Identification and Differentiation of Clinically Relevant *Mycobacterium* Species Directly from Acid-Fast Bacillus-Positive Culture Broth[▽]

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Mycobacterium species cause a variety of clinical diseases, some of which may be species specific. Therefore, it is clinically desirable to rapidly identify and differentiate mycobacterial isolates to the species level. We developed a rapid and high-throughput system, MycoID, to identify *Mycobacterium* species directly from acid-fast bacillus (AFB)-positive mycobacterial culture broth. The MycoID system incorporated broad-range PCR followed by suspension array hybridization to identify 17 clinically relevant mycobacterial complexes, groups, and species in one single reaction. We evaluated a total of 271 AFB-positive culture broth specimens, which were identified by reference standard methods in combination with biochemical and molecular tests. The overall identification agreement between the standard and the MycoID system was 89.7% (perfect match) or 97.8% (one match in codetection). In comparison to the standard, the MycoID system possessed an overall sensitivity of 97.1% and specificity of 98.8%. The 159 *Mycobacterium avium*-*M. intracellulare* complex isolates were further identified to the species level by MycoID as being *M. avium* ($n = 98$; 61.1%), *M. intracellulare* ($n = 57$; 35.8%), and mixed *M. avium* and *M. intracellulare* ($n = 2$; 1.3%). *M. avium* was recovered more frequently from sterile sites than *M. intracellulare* (odds ratio, 4.6; $P = 0.0092$). The entire MycoID procedure, including specimen processing, can be completed within 5 h, providing rapid and reliable identification and differentiation of mycobacterium species that is amenable to automation. Additional differentiation of *Mycobacterium avium*-*M. intracellulare* complex strains into *M. avium* and *M. intracellulare* may provide a tool to better understand the role of *Mycobacterium avium*-*M. intracellulare* complex isolates in human disease.

The increasing numbers of infections caused by mycobacteria other than tuberculosis have intensified the need for the introduction of rapid methods for isolating and identifying clinically encountered mycobacteria. The detection of pathogenic mycobacterial species continues to depend mainly on mycobacterial culture. The use of liquid culture media with continuous monitoring has significantly improved test sensitivity and test turnaround time. The Bactec mycobacterial growth indicator tube (MGIT) 960 system (Becton Dickinson Microbiology Systems, Sparks, MD), which is a fully automated, continuous-monitoring, nonradiometric, noninvasive device, is sensitive and shortens the turnaround time by 2 to 27 days (13, 25, 31).

Current laboratory techniques for the identification of mycobacteria to the species level involve growth in broth, nucleic acid probing, or subculture on solid medium for phenotypic tests, which can take weeks, leading to significant delays in diagnoses. Thus, there is a need for quick and accurate identification of all mycobacteria directly from culture broth. The use of DNA probes (AccuProbe; GenProbe, San Diego, CA) that are specific for the *Mycobacterium tuberculosis* complex, the *M. avium*-*M. intracellulare* complex, *M. kansasii*, and *M.*

gordonae directly from Bactec TB broth culture has dramatically reduced the time to identification and differentiation of mycobacterial infections (1, 6, 23). This approach, however, can identify only a limited number of mycobacterium species and requires multiple reactions. If the probe results are negative, a battery of biochemical tests must be performed with or without extracted mycolic acid analysis for definitive species identification.

Several molecular methods including PCR followed by restriction fragment length polymorphism, melt curve analysis, regular sequencing, and pyrosequencing were previously reported to directly identify and differentiate mycobacterial isolates from acid-fast bacillus (AFB)-producing culture broth (15, 16, 26, 32). In this study, we report the use of a *Mycobacterium* genus-specific broad-range primer to amplify the partial 16S-23S rRNA gene internal transcribed spacer (ITS) region (24). After amplification, we then designed 25 mycobacterial species-specific capture oligonucleotide probes to identify and differentiate 17 commonly encountered mycobacterial species in the clinical setting by use of an xMAP suspension array system (Luminex, Austin, TX) (9). Results were validated in comparison to a reference standard using a combination of biochemical and molecular methods.

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MATERIALS AND METHODS

Reference strains. A panel of mycobacterial reference strains were collected from the American Type Culture Collection (ATCC, Manassas, VA); the Clin-

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TABLE 1. Mycobacterial complex, group, and species identified by the MycoID system with genus- and species-specific capture probes

MycoID system identification	Species covered	Probe (sequence [5'-3']) ^a
Genus control	<i>Mycobacterium</i> genus	MYC (GTG GTG GGG TGT GGT GTT TG)
<i>M. tuberculosis</i> complex	<i>M. africanum</i> , <i>M. bovis</i> , <i>M. bovis</i> BCG, <i>M. microti</i> , and <i>M. tuberculosis</i>	MTB (GCA TGA CAA CAA AGT TGG CC)
<i>M. marinum</i> - <i>M. ulcerans</i> group	<i>M. marinum</i> and <i>M. ulcerans</i>	UMC (AAC ATC TCT GTT GGT TTC GG)
<i>M. chelonae</i> - <i>M. abscessus</i> group	<i>M. abscessus</i> , <i>M. chelonae</i> and <i>M. immunogenum</i>	ABS (GGG AAC ATA AAG TAG GCA TC) CHE1 (GGA ACA TAA AGC GAG TTT CT) CHE2 (AGT GGA TGC ATG CTT GGT GA) FOR1 (CAA ACT TTT TTG ACT GCC AG) FOR2 (TTT GCG GTG ATG GGA CTG CC) FOR3 (GAT TTT GCG GTG ATG GGA CC) FOR4 (ACA ACA AAC TTG TAG AAC TG)
<i>M. fortuitum</i> group	<i>M. fortuitum</i> and <i>M. senegalense</i>	AVI (AAC ACT CGG TCC GTC CGT GT) GOR1 (CGT GCG GGG TCA TCG TCT GT) GOR2 (CGT CTG TAG TGG ACG AAG AC) HAE (CTC AGG CTT GTC CCA TGT TG) INT (GGT CGA TCC GTG TGG AGT CC) KANI (GTG CGC AAC TGT AAA TGA AT) KAN2 (AAA AGT GCC CCA ATT GGT GG) KAN3 (AAA AGC ATC CCA ACA AGT GG) KAN4 (GTG CGC GAC AAC GAA CGA GC)
<i>M. avium</i>	<i>M. avium</i>	MAL (AAC ACT CGG CCA GTC CGC GT)
<i>M. gordonae</i>	<i>M. gordonae</i>	MUC (TCC GCT CTC GTT ATC GAG GT) SCR (ACT CGG CTC GTT CTG AGT GG) SIM (GGC CGA CTT CGG TTG AAG TG) SME (ACG TTG AGA TGC GGT GTG GT) SZU (GGC TTG GCC AGA GCT GTT GT) TER (CAG AGG AAT TAC GAA CAA CA) XEN (GTT GGG CAG CAG GCA GTA AC)
<i>M. haemophilum</i>	<i>M. haemophilum</i>	
<i>M. intracellulare</i>	<i>M. intracellulare</i>	
<i>M. kansasii</i>	<i>M. kansasii</i>	
<i>M. malmoense</i>	<i>M. malmoense</i>	
<i>M. mucogenicum</i>	<i>M. mucogenicum</i>	
<i>M. scrofulaceum</i>	<i>M. scrofulaceum</i>	
<i>M. simiae</i>	<i>M. simiae</i>	
<i>M. smegmatis</i>	<i>M. smegmatis</i>	
<i>M. szulgai</i>	<i>M. szulgai</i>	
<i>M. terrae</i>	<i>M. terrae</i>	
<i>M. xenopi</i>	<i>M. xenopi</i>	

^a Each probe was labeled with C12 amino modifier at the 5' end.

ical Microbiology Service, Columbia University Medical Center (New York, NY) (6); and the Clinical Microbiology Laboratory at Vanderbilt University Medical Center (11, 20, 29). They included the *M. tuberculosis* complex (*M. africanum*, *M. bovis*, *M. bovis* BCG, *M. microti*, and *M. tuberculosis*), *M. abscessus*, *M. asiaticum*, *M. avium*, *M. chelonae*, *M. diernhoferi*, *M. flavescens*, *M. fortuitum*, *M. gastri*, *M. gordonae*, *M. haemophilum*, *M. immunogenum*, *M. intracellulare*, *M. kansasii*, *M. lentiflavum*, *M. malmoense*, *M. marinum*, *M. paratuberculosis*, *M. phlei*, *M. scrofulaceum*, *M. senegalense*, *M. shimoidei*, *M. simiae*, *M. smegmatis*, *M. szulgai*, *M. terrae*, *M. triplex*, *M. ulcerans*, *M. vaccae*, and *M. xenopi*. Nonmycobacterial strains, including *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Nocardia asteroides*, *Nocardia brasiliensis*, *Rhodococcus equi*, *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*, were included for analytical specificity determinations.

Clinical specimens and recombinant plasmids. AFB-positive culture broth specimens from the Bactec MGIT 960 system were collected consecutively from 30 May 2007 through 9 October 2008 at the Vanderbilt University Medical Center and the Veterans Affairs Tennessee Valley Health Care System. Multiple specimens may be included from the same patient during the study period. Both AFB-positive broth culture specimens and the corresponding mycobacterial isolates were stored at -80°C for further study. The amplification fragments specific for *M. gordonae*, *M. intracellulare*, *M. kansasii*, and *M. tuberculosis*, which were generated by PCR (see below), were cloned into the pCR2.1 vector (Invitrogen, Carlsbad, CA). The DNA concentration of recombinant plasmid standards (pCR-Mg, pCR-Mi, pCR-Mk, and pCR-Mt) was calibrated by spectrophotometry at 260 nm (17). Each inserted plasmid was adjusted to 10,000 copies/μl and stored at -80°C.

Specimen processing, mycobacterial culture, and identification. The specimens were processed within 24 h of collection. Standard sample decontamination with *N*-acetyl-L-cysteine (0.5% final concentration)-NaOH (2% final concentration) and concentration by centrifugation were performed for sputum and other nonsterile specimens as described previously (21). The volume of the final sediment was approximately 2 ml. Part of the sediment was used for the AFB smear, which was then stained using auramine-rhodamine fluorochrome staining (21). Part of the *N*-acetyl-L-cysteine-NaOH sediment or sterile specimen was inoculated onto Löwenstein-Jensen solid medium and into 7H12 broth liquid medium that was monitored for growth using the Bactec 960 instrument. Bactec MGIT

960 broth medium that yielded a positive culture signal was considered positive for mycobacteria after confirmation by AFB staining. The clinical mycobacterial isolates were identified to the species level by standard biochemical and DNA probe procedures (3, 33), and their identities were confirmed by high-performance liquid chromatography (HPLC) analysis by Tennessee State Laboratory Services as previously described (4).

Nucleic acid extraction. Total nucleic acids were extracted from the AFB-positive MGIT broth medium specimens by using a NucliSens easyMAG system (bioMerieux, Inc., Durham, NC). Briefly, 200 μl of contents was mixed with 900 μl of lysis buffer (bioMerieux, Inc.) under a biosafety hood within a biosafety level 3 laboratory. After thorough vortexing, the mixture was transferred out and placed into the instrument by using a default extraction protocol (20, 30). Total nucleic acids were eluted in 55 μl of elution buffer (bioMerieux, Inc.). For each reference mycobacterial or nonmycobacterial strain, one loop of purified colony was added into 1 ml of sterile water. The mixture was vortexed, heated at 94°C for 10 min, and briefly centrifuged. The supernatant was considered to be extract and stored at 4°C (11). The volume used for nucleic acid amplification was 5 μl for both clinical specimen and strain extracts.

PCR amplification. A broad-range primer set (Sp1 [5'-ACC TCC TTT CTA AGG AGC ACC-3'] and Sp2 [5'-biotin-GAT GCT CGC AAC CAC TAT YCA-3', where Y is C or T]) was designed to amplify a 221-bp portion of the *M. tuberculosis* 16S-23S ITS modified from that reported previously (24, 34). In brief, 50 μl of a PCR mixture contained the following: 1× buffer, 1.5 mM MgCl₂, 10% glycerol, 200 μM deoxynucleoside triphosphates, 1 μM each primer, 0.025 units/μl AmpliTaq Gold DNA polymerase (Applied Biosystems), and 5 μl of DNA extract. PCR amplification was performed by use of an ABI 9700 thermal cycler (Applied Biosystems, Foster City, CA) with 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s.

Capture probe design and microsphere coupling. One mycobacterium genus-specific (MYC) and 35 species-specific probes were synthesized with a C12 amino modifier modification at the 5' end (Integrated DNA Technologies, Inc., Coralville, IA). Probes were designed to be 20 nucleotides in length and complementary in sequence to the biotinylated strand of the amplification products (Table 1). Probes were coupled to carboxylated microspheres by using a previously described carbodiimide coupling method (8, 9). Briefly, for each coupling, 5 × 10⁶ carboxylated microspheres were resuspended in 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) (pH 4.5) and mixed with 1 nM of amine-substi-

TABLE 2. MycoID system analytical sensitivity for detection of *M. gordonae*, *M. intracellulare*, *M. kansasii*, and *M. tuberculosis*

Test mode	Species	No. of hybridized plasmids/total no. of plasmids tested for no. of copies/reaction of:						Limit of detection	
		0	1	10	100	1,000	10,000	No. of copies/reaction	No. of copies/ml
Single	<i>M. gordonae</i>	0/3	1/3	3/3	3/3	3/3	3/3	10	500
	<i>M. intracellulare</i>	0/3	3/3	3/3	3/3	3/3	3/3	1	50
	<i>M. kansasii</i>	0/3	3/3	3/3	3/3	3/3	3/3	1	50
	<i>M. tuberculosis</i>	0/3	2/3	3/3	3/3	3/3	3/3	10	500
Mixed	<i>M. gordonae</i>	0/3	0/3	3/3	3/3	3/3	3/3	10	500
	<i>M. intracellulare</i>	0/3	3/3	3/3	3/3	3/3	3/3	1	50
	<i>M. kansasii</i>	0/3	3/3	3/3	3/3	3/3	3/3	1	50
	<i>M. tuberculosis</i>	0/3	0/3	3/3	3/3	3/3	3/3	10	500

tuted probe oligonucleotide, followed by the addition of 25 µg of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) and incubation in the dark for 30 min. The addition of EDC and incubation were repeated, and the microspheres were washed. Coupled microspheres were stored in TE (pH 8.0) (10 mM Tris-HCl, 1 mM EDTA) at 2°C to 8°C in the dark. Coupling efficacy was determined by hybridization to synthetic oligonucleotide targets (9).

Amplification product identification. The amplified products were further characterized by an xMAP suspension array on a Luminex (Austin, TX) 200 instrument. In brief, after PCR amplification, 2 µl of each reaction mixture was added to hybridization solution (3 M tetramethylammonium chloride, 50 mM Tris-HCl [pH 8.0], 4 mM EDTA [pH 8.0], 0.1% Sarkosyl) containing a mixture of 5,000 of each probe-coupled microsphere set in a 50-µl total reaction mixture volume. Reaction mixtures were denatured at 95°C for 5 min and hybridized at 45°C for 15 min. Hybridized amplicons were labeled by the addition of 25 µl of hybridization solution containing 250 ng of streptavidin-R-phycoerythrin (Molecular Probes) and incubation at 45°C for 5 min (9). A red laser identified each bead (or target) by its color coding, while a green laser detected the hybridization signal associated with each bead. Results for each channel are expressed as the median fluorescence intensity values (9). The cutoff value for each target was determined as the mean plus 3 times the standard deviation of the negative controls for each target (12, 18).

Identification result reporting. Mycobacterial identification was categorized into the following complexes, groups, and/or species: (i) the *M. tuberculosis* complex, covering *M. africanum*, *M. bovis*, *M. bovis* BCG, *M. microti*, and *M. tuberculosis*; (ii) the *M. marinum*-*M. ulcerans* group, covering *M. marinum* and *M. ulcerans*; (iii) the *M. chelonae*-*M. abscessus* group, covering *M. abscessus*, *M. chelonae*, and *M. immunogenum*; (iv) the *M. fortuitum* group, covering *M. fortuitum* and *M. senegalense*; (v) *M. avium*; (vi) *M. gordonae*; (vii) *M. haemophilum*; (viii) *M. intracellulare*; (ix) *M. kansasii*; (x) *M. malmoense*; (xi) *M. mucogenicum*; (xii) *M. scrofulaceum*; (xiii) *M. simiae*; (xiv) *M. smegmatis*; (xv) *M. szulgai*; (xvi) *M. terrae*; and (xvii) *M. xenopi*. Specimens were considered unusual mycobacterium species if they were reactive to genus-specific probes but not to any species-specific probes.

Housekeeping gene detection. The human β -actin gene was amplified as an internal amplification control. The primers and fluorophore hydrolysis probes for the human β -actin gene and real-time PCR protocol were reported previously (17).

Statistical analysis. Statistical comparisons were performed with Epi Info software (version 6; CDC, Atlanta, GA). The accuracy of the MycoID system for the identification of mycobacteria to the species level was determined in comparison to a reference in a combination of biochemical testing, DNA probe hybridization, and fatty acid HPLC analysis. Sensitivity and specificity were determined. Odd ratios, 95% confidence limits, and *P* values were calculated; *P* values of ≤ 0.05 were considered statistically significant.

RESULTS

The MycoID system contained three components: (i) specimen processing and nucleic acid extraction, (ii) amplification of the *Mycobacterium* genus-specific ITS region by using PCR with a broad-range primer set, and (iii) detection and determination of 17 clinically encountered *Mycobacterium* species

by using an xMAP suspension array with 26 species-specific probes (Table 1). We first used lysis buffer to disinfect specimens, lyse human and bacterial cells, and stabilize released nucleic acids and then adapted the magnetic bead-based easyMAG system for nucleic acid extraction. All DNAs extracted from MGIT broth specimens tested positive for the human β -actin gene by real-time TaqMan PCR, indicating that they were free of amplification inhibitors.

The analytical sensitivity of the MycoID system was determined by using recombinant plasmids pCR-Mg, pCR-Mi, pCR-Mk, and pCR-Mt, to which the amplification product from *M. gordonae*, *M. intracellulare*, *M. kansasii*, or *M. tuberculosis* was inserted. Serial 10-fold dilutions of the four plasmids ranging from 1 to 10,000 copies/reaction were tested by the MycoID system. The four plasmids were tested either separately (single existence) or together in a 1:1:1:1 ratio (mixed existence), and each plasmid and dilution were run in triplicate. All four plasmids were hybridized by the genus-specific MYC probe, and each plasmid was hybridized by the corresponding species-specific probes. The analytical sensitivities for *M. gordonae*, *M. intracellulare*, *M. kansasii*, and *M. tuberculosis* were 10, 1, 1, and 10 copies/reaction, respectively, determined to be either a single or mixed existence (Table 2), which are equivalent to 500, 50, 50, and 500 copies/ml, respectively, when applied to MGIT broth culture specimens.

The analytical specificity of the MycoID system was tested by use of a panel of mycobacterial and nonmycobacterial strains. The genus-specific MYC probe detected all mycobacterial strains, and species-specific probes detected the corresponding mycobacterial species correctly. No cross-reactions among complexes, groups, and species were revealed. The MycoID system did not react with any nonmycobacterium strains including *S. aureus*, *S. pneumoniae*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *L. pneumophila*, *M. pneumoniae*, *N. asteroides*, *N. brasiliensis*, *R. equi*, *C. albicans*, *C. neoformans*, and *A. fumigatus*.

During the 16-month study period, a total of 271 consecutive AFB-positive Bactec 960 broth medium specimens were collected from 181 patients. Specimen types included 208 (76.8%) sputum samples, 18 (6.6%) surgical tissue/wound samples, 15 (5.5%) bronchial washing specimens, 14 (5.2%) bronchial alveolar lavage samples, 10 (3.7%) stool samples, 5 (1.8%) other sterile fluid samples, and 1 (0.4%) gastric aspirate sample. By use of an identification standard using a combination of bio-

TABLE 3. Agreement between the MycoID system and standard reference for identification and identification to the species level of 271 mycobacterial isolates directly from AFB-positive MGIT broth culture media^a

MycoID system identification	No. of isolates determined by reference identification									
	<i>M. avium-M. intracellulare</i> complex (n = 159)	<i>M. abscessus</i> (n = 16)	<i>M. chelonae</i> (n = 1)	<i>M. fortuitum</i> (n = 7)	<i>M. gordonae</i> (n = 11)	<i>M. kansasii</i> (n = 19)	<i>M. simiae</i> (n = 1)	<i>M. szulgai</i> (n = 3)	<i>M. tuberculosis</i> complex (n = 52)	<i>M. avium-M. intracellulare</i> complex and <i>M. gordonae</i> (n = 2)
<i>M. avium</i> (n = 84)	83			1						
<i>M. intracellulare</i> (n = 54)	53									1
<i>M. chelonae-M. abscessus</i> group (n = 18)	2	15	1							
<i>M. fortuitum</i> group (n = 8)	2			6						
<i>M. gordonae</i> (n = 12)					11					1
<i>M. kansasii</i> (n = 18)						18				
<i>M. simiae</i> (n = 1)							1			
<i>M. szulgai</i> (n = 3)								3		
<i>M. tuberculosis</i> complex (n = 52)									52	
<i>M. avium</i> and <i>M. gordonae</i> (n = 14)	14									
<i>M. avium</i> and <i>M. intracellulare</i> (n = 2)	2									
<i>M. intracellulare</i> and <i>M. fortuitum</i> (n = 1)	1									
<i>M. intracellulare</i> and <i>M. gordonae</i> (n = 1)	1									
<i>M. intracellulare</i> and <i>M. chelonae-M. abscessus</i> group (n = 1)		1								
<i>M. kansasii</i> and <i>M. gordonae</i> (n = 1)						1				
Species undetermined (n = 1)	1									

^a Overall numbers for correct identification by the MycoID system were 243 (89.7%, perfect match) and 265 (97.8%, one match in codetection) in comparison to the reference identification method.

chemical testing, DNA probe hybridization (Gen-Probe), and HPLC analysis identification, they were identified to be 159 (58.7%) *M. avium-M. intracellulare* complex specimens, 16 (5.9%) *M. abscessus* specimens, 1 (0.4%) *M. chelonae* specimen, 7 (2.6%) *M. fortuitum* specimens, 11 (4.1%) *M. gordonae* specimens, 19 (7.0%) *M. kansasii* specimens, 1 (0.4%) *M. simiae* specimen, 3 (1.1%) *M. szulgai* specimens, 52 (19.2%) *M. tuberculosis* complex specimens, and 2 (0.7%) mixed *M. avium-M. intracellulare* complex and *M. gordonae* specimens (Table 3). The MycoID system detected all 271 specimens by use of the genus-specific MYC probe. In addition, the system identified 84 (31.0%) *M. avium* specimens, 54 (19.9%) *M. intracellulare* specimens, 18 (6.6%) *M. chelonae-M. abscessus* group specimens, 8 (3.0%) *M. fortuitum* group specimens, 12 (4.4%) *M. gordonae* specimens, 18 (6.6%) *M. kansasii* specimens, 1 (0.4%) *M. simiae* specimen, 3 (1.1%) *M. szulgai* specimens, 52 (19.2%) *M. tuberculosis* complex specimens, 14 (5.2%) mixed *M. avium-M. gordonae* specimens, 2 (0.7%) mixed *M. avium-M. intracellulare* specimens, 1 (0.4%) mixed *M. intracellulare-M. fortuitum* specimen, 1 (0.4%) mixed *M. intracellulare-M. gordonae* specimen, 1 (0.4%) mixed *M. intracellulare-M. chelonae* specimen, 1 (0.4%) mixed *M. kansasii-M. gordonae* specimen, and 1 (0.4%) specimen for which the species was undermined (Table 3). The overall agreements between the MycoID system and the standard for mycobacterial species identification were

87.7% and 97.8% when a perfect match and one match in codetection were used, respectively (Table 3).

The diagnostic performance of the MycoID system for mycobacterium detection and identification of isolates to the species level was determined by using the 271 AFB-positive MGIT broth culture specimens. In all, the MycoID system possessed sensitivity and specificity of 97.1% and 98.8%, respectively, when a standard including a combination of biochemical, probe hybridization, and cell wall fatty acid profiles was used (Table 4). The MycoID system presented perfect performance for the detection and identification of *M. tuberculosis* complex isolates. Fifteen specimens, which were all from sputum samples, falsely tested positive for *M. gordonae* by the MycoID system, resulting in a relatively low specificity of 94.2% for the identification of this species (Table 4).

Among 157 specimens identified as belonging to the *M. avium-M. intracellulare* complex by both methods, 27 (17.2%) and 130 (82.8%) were recovered from sterile (surgical tissue/wound, bronchial washing fluid, bronchoalveolar lavage fluid, and other sterile fluid samples) and nonsterile (sputum, stool, and gastric aspirate samples) sites, respectively. The MycoID system was able to detect and identify 98 (62.4%) *M. avium*, 57 (36.3%) *M. intracellulare*, and 2 (1.3%) mixed *M. avium-M. intracellulare* specimens. *M. avium* was recovered more frequently from sterile sites (23.5%) than was *M. intracellulare*

TABLE 4. Sensitivity and specificity of the MycoID system for identification of 271 mycobacterial isolates directly from AFB-positive MGIT broth culture media

Mycobacterial species, complex, or group	No. of isolates ^a				Sensitivity (%)	Specificity (%)
	R ⁺ M ⁺	R ⁺ M ⁻	R ⁻ M ⁺	R ⁻ M ⁻		
<i>M. avium</i> - <i>M. intracellulare</i> complex	155	6	1	109	96.3	99.1
<i>M. chelonae</i> - <i>M. abscessus</i> group	17	0	3	251	100.0	99.8
<i>M. fortuitum</i> group	6	1	3	261	85.7	98.9
<i>M. gordonae</i>	12	1	15	243	92.3	94.2
<i>M. kansasii</i>	19	0	0	252	100.0	100.0
<i>M. simiae</i>	1	0	0	270	100.0	100.0
<i>M. szulgai</i>	2	0	0	268	100.0	100.0
<i>M. tuberculosis</i> complex	52	0	0	219	100.0	100.0
Total	265	8	22	1,873	97.1	98.8

^a R, reference; M, MycoID system; +, detected; -, not detected.

(7.0% [odds ratio, 4.6; 95% confidence interval, 1.23 to 14.79; $P = 0.0092$]).

DISCUSSION

In this study, we developed a MycoID system, which combines (i) nucleic acid extraction by the bioMerieux NucliSens easyMAG system (20, 30), (ii) broad-range PCR amplification of the partial mycobacterial genus-specific 16S-23S ITS region (24), and (iii) determination of mycobacterial isolates to the species level by the Luminex xMAP suspension array system (9) for the high-throughput detection and identification of *Mycobacterium* species directly from AFB-positive MGIT culture broth. The overall identification agreement between the standard and the MycoID system was 99.3%. The MycoID system was able to further identify and differentiate *M. avium* and *M. intracellulare* within the *M. avium*-*M. intracellulare* complex group. The entire MycoID procedure, including specimen processing, can be completed within 5 h, providing another means for a rapid and reliable identification and differentiation of mycobacterium species that is amenable to automation.

The Luminex analyzer interrogates analytes via a flow cell illuminated with focused laser excitation light. High-speed digital signal processing and advanced computer algorithms provide analysis of the xMAP microspheres as they pass through the analyzer. Sample data are processed via statistical techniques to determine the existence of a given target analyte with high sensitivity. The analyzer then takes every valid data point and determines the median of the reporter fluorophore signals to give a median fluorescence intensity value for that set (9). We have used this platform extensively in the diagnostic microbiology field including for the detection, typing, and semiquantification of 25 human papillomaviruses (12); detection and differentiation of a panel of respiratory viral and bacterial pathogens (7, 18); identification, antibiotic resistance determination, and toxin gene detection of *Staphylococcus aureus* (28); quantitative, multiplexed detection of *Salmonella* and other diarrheal pathogens (8); and screening and differentiation of methicillin-resistant *S. aureus* and vancomycin-resistant enterococcus isolates (22).

In addition to rapid mycobacterial pathogen detection and differentiation directly from the AFB-positive broth medium specimens described in this study, the xMAP suspension array

system has been used for other tuberculosis research and diagnosis. We have adapted this system following target enrichment multiplex PCR amplification for the detection and differentiation of 24 antituberculosis drug resistance-related mutations (11). Others previously reported using this system to study the pattern and diversity of cytokine production between tuberculosis infection and disease (27), perform *M. tuberculosis* spoligotyping on a rapid and high-throughput scale (5), and detect *M. tuberculosis*-specific antibodies for tuberculosis diagnosis (14). As the detection system for several nucleic acid amplification products, this system will provide another rapid and high-throughput system for amplification product identification, differentiation, and characterization.

M. avium and *M. intracellulare* are closely related organisms and comprise the *M. avium*-*M. intracellulare* complex. These organisms share many common characteristics, including the ability to cause life-threatening respiratory infections in people with AIDS. However, pathogenic differences between *M. avium* and *M. intracellulare* have been reported. The ability to invade the mucosa of the gastrointestinal tract and cause disseminated disease in AIDS patients has been epidemiologically linked uniquely to *M. avium* (2, 35). *M. avium* could invade intestinal epithelial cells more efficiently than *M. intracellulare* (19). *M. intracellulare* also appears to have a distinct environmental niche, as it has been found to be more prevalent in biofilms and at significantly higher numbers than *M. avium* (10). The MycoID system described in this study is able to differentiate between *M. avium* and *M. intracellulare* and found that *M. avium* was recovered more frequently from sterile sites than *M. intracellulare*, which may provide a tool to better understand the role of *M. avium*-*M. intracellulare* complex isolates in human disease.

By the incorporation of broad-range PCR amplification, analytical sensitivities of the MycoID system reached 10 copies/reaction for four clinical commonly encountered mycobacterial species, including *M. intracellulare*, *M. gordonae*, *M. kansasii*, and *M. tuberculosis*. In addition to AFB-positive MGIT broth medium specimens, other crude clinical specimens may be used directly by this system for the detection and identification of mycobacterial pathogens. We have successfully detected and identified *M. avium*, *M. intracellulare*, and *M. marinum*-*M. ulcerans* group isolates from formalin-fixed, paraffin-embedded lung tissue specimens collected from patients with respiratory

failure (J. A. Johnson, unpublished data). Larger studies are being planned to explore this assay for the detection and identification to the species level of mycobacterial pathogens directly from a variety of clinical specimens.

Overall, the MycoID system presented satisfactory sensitivity and specificity for the rapid detection and identification of mycobacterial species directly from AFB-positive MGIT broth culture media. Perfect performance was noticed when the system was used to detect and identify *M. tuberculosis* complex isolates. However, it is acknowledged that several mycobacterial species with potential medical relevance, including *M. pinipedii*, *M. bovis* subsp. *caprae*, and "*M. canettii*" in the *M. tuberculosis* complex as well as *M. bolletii* in the *M. chelonae*-*M. abscessus* group, were not included in the study. In addition, we did observe that for 16 sputum specimens, *M. gordonae* was detected together with other mycobacterium species by the MycoID system, which was significantly higher than the 2 samples detected by the standard method. An extended study is being planned to determine the clinical relevance of these codetections and decide whether a separate, higher cutoff value is needed for *M. gordonae*.

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