Identification of nontuberculous mycobacteria (NTM) by conventional phenotypic tests requires long incubation periods that may take up to 12 weeks. The GenoType Mycobacterium CM/AS (Hain Lifescience GmbH, Nehren, Germany) is a test based on the amplification of a 23S rRNA gene region followed by reverse hybridization with specific probes that allows the identification of 40 of the most common NTM. However, the hybridization step requires high sequence homology, and even point mutations in the target regions may hamper the hybridization step, leading to unreliable results (1). Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) has been recently applied to the identification of a wide range of microorganisms (2), mainly clinically significant bacteria and fungi. Experience with MALDI-TOF for the rapid identification of NTM is very limited, mainly because of the number of identified species and because database information is scarce (3–5). In this study, a large collection of NTM clinical isolates and reference strains were analyzed using MALDI-TOF technology in comparison with the GenoType Mycobacterium CM/AS. Identification by 16S rRNA/hsp65 sequencing was considered the gold standard. Agreements between MALDI-TOF and GenoType CM/AS with the reference method were, respectively, 94.4% and 84.0%. In 17 cases (13.6%), results provided by GenoType and MALDI-TOF were discordant; however, the reference method agreed with MALDI-TOF in 16/17 cases (94.1%; \( P = 0.002 \)).

**TABLE 1**

<table>
<thead>
<tr>
<th>Mycobacterium</th>
<th>Reference no.</th>
<th>Concordance with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID by 16S rRNA/hsp65</td>
<td>MALDI-TOF ID</td>
<td>GenoType CM/AS ID</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------</td>
<td>------------------</td>
</tr>
<tr>
<td><em>M. avium</em></td>
<td>NC103034</td>
<td><em>M. avium</em></td>
</tr>
<tr>
<td><em>M. haemophilum</em></td>
<td>DSM44634</td>
<td><em>M. haemophilum</em></td>
</tr>
<tr>
<td><em>M. malmoense</em></td>
<td>DSM44163</td>
<td><em>M. malmoense</em></td>
</tr>
<tr>
<td><em>M. palustre</em></td>
<td>DSM44572</td>
<td><em>M. palustre</em></td>
</tr>
<tr>
<td><em>M. shimoidei</em></td>
<td>DSM44152</td>
<td><em>M. shimoidei</em></td>
</tr>
<tr>
<td><em>M. simiae</em></td>
<td>DSM44165</td>
<td><em>M. simiae</em></td>
</tr>
<tr>
<td><em>M. szulgai</em></td>
<td>MC2155</td>
<td><em>M. szulgai</em></td>
</tr>
<tr>
<td><em>M. triplex</em></td>
<td>DSM44626</td>
<td><em>M. triplex</em></td>
</tr>
<tr>
<td><em>M. xenopi</em></td>
<td>DSM43995</td>
<td><em>M. xenopi</em></td>
</tr>
</tbody>
</table>

Identification of sequencing results was performed considering CLSI recommendations for the genus *Mycobacterium* (9). Results obtained by 16S rRNA/hsp65 sequencing were considered the final microorganism identification.

**GenoType CM/AS identification.** The GenoType assay (Hain Lifescience, GmbH, Nehren, Germany) was applied to the NTM
isolates following the manufacturer’s instructions. The test consists of PCR amplification of a 23S rRNA gene region, a reverse hybridization with specific probes immobilized on nitrocellulose strips, and detection of the banding patterns. The turnaround time for this technique is approximately 8 h.

MALDI-TOF mass spectrometry. Isolates were treated prior to MALDI-TOF analysis following the manufacturer’s instructions. Under biosafety level 3 (BSL3) conditions, samples were inactivated for 30 min at 95°C, resuspended in 300 μl of deionized water plus 900 μl of ethanol, and centrifuged at 13,000 rpm, and the supernatants were discarded. Afterwards, the pellet was taken to a BSL2 laboratory in order to conclude the preprocessing steps (10). The MALDI-TOF settings used have already been described (11). All isolates were analyzed by MALDI-TOF MS, using a Microflex LT benchtop mass spectrometer (Bruker Daltonics, Bremen, Germany). FlexControl 3.3 and Maldi Biotyper 3.1 software (Bruker Daltonics, Bremen, Germany), respectively, were used for the control of the instrument and for spectrum analysis and comparison with the database (updated with 5,627 species plus the specific Mycobacteria Library v2.0 with 313 isolates from 127 species; Bruker Daltonics). When this method was performed, the total turnaround time until MALDI-TOF identification was obtained was 90 min.

**TABLE 2** Mycobacterium isolates identified by 16S rRNA/hsp65 sequencing and their correlation with MALDI-TOF, grouped by score and GenoType results

<table>
<thead>
<tr>
<th>Mycobacterium species ID by 16S rRNA/hsp65</th>
<th>With MALDI-TOF ID at species level with a score of:</th>
<th>With discordant or no MALDI-TOF ID</th>
<th>With concordance with GenoType CM/AS ID</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥2.0</td>
<td>≥1.9</td>
<td>≥1.7</td>
<td>&lt;1.7</td>
</tr>
<tr>
<td>M. abscessus</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>M. arupense</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. avium</td>
<td>6</td>
<td>9</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>M. boehmianicum</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. chelonae</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>M. fortuitum</td>
<td>10</td>
<td>14</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>M. gastri</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. gordonae</td>
<td>4</td>
<td>8</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>M. haemophilum</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. intracellulare</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>M. kansas</td>
<td>3</td>
<td>5</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>M. lentiflavum</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>M. mageritense</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>M. malmoense</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. marinum</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. massilense</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M. mucogenicum</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>M. palustrum</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. peregrinum</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>M. porcinum</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. shimoidai</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. simiae</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>M. smegmatis</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. szulgai</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. terrae</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. triplex</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M. xenopi</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>86</td>
<td>111</td>
<td>7</td>
</tr>
</tbody>
</table>

**TABLE 3** Resolution of discrepancies between MALDI-TOF and the GenoType CM/AS ID according to 16S rRNA/hsp65 ID

<table>
<thead>
<tr>
<th>No. of isolates (n = 17)</th>
<th>MALDI-TOF ID</th>
<th>GenoType CM/AS ID</th>
<th>Mycobacterium ID by 16S rRNA/hsp65 ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>M. abscessus</td>
<td>M. chelonae</td>
<td>M. abscessus</td>
</tr>
<tr>
<td>1</td>
<td>M. abscessus</td>
<td>M. chelonae</td>
<td>M. massilense</td>
</tr>
<tr>
<td>1</td>
<td>M. arupense</td>
<td>Mycobacterium spp.</td>
<td>M. arupense</td>
</tr>
<tr>
<td>1</td>
<td>M. avium</td>
<td>M. kansaii</td>
<td>M. avium</td>
</tr>
<tr>
<td>1</td>
<td>M. boehmianum</td>
<td>M. scrofulaceum</td>
<td>M. boehmianum</td>
</tr>
<tr>
<td>1</td>
<td>M. fortuitum</td>
<td>Mycobacterium spp.</td>
<td>M. fortuitum</td>
</tr>
<tr>
<td>1</td>
<td>M. gordonae</td>
<td>M. szulgai</td>
<td>M. gordonae</td>
</tr>
<tr>
<td>1</td>
<td>M. intracellulare</td>
<td>M. avium complex</td>
<td>M. intracellulare</td>
</tr>
<tr>
<td>1</td>
<td>M. lentiflavum</td>
<td>Mycobacterium spp.</td>
<td>M. lentiflavum</td>
</tr>
<tr>
<td>1</td>
<td>M. mageritense</td>
<td>M. fortuitum</td>
<td>M. mageritense</td>
</tr>
<tr>
<td>1</td>
<td>M. palustrum</td>
<td>M. avium</td>
<td>M. palustrum</td>
</tr>
<tr>
<td>2</td>
<td>M. peregrinum</td>
<td>M. fortuitum</td>
<td>M. peregrinum</td>
</tr>
<tr>
<td>1</td>
<td>M. porcinum</td>
<td>M. fortuitum</td>
<td>M. porcinum</td>
</tr>
<tr>
<td>1</td>
<td>M. simiae</td>
<td>M. gordonae</td>
<td>M. simiae</td>
</tr>
<tr>
<td>1</td>
<td>M. triplex</td>
<td>M. avium</td>
<td>M. triplex</td>
</tr>
</tbody>
</table>

* The line in bold shows isolates with discordant ID by MALDI-TOF and the reference method.
Identification of the 125 NTM isolates by MALDI-TOF and GenoType CM/AS

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>% sensitivity (95% CI)</th>
<th>% specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow growers, nonchromogens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% sensitivity (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid growers</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nontuberculous Mycobacteria Identified by MALDI-TOF

Sensitivity and specificity values were compared using the McNemar test for paired samples, with two tails. Validity values were calculated with a 95% confidence interval. Data were analyzed using Excel 2016 (Microsoft, Redmond, WA) and R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).
of the specific mycobacterial database allowed the correct identification of 118 isolates from 26 different NTM species. The agreement with the reference method was 94.4% at the species level. Similar results have been reported, but the studies included fewer mycobacterial species (15, 16). Our results remain below the 97.2% reported by Balada-Llasat et al. (5), although their study included 13 different NTM species, half the number identified in this study.

In summary, MALDI-TOF identification of NTM species proved decisively superior to GenoType CM/AS both in accuracy (94.4% versus 84.0% according to the genomic sequencing) and in turnaround time (90 min versus 8 h). The high agreement of MALDI-TOF with 16S rRNA/hsp65 sequencing allows its implementation in the microbiology laboratory for NTM identification, especially for rapidly growing NTM (Table 4), where MALDI-TOF has shown a very low rate of discrepant results (3 isolates [0.6%] in this study). MALDI-TOF is also cheaper than GenoType CM/AS (1.5€ versus 42€ per sample). Thus, our recommendation is to perform MALDI-TOF, when it is available, for all the samples suspected of being NTM and sequence only the isolates with unreliable or no MALDI-TOF ID, which may represent only 5.6% of isolates subjected to MALDI-TOF, according to our results.

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REFERENCES

Correction for Rodríguez-Sánchez et al., Evaluation of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of Nontuberculous Mycobacteria from Clinical Isolates

Belén Rodríguez-Sánchez, a,b,c María Jesús Ruiz-Serrano, a,b,c Mercedes Marín, a,b,c,d Paula López Roa, a Marta Rodríguez-Créixems, a

Emilio Bouza a,b,c,d

Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain a; CIBER de Enfermedades Respiratorias (CIBERES CB06/06/0058), Madrid, Spain b; Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain c; Medicine Department, School of Medicine, Universidad Complutense de Madrid, Madrid, Spain d

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