples tested (one calibrator per acquisition group of 16 spots). The VITEK MS goes to the calibration spot in an acquisition group and performs a calibration. If the calibration passes, the instrument goes to the first spot in the acquisition group. If the calibration fails, an error is reported and VITEK MS proceeds to the next acquisition group without collecting sample spectra. After spectra have been acquired from each sample spot in an acquisition group, the calibration spot is checked again. The calibration sample should provide *E. coli* identification at 99.9% in Myla ™ software.

Stability

VITEK MS MYCOBACTERIUM/NOCARDIA KIT: 12 months (365 days) shelf life for VITEK -MS Mycobacterium/Nocardia IVD Ref: 415659, when stored at 2-25°C..

VITEK MS MOULD KIT: 12 months (365 days) shelf life for VITEK MS MOULD KIT IVD Re:.415680, when stored at 2-25°C.

Summary: Conditions and Days Post Extraction-Prior to Spotting*

Genus	Condition	Recommended
Mycobacterium	Room Temperature (15-25°C)	3 Days
	Refrigerated (2 8°C)	7 Days
	Frozen (-19 to -31°C)	14 Days
	Freeze-Thaw cycles	2 Freeze-thaw
Nocardia	Room Temperature (15-25°C)	3 Days
	Refrigerated (2-8°C)	7 Days
	Frozen (-19 to -31°C)	21 Days
	Freeze-Thaw cycles	3 Freeze-thaw
Moulds	The extracts of moulds are not stable regardless of the storage conditions and must be spotted within 8 hours on the day of extraction.	

*For details see M.1.h-l and M.1.o-s below.

Controls

During the reproducibility, proficiency and clinical isolate testing, three organisms were used for positive quality control, with reagents alone used for the negative control. Quality control organism were tested by VITEK MS each day a mold,

mycobacterium or nocardia isolate was tested. The quality control strains were as follows:

- *Aspergillus brasiliensis*, ATCC 16404
- *Mycobacterium smegmatis*, ATCC 19420
- *Nocardia farcinica*, ATCC 3308
- Negative Control (reagents alone)

Table 3: Number and Percent of Repeat QC Testing

QC Species	N	1 Repeat (Percent)	2 Repeats (Percent)
Aspergillus brasiliensis	162	19 (11.73%)	11 (6.79%)
Mycobacterium smegmatis	189	11 (5.82%)	1 (0.53%)
Nocardia farcinica	113	5 (4.42%)	1 (0.88%)
Neg control (reagents alone)	391	0 (0.00%)	0 (0.00%)
Total	855	35 (4.09%)	13 (1.52%)

d. Detection limit: See DEN130013 (K124067)

e. Biological Performance Equivalency Study Summary Report VITEK MS v2.2 vs VITEK MS v3

Biological performance equivalency was demonstrated for the 193 FDA originally claimed species using one well characterized strain per species, i.e. type or sequenced was sub-cultured on solid media and tested triplicate (from three separate subcultures) on the same slide after 18h to 24h of growth. In total, 627 distinct samples representing the 193 FDA claimed species were tested with both VITEK MS systems (V2.2 and V3.0) during the equivalency testing included:

- Gram negative Bacteria: 333 samples (111 strains x 3 replicates)
- Gram positive bacteria: 204 samples (68 strains x 3 replicates)
- Yeasts: 90 samples (30 strains x 3 replicates)

The global biological performance calculated from 627 samples (209 strains x 3 replicate) tested during this study (for each VITEK MS systems) is presented in Table 4 below. No calibration spot failures were observed with either VITEK MS system. The rate of overall correct identification rate is 94.3% (591/627) from VITEK MS V2.2 and 97.3% (610/627) from VITEK MS V3.0.

TABLE 4: Biological Performance Equivalency Study*

All families (after re-test)																			
VITEK MS V3.0 vs VITEK MS V2.2	Failure										Success								Total (ALL)
	Misidentification				No Identification				Total (ALL)		Combined Correct Identification						Total (ALL)		
	LD Incorrect ID		SC Incorrect ID		LD Multiple Genera		No Matching Class				LD Correct ID		LD Same Genus		SC Correct ID				
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N
VITEK MS V2.2	1	0.16	4	0.64	7	1.12	24	3.83	36	5.74	25	3.99	30	4.78	536	85.49	591	94.26	627
VITEK MS V3.0	2	0.32	5	0.8	.	.	10	1.59	17	2.71	29	4.63	15	2.39	566	90.27	610	97.29	627

*Abbreviations: Low discrimination (LD); Single choice (SC)

The McNemar's test, applied on a 2x2 contingency table (Failure vs. Success) was used to attest the equivalency of both systems. In this test, the null hypothesis (H_0) is that the two proportions of success (PV3.0 and PV2.2) are at the same rate (PV3.0 = PV2.2) or that the contingency 2x2 table is symmetric.

Total (All families)						
	2x2 table for global status				McNemar's Test	
		VITEK MS V2.2			Statistic (S)	7.367
		Failure	Success	Total	DF	1
VITE KS MS V3.0	Failure	2	15	17	Pr > S	0.007
		0.32	2.39	2.71		
	Success	34	576	610		
		5.42	91.87	97.29		
	Total	36	591	627		
		5.74	94.26	100.00		

The McNemar test resulted in the rejection the hypothesis of equivalency between both systems (p-value < 5%). VITEK MS V3.0 (97.3% of success) produces better global performance than VITEK MS V2.2 (93.8% of success). The McNemar's test was also applied to Gram Negative, Gram Positive and Yeast groups. It was concluded that for gram negative organisms and yeast, VITEK MS V3.0 performed as good as VITEK MS V2.2 on this set of data. For Gram positive organisms, the VITEK MS V3.0 system gave higher performance than VITEK MS V.2.2 on this set of data.

With the new acquisition station (new system), the instrument was fine-tuned in order to produce 20 more peaks (in average). A Bland-Altman scatter-plot was built for all the samples for which provided a correct identification from both VITEK MS systems, in order to measure the average bias observed between both systems during this study. For each couple of values (number of peaks V3.0, number of peaks V2.2), the average was calculated and reported on the X-axis, and then the difference between the number of peaks from V3.0 and the number of peaks from V.2.2 was calculated and reported on the Y-axis. (Plot not shown.) The average was 19.76 peaks, which is very close to the expected value (20 peaks). All the individual observations were well distributed over and under the mean.

Due to update in the database (KB V3.0 0), the following bacterial identification notes have been placed:

- In KB V3.0.0, *Aeromonas sobria* is displayed as a low discrimination result with *Aeromonas veronii* but only*Aeromonas sobria* has been clinically validated. *Aeromonas sobria*, has a high level of unidentified results
- In KB V3.0.0, *Bacteroides ovatus* is grouped in a slashline with *Bacteroides xylanisolvens*. It is not possible to distinguish between the two species. *B. ovatus* is more commonly associated with human infection.
- In KB V3.0.0, *Lactococcus lactis ssp lactis* is integrated in a new group *Lactococcus lactis* (with two other subspecies *Lactococcus lactis ssp cremoris* and *Lactococcus lactis ssp hordniae*). In the *Lactococcus lactis* group, only subspecies *Lactococcus lactis ssp lactis* has been clinically validated.
- In KB V3.0.0, *Neisseria mucosa* is grouped in a slashline with *Neisseria sicca*. It is not possible to distinguish between the two species. Both species have been associated with human infection.
- In KB V3.0.0, *Staphylococcus hominis ssp hominis* is integrated in a new group *Staphylococcus hominis* (with another subspecies *S. hominis ssp novobiosepticus*). In the *Staphylococcus hominis* group, only subspecies *Staphylococcus hominis ssp hominis* has been clinically validated.
- In KB V3.0.0, *Streptococcus salivarius ssp salivarius* is displayed as a low discrimination result with *Streptococcus salivarius ssp thermophilus* and *Streptococcus vestibularis* but only *Streptococcus salivarius ssp salivarius* has been clinically validated.

f. Age of Culture for Mycobacteria grown on Solid Media for VITEK MS Testing

A total of 734 spectra were generated through 12 testing time points from the original test panel (*M. abscessus, M. avium, M. chelonae, M. fortuitum, M. kansasii, M. intracellulare, M. scrofulaceum* and *M. tuberculosis*) and 80 additional spectra generated through 10 testing time points (Day 0 to 42 days, with Day 0 being the time sufficient growth of the culture was observed) with the additional testing of *M. smegmatis*, for a total of 814 spectra. Of those 814 spectra 42 did not pass quality control checks (number of peaks and amount of noise). Results demonstrated that the increasing age of cultures for mycobacteria species had an impact on the quality of spectra, number of peaks and correct identification results. Recommended testing for correct identification for testing mycobacteria was between 0-14 days of culture growth; with 0 days being the time sufficient growth of the culture was observed.

g. Age of culture for Mycobacteria from Liquid Media for VITEK MS Testing

The results of prolonged incubation (age of culture) on a positive liquid mycobacteria culture used for protein extraction and identification on the VITEK MS were evaluated. Positive liquid media mycobacteria cultures were held at 35-37^{o}C for 0, 1, 2 and 3 days (0-24, 24-48, 48-72 and 72-96 hours) post positivity by the liquid media detection instrument. A total of 8 species (30 strains) were tested in each of the three

automated liquid media systems (MGIT, VersaTREK, and MP). VITEK MS identification results were compared to the expected reference identification and percent identification was calculated per testing time point. Additionally, protein extracts prepared for age of culture testing were used to evaluate if the age of culture from liquid media has an impact on Time To result (TTR) for total acquisition time on the VITEK MS. A total of 358 samples were tested generating 716 spectra on the VITEK MS (119 MGIT, 119 VersaTREK and 120 MP samples).

Overall, 90% correct identification was achieved through three days of testing when compared to the expected reference result. Day 0 testing in the MGIT system resulted in a high number of failed acquisition spots due to low biomass at time of positivity by the detection system. To mitigate the risk of low biomass at time of positivity especially in the MGIT system, it is recommended to incubate a positive sample for a minimum of 24 hours after positivity for all liquid media types. Although all mycobacteria species tested in this report had good performance through 96 hours of testing; to mitigate the risk of mycobacteria transitioning to a stationary growth phase and altering protein profiles, the maximum recommended incubation time post positivity is 72 hours. Assessment of spot to spot variation showed no improvement as biomass increased indicating this issue is independent of biomass. No correlation was observed between age of culture and TTR. Therefore, an increase in biomass will not improve TTR.

h. Nocardia Age of Culture Study for VITEK MS Testing

A set of 10 nocardia isolates were tested in duplicate at 3 time points (in hours): T24h, T48h, T72h on three different media (Columbia Blood Agar, Chocolate Agar with Polyvitex and Buffered Charcoal Yeast Agar). Results demonstrated the age of culture recommended for nocardia isolates for VITEK MS acquisition is from 24 to 72 hours.

i. Mould Age of Culture Study for VITEK MS Testing

Two hundred and sixty-seven organisms were inoculated onto different media according to their growth requirements and incubated in appropriate conditions. Two growth times were tested per mould isolate according to their speed of growth (i.e., 2 and 8 days for rapid-growing, 5 and 10 days for medium growers and 10 and >=15 days for slow-growing organisms). 2040 spectra from 267 distinct strains, representative of 53 moulds species from 14 genera using a variety of media were included in the study. Based on these results, the age of culture recommended for mould isolates for VITEK MS acquisition is from sufficient growth until ~5 days after sufficient growth.

j. Impact of Mixed Cultures on VITEK MS ID of Mycobacteria and Nocardia From Solid Media

To evaluate the impact of mixed cultures on the identification of mycobacteria and

nocardia using VITEK MS, Four mycobacteria and two nocardia were grown on solid media and protein extracts were prepared following the instructions for VITEK MS MYCOBACTERIUM/ NOCARDIA KIT. Non-diluted protein extracts (High Positive Sample and Moderate Positive Sample) were tested from each organism as well as in mixed combinations. While the spectra profiles generated from mixed protein extracts did not produce incorrect identifications, the algorithm could not consistently distinguish when two organisms were present in the sample with low discrimination. In over half of the combinations tested the High Positive Sample type outcompeted the Moderate Positive Sample type resulting in a single choice identification of the High Positive Sample. As a result pure cultures should always be used for protein extraction to obtain a sample identification on the VITEK MS.

k. Impact of Mixed cultures on ID of Mycobacteria From Liquid Media

For each organism combination tested (see Table 4 below), BacT/ALERT MP culture bottles were inoculated with the mixed culture and tested.

Table 4: Test Organism Combinations

Combination	Organism 1	Organism 2
1	*M.fortuitum*	*M. smegmatis*
2	*M.tuberculosis*	*M. lentiflavum*
3	*Nocardia asteroids*	*M. fortuitum*
4	*Staphylococcus aureus*	*M. smegmatis*
5	*C.albicans*	*M. intracelluare*
6	*P.aeruginosa*	*N. asteroides*

Out of the 24 spectra generated for mixed cultures, 22 resulted in single choice identification of one of the two species present. The other two spectra generated 'Not Enough Peaks' and 'Low Discrimination' calls. Testing of pure cultures is recommended.

l. Impact of Mixed Cultures on Mould ID

Five molds, representative of clinically prevalent classes, were grown at 30^{o}C on Sabouraud Dextrose Agar, extracted and tested in combinations of high and moderate concentrations. Each combination was tested in quadruplicate. The eight mixes were always identified to at least one of the two species or both species present in the mix. Testing of pure cultures is recommended.

m. Mycobacteria Carry Over and Cross Contamination Study

The potential for carry over and cross contamination of protein extracts when testing mycobacteria on the VITEK MS was evaluated. Five mycobacteria species were grown on solid medium and protein extracts were prepared following manufactures instructions for VITEK MS MYCOBACTERIUM/NOCARDIA KIT. High Positive, Moderate Positive, and Negative samples were spotted in an alternating fashion in order to assess the carry over and cross contamination. All negative samples tested resulted in 'No Identification' on the VITEK MS. indicating no carry over or cross

contamination was observed during this study. All High and Moderate Positive samples provided the expected results.

n. Mould and Nocardia Carry Over and Cross Contamination Study

Six moulds and six nocardia were grown on solid medium and protein extracts were prepared following manufactures instructions. High Positive, Moderate Positive, and Negative samples were spotted in an alternating fashion in order to assess the carry over and cross contamination. All negative samples tested resulted in No Identification on the VITEK MS. indicating no carry over or cross contamination was observed during this study. All High and Moderate Positive samples provided the expected results.

o. Protein Stability for Mycobacteria and Nocardia Solid Media Extractions

The stability of protein extracts of mycobacteria and nocardia prior to spotting on VITEK MS DS target slides was evaluated. The stability of a protein extract was defined as acceptable storage temperature and time after the extraction from the respective culture. Two solid media types were used for mycobacteria and one solid medium type for *Nocardia* species. Three extracts per organism and per media type were prepared. Protein extracts were held at refrigerated (2 - 8°C), frozen (-19 to -31°C), and room temperatures (15 - 25°C). Protein extracts were tested at different time points ranging from 0 to 21 days post extraction. Additionally, freeze-thaw cycles were also tested to determine if freezing and thawing the extracts would degrade protein extracts. Based on the data generated from this study, the preferred holding temperature and time is frozen (-19 to -31°C} up to 14 days for both mycobacteria and *Nocardia* species. At other temperatures, the protein extracts tend to evaporate and become difficult to obtain the sufficient volume required for spotting on VITEK MS DS target slides leading to inconsistent identification. Freeze-thaw cycle results indicate that mycobacteria and nocardia samples are stable for up to two freeze-thaw cycles.

p. Protein Stability for Mycobacteria from Liquid Media Extractions

The stability of protein extracts of mycobacteria from liquid medium prior to spotting on VITEK MS DS target slides was evaluated. The stability of a protein extract was defined as acceptable storage temperature and time after the extraction from the respective culture.

BacT/ALERT® MP bottles (MP) and BACTEC MGIT™ 960 tubes (MGIT) were evaluated in the study. Four extracts per organism and per media type were prepared. Protein extracts were held at room temperature (15°C to 25°C), refrigerated (2°C to 8°C), and frozen (-19°C to -31°). Protein extracts were tested at different time points ranging from 0 to 21 days post extraction. Additionally, freeze-thaw cycles were also tested to determine if freezing and thawing the extracts would degrade protein extracts.

Based on the data generated from this study, the extract was determined to be stable when frozen (-19 to - 31°C) for up to 21 days. At refrigerated and room temperature, samples remained stable for at least 36 hours but sample degradation caused a reduced rate of correct identifications by day 3.

Based on the results of this study, it was recommended to test protein extracts on the same day of the extraction; however if this is not possible, it is recommended to stay consistent with extract stability recommendation for solid media mycobacterium and solid media nocardia. As such, mycobacterium protein extracts from liquid medium can be stored at frozen temperatures (-19 to -31°C) up to 14 days. Frozen extracts can be used up to two freeze thaw cycles for consistent identification results.

q. Stability of Moulds Extracts

Initially, a set of 10 molds were tested at 0,1,3,7,14, and 21 days post extraction using 3 storage temperatures : Room Temperature (RT): 15 - 25°C ; refrigerated: 2 - 8°C ; frozen: -19 to -31°C. Four freeze-thaw cycles were also performed for each mould extract. An additional set of eight molds was tested at the frozen storage temperature after obtaining inconsistent results on the first set of 10 strains. The freeze-thaw cycles experiment was performed on 18 strains that were kept in the freezer.

It was determined that the extracts were not stable after one day at RT or refrigerated. Higher mould identification rates were obtained when the extracts are frozen, but the results were inconsistent and varied depending on the species and strains tested even after multiple testing on an extensive dataset. The extracts were also not stable after one freeze-thaw cycle. Therefore, it was concluded that the extracts of molds are not stable regardless of the storage conditions and must be spotted within 8 hours on the day of extraction. The package insert indicates to spot 1 µL of the supernatant immediately on a target slide.

r. Stability of Extract Deposits of Moulds, Nocardia, and Mycobacteria

A study was performed to determine the stability of the mycobacteria, nocardia and mould deposits on the target slide after matrix addition and before spectra acquisition. It is recommended that data acquisition be performed at a maximum of 3 days after matrix addition.

s. Stability of Extract Deposits of Mycobacterium from Liquid Media

The stability of mycobacteria protein extracts from liquid media deposited on a target slide with the addition of CHCA matrix was determined. Mycobacteria species were grown in different automated liquid media systems and protein extracts were prepared manufacturer's instructions. Target slides were left at room temperature in original packaging and sample acquisition of protein extracts occurred initially and every day for up to four days. Identification results of protein deposit s were compared to initial

results to assess the effects of prolonged acquisition.

Overall, 90% correct identification when compared to the expected reference results was achieved through four days of testing. An increase in failed acquisition spots was observed at T4 and average peaks counts decreased from T0 - T4. Therefore, recommended storage for prepared target slides will be up to 3 days at room temperature in the original packaging.

t. VITEK MS Liquid Media Interfering Substances

The potential for sputum digestion/decontamination reagents and/or liquid media components to cause an interference on the VITEK MS and/or impact identification of mycobacteria protein extracts on the VITEK MS was evaluated. Mycobacteria species were grown in automated liquid media detection systems and protein extracts were prepared per manufacturer's instructions. Interference was tested using negative and positive samples with and without growth (GS) and antimicrobial (AS) supplements testeq in combination with sputum processing reagents N-acetyl-L-cysteine (NALC)- sodium hydroxide (NaOH) (NALC-NaOH). Impact on identification was tested using VersaTRE K® Myco bottles and BBL™ MGIT™ 960 tubes inoculated with patient samples processed with NALC-NaOH sputum digestion/decontamination method. Samples inoculated into VersaTREK bottles were freshly processed (never frozen). For MGIT tubes frozen processed sputum samples exposed to one or more freeze thaw cycles were used.

All positive samples tested resulted in correct species level identification and all negative samples resulted in 'No Identification' due to 'Not Enough Peaks'. Results indicate that if the protocol is followed as designed, neither sputum digestion/decontamination nor liquid media components cause an interference on the VITEK MS.

Identification results for samples tested in VersaTREK bottles met acceptance criteria of 90% correct identification indicating no impact on identification when using fresh (never frozen) sputum samples. Samples tested from MGIT tubes resulted in a high number of 'No Identification' results and did not meet the acceptance criteria. Upon further investigation, results indicated using frozen processed sputum samples inoculated in liquid media and then used for identification impacts identification. It is recommended to use the fresh processed sputum sample and not to use a frozen processed sputum sample as an inoculation source for liquid media to minimize the risk of 'No identification' on the VITEK MS.

u. Verification of Inactivation and Extraction of Mycobacteria from Liquid Media

This study was performed to verify the modifications selected for improving the robustness of mycobacteria liquid media extraction workflow. The combination of sample processing between 24-72 hour post positivity, 3 ml sample volume, and use of the 5 ml tube improved the identification success for all liquid media types.

This study included eight (8) clinically prevalent *Mycobacterium* species from different automated liquid media systems including BacT/ALERT® MP bottles (MP), BACTEC™ MGIT™ 960 tubes (MGIT), and VersaTREK® Myco bottles (VersaTREK) on the VITEK MS.

Testing 24-48 hours post positivity performed above 90% correct species level identification and met the acceptance criterion; however some spectra failed original quality checks (Not Enough Peaks, Too Many Peaks) and either had to be re-spotted (same extract) or re-extracted from a new culture. Testing at 48-72 hours post positivity improved identification success resulting in no final results of No ID or sample re- extractions. None of the spectra failed initial quality checks.

The recommended window for testing mycobacterium from liquid medium is 24-72 hours post instrument declaring bottle/tube positive. If spectral quality checks do not pass (Not Enough Peaks, Too Many Peaks) or No Identification is resulted on the original deposit, it is recommended to re-spot from the same extract. If spectral checks still do not pass, it is recommended that the sample be re-extracted if the bottle/tube is still within 24-72 hour window.

v. *Validation of Inactivation of Mycobacterium and Nocardia from solid media*

The sample preparation of mycobacteria and nocardia requires additional steps as compared to the standard procedure due to their infectious nature. It is important to inactivate the cultures to make the samples safe for handling outside of a Biosafety Level-3 (BSL-3/P3) environment. Several studies were conducted for establishing a protocol for inactivation of mycobacteria and nocardia for VITEK MS. Inactivation studies were performed on solid media (LJ, 7H11 and TSB agar plates) with various species of mycobacteria and nocardia including drug sensitive and resistant strains of *M. tuberculosis*.

The optimal inactivation was obtained by five minutes mechanical disruption with bead beating in ethanol using glass beads in a pre-sterile tube. This step breaks up clumps of cells allowing the ethanol to come in contact with all cells, as well as disrupting the thick waxy mycobacterial cell walls. The sample tube is then incubated at room temperature for 10 minutes in order to allow the ethanol to inactivate all cells. After inactivation, the suspension is transferred into another tube and centrifuged to create a pellet of the cell matter and the ethanol supernatant is discarded.

w. *Validation of Inactivation of Moulds*

The inactivation step was verified with 16 mould species among 12 different genera. Following manufacturer extraction instructions, it was found that the inactivation is effective after the second centrifugation step using 70% formic acid and 100% acetonitrile addition. The first step using 70% ethanol is not sufficient to allow a complete inactivation of the sample.

The inactivation was verified after the second step of centrifugation by mixing the supernatant and the pellet. A 10 ul sample of this suspension was subcultured on Sabouraud Dextrose Agar plate and incubated from 8 days to 8 weeks depending on the tested strains. Tests were performed at two different colony ages or at least using the older culture in order to work on conidia/spores representing the more resistant stage. No growth was demonstrated after the incubation period.

x. Identification of Mycobacterium spp. from BacT ALERT MP Using Simulated Sputum Processing

This study was designed to demonstrate the ability of the VITEK MS to generate spectra from simulated clinical samples inoculated into liquid media and identify mycobacteria at the species level. Simulated sputum was spiked with mycobacteria and contaminating respiratory flora organisms and underwent sputum processing prior to inoculation into BacT/ALERT® MP bottles.

Out of a test panel including 20 species and 77 strains, at total of 385 spectra were collected. Overall results were 99.7% correct identification (excluding no identification results) and 1.0% no identification.

Based on the results of this study, the VITEK MS system performs acceptably when analyzing simulated clinical samples from BacT/ALERT® MP bottles.

y. Reproducibility for Mycobacteria from Liquid Media Summary Report

This study examined the reproducibility of mycobacterium identifications on the VITEK MS system from liquid media. A panel of representative mycobacteria species were grown in three different liquid media detection systems over five days on VITEK MS instruments at two different sites with multiple operators.

Mycobacteria species were grown in BacT/ALERT® MP (MP) bottles at Site 1 and both BBL MGIT™ 960 tubes (MGIT) and VersaTREK™ Myco bottles(Versa Trek) at Site 2. On each day of testing, two extracts from positive liquid samples were performed for each species tested. Each technologist prepared two (2) slides per day of testing, one from each extract. Extracts were deposited in sequential order on one slide and in randomized order on the second slide for each day of testing. Extracts were prepared following manufacturer's instructions for VITEK MS MYCOBACTERIUM/NOCARDIA KIT.

The organism-specific and overall rates of correct identification results for both sites combined are ≥95%. The confidence intervals for the organism specific and overall rates of correct identification contain 95%.

z. Analytical specificity:

Also see DEN130013 / K124067

Rhodococcus, Tsukamurella, Streptomyces, Segniliparus, Williamsia Study: In this study, a panel of 14 strains (including two to three representative strains of each genus) were tested with VITEK MS V3.

These strains were tested in parallel using the extraction protocol and direct deposit. When tested with the extraction protocol, none of the 14 strains were identified as a mycobacteria or nocardia, which was in agreement with the acceptance criterion. When tested by direct deposit, only one strain (*Segniliparus rugosus*) of the 14 strains was identified as *Mycobacterium szulgai*. The remaining 13 strains were not identified as a mycobacterium or nocardia. A note was added to the user manual indicated that a result of mycobacteria obtained by direct deposit should be confirmed by retesting the strain using the extraction kit. When considering both approaches together(direct deposit and extraction), the panel of 14 strains representing *Rhodococcus, Tsukamurella, Streptomyces, Segniliparus* and *Williamsia* genus were not identified as a mycobacteria or nocardia.

Additional exclusivity testing was performed for *Actinomadura, Amycolatopsis and Nocardia* species extracts (one per strain). All were prepared following manufacturer instructions for nocardia colonies. Each extract is spotted in triplicate on the VITEK MS slide. Results are found in Tables 5 and 6 below.

TABLE 5: *Actinomadura* and *Amycolatopsis* species tested;VITEK MS result 'No Identification'

Reference Result	# No ID	# tests	# isolates
Actinomadura chibensis	3	3	1
Actinomadura cremea	3	3	1
Actinomadura latina	3	3	1
Actinomadura vinacea	2	3	1
Amycolatopsis benzoatilytica	3	3	1
Amycolatopsis orientalis ssp orientalis	3	3	1
Amycolatopsis palatopharyngis	3	3	1

TABLE 6: *Nocardia* species tested; VITEK MS result 'No Identification'

Reference Result	**# No ID**	**# tests**	**# isolates**
Nocardia anaemiae	3	3	1
Nocardia arthritidis	5	6	6
Nocardia asiatica	1	5	5
Nocardia carnae	3	3	1
Nocardia concava	3	3	1

Reference Result	# No ID	# tests	# isolates
Nocardia corynebacterioides = Rhodococcus corynebacterioides	3	3	1
Nocardia exalbida	3	3	1
Nocardia gamkensis[1]	2	5	1
Nocardia higoensis	1	3	1
Nocardia ignorata	3	3	1
Nocardia inohanensis	3	3	1
Nocardia niigatensis	3	3	1
Nocardia ninae	3	3	1
Nocardia jiangxiensis	3	3	1
Nocardia mexicana	1	1	1
Nocardia niwae	3	3	1
Nocardia pneumoniae	3	3	1
Nocardia puris	3	3	1
Nocardia rhamnosiphila	2	2	2
Nocardia sienata[1]	3	6	1
Nocardia takedensis	3	3	1
Nocardia terpenica	3	3	1
Nocardia testacea	1	1	1
Nocardia thailandica	3	3	1
Nocardia vermiculata	3	3	1
Nocardia vinacea	3	3	1
Nocardia yamanashiensis	3	3	1
Nocardia flavorosea	3	3	1
Nocardia gamkensis	2	6	1
Nocardia brevicatena	10	10	1

[1] = Also see Table 9: Cross-identification of Clinically Validated Taxa and Unclaimed Taxa

The following mycobacteria were tested and provided no identification with the VITEK MS V3.0 Knowledge Base. Results are summarized in Table 7 below.

TABLE 7: *Mycobacteria* species tested; VITEK MS result 'No Identification'

Reference Result	# No ID	# tests	# isolates
M. bouchedurhonense/colombiense	3	3	3
M. bouchedurhonense/colombiense/vulneris	1	1	1
M. chimaera[1]	1	24	16
M. colombiense/ yongonense/marseillense	1	1	1
M. gordonae/paragordonae[2]	1	2	1
M. paraense	1	1	1
M. paraffinicum[1]	7	9	9
M. parascrofulaceum[1]	1	2	2
M. phocaicum[1]	1	21	21
M. shigaense	3	3	3
M. yongonense/marseillense[1]	2	21	13

[1] = Also see Table 9: Cross-identification of Clinically Validated Taxa and Unclaimed Taxa

TABLE 8: Moulds tested; VITEK MS result 'No Identification'

Reference Result	# No ID	# tests	# isolates
Alternaria infectoria	3	3	1
Aspergillus aculeatus	1	1	1
Aspergillus alabamensis	1	1	1
Aspergillus brevipes	3	3	1
Aspergillus creber	2	2	2
Aspergillus fumigatiaffinis	3	3	1
Aspergillus glaucus	3	3	1
Aspergillus jensenii	5	5	5
Aspergillus niveus	1	1	1
Aspergillus nomius[1]	1	2	2
Aspergillus ochraceus	19	19	4
Aspergillus sclerotiorum	3	3	1
Aspergillus section *Usti*	1	1	1
Aspergillus striatus / Aspergillus cleistominutus	1	1	1

Reference Result	# No ID	# tests	# isolates
Aspergillus tabacinus	1	1	1
Aspergillus tamarii	1	1	1
Aureobasidium pullulans	19	19	4
Byssochlamys nivea	21	22	3
Cladophialophora carrionii	1	1	1
Cladophialophora minourae	2	2	2
Cladosporium cladosporioides	1	1	1
Cladosporium halotolerans	1	1	1
Cladosporium herbarum	62	65	10
Cladosporium macrocarpum	3	3	1
Cladosporium oxysporum	11	38	4
Cladosporium sphaerospermum	20	20	4
Coprinellus xanthothrix	1	1	1
Curvularia aeria	1	1	1
Curvularia hominis	2	2	2
Curvularia lunata[1]	1	2	2
Curvularia pseudolunata[1]	2	3	3
Exophiala bergeri	1	1	1
Exophiala oligosperma	1	1	1
Fusarium brachygibbosum	1	1	1
Fusarium dimerum	2	2	2
Fusarium falciforme[1]	1	3	3
Fusarium formosus	2	2	2
Fusarium incarnatum-equiseti species complex	1	2	2
Fusarium lactis	1	1	1
Fusarium lichenicola[1]	1	2	2
Fusarium fujikuroi spp. complex	1	1	1
Fusarium keratoplasticum[1]	1	3	1
Fusarium oxysporum f.sp. lypopersici[1]	2	2	1
Fusarium sublutinans	1	1	1

Reference Result	# No ID	# tests	# isolates
Fusarium verticillioides	1	1	1
Lecythophora canina	3	3	1
Lecythophora lignicola	3	3	1
Lecythophora mutabilis	3	3	1
Leptosphaerulina chartarum	68	68	10
Lichtheimia ramosa	3	3	3
Lichtheimia ramosa vs corymbifera	2	2	2
Mucor circinelloides	1	1	1
Mucor plumbeus	1	1	1
Mucor racemosus f. sphaerosporus	5	5	1
Neosartorya fisheri[1]	4	8	1
Neosartorya pseudofisheri[1]	25	34	6
Paecilomyces brunneolus	1	1	1
Paecilomyces formosus	2	2	2
Paecilomyces fulvus	13	13	2
Penicillium commune	18	28	3
Penicillium copticola	1	1	1
Penicillium crustosum	2	2	2
Penicillium decumbens	1	1	1
Penicillium glabrum	35	35	31
Penicillium janthinellum	1	1	1
Penicillium marneffei	4	4	1
Penicillium paneum	1	1	1
Penicillium polonicum	1	1	1
Penicillium sizovae	1	1	1
Rasamsonia aegroticola	2	2	1
Rhinocladiella similis	1	1	1
Rhizomucor pusillus	3	3	1
Rhizomucor miehei	3	3	1
Rhizopus chinensis	1	1	1

Reference Result	# No ID	# tests	# isolates
Rhizopus oligosporus	1	1	1
Rhizopus rhizopodiformis	1	1	1
Sarocladium bifurcatum	1	1	1
Scopulariopsis brevicaulis	9	9	3
Sporothrix albicans	2	2	1
Sporothrix mexicana	2	2	1
Stachybotrys chartarum	7	9	1
Trichoderma atroviride	26	28	4
Trichoderma longbrachiatum	21	21	4
Trichophyton concentricum	3	3	1
Trichophyton ferrugineum	3	3	1
Trichophyton schoenleinii	3	3	1
Wallemia sebi	45	45	7

[1] = Also see Table 9: Cross-identification of Clinically Validated Taxa and Unclaimed Taxa

TABLE 9: Cross-identification of VITEK MS claimed displayed taxa and Unclaimed Taxa

Clinical Claimed Displayed Taxon	Reference ID of Unclaimed Taxa	# cross-ID	# tests	# isolates
Aspergillus calidoustus	*Aspergillus ustus*	*	*	*
Aspergillus flavus / oryzae	*Aspergillus nomius*	1	1	1
	Neosartorya pseudofischeri	1	34	6
Aspergillus fumigatus	*Neosartorya fischeri*	3	8	1
	Neosartorya pseudofischeri	7	34	6
Aspergillus lentulus	*Neosartorya fischeri*	3	8	1
	Neosartorya pseudofischeri	3	34	6
Aspergillus nidulans	*Aspergillus delacroxii*	3	3	3
	Aspergillus quadrilineatus	3	3	3
	Emericella variecolor	1	1	1
Aspergillus niger complex	*Aspergillus tubingensis*	1	1	1
	Aspergillus awamori	10	10	10
	Aspergillus foetidus	1	1	1
Aspergillus terreus complex	*Aspergillus hortae*	1	1	1
Aspergillus versicolor	*Aspergillus amoenus*	2	2	2
	Aspergillus fructus	1	1	1
Curvularia hawaiiensis	*Curvularia lunata*	6	11	1
	Curvularia senegalensis	1	1	1
Curvularia spicifera	*Curvularia lunata*	7	12	2
	Curvularia pseudolunata	1	1	1

Clinical Claimed Displayed Taxon	Reference ID of Unclaimed Taxa	# cross-ID	# tests	# isolates
Fusarium oxysporum complex	*Fusarium nygamai*	1	1	1
	Fusarium oxysporum f.sp. radicis-lycopersici	2	2	1
Fusarium proliferatum	*Fusarium fujikuroi*	2	2	2
Fusarium solani complex	*Fusarium falciforme Fusarium keratoplasticum Fusarium lichenicola Fusarium petroliphilum*	2	3	3
		2	3	1
		1	2	2
		4	4	4
Mycobacterium abscessus	*Mycobacterium abscessus* ssp. *abscessus*	34	34	34
	Mycobacterium abscessus ssp. *bollettii*	3	3	1
	Mycobacterium abscessus ssp. *massiliense*	5	5	3
Mycobacterium avium	*Mycobacterium avium* ssp. *avium*	3	3	1
	Mycobacterium avium ssp. *paratuberculosis*	3	3	1
	Mycobacterium avium ssp. *silvaticum*	3	3	1
	Mycobacterium chimaera	3	24	16
Mycobacterium gordonae	*Mycobacterium paragordonae*	1	1	1
Mycobacterium intracellulare	*Mycobacterium chimaera*	20	24	16
	Mycobacterium colombiense	3	3	2
	Mycobacterium yongonense/ marseillense	19	21	13
	Mycobacterium yongonense/ marseillense/vulneris	1	1	1
Mycobacterium marinum	*Mycobacterium ulcerans*	3	3	1
Mycobacterium mucogenicum	*Mycobacterium phocaicum*	20	21	21
Mycobacterium scrofulaceum	*Mycobacterium paraffinicum*	2	9	9
	Mycobacterium paragordonae	1	1	1
	Mycobacterium parascrofulaceum	1	2	2
Mycobacterium simiae	*Mycobacterium palustre*	3	3	2
Mycobacterium tuberculosis complex	*Mycobacterium pinnipedii*	2	2	1
Nocardia abscessus	*Nocardia asiatica*	2	2	2
Nocardia africana[1]	*Nocardia aobensis*	3	3	1
	Nocardia cerradoensis	3	3	1
Nocardia nova	*Nocardia elegans*	2	2	2
	Nocardia kruczakiae	1	1	1
Nocardia brasiliensis	*Nocardia iowensis*	3	3	1

Clinical Claimed Displayed Taxon	Reference ID of Unclaimed Taxa	# cross-ID	# tests	# isolates
	Nocardia vulneris	1	1	1
Nocardia farcinica	*Nocardia higoensis*	2	3	1
	Nocardia shimofusensis	3	3	1
Nocardia paucivorans	*Nocardia sienata*	1	6	1
Nocardia wallacei	*Nocardia blacklockiae*	6	6	6
Penicillium chrysogenum	*Penicillium rubens*	2	2	2
Rasamsonia argillacea complex	*Rasamsonia piperina*	2	2	1
Sporothrix schenckii complex	*Sporothrix brasiliensis*	2	2	1
	Sporothrix globosa	1	1	1
	Sporothrix luriei	2	2	1
Trichophyton interdigitale	*Trichophyton mentagrophytes*	27	30	1

[1] *Nocardia nova* is displayed as a low discrimination result with *Nocardia africana* but only *Nocardia nova* has been clinically validated

TABLE 10: Cross-identification between VITEK MS claimed displayed taxa

Displayed Taxon	Reference ID	# cross-ID	# tests	# isolates
Mycobacterium avium	*Mycobacterium intracellulare*	1	70	43
Mycobacterium intracellulare	*Mycobacterium avium*	2	115	67
Nocardia abscessus	*Nocardia beijingensis*	1	1	1
Nocardia asteroides	*Nocardia neocaledoniensis*	3	31	31
Trichophyton tonsurans	*Trichophyton interdigitale*	2	34	34
Trichophyton verrucosum	*Trichophyton erinacei* *Trichophyton interdigitale*	3	36	36
Trichophyton violaceum	*Trichophyton rubrum*	3	36	36

aa. Assay cut-off:

After the calibration is accepted for an acquisition group, the VITEK MS acquires the spectra for the samples in that group. A perfect match between the spectrum and the unique spectrum of a single organism or organism group would provide a percent probability of 99.9. Results are displayed as follows:

- A single identification is displayed with confidence value of 60 to 99.9 when one significant organism or organism group is retained.
- Low Discrimination identifications are displayed when more than one significant organism or organism group are retained, but not for more than 4 organisms. In this case, the sum of confidence values is equal to 100.
- When more than 4 organisms or organism groups are found, the organism is considered as non-identified. In this case, a list of possible organisms is displayed and the sum of confidence values is less than 100.
- When no match is found, the organism is considered as non-identified.

2. Comparison studies:

a. Method comparison with predicate device: N/A

b. Matrix comparison: N/A

3. Clinical studies:

a. Clinical Sensitivity:

Challenge Study: One hundred well-characterized challenge strains including 50 moulds, 35 mycobacteria and 15 nocardia strains were evaluated. Three trial sites tested the mould challenge strains and three different trial sites tested the mycobacteria and nocardia challenge strains. Organism identifications were masked to the technologist performing the testing. Results from this study are summarized in Table 11 below.

TABLE 11: Challenge Study Results with VITEK MS V3.0 Knowledge Base.

Site	Organism	# of Claimed Isolates Tested	Correct Single Choice (SC)	Low Discrim (LD) Correct Genus	Correct OC +LD Correct Genus	MsID	LD Incorrect Genus	MsID +LD Incorrect Genus	NoID	NoID Multiple Genera	NoID+ Multiple Genera
All	Moulds	150	94.0% (141)	0.0% (0)	94.0% (141)	0.0% (0)	0.0% (0)	0.0% (0)	6.0% (9)	0.0% (0)	6.0% (9)
	Mycobacteria	105	99.0% (104)	0.0% (0)	99.0% (104)	0.0% (0)	0.0% (0)	0.0% (0)	1.0% (1)	0.0% (0)	1.0% (1)
	Nocardia	45	93.3% (42)	6.7% (3)	100.0% (45)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)
	All	300	95.7% (287)	1.0% (3)	96.7% (290)	0.0% (0)	0.0% (0)	0.0% (0)	3.3% (10)	0.0% (0)	3.3% (10)

The following performance characteristics were obtained by testing routine fresh strains from patient cultures, including moulds, mycobacteria and nocardia, in five clinical microbiology laboratories in the United States. Each VITEK MS identification was compared to a reference identification determined by molecular sequencing supplemented as needed by additional pre-defined testing.

TABLE 12: Overall Study Performance Results for Moulds, Mycobacteria and Nocardia with VITEK MS V3.0 Knowledge Base.

Organism Group	Correct Identification (ID)						
	Correct Single Choice (no. of results)	**Low Discrimination Correct Genus (no. of results)**	**Combined Correct Single Choice and Low Discrimination Correct Genus (no. of results)**	**Single Choice Incorrect ID[1] (no. of results)**	**Low Discrim. Incorrect Genus (no. of results)**	**Low Discrim. Multiple Genera (no. of results)**	**No ID[2] (no. of results)**
Moulds	91.2% (1376/1508)	1.5% (22/1508)	92.7% (1398/1508) 95% CI [91.3 ; 94.0]%	0.9% (13/1508)	0.0% (0/1508)	0.4% (6/1508)	6.0% (91/1508)
Mycobacterium	96.5% (777/805)	0.0% (0/805)	96.5% (777/805) 95% CI [95.0 ; 97.7]%	0.4% (3/805)	0.0% (0/805)	0.0% (0/805)	3.1% (25/805)
Mycobacterium Solid	97.4% (713/732)	0.0% (0/732)	97.4% (713/732) 95% CI [96.0 ; 98.4]%	0.1% (1/732)	0.0% (0/732)	0.0% (0/732)	2.5% (18/732)
Mycobacterium Liquid	87.7% (64/73)	0.0% (0/73)	87.7% (64/73) 95% CI [77.9 ; 94.2]%	2.7% (2/73)	0.0% (0/73)	0.0% (0/73)	9.6% (7/73)
Nocardia	89.3% (341/382)	8.6% (33/382)	97.9% (374/382) 95% CI [95.9 ; 99.1]%	0.8% (3/382)	0.0% (0/382)	0.0% (0/382)	1.3% (5/382)

[1] The Single Choice Incorrect column includes single choice incorrect identifications and low discrimination results with >1 choice in same genus but genus does not match the reference genus. See Single Choice Discordant (Incorrect) Results (Table 16) below.
[2] The No Identification column includes Low Discrimination with multiple genera or No ID (Bad Spectra, Not Enough Peaks, Too Many Peaks (Bad spectrum), or No ID (Good spectrum).

Please note for tables 13-15, the following column descriptions apply:

- The Discordant column includes single choice incorrect identifications and low discrimination results with >1 choice in same genus but genus does not match the reference genus. (See Single Choice Discordant (Incorrect) Results (Table 16) below.)
- The No Identification column includes Low Discrimination with multiple genera or No ID (Bad Spectra, Not Enough Peaks, Too Many Peaks (Bad spectrum), or No ID (Good spectrum).

TABLE 13: Mould Performance with VITEK MS V3.0 Knowledge Base

Species	Number of Unique Isolates	Correct Identification (ID)			Discordant	No Identification
		Correct for Genus & Species (1 choice)	Low Discrimination (>1 Choice in Same Genus)	Combined (1 Choice + > 1 Choice in Same Genus)		
Acremonium sclerotigenum	3	100% 30/30	0% 0/30	100% 30/30	0% 0/30	0% 0/30
Alternaria alternata	12	93.8% 30/32	0% 0/32	93.8% 30/32	0% 0/32	6.3% 2/32
Aspergillus brasiliensis	6	93.5% 29/31	0% 0/31	93.5% 29/31	0% 0/31	6.5% 2/31
Aspergillus calidoustus	14	87.9% 29/33	0% 0/33	87.9% 29/33	0% 0/33	12.1% 4/33
Aspergillus flavus/oryzae	33	100% 33/33	0% 0/33	100% 33/33	0% 0/33	0% 0/33
Aspergillus fumigatus	32	100% 32/32	0% 0/32	100% 32/32	0% 0/32	0% 0/32
Aspergillus lentulus	7	100% 30/30	0% 0/30	100% 30/30	0% 0/30	0% 0/30
Aspergillus nidulans	16	97% 32/33	0% 0/33	97.0% 32/33	0% 0/33	3.0% 1/33
Aspergillus niger complex [1]	33	86.5% 32/37	0% 0/37	86.5% 32/37	0% 0/37	13.5% 5/37
Aspergillus sydowii	11	96.8% 30/31	0% 0/31	96.8% 30/31	0% 0/31	3.2% 1/31
Aspergillus terreus complex	34	94.1% 32/34	0% 0/34	94.1% 32/34	0% 0/34	5.9% 2/34
Aspergillus versicolor	3	71.0% 22/31	3.2% 1/31	74.2% 23/31	0% 0/31	25.8% 8/31
Blastomyces dermatitidis	19	100% 40/40	0% 0/40	100% 40/40	0% 0/40	0% 0/40
Cladophialophora bantiana	10	96.6% 28/29	0% 0/29	96.6% 28/29	0% 0/29	3.4% 1/29
Coccidioides immitis/posadasii	19	100% 38/38	0% 0/38	100% 38/38	0% 0/38	0% 0/38
Curvularia hawaiiensis	4	96.2% 25/26	0% 0/26	96.2% 25/26	0% 0/26	3.8% 1/26
Curvularia spicifera	10	97.1% 34/35	0% 0/35	97.1% 34/35	0% 0/35	2.9% 1/35
Epidermophyton floccosum	4	96.8% 30/31	0% 0/31	96.8% 30/31	0% 0/31	3.2% 1/31
Exophiala dermatitidis	25	100% 31/31	0% 0/31	100% 31/31	0% 0/31	0% 0/31
Exophiala xenobiotica [2]	8	78.1% 25/32	0% 0/32	78.1% 25/32	0% 0/32	21.9% 7/32
Exserohilum rostratum [2]	14	54.3% 19/35	0% 0/35	54.3% 19/35	0% 0/35	45.7% 16/35
Fusarium oxysporum complex	21	96.8% 30/31	0% 0/31	96.8% 30/31	0% 0/31	3.2% 1/31
Fusarium proliferatum	10	100% 30/30	0% 0/30	100% 30/30	0% 0/30	0% 0/30
Fusarium solani complex	22	82.9% 29/35	0% 0/35	82.9% 29/35	0% 0/35	17.1% 6/30
Histoplasma capsulatum	17	100% 32/32	0% 0/32	100% 32/32	0% 0/32	0% 0/32
Lecythophora hoffmannii	2	90.0% 27/30	0% 0/30	90.0% 27/30	0% 0/30	10.0% 3/30
Lichtheimia corymbifera	7	93.5% 29/31	0% 0/31	93.5% 29/31	0% 0/31	6.5% 2/31
Microsporum audouinii	3	90.9% 30/33	3% 1/33	93.9% 31/33	0% 0/33	6.1% 2/33
Microsporum canis	12	96.8% 30/31	0% 0/31	96.8% 30/31	0% 0/31	3.2% 1/31
Microsporum gypseum	5	91.4% 32/35	0% 0/35	91.4% 32/35	0% 0/35	8.6% 3/35
Mucor racemosus complex [3]	2	80.0% 24/30	0% 0/30	80.0% 24/30	0% 0/30	20.0% 6/30
Paecilomyces variotii complex	8	100% 30/30	0% 0/30	100% 30/30	0% 0/30	0% 0/30
Penicillium chrysogenum	5	100% 30/30	0% 0/30	100% 30/30	0% 0/30	0% 0/30
Pseudallescheria boydii	22	93.8% 30/32	0% 0/32	93.8% 30/32	3.1% 1/32	3.1% 1/32
Purpureocillium lilacinum	17	93.5% 29/31	0% 0/31	93.5% 29/31	0% 0/31	6.5% 2/31
Rasamsonia argillacea complex [4]	9	85.3% 29/34	0% 0/34	85.3% 29/34	0% 0/34	14.7% 5/34
Rhizopus arrhizus complex	18	78.6% 22/28	0% 0/28	78.6% 22/28	0% 0/28	21.4% 6/28

Species	Number of Unique Isolates	Correct Identification (ID)						Discordant		No Identification	
		Correct for Genus & Species (1 choice)		Low Discrimination (>1 Choice in Same Genus)		Combined (1 Choice + > 1 Choice in Same Genus)					
Rhizopus microsporus complex	19	89.7%	26/29	0%	0/29	89.7%	26/29	3.4%	1/29	6.9%	2/29
Sarocladium kiliense	6	100%	30/30	0%	0/30	100%	30/30	0%	0/30	0%	0/30
Scedosporium apiospermum	41	100%	41/41	0%	0/41	100%	41/41	0%	0/41	0%	0/41
Scedosporium prolificans	11	100%	32/32	0%	0/32	100%	32/32	0%	0/32	0%	0/32
Sporothrix schenckii complex	6	100%	30/30	0%	0/30	100%	30/30	0%	0/30	0%	0/30
Trichophyton interdigitale	22	96.7%	29/30	3.3%	1/30	100%	30/30	0%	0/30	0%	0/30
Trichophyton rubrum	25	100%	31/31	0%	0/31	100%	31/31	0%	0/31	0%	0/31
Trichophyton tonsurans	18	90.9%	30/33	3.0%	1/33	93.9%	31/33	6.1%	2/33	0%	0/33
Trichophyton verrucosum	3	58.1%	18/31	16.1%	5/31	74.2%	23/31	12.9%	4/31	12.9 %	4/31
Trichophyton violaceum [5]	5	41.2%	14/34	41.2%	14/34	82.4%	28/34	14.7%	5/34	2.9%	1/34

[1] = Both *Aspergillus alabamensis* and *Aspergillus niveus* are not identified as *Aspergillus terreus* complex. No identification is expected with VITEK MS.

[2] = All of the no identifications for this organism were from multiple replicates of the same isolate.

[3] = *Mucor racemosus f. sphaerosporus* is not identified as *Mucor racemosus* complex

[4] = 3 out of the 5 no identifications for this organism were from multiple replicates of the same isolate.

[5] = All of the discordant identifications for this organism were from multiple replicates of the same isolate.

TABLE 14: Mycobacterium Performance with VITEK MS V3.0 Knowledge Base

Species	Number of Unique Isolates	Correct Identification (ID)						Discordant		No Identification	
		Correct for Genus & Species (1 choice)		Low Discrimination (>1 Choice in Same Genus)		Combined (1 Choice + > 1 Choice in Same Genus)					
Mycobacterium abscessus 41 tested isolates - 40 from solid medium - 1 from liquid medium	40	100%	41/41	0%	0/41	100%	41/41	0%	0/41	0%	0/41
Mycobacterium avium 115 tested isolates - 75 from solid medium - 40 from liquid medium	67	95.7% 110/115		0%	0/115	95.7%	110/115	1.7%	2/115	2.6%	3/115
*Mycobacterium chelonae**	29	97.0%	32/33	0%	0/33	97.0%	32/33	0%	0/33	3%	1/33
Mycobacterium fortuitum group 50 tested isolates - 49 from solid medium - 1 from liquid medium	49	98.0%	49/50	0%	0/50	98.0%	49/50	0%	0/50	2%	1/50
Mycobacterium gordonae 40 tested isolates - 36 from solid medium - 4 from liquid medium	30	95.0%	38/40	0%	0/40	95.0%	38/40	0%	0/40	5%	2/40
*Mycobacterium haemophilum**	9	97.1%	34/35	0%	0/35	97.1%	34/35	0%	0/35	2.9%	1/35
*Mycobacterium immunogenum**	19	100%	32/32	0%	0/32	100%	32/32	0%	0/32	0%	0/32
Mycobacterium intracellulare 70 tested isolates - 54 from solid medium - 16 from liquid medium	43	95.7%	67/70	0%	0/70	95.7%	67/70	1.4%	1/70	2.9%	2/70

Species	Number of Unique Isolates	Correct Identification (ID)			Discordant	No Identification
		Correct for Genus & Species (1 choice)	Low Discrimination (>1 Choice in Same Genus)	Combined (1 Choice + > 1 Choice in Same Genus)		
*Mycobacterium kansasii**	31	100% 31/31	0% 0/31	100% 31/31	0% 0/31	0% 0/31
Mycobacterium lentiflavum 45 tested isolates - 44 from solid medium - 1 from liquid medium	37	88.9% 40/45	0% 0/45	88.9% 40/45	0% 0/45	11.1% 5/45
*Mycobacterium malmoense**	4	94.1% 32/34	0% 0/34	94.1% 32/34	0% 0/34	5.9% 2/34
*Mycobacterium marinum**	25	100% 32/32	0% 0/32	100% 32/32	0% 0/32	0% 0/32
*Mycobacterium mucogenicum**	20	93.5% 29/31	0% 0/31	93.5% 29/31	0% 0/31	6.5% 2/31
*Mycobacterium scrofulaceum**	6	93.5% 29/31	0% 0/31	93.5% 29/31	0% 0/31	6.5% 2/31
*Mycobacterium simiae**	28	100% 31/31	0% 0/31	100% 31/31	0% 0/31	0% 0/31
*Mycobacterium smegmatis**	5	93.3% 28/30	0% 0/30	93.3% 28/30	0% 0/30	6.7% 2/30
Mycobacterium szulgai 37 tested isolates - 36 from solid medium - 1 from liquid medium	24	97.3% 36/37	0% 0/37	97.3% 36/37	0% 0/37	2.7% 1/37
Mycobacterium tuberculosis complex 54 tested isolates - 45 from solid medium	45	98.1% 53/54	0% 0/54	98.1% 53/54	0% 0/54	1.9% 1/54
*Mycobacterium xenopi**	24	100% 33/33	0% 0/33	100% 33/33	0% 0/33	0% 0/33

*Solid Media Only

TABLE 15: Nocardia Performance with VITEK MS V3.0 Knowledge Base

Species	Number of Unique Isolates	Correct Identification (ID)			Discordant	No Identification
		Correct for Genus & Species (1 choice)	Low Discrimination (>1 Choice in Same Genus)	Combined (1 Choice + > 1 Choice in Same Genus)		
Nocardia abscessus	16	96.7% 29/30	0% 0/30	96.7% 29/30	0% 0/30	3.3% 1/30
Nocardia asteroides	9	88.6% 31/35	0% 0/35	88.6% 31/35	8.6% 3/35	2.9% 1/35
Nocardia brasiliensis	37	94.6% 35/37	0% 0/37	94.6% 35/37	0% 0/37	5.4% 2/37
Nocardia cyriacigeorgica	33	100% 33/33	0% 0/33	100% 33/33	0% 0/33	0% 0/33
Nocardia farcinica	32	100% 34/34	0% 0/34	100% 34/34	0% 0/34	0% 0/34
Nocardia nova[1]	33	0% 0/33	100% 33/33	100% 33/33	0% 0/33	0% 0/33
Nocardia otitidiscaviarum	18	100% 30/30	0% 0/30	100% 30/30	0% 0/30	0% 0/30
Nocardia paucivorans	18	100% 30/30	0% 0/30	100% 30/30	0% 0/30	0% 0/30
Nocardia pseudobrasiliensis	13	96.7% 29/30	0% 0/30	96.7% 29/30	0% 0/30	3.3% 1/30
Nocardia transvalensis	2	100% 30/30	0% 0/30	100% 30/30	0% 0/30	0% 0/30
Nocardia veterana	23	100% 30/30	0% 0/30	100% 30/30	0% 0/30	0% 0/30
Nocardia wallacei	13	100% 30/30	0% 0/30	100% 30/30	0% 0/30	0% 0/30

[1] = In KB V3.0.0, Nocardia nova is displayed as a low discrimination result with *Nocardia africana* but only *Nocardia nova* has been clinically validated.

TABLE 16: Single Choice Discordants

Number of Isolates	Reference Result	VITEK MS Result
2	*Mycobacterium avium*	*Mycobacterium intracellulare*
1	*Mycobacterium intracellulare*	*Mycobacterium avium*
3	*Nocardia asteroides*	*Nocardia neocaledoniensis*
1	*Pseudallescheria boydii*	*Scedosporium apiospermum*
5	*Trichophyton violaceum*	*Trichophyton rubrum*
2	*Trichophyton tonsurans*	*Trichophyton interdigitale*
1	*Trichophyton verrucosum*	*Trichophyton erinacei**
3	*Trichophyton verrucosum*	*Trichophyton interdigitale*
1	*Vibrio cholerae*	*Vibrio parahaemolyticus*
1	*Yersinia frederiksenii*	*Yersinia pseudotuberculosis*

TABLE 17: Repeat rate in clinical study

Group	**Number of Samples Tested**	**Number of Samples Same Extraction Tested (Repeat 1)**	**Number of Samples New Extraction Tested (Repeat 2)**
Mycobacteria	805	109 (13.54%)	46 (5.71%)
Nocardia	382	26 (6.81%)	13 (3.40%)
Moulds	1508	241 (15.98%)	126 (8.36%)
Unclaimed	287	154 (53.66%)	123 (42.86%)
All Organisms	2695	376 (13.95%)	185 (6.86%)

b. Clinical specificity: See clinical sensitivity above.

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

4. Clinical cut-off: N/A

5. Expected values/Reference range: See M.1.c

N. Instrument Name:

VITEK MS

O. System Descriptions:

1. Modes of Operation:
   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ____X____ or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?
Yes ___X_____ or No _____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
   Yes ___X_____ or No _____

   FDA has reviewed applicant's cybersecurity processes.

   VITEK MS acquisition station:

   For VITEK MS v3 / KB v3.0.0, the Acquisition Station Software was optimized to account for the detection of higher mass peaks, relevant for some moulds and *Mycobacterium*. The Acquisition Station Software was updated from v1.4.2 to v1.5.0. The most significant change to the Acquisition Station algorithms in v1.5.0 is the rastering strategy. As the spectrum is acquired for each target spot, it is visible in the Spectrum Display on the Acquisition Screen with the number of profiles incrementing as they are collected.

   In the Acquisition Station v1.5.0, the acquisition process stops when the target of 100 good profiles is achieved, or when all raster points have been visited. As with v1.4.2, if the final total of good profiles is less than 30 then the acquisition has failed, and if more than 30 good profiles are obtained then the spectrum is acceptable.

   The following software has remained unchanged from VITEK MS v2; see DEN130013 (K124067).
   - VITEK MS Sample Prep Station software
   - VITEKS Analysis Server / Software and
   - VITEK MS Computation Engine
   - Myla Middleware

3. Specimen Identification:

   Same as DEN130013 (K124067).

4. Specimen Sampling and Handling:

   Cultures of mould must be processed and inactivated before spotting to the target slide. Cultures of Mycobacterium (liquid and solid) and Nocardia must be processed and inactivated before spotting to the target slide.

   Also see DEN130013(K124067)

5. Calibration:

   Same as DEN130013(K124067)

6. Quality Control:

   See M.1.c above.

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

N/A

Q. Proposed Labeling:

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

R. Conclusion:

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