18 November 2022

To: Recipients of MM18, 2nd ed.

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

Subject: Correction

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

Table 12. Gram-Positive Anaerobes

The spelling of "***Actinobaculum-Actininotignum***" is listed incorrectly and has been corrected to read "***Actinobaculum-Actinotignum***."

Table 12. Gram-Positive Anaerobes

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
Actinobaculum-Actinotignum	Resolution to genus and species.	These genera can be distinguished and species differentiated by mismatches at positions ≈ 50–220, ≈ 450–500, and ≈ 950–1030 bp.	Of limited additional benefit.	MALDI-TOF MS may aid in identification to genus and some species.

Subchapter 3.1.11, Mycobacteria

An omitted cutoff table and additional text have been added to Subchapter 3.1.11. The additions are highlighted:

The information content of the 5′ end of the 16S rRNA gene is sufficient for specifically identifying most mycobacteria. 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*. 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 key points are relevant for identification 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

For the microorganisms listed in Table 16, the following algorithm can be applied for identification of 16S rRNA sequences:

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."

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.

If you require any additional clarification regarding these corrections, please contact CLSI Customer Service (customerservice@clsi.org).

We appreciate your commitment to CLSI and regret any inconvenience.