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MYCOBACTERIOLOGY AND AEROBIC ACTINOMYCETES

Performance of Vitek MS v3.0 for Identification of *Mycobacterium* Species from Patient Samples by Use of Automated Liquid Medium Systems

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ABSTRACT The accuracy and robustness of the Vitek MS v3.0 matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) system was evaluated by identifying mycobacteria from automated liquid-medium systems using patient samples. This is the first report to demonstrate that proteins within the liquid medium, its supplements, and decontamination reagents for nonsterile patient samples do not generate misidentification or false-positive results by use of the Vitek MS v3.0 system. Prior to testing with patient samples, a seeded-culture study was conducted to challenge the accuracy of the Vitek MS system at identifying mycobacteria from liquid medium by mimicking a clinical workflow. Seventy-seven *Mycobacterium* strains representing 21 species, seeded in simulated sputum, were decontaminated, inoculated into BacT/Alert MP liquid culture medium, incubated until positivity, and identified using the Vitek MS system. A total of 383 liquid cultures were tested, of which 379 (99%) were identified correctly to the species/complex/group level, 4 (1%) gave a "no-identification" result, and no misidentifications were observed. Following the simulated-sputum study, a total of 73 smear-positive liquid-medium cultures detected using BD BBL MGIT and VersaTREK Myco liquid media were identified by the Vitek MS system. Sixty-four cultures (87.7%) were correctly identified to the species/complex/group level; 7 (9.6%) resulted in no identification; and 2 (2.7%) were misidentified at the species level. These results indicate that the Vitek MS v3.0 system is an accurate tool for routine diagnostics of *Mycobacterium* species isolated from liquid cultures.

KEYWORDS liquid media, MALDI-TOF, mycobacteria

Mycobacterium tuberculosis persists as a global health problem, with the World Health Organization (WHO) reporting 10.4 million new cases and 1.4 million related deaths in 2015 (1). Alongside the significant threat of infection and death caused by *M. tuberculosis*, infections with nontuberculous mycobacteria (NTM) are increasingly prevalent (2). NTM are primarily opportunistic pathogens found in water and soil that also cause infections in humans and animals (3). With mycobacterial infections increasing worldwide, the development of a highly accurate and specific identification method is crucial.

Identification of the species or complex of mycobacteria from patient samples relies heavily on molecular diagnostics. In the United States, DNA probes are widely used for

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TABLE 1 Contaminating respiratory microbiota panel in simulated sputum

Organism	Strain	Relevance	Culture conditions
Staphylococcus aureus	ATCC 6538	Drug-resistant respiratory pathogen	TSAB, 37°C, 18–24 h
Pseudomonas aeruginosa	ATCC 27853	Respiratory pathogen isolated from cystic fibrosis patients	
Staphylococcus epidermidis	ATCC 12228	Common skin bacterium	
Streptococcus oralis	ATCC 11663	Common oral bacterium	TSAB, 37°C with CO_2, 18–24 h
Neisseria sicca	501593[a]	Common respiratory bacterium	Chocolate agar, 37°C with CO_2, 18–48 h
Candida albicans	ATCC 11006	Common respiratory or oral organism	Sabouraud agar, 30°C, 18–48 h

[a]Clinical isolate.

rapid identification but are limited to frequently isolated mycobacterial species (4). Additionally, sequencing of the *hsp65*, 16S rRNA, *sod*, and *rpoB* genes can identify mycobacteria to the species level, but these methods are expensive and are generally performed only in large reference or academic laboratories (5). In recent years, matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) has emerged as a tool for the rapid identification of mycobacteria, and many studies testing seeded cultures show accurate results (6, 7). However, most studies have not applied MALDI-TOF MS technology to the identification of mycobacteria grown from clinical samples—particularly nonsterile specimens—in liquid media (8). Seeded-culture studies published on liquid-medium testing do not address the effects of sputum-processing reagents and the patient microbiota (5, 9, 10).

This is the first report to demonstrate that proteins within the liquid medium, its supplements, and decontamination reagents for nonsterile patient samples do not generate misidentification or false-positive results by use of the Vitek MS v3.0 system. Furthermore, the accuracy, robustness, and performance of direct identification of *Mycobacterium* species isolated from patient samples in liquid media by use of the Vitek MS v3.0 system were demonstrated to be suitable for clinical use.

(A portion of these data pertaining to seeded simulated-sputum studies was presented as a poster at ASM Microbe, 1 to 5 June 2017, New Orleans, LA [11].)

MATERIALS AND METHODS

Study sites. The performance of the Vitek MS v3.0 system was evaluated at two clinical laboratories within the United States: LabCorp (Burlington, NC) (LC) and Memorial Sloan Kettering Cancer Center (New York, NY) (MSKCC). Bactec MGIT 960 tubes (Becton Dickinson Microbiology Systems [BD]) were tested at Memorial Sloan Kettering Cancer Center, whereas VersaTREK Myco bottles (Trek Diagnostic Systems, Cleveland, OH) were tested at LabCorp. All seeded-culture studies were performed by the Research and Development (R&D) Laboratory at bioMérieux Inc., Durham, NC, using BacT/Alert MP culture bottles (bioMérieux Inc., Durham, NC). The components and formulations of all three reagent systems were compared based on the manufacturers' instructions for use, and the concentrations were similar; therefore, the study designs and results for one system can be applied to the systems not evaluated.

Growth conditions. Mycobacterial strains were cultured on Lowenstein-Jensen (LJ) slants or Middlebrook 7H11 solid medium plates (Remel, Lenexa, KS) at 35 to 37°C under 5% CO_2, except for *Mycobacterium haemophilum* and *Mycobacterium malmoense*, which were grown at 30°C under 5% CO_2 using LJ medium supplemented with ferric ammonium citrate (FAC) and Herrold's egg yolk agar (Becton Dickinson, Cockeysville, MD), respectively. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus oralis*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Neisseria sicca* were cultured on different media as listed in Table 1. For the seeded simulated-sputum study, species were inoculated and incubated in automated detection systems as described in the study design below. *Escherichia coli* ATCC 8739 grown on tryptic soy agar with 5% sheep blood (TSAB; bioMérieux Inc., Durham, NC) was used as a calibrant, and *Mycobacterium smegmatis* ATCC 19420 grown on LJ medium was used as the quality control (QC) strain for the Vitek MS system.

Vitek MS sample preparation from liquid media. Liquid cultures were inactivated as described previously by Dunne et al. (12) and Totty et al. (13) and were prepared for extraction as described previously by Moreno et al. (14). All steps prior to inactivation were performed using biosafety level 3 lab practices. A 3-ml aliquot from the liquid culture was centrifuged at 3,000 $\times$ *g* for 10 min, and the supernatant was decanted. The centrifuge tube was blotted dry onto single-use, safety backed absorbent pads to remove residual medium. The pellet was resuspended in 500 μl of 70% ethanol and was transferred to a 2-ml tube containing 0.5-mm glass beads. The sample was mixed on a vortexer for 15 min in a horizontal position (maximum speed), or a bead beater was used for 5 min (maximum speed), followed by incubation for 10 min at room temperature. At this point, the samples were inactivated, and subsequent steps were performed outside the biosafety level 3 laboratory. The liquid was then transferred to a 2-ml tube and was centrifuged at 14,000 $\times$ *g* for 2 min. By use of a pipette, the supernatant

was discarded, and the pellet was suspended in 10 μl of 70% formic acid, followed by the addition of 10 μl of 100% acetonitrile. The resulting sample was then centrifuged at 14,000 $\times$ *g* for 2 min, and 1 μl of the supernatant was spotted onto a Vitek MS disposable (DS) target slide. When the sample was dry, 1 μl of an α-cyano-4-hydroxycinnamic acid (CHCA) matrix was added, and after drying, the slide was loaded onto the Vitek MS system for MALDI-TOF analysis.

Vitek MS configuration, calibration, and quality control. The spectra of target slides were acquired using the Vitek MS Prep Station, v2.3, and the Vitek MS Acquisition Station, v1.5. Spectra were analyzed using Myla 4.0 software with Knowledge Base (KB) v3.0. For the calibration of the Vitek MS system and QC of every DS target slide, a thin layer (1-μl loop) of *E. coli* ATCC 8739 was spotted onto each calibrant position and was immediately covered with 1 μl of the CHCA matrix. For mycobacterial QC, *M. smegmatis* ATCC 19420 was used according to the manufacturer's recommendation (Vitek MS *Mycobacterium/Nocardia* reagent kit and Vitek MS Liquid Myco supplemental kit; bioMérieux, Marcy l'Étoile, France).

Reagent interference study. The reagent interference study was designed to determine whether negative liquid cultures containing supplements and sputum-processing reagents would generate mass peaks in the overall MALDI-TOF spectrum leading to false-positive results on the Vitek MS system (Table 2). Three different liquid-medium systems were evaluated: BacT/Alert MP bottles, MGIT tubes, and VersaTREK bottles. Bottles and tubes were supplemented with their respective antimicrobial and growth supplements (AS and GS, respectively) according to the manufacturers' instructions: MB/BACT antibacterial supplement (MAS) and reconstitution fluid (RF) for BacT/Alert MP bottles; Bactec MGIT growth supplement and BBL MGIT PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin) antibiotic mixture for MGIT tubes; and VersaTREK Myco GS and VersaTREK Myco AS for VersaTREK bottles. Supplemented liquid cultures were then incubated in automated detection systems (BacT/Alert 3D system [bioMérieux Inc.], Bactec MGIT 960 mycobacterial detection system [BD], VersaTREK automatic microbial detection system [Trek Diagnostic Systems]) until they were declared negative. Additional liquid cultures were supplemented and spiked (Table 2) with 2 ml of deionized (DI) water processed using the NAC-PAC RED AFB digestion and decontamination system according to the manufacturer's instructions (Alpha-Tec Systems, Inc., Vancouver, WA) to evaluate sputum-processing reagents. Negative liquid cultures were unloaded and were prepared for protein extraction and identification by following the Vitek MS sample preparation method described above. One extract was used to perform two individual samples on the Vitek MS system.

Seeded simulated-sputum study. The seeded simulated-sputum study was conducted to evaluate the robustness of the method and the ability of the Vitek MS algorithm to remove background noise from sputum-processing reagents, liquid-medium components, and/or patient microbiota before clinical testing. A methylcellulose solution was prepared as described by Demers et al. (15) to mimic a patient sputum sample. Ten grams of methylcellulose was added to 1 liter of boiling water (final concentration, 1%) and was stirred until fully dissolved (15 min), followed by sterilization. The autoclaved solution was agitated on an orbital shaker until it was equilibrated to room temperature and was held overnight. The homogeneity of the solution was visually confirmed before storing at 2 to 8°C. Prior to testing, a chicken egg was emulsified by whisking with a fork and was then incorporated into the solution to mimic the viscosity of a patient sputum sample.

A total of 77 strains representing 21 mycobacterial species were seeded at a target of 1×10^3 CFU/ml into the simulated sputum in combination with contaminating bacteria and yeast organisms at a total concentration of 18,000 CFU/ml to represent a clinical microbiota load (Table 1). The seeded specimens underwent digestion and decontamination using *N*-acetyl-L-cysteine combined with sodium hydroxide (NALC-NaOH) at a final concentration of 1% according to the manufacturer's instructions (Remel TB base digestant; Remel, Lenexa, KS) and were inoculated into BacT/Alert MP bottles supplemented with MAS and RF according to the manufacturer's instruction. The bottles were incubated in the BacT/Alert 3D system until they were declared positive. Once declared positive, bottles were incubated for an additional 24 to 72 h, and the contents were then extracted for identification by following the Vitek MS sample preparation method.

Clinical study. Clinical evaluation of the Vitek MS system was performed in MGIT and VersaTREK liquid cultures. BacT/Alert MP liquid cultures were not tested, because the results from the simulated-sputum study were deemed sufficient to show that mycobacteria recovered from BacT/Alert MP liquid cultures were correctly identified by the Vitek MS system. This study was performed at LC and MSKCC using acid-fast bacillus (AFB) smear-positive patient samples that were decontaminated and digested with the NAC-PAC RED AFB and BD BBL MycoPrep kits, respectively, and were inoculated into VersaTREK bottles and MGIT tubes, respectively. Once declared positive, bottles or tubes were incubated for an additional 24 to 72 h and were then processed for identification according to the manufacturer's instructions for the Vitek MS *Mycobacterium/Nocardia* reagent kit and the Vitek MS Liquid Myco supplemental kit (bioMérieux, Marcy l'Étoile, France). In parallel, the liquid cultures were subcultured onto 7H11 or LJ solid medium and were sent to the University of Texas Health Science Center at Tyler for gene sequencing.

Reference method for organism identification. The 16S rRNA gene and several additional housekeeping genes were sequenced to identify isolates to the species level. Slow-growing mycobacteria and pigmented rapidly growing mycobacteria (RGM) were initially identified using partial 16S rRNA gene sequencing. The *rpoB* gene was sequenced for initial identification of RGM, followed by *erm*(41) for *M. abscessus* complex isolates. *M. tuberculosis* isolates were identified using *gyrB* sequencing. Isolates that could not be resolved to species level using the primary gene target were identified using complete 16S rRNA gene sequencing as a secondary gene target.

TABLE 2 Reagent interference test matrix

Test set	Potential interference source[a]	No. of samples tested	Test description	Test plan	Result
1	Residual NALC-NaOH	6	Sputum-processing reagents (NALC-NaOH), processed DI water (negative sample)	Process DI water with NALC-NaOH and inoculate into liquid medium; process for identification	No identification for all samples
2	Various growth supplements (oleic acid, glycerol, BSA, sodium pyruvate, dextrose, catalase, polyoxyethylene stearate, sodium chloride, casein digest)	5	Liquid medium and GS (negative sample)	Inoculate GS into liquid medium; process for identification	No identification for all samples
3	Various antimicrobials (amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, vancomycin, fosfomycin)	3	Liquid medium, GS, and AS (negative sample)	Inoculate GS and AS into liquid medium; process for identification	No identification for all samples

[a]BSA, bovine serum albumin.

Partial 16S rRNA gene sequencing was performed on the samples using MicroSeq 16S rDNA PCR and sequencing kits according to the manufacturer's recommendations (Life Technologies, Carlsbad, CA). The resulting ~500-bp gene was analyzed using RipSeq software (Pathogenomix, Santa Cruz, CA). Complete 16S rRNA gene sequencing was performed as described previously by Edwards et al. (16) with slight modifications.

Gene sequence analysis was performed using RipSeq software. Sequences were compared to validated type strains and all available sequences using RipSeq. Interpretation was carried out in accordance with the interpretive criteria for DNA target sequencing of the Clinical and Laboratory Standards Institute (CLSI) (17). Sequencing of region V of the *rpoB* gene (720 bp excluding the primer regions) was performed on the isolates utilizing primers Myco F and Myco R as described by Adékambi et al. (18).

erm(41) gene sequencing was performed on *M. abscessus* complex isolates by using primers ermF (5′-GACCGGGGCCTTCTTCGTGAT-3′), ermR1 (5′-GACTTCCCCGCACCGATTCC-3′), and erm(41)-4 (5′-CCGG CCCGTAGCGTCCAATG-3′) as described previously (19). *gyrB* gene sequences were determined by amplifying a 1,020-bp fragment using primers MTUBf and MTUBr by a protocol modified from that published by Kasai et al. in 2000 (20) as described by Niemann et al. (21).

Data analysis. Vitek MS v3.0 MALDI-TOF MS results were considered accurate to the species/complex/group level if a single-choice identification matched the reference method result. When a single result was identified as multiple species from the same genus and matched the sequencing results for the genus, the result was considered correct to the genus level. If the species- or genus-level identification did not match the sequencing result, it was considered an incorrect result (misidentification). If a result was "no identification," repeat testing of a single spot was performed using the same extract; if a "no identification" result was obtained again, a new extract was prepared from the sample, and a single spot was tested. If the repeat result was still no identification, this was used as the final result. "No identification" results included low discrimination with multiple genera and the following results reported by Vitek MS: "not enough peaks" or "too many peaks."

RESULTS

Reagent interference study. Negative liquid cultures with various combinations of supplements (Table 2) were analyzed on the Vitek MS system to evaluate whether the mass peaks generated in the overall MALDI-TOF spectrum led to false-positive results. All 27 spectra tested resulted in "not enough peaks" on the Vitek MS system (data not shown), with an average peak count of six. The Vitek MS KB requires a minimum detection of 30 peaks for a sample to pass quality checks and for assignment of an identification; thus, as expected, all negative liquid cultures tested failed to pass quality checks. The results demonstrated that liquid-medium components, such as fatty acids and proteins or sputum-processing reagents, do not cause any interference in the KB when one adheres to the Vitek MS liquid-medium extraction method.

Seeded simulated-sputum liquid cultures. A total of 383 liquid cultures representing 77 strains and 21 species were tested. Identification rates per species are shown in Table 3. Overall, 379 (99%) resulted in correct identification to the species/complex/group level and 4 (1%) resulted in "no identification," including *Mycobacterium lentiflavum* ($n = 1$), *Mycobacterium marinum* ($n = 2$), and *M. smegmatis* ($n = 1$) (Table 3). No misidentifications were observed.

Clinical liquid cultures. A total of 89 AFB smear-positive liquid cultures were extracted and tested on the Vitek MS system. Sixteen were removed because the mycobacterial species were not in the KB, and 73 were used for analysis (Table 4). Sixty-four liquid cultures (87.7%) were identified correctly to the species/complex/group level, and 7 (9.6%) resulted in "no identification," representing 3 species and 1 complex: *M. avium* ($n = 3$), *M. intracellulare* ($n = 2$), *M. lentiflavum* ($n = 1$), and *M. tuberculosis* complex ($n = 1$). Two *M. avium* cultures (2.7%) were misidentified at the species level as *M. intracellulare*. For analysis based on the medium type, 31 MGIT tubes representing 3 species and 2 complexes or groups were tested. Twenty-six were correctly identified to the species/complex/group level: *M. avium* ($n = 20$), *M. intracellulare* ($n = 3$), *M. gordonae* ($n = 1$), *M. fortuitum* group ($n = 1$), and *M. tuberculosis* complex ($n = 1$). In the testing of *M. avium*, three "no identification" results (9.5%) and two misidentifications (6.5%) were obtained. Forty-two VersaTREK bottles representing 6 species and 1 complex were tested. Thirty-eight were correctly identified to the species/complex level: *M. avium* ($n = 15$), *M. intracellulare* ($n = 11$), *M. gordonae* ($n = 3$), *Mycobacterium szulgai* ($n = 1$), *M. abscessus* ($n = 1$), and *M. tuberculosis* complex ($n = 7$). Four "no identification" results

TABLE 3 Identification results by species for simulated-sputum samples

Species	Total no. of samples	No. (%) with the following result:		
		Correct	No ID[a]	MisID
M. abscessus	20	20 (100)	0 (0)	0 (0)
M. africanum	10[b]	10 (100)	0 (0)	0 (0)
M. avium	20	20 (100)	0 (0)	0 (0)
M. chelonae	20	20 (100)	0 (0)	0 (0)
M. fortuitum group	20	20 (100)	0 (0)	0 (0)
M. gordonae	20	20 (100)	0 (0)	0 (0)
M. haemophilum	5[c]	5 (100)	0 (0)	0 (0)
M. immunogenum	20	20 (100)	0 (0)	0 (0)
M. intracellulare	20	20 (100)	0 (0)	0 (0)
M. kansasii	20	20 (100)	0 (0)	0 (0)
M. lentiflavum	20	19 (95)	1 (5)	0 (0)
M. malmoense	20	20 (100)	0 (0)	0 (0)
M. marinum	18[d]	16 (89)	2 (11)	0 (0)
M. mucogenicum	20	20 (100)	0 (0)	0 (0)
M. peregrinum	20	20 (100)	0 (0)	0 (0)
M. scrofulaceum	20	20 (100)	0 (0)	0 (0)
M. simiae	20	20 (100)	0 (0)	0 (0)
M. smegmatis	20	19 (95)	1 (5)	0 (0)
M. szulgai	20	20 (100)	0 (0)	0 (0)
M. tuberculosis complex	10[b]	10 (100)	0 (0)	0 (0)
M. xenopi	20	20 (100)	0 (0)	0 (0)
Total	**383**	**379 (99)**	**4 (1)**	**0 (0)**

[a]ID, identification.
[b]Testing of *M. africanum* and *M. tuberculosis* represents 20 total samples tested for *M. tuberculosis* complex.
[c]There was limited recovery in liquid medium, since *M. haemophilum* requires supplementation with hemin.
[d]Two samples for which the respotting procedure was not completed were removed from analysis.

(9.5%), with *M. lentiflavum* ($n = 1$), *M. intracellulare* ($n = 2$), and *M. tuberculosis* complex ($n = 1$), and no misidentifications were obtained.

DISCUSSION

Vitek MS KB, v3.0, is an FDA-approved database for the identification of *Mycobacterium* species. The Vitek MS system generates raw protein spectral data in a mass range of 2,000 to 20,000 Da. A baseline is applied to the raw spectra, reducing the background noise of nonprominent peaks, such as liquid-medium components or the cellular debris of patient microbiota, creating a peak list. The peak list is then processed through a mass-binning proprietary algorithm, and peak intensities are normalized to further

TABLE 4 Identification results from clinical samples by species and medium type

Reference ID[a] by medium type	Total no. of samples	No. (%) with:		
		Correct ID	No ID	MisID
MGIT				
M. avium	25	20 (80)	3 (12)	2 (8)
M. fortuitum group	1	1 (100)	0 (0)	0 (0)
M. gordonae	1	1 (100)	0 (0)	0 (0)
M. intracellulare	3	3 (100)	0 (0)	0 (0)
M. tuberculosis complex	1	1 (100)	0 (0)	0 (0)
VersaTREK				
M. abscessus	1	1 (100)	0 (0)	0 (0)
M. avium	15	15 (100)	0 (0)	0 (0)
M. gordonae	3	3 (100)	0 (0)	0 (0)
M. intracellulare	13	11 (85)	2 (15)	0 (0)
M. lentiflavum	1	0 (0)	1 (100)	0 (0)
M. szulgai	1	1 (100)	0 (0)	0 (0)
M. tuberculosis complex	8	7 (87.5)	1 (12.5)	0 (0)
Total	**73**	**64 (87.7)**	**7 (9.6)**	**2 (2.7)**

[a]ID, identification.

reduce noisy spectra. Based on the binning algorithm, which accounts for the presence and absence of peaks, a species-specific pattern of weights is assigned to the spectra. This is compared to a database of known microorganisms, and an identification is determined (22).

The ability to readily obtain a *Mycobacterium* identification directly from a positive liquid culture through an automated detection system can significantly reduce the time to diagnosis from that with identification by conventional microbiology methods. Conventional identification of *Mycobacterium* relies on growth-based parameters and includes pigmentation, growth rate, and temperature preferences, as well as phenotypic and biochemical characteristics that may require incubation for as long as 12 weeks (23–25). It has been reported that identification using MALDI-TOF MS from solid cultures can reduce the time to reporting by 3 to 5 days for RGM and by 14 to 21 days for slow-growing mycobacteria (24). The method described can produce an identification directly from a positive liquid culture within 24 to 72 h after the culture has been declared positive by the automated detection system (14).

Differences in automated detection technology, such as detection by oxygen consumption, carbon dioxide production, or the change in pressure all yield different levels of biomass upon positivity. Variable biomass creates a challenge to the standardization of a MALDI-TOF MS method that is compatible with different automated detection systems (5). Inconsistent identification results at the time of positivity were demonstrated using the Vitek MS method from positive seeded liquid cultures (data not shown); therefore, an additional 24 h of incubation was necessary prior to extraction in order to achieve consistent identification results for the different detection technologies. A maximum of 72 h postpositivity was selected to ensure that mycobacteria had not entered stationary phase, since the Vitek MS database was developed on protein profiles from log phase. This is especially important for RGM.

The additional incubation time, in combination with the 3.0-ml extraction volume, provides sufficient biomass, resulting in consistent, reliable, and reproducible identification. This further supports a recent study by Huang et al. that showed improved identification of *Mycobacterium* species with a 3-ml sample volume from a positive liquid culture for use with the Vitek MS v3.0 system (26).

Negative samples containing liquid-medium supplements (i.e., growth and antimicrobial supplements), in combination with sputum-processing reagents, were tested on the Vitek MS system to ensure that the reagents do not result in an identification. The average number of peaks detected for all negative samples was six; thus, the result was "not enough peaks." The low number of peaks detected indicates that the components tested do not generate a false identification and will not interfere with the detectable range of the Vitek MS system due to the removal of background noise.

A seeded-culture study that incorporated clinically prevalent respiratory microbiota further challenged the database in order to determine the impact of residual microbiota protein with a low concentration of mycobacteria in a positive liquid culture. The overall percentage of correct identifications for the seeded-culture study was 99%, with 1% of samples resulting in "no identification." No misidentification was observed. The high level of correct identification indicates that the presence of patient microbiota will be reduced by the Vitek MS algorithm and hence will not impact the mycobacterial identification results. Similar results were observed from seeded-culture studies conducted by Leyer et al., reporting 91% correct identification when testing in MGIT tubes, and Girard et al., reporting 90% correct identification of mycobacteria on the Vitek MS v3.0 system (27, 28).

To further challenge the liquid extraction method and the Vitek MS v3.0 system, clinical sites that use VersaTREK bottles and MGIT tubes to test patient samples on other widely used detection systems were selected. Overall, the percentage of correct identifications was 87.7% when other clinical liquid cultures were tested, in contrast to 99% in the seeded-culture studies using BacT/Alert MP culture bottles. The decrease was primarily due to the number of "no identification" results observed, which increased from 1.0% in the seeded-culture studies to 9.6% but was still within the range

TABLE 5 Unclaimed species from clinical samples by medium type

Vitek MS identification by medium type	Reference identification
MGIT	
M. intracellulare	*M. yongonense/M. marseillense*
M. intracellulare	*M. chimaera*
M. intracellulare	*M. yongonense/M. marseillense*
M. intracellulare	*M. yongonense/M. marseillense*
M. avium	*M. chimaera*
M. intracellulare	*M. chimaera*
M. avium	*M. chimaera*
M. intracellulare	*M. yongonense/M. marseillense*
M. intracellulare	*M. chimaera*
M. intracellulare	*M. yongonense/M. marseillense*
VersaTREK	
M. intracellulare	*M. arosiense/M. intracellulare*
M. intracellulare	*M. chimaera*
M. intracellulare	*M. yongonense/M. marseillense*
M. intracellulare	*M. yongonense/M. marseillense*
M. intracellulare	*M. chimaera*
M. intracellulare	*M. chimaera*

of acceptable clinical performance. Although the "no identification" rate increased, there was still a substantial improvement in turnaround time for identification. The laboratories should maintain standard clinical workflows, such as solid cultures alongside the liquid culture. An identification can be performed either on the Vitek MS system from solid medium or by conventional methods (e.g., microscopic morphology, AFB staining) when a "no identification" result is obtained from liquid cultures. Excluding "no identification" results, 96.9% of *Mycobacterium* species were correctly identified.

Two *M. avium* cultures, extracted from MGIT tubes, were misidentified to the species level as *M. intracellulare* (Table 4). To our knowledge, this is the first report of the misidentification of *M. avium* using the Vitek MS v3.0 system. Several studies demonstrate the ability of the Vitek MS v3.0 system to distinguish between *M. avium* and *M. intracellulare* (6, 12, 27, 28). The low rate of misidentification (2.7%) is of relatively low importance, because *M. avium* and *M. intracellulare* are part of the *Mycobacterium avium-intracellulare* complex (MAC), for which diagnosis of infection is treated similarly with the use of multidrug regimens, including macrolides and amikacin regimens (29). However, the differentiation is important epidemiologically, since *M. intracellulare* has not been recovered from tap water systems, unlike the closely related species *M. chimaera* and *M. avium*. The unclaimed MAC species *M. arosiense*, *M. chimaera*, *M. yongonense*, and *M. marseillense* were recovered during clinical testing and were removed from the data analysis. These species are closely related to claimed species of the MAC, *M. avium and M. intracellulare*, and cross-identification to these claimed taxa occurred (Table 5). In the future, the addition of *M. chimaera* and other MAC species to the database will be considered.

Only pure mycobacteria isolated from liquid cultures were included in these studies, since MALDI-TOF MS is intended for the identification of pure cultures (30). Mixed *Mycobacterium* cultures present a challenge for MALDI-TOF MS, because both species may not be detected in the sample, especially if the mixed infection is from closely related taxa. An identification result will also depend on the concentration and protein content of the faster-growing organism. It is also possible to obtain low discrimination between equally present species. Clinical laboratories should continue to follow standard practices, such as subcultures on solid medium, microscopic morphology, staining, or other methods, when a mixed culture is suspected.

The liquid-medium extraction method in conjunction with the Vitek MS v3.0 system is reliable for identifying mycobacteria. The ability to identify a range of mycobacteria, including *M. tuberculosis* complex and NTM, as well as the low misidentification rate, supports the use of this method with multiple automated liquid-medium systems in a

clinical laboratory. The identification of mycobacteria directly from positive liquid cultures significantly reduces the turnaround time of diagnosis and hence enables timely intervention with proper therapy for the patient.

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