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29 November 2018

To: Recipients of MM18, 2nd ed.

From: Jennifer K. Adams, MT(ASCP), MSHA
Vice President, Standards and Quality

Subject: Correction

This notification is to inform you of corrections made to CLSI document MM18, *Interpretive Criteria for Identification of Bacteria and Fungi by Targeted DNA Sequencing,* 2nd ed.

Tables 15 and 16 are reprinted below, along with the introductory text that precedes each table. In the tables, the corrections and additions are highlighted. In the introductory text, the corrections are shown as highlighted and/or stricken text. Other minor clarifications were also made throughout the guideline.

NOTE: Tables 15 and 16 have been significantly revised and now contain more detailed information regarding targeted DNA sequencing of aerobic actinomycetes and mycobacteria, respectively. The revised versions below should replace the original published versions of Tables 15 and 16.

Subchapter 3.1.10, Aerobic Actinomycetes:

Aerobic actinomycete taxonomy has evolved significantly, with new species identified. For example, for the genus *Nocardia,* sequencing 16S rRNA, *secA1,* and other loci has led to improved complex and species differentiation, with better correlation to human pathogenic potential and antimicrobial susceptibility profiles.[237-241] Additional description and analysis of phylogenetic relationships for ~~*Dietzia,*~~ *Gordonia, Rhodococcus, Nocardia, Skermania, Tsukamurella,* and *Turicella*~~, and *Williamsia*~~ is available.[242]

For the microorganisms or groups listed in Table 15, the following ~~algorithm~~key points are relevant for identification ~~can be applied~~using 16s rRNA sequences:

- Many species within the aerobic actinomycetes genera are closely related by the 16S rRNA gene. Closely related species may show only a few mismatches across the entire 16S gene or no mismatches at all. Chromatograms should therefore be carefully reviewed and edited when necessary to reliably capture the few divergent positions.

- Full-length 16S rRNA gene sequencing is recommended to reliably separate many of these genera.

- Performing full-length sequence alignments of a sample sequence against one or several reference sequences of possibly matching species is helpful to detect mismatches for differentiation and species identification.

- If mismatches between several closely related species occur at the same positions or within the same variable regions, this finding adds credibility to the mismatches being potentially discriminatory.

~~≥ 99.6% identity for genus and species identification (with > 0.4% separation between different species); report "[*Genus species*]."~~

~~99.0% to 99.5% identity for genus identification; consider reporting "[*Genus*], most closely related to [*species*]."~~

~~≥ 95% cannot be definitively identified by 16S rRNA gene sequencing; consider reporting "Unable to identify by 16S rRNA gene sequencing, most closely related to [*Genus*]."~~

~~The cutoff values for percent identity scores are suggested as tools for medical laboratories to identify microorganisms in a consistent, pragmatic manner. They do not reflect strict taxonomical classifications.~~

Table 15. Aerobic Actinomycetes[*]

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
Actinomadura **spp.**[243-246]	Resolution to genus and some to species.	*Actinomadura* is a highly homologous genus that contains many environmental species. Resolution to genus occurs by mismatches within the ≈ 150–250 bp (V2), ≈ 420–500 bp (V3), and ≈ 570–700 bp (V4) regions. A full-length 16S sequence is required for accurate differentiation of many *Actinomadura* spp. *A. madurae* and *A. pelletieri* are the most common species in mycetoma. Other clinical species primarily recovered from sputa include *A. sputi*, *A. cremea*, and *A. nitrigenes*. *A. madurae* is closely related to *A. bangladeshensis* but can be separated by mismatches at ≈ 150–250 bp. *A. nitritigenes* is separated by mismatches within the same 16S region.	Of limited additional benefit.	Limited MALDI-TOF MS data.[247] Resolution to genus is usually sufficient.
Dermatophilus congolensis	Resolution to genus and species.	This organism can be identified by mismatches within the first ≈ 500 bp of 16S.	Of limited additional benefit.	

Table 15. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
Gordonia spp.[243,248-256] Many environmental species.	Resolution to genus and some to species.	*Gordonia* is a highly homologous genus. Resolution to genus occurs by mismatches within the ≈ 150-250 bp (V2), ≈ 420-470 bp (V3), and ≈ 570-650 bp (V4) regions. A full-length 16s sequence is required for accurate differentiation of many *Gordonia* spp. *G. terrae* is commonly recovered from sputa, but *G. bronchialis, G. sputi,* and *G. otitidis* may also be recovered from clinical samples. *G. terrae* is closely related to *G. lacunae, G. hongkongensis,* and *G. didemni*. These species may be separated by only a few mismatches within the V2 and V4 at ≈ 150 bp and ≈ 450 bp. The species outlined below can be identified by mismatches within the first ≈ 500 bp. *G. bronchialis* can be separated by mismatches within the V2, V3, and V4 regions. *G. sputi* and *G. aichiensis* have a few mismatches in the V3 region. *G. otitidis* and *G. polyisoprenivorans* have 4 mismatches in V3 and some mismatches in the V4 region. A longer 16S sequence is required to separate some species.	*gyrB* and *secA1* provide better resolution to species (*gyrB* is observed to exhibit greater sequence divergence between species than *secA1*).[257]	Limited MALDI-TOF MS data.[81,247] Resolution to genus is usually sufficient.

Table 15. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
Nocardia **spp.**[258] (in general)	Resolution to genus, with limited resolution to species.	Many *Nocardia* spp. are closely related with limited diversity across the 16S rRNA gene. A few mismatches at positions ≈ 160–220, ≈ 560–650, and in the V5 region at ≈ 970–1020 can allow species-level identification. A longer sequence up to ≈ 1200 bp of 16S provides the optimal species resolution.	*secA1* and *gyrB* have been shown to have much better resolution to species.[241,259,260]	MALDI-TOF MS aids in resolution to genus and some species. Report to species level or if not possible to group or complex level.
N. abscessus **complex**[237,243,252,253,258,261] *N. abscessus* *N. arthritidis* *N. asiatica* *N. beijingensis* *N. pneumoniae*	Resolution to group, with limited resolution to species.	Species within this complex are closely related. *N abscessus, N. asiatica, N. gamkensis, N. exalbida,* and *N. arthritidis* cannot be differentiated within the first ≈ 500 bp of 16S. Some species, including *N. abscessus, N. asiatica,* and *N. arthritidis,* share almost complete sequence identity over the entire 16S and are closely related to *N. beijingensis*. Some species may be differentiated by a few mismatches at positions ≈ 580–650 bp. To attempt differentiating species of this complex, a longer sequence covering at least the V4–V6 regions (up to ≈ 1200 bp) should be analyzed.	*gyrB* and *secA1* may provide better resolution to species.[241,259,260]	MALDI-TOF MS aids in resolution to complex and limited resolution to species. Report to species when possible.

Table 15. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
N. asteroides sensu stricto	Obsolete name. The only currently valid taxon that matches its type strain is *N. asteroides* (which is not pathogenic).			MALDI-TOF MS aids in the resolution to genus and species.[262]
N. exalbida	Resolution to genus and sometimes species.	*N. gamkensis* cannot be separated from *N. exalbida*. Some *N. arthritidis* variants are close to *N. exalbida*, whereas some variants of *N. abscessus* complex differentiate only in the V4 region at positions ≈ 580–650 bp. A longer sequence up to ≈ 1200 bp is helpful for species-level identification due to homology.	*gyrB* and *secA1* may provide better resolution to species.[241,259,260]	Report to species when possible.
N. beijingensis[253,261,263,264]	Resolution to genus and species.	Some *N. beijingensis* references share high identity with *N. araoensis*, *N. arthritidis*, and other species. Differentiation can be attempted in the V2 region at positions ≈ 160–200 bp.	*gyrB* and *secA1* provide better resolution to species.[241,259,260]	MALDI-TOF MS aids resolution to genus and variable resolution to species. Report to species.
N. brasiliensis[243,253,261,264]	Resolution to genus and species.	*N. brasiliensis* is closely related to *N. vulneris*, but they can be differentiated by a few mismatches at positions ≈ 160–220 bp and ≈ 580–650 bp. A longer sequence up to ≈ 1200 bp may be needed for species-level identification.	*gyrB* and *secA1* provide better resolution to species.	MALDI-TOF MS aids resolution to genus and species. Report to species when possible.

Table 15. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
N. brevicatena/ N. paucivorans **complex**[258]	Resolution to genus and sometime species.	These species are closely related, but some strains may be differentiated by a few mismatches at ≈ 170–220 bp. A longer sequence up to ≈ 1200 bp is useful for species-level identification.	*gyrB* and *secA1* may provide better resolution to species.[241,259,260]	Limited MALDI-TOF MS data.
N. cyriacigeorgica[261]	Resolution to genus and species.	*N. cyriacigeorgica* is closely related to *N. farcinica* and *N. kroppenstedtii* within the first ≈ 500 bp of 16S. This species can be differentiated by a few mismatches at positions ≈ 160–220 bp and ≈ 580–650 bp. *N. cyriacigeorgica* also contains at least 3 closely related genomic subgroups.	*gyrB* and *secA1* provide better resolution to species.[241,259,260]	MALDI-TOF MS aids resolution to genus and species. Report as "*N. cyriacigeorgica.*"
N. farcinica[243,252,253,261,264]	Resolution to genus and species.	*N. farcinica* is closely related to *N. kroppenstedtii*. This species can be differentiated by a few mismatches at positions ≈ 160–220 bp and ≈ 580–650 bp.	*gyrB* and *secA1* provide better resolution to species.	MALDI-TOF MS resolution to genus and species. Report to species.

Table 15. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
***N. nova* complex**[9,243,252,253,261] *N. africana* *N. aobensis* *N. cerradoensis* *N. elegans* *N. kruczakiae* *N. mikamii* *N. nova* *N. vermiculata* *N. veterana*	Resolution to genus and complex with limited resolution to species.	Many species within this complex are closely related and cannot be differentiated by 16S. A longer sequence up to ≈ 1200 bp may be needed for species-level identification. *N. veterana*, *N. africana*, and *N. elegans* share high-sequence identity. *N. nova* cannot be differentiated from *N. vermiculata;* nor can *N. cerradoensis* and *N. africana* be separated. Some species of this complex can be differentiated by a few mismatches at positions ≈ 160–220 bp and ≈ 580–650 bp.	*gyrB* and *secA1* provide better resolution to species.	MALDI-TOF MS resolution to genus and complex and sometimes species. Report as "*N. nova* complex" unless species-level identification is needed.
N. otitidiscaviarum[243,261,264]	Resolution to genus and species.	This species can be differentiated by a few mismatches at positions ≈ 160–220 bp and ≈ 580–650 bp.	*gyrB* and *secA1* provide better resolution to species.	MALDI-TOF MS resolution to genus and species. Report to species.
N. pseudobrasiliensis[243,253,261]	Resolution to genus and species.	This species can be differentiated by a few mismatches at positions ≈ 160–220 bp and ≈ 580–650 bp. Differentiation from *N. rayongensis* cannot be achieved within the ≈ 500 bp of 16S.	*gyrB* and *secA1* provide better resolution to species.[241,259,260]	MALDI-TOF MS resolution to genus and species. Report to species.

Table 15. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
N. transvalensis **complex** *N. blacklockiae* *N. transvalensis* *N. wallacei*	Resolution to genus and complex.	*N. transvalensis* and *N. wallacei* share sequence identity and cannot be resolved by 16S sequencing. *N. blacklockiae* may be differentiated by a few mismatches at positions ≈ 160–220 bp and ≈ 970–1020 bp.	*gyrB* and *secA1* provide better resolution to species.	MALDI-TOF MS resolution to genus and complex and sometimes species. Reporting to complex is usually sufficient.
Nocardiopsis dassonvillei	Resolution to genus and species.	This organism can be identified within the first ≈ 500 bp of 16S. *N. dassonvillei* is closely related to *N. synnemataformans*, with only a few mismatches at ≈ 450–500 bp within the V3 region.	Of limited additional benefit.	Limited MALDI-TOF MS data. May report genus and species based on 16S sequencing.
Rhodococcus hoagii (equi)[243,265,266] *R. erythropolis* *R. globerulus*[267] Many environmental species.	Resolution to genus and few species.	*Rhodococcus* is a highly homologous genus. Some *Rhodococcus* spp. are closely related to *Nocardia* spp. Resolution to genus occurs by mismatches within the ≈ 50–100 bp (V1), ≈ 450–620 bp (V4), and ≈ 950–1000 bp (V6) regions. A full-length sequence of 16S is required for accurate differentiation of many *Rhodococcus* spp.	*choE* provides a specific target for *R. equi*.	MALDI-TOF MS resolution to genus and species.[81,247] Report to species.

Table 15. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
Rhodococcus hoagii (equi) *R. erythropolis* *R. globerulus* **(Continued)**		*R. hoagii* and *R. equi* are the same species. *R. hoagii* and *R. soli* are closely related, with only 3 mismatches in the V1 region. A full-length 16S sequence is required to separate them by mismatches in the V6 region. *R. agglutinans* is also closely related but may be separated by a few mismatches within the V4 (≈ 600 bp) and V6 regions. *R. erythropolis* 16S sequence is very similar to that of *Nocardia coeliaca* but can be differentiated from other *Rhodococcus* spp. by mismatches within the first ≈ 500 bp of 16S in the V1 and V4 regions. *R. globerulus* and *Nocardia globerula* are also homologous and cannot be differentiated. These species are also closely related to *R. baikonurensis*, *R. degradans*, and *R. gingshengii*. *R. degradans* and *R. gingshengii* share identical 16S sequences, but *R. baikonurensis* can be differentiated by 2 mismatches at ≈ 560–650 bp in the V4 region.		

Table 15. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
Segniliparus rugosus[268]	Resolution to genus and species.	This organism can be identified by mismatches within the first ≈ 500 bp of 16S. *S. rugosus* is closely related to *S. rotundus* but can be differentiated by several mismatches in the V1 (≈ 70–100 bp) and V2 region (≈ 170–260 bp). Mismatches within these regions of 16S also allow separation of *Segniliparus* from *Rhodococcus*.	Of limited additional benefit.	Limited MALDI-TOF MS data. May report to genus and species by 16S sequencing.
Streptomyces **spp.**[243,266] Very large genus that contains more than 600 environmental species.	Resolution to genus, with limited resolution to species.	Limited sequence information available in reference databases. *S. somaliensis* is homologous with *S. flavofungini* and cannot be differentiated. *S. albidoflavus* and *S. violascens* are also closely related.	Of limited additional benefit.	Limited MALDI-TOF MS data. Reporting to genus only is usually sufficient.

Table 15. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
Tsukamurella paurometabola[243] *T. pulmonis* *T. inchonensis* Several environmental species.	Resolution to genus and some to species.	*Tsukamurella* is a highly homologous genus. *T. paurometabola* is closely related to *T. strandjordii* and *T. inchonensis* and cannot be reliably separated within the first ≈ 500 bp of 16S. There are only single mismatches in the V3 and V6 regions. *T. pulmonis* is also closely related to *T. tyrosinosolvens*, *T. sinensis*, and *T. strandjordii*, with only a few mismatches throughout 16S. A full-length 16S sequence may differentiate these species with only a few mismatches occurring in the V2, V3, V4, V6, and V7 regions.	Of limited additional benefit.	Limited MALDI-TOF MS data. Report to species when possible.

[*] All 16S rRNA gene positions outlined for microorganisms or groups in this table were derived by multisequence alignment using a representative reference strain designated by species and GenBank AC: *Actinomadura sediminis* (JF272484), *Dermatophilus congolensis* (AJ243918), *Gordonia terrae* (CP016594), *Nocardia farcinica* (AP006618), *Nocardiopsis dassonvillei* (CP017965), *Rhodococcus opacus* (CP003949), *Segniliparus rotundus* (CP001958), *Streptomyces albus* (DQ026669), and *Tsukamurella paurometabola* (CP001966). **NOTE:** 16S rRNA gene positions in this table indicate variable regions only, because positioning depends on the reference sequences chosen.

[†] The references cited in this table are provided as resources only and do not necessarily substantiate the proposed interpretive guidelines. The appropriateness of DNA targets and their limitations were determined by the consensus process. MALDI-TOF MS use for the identification of each microorganism or group has been included, along with clinically relevant references.

[‡] See CLSI document M58.[59] Data evaluating MALDI-TOF MS use for the identification of aerobic actinomycetes are limited.

Abbreviations: DNA, deoxyribonucleic acid; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; rDNA, ribosomal DNA; rRNA, ribosomal ribonucleic acid.

Subchapter 3.1.11, Mycobacteria:

The information content of the 5′ end of the 16S rRNA gene is sufficient for specifically identifying most mycobacteria.[20] Identification focuses on the signature sequences in the hypervariable regions A and B, which correspond to *E. coli* positions around 129 to 267 bp and 430 to 500 bp, respectively. Many species can be unequivocally defined by this signature sequence. Closely related species often differ by only a few bases. However, some species share identical 16S rRNA gene sequences (eg, *Mycobacterium tuberculosis* complex species) and can be identified only biochemically or with alternative DNA targets. In contrast, some species have intraspecies heterogeneity, such as *Mycobacterium avium, Mycobacterium fortuitum,* and *Mycobacterium gordonae*.[20,273] Caution should be used with references in public databases, including sequences that have been previously published in the peer-reviewed literature.

For the microorganisms or groups listed in Table 16, the following ~~algorithm~~key points are relevant for identification ~~can be applied for~~using 16S rRNA sequences:

- *Mycobacterium* spp. are closely related by the 16S rRNA gene. Closely related species may show only a few mismatches across the entire 16S gene or no mismatches at all.

- Full-length 16S rRNA gene sequencing is recommended to reliably separate many *Mycobacterium* spp.

- Full-length sequence alignments against a reference sequence are helpful to identify mismatches between closely related mycobacterial complexes and species.

- Rapidly growing *Mycobacterium* spp. often have a characteristic deletion of several nucleotides in the V3 region of 16S at ≈ 400–450 bp.

~~100% identity for genus and species identification; report "[*Genus* and *species*]."~~

~~99.0% to 99.9% identity for genus identification; consider reporting "[*Genus*], most closely related to [*species*]."~~

~~≥ 95% cannot be definitively identified by 16S rRNA gene sequencing; consider reporting "Unable to definitively identify by 16S rRNA gene sequencing, most closely related to *Mycobacterium* spp."[29]~~

~~**NOTE:** Although 100% identity is mandatory for signature sequences, one or very few mismatches at other positions may be acceptable for species identification.~~

~~The cutoff values for percent identity scores are suggested as tools for medical laboratories to identify microorganisms in a consistent, pragmatic manner. They do not reflect strict taxonomical classifications.~~

Table 16. Mycobacteria*,[247,270-272]

Microorganism or Group†	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution‡
Mycobacterium **spp.** (in general) [20,21,273-276]	Resolution to genus and usually to species.	No separation within the *M. tuberculosis* complex and: • Between *M. kansasii* and *M. gastri* • Between *M. marinum* and *M. ulcerans* • Between *M. chelonae* and *M. abscessus* Poor separation within *M. fortuitum* group.	*rpoB*, *hsp65*, and *sodA* are often used as alternative targets for identification.	MALDI-TOF MS resolution to genus and usually complex and/or group or species.
M. tuberculosis **complex**[277-279] *M. tuberculosis* *M. africanum* *M. canettii* *M. bovis* *M. bovis* BCG *M. microti* *M. orygis* *M. caprae* *M. pinnipedii* *M. suricattae* *M. mungi*	Resolution to genus and complex but none to species.	*M. tuberculosis* complex species, including *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, and *M. africanum*, are homologous and cannot be differentiated by 16S sequencing.	*gyrB* provides resolution of species within the *M. tuberculosis* complex, except for *M. tuberculosis* and *M. africanum* subtype II.[277]	MALDI-TOF MS resolution to *M. tuberculosis* complex, with no resolution to species in this complex.[271] Report as *M. tuberculosis* complex. Speciation may be clinically indicated by phenotypic testing or other molecular-based methods.[277,280]

Table 16. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
M. avium **complex** (MAC) includes *M. avium* and related species (*M. paratuberculosis and M. silvaticum*); *M. intracellulare* and related species (*M. arosiense, M. bouchedur-honense, M. yongonense, M. marseillense, M. colombiense, M. chimaera, M. timonense, M. vulneris*)	Resolution of the MAC group into *M. avium* and related species and *M. intracellulare* and related species.	16S sequencing broadly separates the MAC group from other *Mycobacterium* spp. and the *M. avium* and related species from *M. intracellulare* and related species within the MAC group.	Of limited additional benefit for routine workup. Species-level identification is recommended for outbreak investigations (ie, health care-associated *M. chimaera* infection). ITS region, *rpoB*, and *hsp65* sequencing can provide resolution at the species level within the MAC complex.	MALDI-TOF MS resolution to complex (MAC) and some species within the MAC complex. Report as "*Mycobacterium avium* complex."
	Resolution to *M. avium* group but not to species.	*M. avium* and related species, including *M. avium, M. paratuberculosis,* and *M. silvaticum,* are homologous across 16S and cannot be differentiated. *M. lepraemurium* is closely related but separate from *M. avium,* and related species may be separated by only a few mismatches over the entire 16S gene. Many other species within this complex share similar sequences, with only a few mismatches at ≈ 180 bp (V2) and at ≈ 450 bp (V3). Full-length 16S analysis is recommended for differentiation of species of this complex.	ITS, *rpoB,*[281-283] *hsp65*, and the presence or absence of specific insertion sequences and large sequence polymorphisms distinguish subsets of the *M. avium* complex.[284,285]	MALDI-TOF MS can identify *M. avium* but cannot differentiate within members of this group. Limited MALDI-TOF MS data are available for species other than *M. avium.*[270]

Table 16. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
M. avium complex (Continued)	Resolution to *M. intracellulare* group but not to species.	Species within the *M. intracellulare* group are homologous across the entire 16S. *M. intracellulare* and *M. paraintracellulare* have identical sequences, and *M. chimaera* differentiates in only 1 position (≈ 450 bp). *M. vulneris* and *M. colombiense* are closely related, with a few mismatches over the entire 16S gene (positions ≈ 70–100 bp and around position ≈ 1250 bp). *M. youngonense*, *M. marseillense*, and *M. bouchedurhonense* differ from *M. intracellulare* in a few positions (≈ 170–200 bp, around position ≈ 450 bp) but cannot be separated with certainty. It is important to note that *M. arosiense* also shares a very similar sequence.	ITS, *rpoB*, and *hsp65* sequencing distinguish among subsets of this complex.[281-283,286,287]	MALDI-TOF MS cannot differentiate *M. intracellulare* from *M. chimaera*. Limited data are available for species in this group.[270] Use of alternative DNA targets is recommended when species-level identification is warranted (ie, outbreak investigations).
M. asiaticum	Resolution to genus and species.	*M. asiaticum* can be differentiated from closely related species that include *M. alsense, M. conspicuum,* and *M. szulgai*/ *M. angelicum* by mismatches at ≈ 150–250 bp (V2).	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.

Table 16. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
M. canarisense	Resolution to genus and species.	This species is closely related to *M. cosmeticum* and *M. diernhoferi* but can be differentiated by mismatches at ≈ 150–200 bp (V2).	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.
M. celatum[24]	Resolution to genus and species.	*M. celatum* has two different 16S operons that differentiate by a few deletions in the V2 region (ie, generates a typical "mixed" pattern in Sanger chromatogram alignments downstream).	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.
M. chelonae–M. abscessus **complex**[20,28,286,288-291] *M. abscessus* has several subspecies.	Resolution to complex but no resolution to species.	16S cannot discriminate among *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletti*, *M. chelonae*, and *M. franklinii*.	*hsp65*, *rpoB*, ITS region, *erm41*, or *secA1* genes provide better resolution to species and subspecies. Combination of above genes with *erm41* sequencing provide species differentiation within *M. abscessus* group and information on inducible macrolide resistance.[292]	MALDI-TOF MS enables resolution to genus and species (*M. chelonae* vs *M. abscessus*). No resolution of *M. abscessus* at the subspecies level. Species identification may be useful for predicting antimicrobial resistance patterns. No reliable phenotypic indicators for species resolution.
M. cosmeticum	Resolution to genus and species.	*M. cosmeticum* can be separated from *M. canariasense* by mismatches at ≈ 150–200 bp (V2).	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.
M. flavescens Three sequevars were described for *M. flavescens.*[293]	Resolution to genus and species.	*M. flavescens* can be identified by mismatches in the first ≈ 500 bp across the V1–V3 regions.	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.

Table 16. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
M. fortuitum **group**[278,294]	Resolution to genus, with some resolution to species.	*M. fortuitum* can be differentiated from several other closely related species by a few mismatches at ≈ 180 bp (V2) and ≈ 1000 bp (V6). However, the following species and/or subspecies cannot be separated due to homology across the 16S gene: • *M. fortuitum* subsp. *acetamidolyticum* and *M. fortuitum* subsp. *fortuitum* cannot be differentiated on the basis of 16S sequencing. • *M. houstonense* and *M. farcinogenes,* as well as *M. senegalense* and *M. conceptionense,* share identical 16S sequences. Furthermore, these pairs cannot be differentiated within the first ≈ 500 bp of the 16S; a full-length sequence enables some differentiation in the V6 region (around position 1000 bp) by few mismatches.	*rpoB* provides better resolution to species.	Resolution to genus, with some resolution to species within the group. Species identification may be useful for predicting antimicrobial resistance patterns.

Table 16. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
M. fortuitum group (Continued)		• *M. peregrinum*, *M. septicum*, *M. lutetiense*, and *M. montmartrense* can be differentiated only by full-length sequencing of the 16S in the V6 region (≈ 1030–1100 bp). • *M. porcinum*, *M. neworleansense*, and *M. boenickei* share almost identical 16S sequences and cannot be differentiated by 16S sequencing with certainty.		
M. gordonae *M. paragordonae*	Resolution to genus and species.	These species can be separated from each other by mismatches at ≈ 170–180 bp (V2), ≈ 260 bp (V3), and ≈ 460 bp (V4).	Of limited additional benefit.	MALDI-TOF MS aids resolution to genus and species.
M. haemophilum	Resolution to species.	This species can be differentiated from *M. malmoense* and *M. bohemicum*, *M. szulgai*, and *M. angelicum* by mismatches at ≈ 60–120 bp (V1) and ≈ 150–220 bp (V2).	Of limited additional benefit.	MALDI-TOF MS aids resolution to genus and often to species.
M. iranicum	Resolution to species.	*M. iranicum* can be identified by mismatches in the first ≈ 500 bp across the V1–V3 regions. Several nucleotide deletions occur at ≈ 60–100 bp (V1).	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.

Table 16. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
M. kansasii *M. gastri* [28,279,286,290,291,295-298]	Resolution to genus, with poor resolution between species.	*M. kansasii* has significant genotypic heterogeneity. Some variants of *M. kansasii* and *M. gastri* are homologous across the entire 16S gene. *M. nebraskense* is closely related but can be differentiated from *M. kansasii* and *M. gastri* by mismatches at ≈ 170 bp (V2) and ≈ 460 bp (V4).	*dnaA*, *gyrB*, *hsp65*, *recA*, *rpoB*, ITS region, and *secA1* genes provide better resolution to species.	MALDI-TOF MS aids resolution to genus and species for *M. kansasii*. Some strains need sequence confirmation (if low scores or low confidence level). Use photochromogenicity or alternative DNA targets to differentiate *M. kansasii* and *M. gastri*. *M. gastri* is rarely a pathogen.[299]
M. leprae	Resolution to species.	A multinucleotide insertion at ≈ 100 bp (V1) enables identification of *M. leprae*.	Limited data currently available.	Limited MALDI-TOF MS data currently available.
M. malmoense	Resolution to genus and species.	*M. malmoense* can be identified by mismatches in the first ≈ 500 bp across the V1–V3 regions.	Of limited additional benefit.	MALDI-TOF MS aids resolution to genus and species. Some strains need sequence confirmation.[300]
M. marinum *M. ulcerans*[286,291,295,298]	Resolution to genus, with no resolution to species.	*M. marinum* and *M. ulcerans* are highly homologous across 16S. A few mismatches occur at ≈ 1200–1300 bp. *M. shottsi* and *M. pseudoshottsi* are also closely related to these species with only a few mismatches across the 16S gene.	*dnaA*, *hsp65*, *rpoB*, and *secA1* genes provide better resolution to species.	MALDI-TOF MS aids resolution to genus and species for *M. marinum*. Some strains need sequence confirmation (if low scores or low confidence level). Use photochromogenicity or alternative DNA targets to differentiate *M. marinum* and *M. ulcerans*.

Table 16. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
M. mucogenicum **group**	Resolution to genus but not to species.	*M. mucogenicum* and *M. phocaicum* cannot be differentiated by 16S sequencing. *M. aubagnense* is closely related but can be separated from these 2 species by mismatches at ≈ 170-200 bp (V2).	Of limited additional benefit.	MALDI-TOF MS aids resolution to genus and group but not to species.
M. neoaurum *M. bacteremicum*	Resolution to genus but not to species.	*M. neoaurum* and *M. bacteremicum* are homologous across the 16S gene and cannot be separated. Other closely related species such as *M. cosmeticum*, *M. diernhoferi*, and *M. canariesense* can be differentiated from these 2 species by mismatches within the first ≈ 500 bp.	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.
M. nonchromogenicum	Resolution to genus and species.	This species is closely related to *M. arupense*, *M. heraklionense*, *M. engbaekii*, and *M. hiberniae*, with only a few mismatches at ≈ 50–80 bp (V1), ≈ 150–250 bp (V2), and ≈ 450–550 bp (V3).	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.
M. scrofulaceum	Resolution to genus but not to species.	This species is closely related to *M. paraffinicum*, with 1 mismatch at ≈ 200 bp (V2) and a few mismatches at ≈ 440–500 bp (V3). *M. scofulaceum* is also closely related to *M. mantenii* and *M. paraseoulense* but can be differentiated by mismatches in the V3 region.	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.

Table 16. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
M. shimoidei	Resolution to genus and species.	*M. shimoidei* can be identified within the first ≈ 500 bp.	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.
M. simiae **group** *M. lentiflavum* *M. sherissi* *M. triplex*	Resolution to genus and species.	*M. simiae* can be separated from other species within this group by mismatches at ≈ 170–270 bp (V2). *M. europaeum* and *M. parascrofulaceum* are closely related to the *M. simiae* group but can be differentiated by mismatches at ≈ 1030 bp (V6).	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.
M. smegmatis	Resolution to genus and species.	*M. smegmatis* can be differentiated from *M. goodii* by a few mismatches at ≈ 170–200 bp (V2). *M. anyangense*, *M. moriokaense*, and other closely related species can be separated from *M. smegmatis* by mismatches at ≈ 80 bp (V1) and ≈ 440–490 bp (V3).	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.
M. szulgai	Resolution to genus but not to species.	*M. szulgai* and *M. angelicum* have homologous 16S sequences and cannot be differentiated. This species is also closely related to *M. riyadhense*, *M. malmoense*, and *M. bohemicum*, with only a few mismatches at ≈ 60–100 bp (V1) and ≈ 150–250 bp (V2). Full-length 16S analysis does not improve species-level resolution.	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.

Table 16. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
M. thermoresistibile	Resolution to genus and species.	This species can be separated from *M. moriokaense*, *M. celeriflavum*, and *M. goodii* by mismatches at ≈ 50–80 bp (V1), ≈ 150–250 bp (V2), and ≈ 400–550 bp (V3).	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.
M. triviale	Resolution to genus and species.	This species is closely related to *M. koreense* and *M. parakoreense* but can be differentiated by mismatches at ≈ 150–250 bp (V2), ≈ 450–550 bp (V3), and ≈ 1000 bp (V6). Full-length sequencing of 16S is required to differentiate the above closely related species.	Of limited additional benefit.	Limited MALDI-TOF MS data currently available.
M. wolinskyi	Resolution to genus and species.	*M. rutilum* and *M. mageritense* are closely related but can be differentiated by mismatches at ≈ 450–520 bp (V3).	Of limited additional benefit.	Limited data currently available.

Table 16. (Continued)

Microorganism or Group[†]	Appropriateness of 16S rRNA (V1-V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[‡]
M. xenopi	Resolution to species.	*M. xenopi* can be identified by mismatches in the first ≈ 500 bp across the V1–V3 regions. There is a 2-nucleotide insertion at ≈ 60–100 bp (V1) and ≈ 150–250 bp (V2) that allows differentiation from other closely related species such as *M. botniense*, *M. wolinskyi*, and *M. heckershornense*.	Of limited additional benefit.	MALDI-TOF MS aids resolution to genus and species.

[*] All 16S rRNA gene positions outlined for microorganisms or groups in this table were derived by multisequence alignment using a representative reference strain designated by species and GenBank AC: *Mycobacterium tuberculosis* (AB0001561). **NOTE:** 16S rRNA gene positions in this table indicate variable regions only, because positioning depends on the reference sequences chosen.

[†] The references cited in this table are provided as resources only and do not necessarily substantiate the proposed interpretive guidelines. The appropriateness of DNA targets and their limitations were determined by the consensus process. MALDI-TOF MS use for the identification of each microorganism or group has been included, along with clinically relevant references.

[‡] See CLSI document M58.[59] Data evaluating MALDI-TOF MS use for the identification of *Mycobacterium* spp. are limited.

Abbreviations: 16S–23S spacer, 16S–23S ribosomal RNA gene intergenic spacer; BCG, bacille Calmette-Guérin; DNA, deoxyribonucleic acid; MAC, *Mycobacterium avium* complex; rRNA, ribosomal ribonucleic acid.

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