
C. Bouakaze,1* C. Keyser,1,2 A. Gonzalez,1 W. Sougakoff,3,4 N. Veziris,3,4 H. Dabernat,2 B. Jaulhac,1,5 and B. Ludes1,2,5

EA4438 Physiopathologie et Médecine Translationnelle, Faculté de Médecine, Université de Strasbourg, Strasbourg, France;1 Laboratoire d’Anthropologie Moléculaire et Imagerie de Synthèse (AMIS), CNRS/UMR5288, Université Paul Sabatier de Toulouse, Toulouse, France;2 Laboratoire de Bactériologie-Hygienne, ERS (EA1541), UPMC, Université Paris 06, Paris, France;3 Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux AntiTuberculeux (CNR MyRMA), AP-HP, Hôpital Pitie-Salpêtrière, Paris, France;4 and Hôpitaux Universitaires de Strasbourg (HUS), Strasbourg, France.

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The major goal of the present study was to investigate the potential use of a novel single nucleotide polymorphism (SNP) genotyping technology, called iPLEX Gold (Sequenom), for the simultaneous analysis of 16 SNPs that have been previously validated as useful for identification of Mycobacterium tuberculosis complex (MTBC) species and classification of MTBC isolates into distinct genetic lineages, known as principal genetic groups (PGGs) and SNP cluster groups (SCGs). In this context, we developed a 16-plex iPLEX assay based on an allele-specific-primer single-base-extension reaction using the iPLEX Gold kit (Sequenom), followed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) analysis on the commercially available Sequenom MassARRAY platform. This assay was tested on a panel of 55 well-characterized MTBC strains that were also genotyped for the same loci using the previously reported SNaPshot assay, as well as 10 non-MTBC mycobacteria and 4 bacteria not belonging to the genus Mycobacterium. All MTBC samples were successfully analyzed with the iPLEX assay, which yielded clear allelic data for 99.9% of the SNPs (879 out of 880). No false-positive results were obtained with the negative controls. Compared to the SNaPshot assay, the newly developed 16-plex iPLEX assay produced fully concordant results that allowed reliable differentiation of MTBC species and recognition of lineages, thus demonstrating its potential value in diagnostic, epidemiological, and evolutionary applications. Compared to the SNaPshot approach, the implementation of the iPLEX technology could offer a higher throughput and could be a more flexible and cost-effective option for microbiology laboratories.

The Mycobacterium tuberculosis complex (MTBC) is composed of causative agents of tuberculosis, a disease that remains a leading cause of human morbidity and mortality worldwide, with approximately 2 million deaths each year (World Health Organization, Tuberculosis Facts 2010 [http://www.who.int/tb/publications/factsheets/en/]). This complex comprises eight closely related bacterial species with distinct host tropisms, including the human pathogens M. tuberculosis, M. africanum, and M. canetti and the animal-adapted pathogens M. bovis, M. microti, M. caprae, and M. pinnipedii and the recently identified species M. mungi (1, 8, 12, 37). Although M. tuberculosis is the predominant causative agent of human tuberculosis, each member of this complex has been implicated in human infection, except M. mungi so far (8, 26). Moreover, two members, M. bovis, the causative agent of zoonotic bovine tuberculosis, and M. canetti, an unusual member responsible for rare tuberculosis cases almost always exposed to Africa, are naturally resistant to pyrazinamide, a first-line antituberculous drug (18, 34). Therefore, the rapid and reliable identification of MTBC isolates to the species level is of prime importance for timely selection of appropriate patient antibiotic treatment and also for epidemiological and public health considerations (36). Furthermore, various studies have recently identified distinct phylogenetic groupings within the human-adapted members of the MTBC (i.e., M. tuberculosis and M. africanum species), all of which are congruent (2, 5, 10, 14–16, 19, 20, 32). As shown in Table 1, these MTBC members are currently classified into six major phylogenetic lineages, two of which are composed of M. africanum strains. These six major lineages were first identified by analysis of genome deletions or large sequence polymorphisms (LSPs), but they are highly congruent to the ones defined by single nucleotide polymorphisms (SNPs), such as principal genetic groups (PGGs) defined by Sreevatsan et al. (37) and SNP cluster groups (SCGs) defined by Filliol et al. (14). Some of the traditional groupings defined by the use of epidemiological tools (e.g., spoligotyping) also correlate with these lineages (9). These lineages are associated with particular geographical regions and show differences in
TABLE 1. Major phylogenetic lineages within human-adapted MTBC members

<table>
<thead>
<tr>
<th>Lineage</th>
<th>MTBC species</th>
<th>Presence/absence of TbD1</th>
<th>Gagneux's nomenclature (LSP based)</th>
<th>PGG (SNP based)</th>
<th>SCG (SNP based)</th>
<th>Spoligotype-defined families</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M. tuberculosis</td>
<td>Intact</td>
<td>Indo-Oceanic lineage</td>
<td>1b</td>
<td>1</td>
<td>EAI</td>
</tr>
<tr>
<td>2</td>
<td>M. tuberculosis</td>
<td>Deleted</td>
<td>East Asian lineage</td>
<td>1b</td>
<td>2</td>
<td>Beijing</td>
</tr>
<tr>
<td>3</td>
<td>M. tuberculosis</td>
<td>Deleted</td>
<td>East African-Indian lineage</td>
<td>1b</td>
<td>3a</td>
<td>CAS</td>
</tr>
<tr>
<td>4</td>
<td>M. tuberculosis</td>
<td>Deleted</td>
<td>Euro-American lineage</td>
<td>2, 3</td>
<td>3b, 3c, 4, 5, 6a, 6b</td>
<td>H, LAM, X, T, S, others</td>
</tr>
<tr>
<td>5</td>
<td>M. africanaum</td>
<td>Intact</td>
<td>West African lineage I</td>
<td>1b</td>
<td>Not described</td>
<td>AFR12</td>
</tr>
<tr>
<td>6</td>
<td>M. africanaum</td>
<td>Intact</td>
<td>West African lineage II</td>
<td>1a</td>
<td>Not described</td>
<td>AFR11</td>
</tr>
</tbody>
</table>

* TbD1, M. tuberculosis-specific deletion region 1 described by Brosch et al. (8); PGGs are as defined by Sreevatsan et al. (37); SCG subgroups are as defined by Filliol et al. (14) and Alland et al. (2); EAI, East African-Indian family; CAS, Central Asian family; H, Haarlem family; LAM, Latin American-Mediterranean family.

FIG. 1. Principle of SNP analysis by using two independent SNP genotyping technologies: the SNaPshot technology (Applied Biosystems) and the iPLEX technology (Sequenom). These genotyping methods comprise three main steps, namely, PCR amplification of the DNA region containing the polymorphic site (step 1), an allelic discrimination reaction consisting of an SBE reaction (step 2), and detection of allele-specific products (step 3).
the sensitivity, rapidity, and accuracy of MALDI-TOF MS detection. The newly developed 16-plex iPLEX assay was evaluated in comparison to a modified version of the previously developed SnPshot approach, allowing the simultaneous analysis of the 16 SNPs in a single 16-plex SnPshot assay.

**MATERIALS AND METHODS**

**Bacterial samples.** This study is based on the collection of MTBC DNA samples analyzed in our previous work (6). A total of 55 MTBC DNA samples from reference strains *M. tuberculosis* H37Rv ATCC 27294, *M. bovis* CIP 102426, and *M. bovis* BCG CIP 105226 and clinical isolates of *M. tuberculosis* (n = 34), *M. bovis* (n = 6), *M. bovis* BCG (n = 4), *M. africanum* (n = 4), *M. canetti* (n = 1), *M. caprae* (n = 1), *M. microti* (n = 1), and *M. pinnipedii* (n = 1) were used in the present study. These isolates were identified to the species level by phenotypic and biochemical characterization methods and/or by use of a gene probe assay and, for some of them, by mycobacterial interspersed repetitive unit-variable-number tandem-repeat (MIRU-VNTR) analysis, as previously described (6). In total, 37 of these samples (*M. tuberculosis* H37Rv, *M. bovis* CIP 102426, one clinical *M. africanum* isolate, and all clinical *M. tuberculosis* isolates) had previously been successfully genotyped for the 16 SNP loci targeted in this study, and the other ones has been successfully genotyped for 8 of these SNPs in each of these 55 MTBC samples. The genotyping analysis was performed as recommended by the manufacturer with reagents included in the iPLEX Gold SNP genotyping kit (Sequenom). The 16 target sequences were simultaneously amplified from a 5-μl PCR volume composed of 1 μl final 16-plex iPLEX mix, 1 μl of an iPLEX Gold extension reaction mix, 1 μl of HotStarTaq enzyme, and 3.4 μl bacterial DNA extract. The thermal cycling conditions consisted of a first denaturation step at 95°C for 2 min, followed by 45 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 1 min, with a final extension step at 72°C for 5 min. To neutralize unincorporated dNTPs, PCR products were treated with 0.5 U shrimp alkaline phosphatase by incubation at 37°C for 40 min, followed by enzyme inactivation by heating at 85°C for 5 min. By adding 2 μl of an iPLEX Gold extension reaction cocktail to the purified PCR products, the 16-plex PCR product was then carried out in a final volume of 9 μl containing 0.222 μl iPLEX buffer, 1 μl iPLEX termination mix, 1 μl iPLEX enzyme, and the PCR primer mix that contained the 16 SBE primer mix divided into 4 groups from low to high masses (each group was composed of 4 primers). In the final SBE reaction, the concentration of the low-mass primers mix was 7 μM, the concentrations of the two medium-mass primer mixes were 9.3 μM and 11.6 μM, and the concentration of high-mass primers was 14 μM. The iPLEX extension reaction was performed under the following thermal conditions: an initial denaturation step at 94°C for 30 s, followed by 40 cycles of a denaturation step at 94°C for 5 s, 5 cycles of annealing at 52°C for 5 s and extension at 80°C for 30 s, and finally, a final extension step at 72°C for 3 min. After desalting of the products by using SpectroCLEAN resin following the

**TABLE 2. General information on SNPs analyzed in this study**

<table>
<thead>
<tr>
<th>SNP name</th>
<th>H37Rv</th>
<th>Polymorphism</th>
<th>Usefulness</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>katG&lt;sup&gt;463&lt;/sup&gt;</td>
<td>2154724</td>
<td>C</td>
<td>C/A</td>
<td>PGG and SCG assignment</td>
</tr>
<tr>
<td>gyrB&lt;sup&gt;65&lt;/sup&gt;</td>
<td>7558</td>
<td>G</td>
<td>G/C</td>
<td>PGG assignment</td>
</tr>
<tr>
<td>katG&lt;sup&gt;329&lt;/sup&gt;</td>
<td>2155503</td>
<td>G</td>
<td>G/A</td>
<td>Segregation of PGG-1a from PGG-1b isolates</td>
</tr>
<tr>
<td>hsp65&lt;sup&gt;31&lt;/sup&gt;</td>
<td>592006</td>
<td>C</td>
<td>C/T</td>
<td>Differentiation of <em>M. canetti</em> from other MTBC species</td>
</tr>
<tr>
<td>16S rRNA&lt;sup&gt;12&lt;/sup&gt;</td>
<td>1473094</td>
<td>T</td>
<td>T/C</td>
<td>Differentiation of <em>M. pinnipedii</em> from other MTBC species</td>
</tr>
<tr>
<td>gyrB&lt;sup&gt;675&lt;/sup&gt;</td>
<td>5671</td>
<td>C</td>
<td>C/T</td>
<td>Differentiation of <em>M. microti</em> from other MTBC species</td>
</tr>
<tr>
<td>gyrB&lt;sup&gt;765&lt;/sup&gt;</td>
<td>5752</td>
<td>G</td>
<td>G/A</td>
<td>Differentiation of <em>M. caprae</em> and <em>M. bovis</em> from other MTBC species</td>
</tr>
</tbody>
</table>

<sup>a</sup> The two SNPs 144390 and 2460626 are phylogenetically coincident since the A in both cases is characteristic of *M. tuberculosis* SCG-4 lineage.

<sup>b</sup> Positions and alleles are relative to the plus strand on the *M. tuberculosis* H37Rv genome sequence, GenBank accession no. NC_000962.2.

3500 genetic analyzer (AB, Foster City, CA), and automated allele calling was done using GeneMapper (version 4) software (AB) (6). However, to enable the automated allele calling for all 16 SNPs, we had to modify the sequences of three SBE primers by either increasing or reducing the size of their 5′ tail so that the peak for each extension product was easily distinguishable on the electropherograms. The sizes of the SBE primers for SNP loci 2460626 and 105139 were increased to 65 nucleotides (nt) instead of 55 nt and 52 nt instead of 50 nt, respectively, while the SBE primer size for SNP locus gyrB(675) was reduced from 64 nt to 52 nt. All 55 MTBC samples were finally analyzed again for these 16 loci using the 16-plex SnPshot assay with the newly ordered primers.

**Novel 16-plex iPLEX assay.** PCR and SBE primers for each SNP investigated were designed using the MassARRAY design software, version 4.0 (Sequenom Inc., San Diego, CA), with the exception of primers for SNP loci hsp65<sup>31</sup> and 144390, which were designed using Primer3 software (http://frodo.wi.mit.edu/primer3). The optimal amplicon size was set to 80 to 120 bp. A 10-mer tag (5′-ACGTG'TTGA-3′) was added to the 5′ end of each PCR primer to avoid confusion in the mass spectrum, and SBE primers were 5′ tailed with nonhomologous sequences varying in length to create large enough mass differences between the different SBE products to be detected by MALDI-TOF MS. PCR and SBE primer sequences are shown in Table 3.

The genotyping analysis was performed as recommended by the manufacturer with reagents included in the iPLEX Gold SNP genotyping kit (Sequenom) and the software and equipment provided with the MassARRAY platform (Sequenom). The 16 target sequences were simultaneously amplified from a 5-μl final PCR volume composed of 1× PCR buffer, 2 mM MgCl<sub>2</sub>, 500 μM deoxynucleoside triphosphates (dNTPs), 0.1 μM each PCR primer, 0.5 U of HotStarTaq enzyme, and 3.4 μl bacterial DNA extract. The thermal cycling conditions consisted of a first denaturation step at 95°C for 2 min, followed by 45 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 1 min, with a final extension step at 72°C for 5 min. To neutralize unincorporated dNTPs, PCR products were treated with 0.5 U shrimp alkaline phosphatase by incubation at 37°C for 40 min, followed by enzyme inactivation by heating at 85°C for 5 min. By adding 2 μl of an iPLEX Gold extension reaction cocktail to the purified PCR products, the 16-plex PCR product was then carried out in a final volume of 9 μl containing 0.222 μl iPLEX buffer, 1 μl iPLEX termination mix, 1× iPLEX enzyme, and the PCR primer mix that contained the 16 SBE primers divided into 4 groups from low to high masses (each group was composed of 4 primers). In the final SBE reaction, the concentration of the low-mass primers mix was 7 μM, the concentrations of the two medium-mass primer mixes were 9.3 μM and 11.6 μM, and the concentration of high-mass primers was 14 μM. The iPLEX extension reaction was performed under the following thermal conditions: an initial denaturation step at 94°C for 30 s, followed by 40 cycles of a denaturation step at 94°C for 5 s, 5 cycles of annealing at 52°C for 5 s and extension at 80°C for 30 s, and finally, a final extension step at 72°C for 3 min. After desalting of the products by using SpectroCLEAN resin following the
The 16 SNPs that were targeted by the previously described two 8-plex SNaPshot assays for identification of *M. tuberculosis* complex species and lineages (6) were successfully analyzed by using the modified 16-plex SNaPshot assay described in this study. The 55 MTBC samples tested with this single 16-plex SNaPshot assay yielded allelic data fully concordant with those obtained previously with the two 8-plex SNaPshot assays. The *M. canetti* sample still unexpectedly failed to amplify 3 loci (gyrA^95^, 1977, and 105139). Thus, 877 alleles were assigned over the 880 alleles that were expected, resulting in an allele call rate of 99.7%. Negative controls analyzed with the 16-plex SNaPshot assay failed to amplify all SNP loci, except sometimes for some loci (hsp65^631^, 16S rRNA^232574^, and 232574), as also previously observed. It must be noted that unexpected peaks were seen for some SNPs in negative-control samples, some of them being systematically observed even in water blanks, but this never interfered with data interpretation.

The 16-plex iPLEX assay developed in this study is suitable for identification of MTBC species, except *P. canetti* sample still unexpectedly failed to amplify 3 loci (gyrA^95^, 1977, and 105139). Thus, 877 alleles were assigned over the 880 alleles that were expected, resulting in an allele call rate of 99.7%. Negative controls analyzed with the 16-plex SNaPshot assay failed to amplify all SNP loci, except sometimes for some loci (hsp65^631^, 16S rRNA^232574^, and 232574), as also previously observed. It must be noted that unexpected peaks were seen for some SNPs in negative-control samples, some of them being systematically observed even in water blanks, but this never interfered with data interpretation.

Figure 2 shows examples of electropherograms and mass spectra obtained for the reference strains *M. tuberculosis* H37Rv and *M. bovis* CIP 102426 using the 16-plex SNaPshot assay and the 16-plex iPLEX assay, respectively. As illustrated, these two species yielded distinct electropherograms and mass
<table>
<thead>
<tr>
<th>MTBC sample (n = 55)</th>
<th>PGG(^a)</th>
<th>SCG(^b)</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kbp6563(^#)</td>
<td>katG(^{463})</td>
<td>gyrA(^{95})</td>
</tr>
<tr>
<td>Clinical M. canetti (n = 1)</td>
<td>lb 1</td>
<td>T</td>
<td>A</td>
</tr>
<tr>
<td>Clinical M. tuberculosis (n = 2)</td>
<td>lb 1</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>M. africanum (n = 1)</td>
<td>lb 1</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Clinical M. tuberculosis (n = 3)</td>
<td>lb 1b</td>
<td>2</td>
<td>C</td>
</tr>
<tr>
<td>Clinical M. tuberculosis (n = 2)</td>
<td>lb 1b</td>
<td>3a</td>
<td>C</td>
</tr>
<tr>
<td>Clinical M. tuberculosis (n = 9)</td>
<td>2</td>
<td>3b</td>
<td>C</td>
</tr>
<tr>
<td>Clinical M. tuberculosis (n = 13)</td>
<td>2</td>
<td>5</td>
<td>C</td>
</tr>
<tr>
<td>Clinical M. tuberculosis (n = 5)</td>
<td>3</td>
<td>6a</td>
<td>C</td>
</tr>
<tr>
<td>M. tuberculosis H37Rv (n = 1)</td>
<td>3</td>
<td>6b</td>
<td>C</td>
</tr>
<tr>
<td>Clinical and reference M. bovis (n = 7)</td>
<td>la 1a</td>
<td>7</td>
<td>C</td>
</tr>
<tr>
<td>M. bovis BCG (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical M. caprae (n = 1)</td>
<td>la 1a</td>
<td>7</td>
<td>C</td>
</tr>
<tr>
<td>Clinical M. microti (n = 1)</td>
<td>la 1</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Clinical M. pinnipedii (n = 1)</td>
<td>la 1a</td>
<td>1</td>
<td>C</td>
</tr>
<tr>
<td>Clinical M. africana (n = 3)</td>
<td>la 1a</td>
<td>1</td>
<td>C</td>
</tr>
</tbody>
</table>

\(^a\) MTBC isolates were assigned to one of the three PGGs delineated by Sreevatsan et al. (37) on the basis of allelic combination at 3 loci (katG\(^{463}\), gyrA\(^{95}\), and kag\(^{203}\)).

\(^b\) MTBC isolates were assigned to one of the seven SCGs delineated by Filliol et al. (14) on the basis of allelic combination at 9 loci (polymorphisms marked with an asterisk).
**M. tuberculosis H37Rv**

**A.a.**

![Electropherogram A.a.](image1)

**A.b.**

![Mass Spectrum A.b.](image2)

**M. bovis CIP102426**

**B.a.**

![Electropherogram B.a.](image3)

**B.b.**

![Mass Spectrum B.b.](image4)

**FIG. 2.** *M. tuberculosis* H37Rv and *M. bovis* CIP102426 electropherograms and mass spectra obtained using the 16-plex SNaPshot assay and 16-plex iPLEX assay, respectively. Electropherograms (A.a. and B.a.) were generated with GeneMapper (version 4) software (AB) and show the relative fluorescence units (RFUs) versus the measured size (in nucleotides) of the SBE products relative to the GeneScan-120 LIZ internal size standard (AB). Mass spectra (A.b. and B.b.) were generated using MassARRAY Typer (version 4.0.5) software (Sequenom) and show the relative intensity versus the mass of the analytes. Mutated alleles are indicated by arrows.
spectra, and for each species, the genotyping results obtained with the two different SNP genotyping assays were concordant with each other and with data in the literature (14, 37). Indeed, the M. tuberculosis H37Rv sample was always found to have the combinations of polymorphisms characteristic of PGG-3 (i.e., C7G → C6G at katG codon 463 and ACC → AGC at gyrB codon 95) and SCG-6b (1977G → A, 335293G → C, and 311613T → G). In contrast, the M. bovis sample possessed the polymorphisms associated with PGG-1a (i.e., ACC → ACT at katG codon 203) and SCG-7 (74092C → T and 913274C → G), as well as the mutated alleles for two gyrB gene SNPs, gyrB (756G → A) and the M. bovis-specific gyrB polymorphism (1410C → T).

The DNA regions surrounding the three loci that failed to be analyzed from the M. canettii sample with the SNaPshot assay and/or the iPLEX assay (i.e., 105139, 1977, and gyrA95) were further investigated by DNA sequencing. The sequencing results revealed that the M. canettii sample used in this study showed sequence variations in the SNaPshot SBE primer binding sites for these three loci and also in the iPLEX SBE primer binding site for the 105139 locus. Thus, the primer extension failures were very likely caused by mismatches between the SBE primers and their targets, preventing the extension reactions. Although we failed to detect these 3 loci from the M. canettii DNA sample, the two assays described in this study easily differentiated M. canettii from the other species thanks to the C-to-T substitution at locus hsp65631, which is a polymorphism previously reported to be specific for the M. canettii species (17).

**DISCUSSION**

In a previous study, we reported the development of two complementary 8-plex SNaPshot assays for the analysis of 16 SNPs that enable (i) the identification of MTBC members (except PGG-1b M. africanum and PGG-1b M. tuberculosis and M. mungi), (ii) the recognition of PGG lineages as defined by Sreevatsan et al. (37), and (iii) the classification of M. tuberculosis isolates into one of the six SCG lineages defined by Filliol et al. (14). The present study confirms that the simultaneous analysis of these 16 SNPs can be achieved in an equally efficient and reliable manner using a single 16-plex SNaPshot assay according to the protocol described in our previous article and modified as described herein. Thus, our results support the fact that SNaPshot assays can be readily multiplexed to a level higher than that suggested by the manufacturer, which recommends a limit of 10 SNPs per assay. As multiplexing is an efficient way to reduce costs and increase throughput, the use of this 16-plex SNaPshot assay is a cost-efficient and time-saving alternative option for laboratories that are interested in both identification of MTBC species and recognition of PGGs and SCGs by using a capillary electrophoresis platform.

This study also describes the development of a new 16-plex iPLEX assay for genotyping of these species- and lineage-specific SNPs using the commercially available Sequenom MassARRAY MALDI-TOF MS platform. This 16-plex iPLEX assay generated a high SNP call rate and showed a high degree of reproducibility. A perfect concordance was observed compared to the data generated by the 16-plex SNaPshot assay. Therefore, the newly developed 16-plex iPLEX assay can also be used for reliable identification of MTBC species and recognition of PGGs and SCGs from cultured MTBC strains.

The SNaPshot and iPLEX assays described in this study are both effective and easy to use and produce data that are easy to interpret since alleles are automatically called by *ad hoc* analysis softwares. Nevertheless, each assay presents specific advantages and disadvantages since the assays are based on different SNP genotyping technologies. The major advantage of the SNaPshot-based assay is that it can easily be introduced in a laboratory having access to an automated sequencer, which is equipment now commonly found in many microbiology laboratories for other common applications, such as classical DNA sequencing and MIRU-VNTR typing. However, this approach requires the use of fluorescently labeled terminators, which is not the case for the iPLEX assay. The iPLEX assay offers many additional benefits with respect to the SNaPshot assay. For instance, an analysis by MALDI-TOF MS is much faster than an analysis by capillary electrophoresis, requiring a few seconds for the former one and up to several minutes for the latter one. In addition, the iPLEX assay is suitable for high-throughput analysis, as either 96 or 384 samples can be analyzed on the same chip, depending on the MassARRAY platform used. Furthermore, a recent study has shown that spoligotyping of the MTBC members by analysis of the 43 spacers found in the direct repeat region can be done on the MassARRAY platform (22). The analysis of these markers, which is currently routinely performed by a reverse line blot hybridization assay, is very useful for the molecular characterization/genotyping of MTBC strains for epidemiological purposes (9, 24). Thus, the MassARRAY platform is also able to provide valuable information for molecular typing of MTBC strains. Although the major drawback of the iPLEX assay lies in the requirement for specific equipment, investment in a MassARRAY platform can be very appealing for microbiology centers with medium- to high-throughput activities, as previously noticed (33).

To conclude, this study demonstrated that the iPLEX technology with the MassARRAY platform can be used for accurate genotyping of 16 SNPs that enable simultaneous differentiation of MTBC species and characterization of the main phylogenetic lineages. Compared to the 16-plex SNaPshot assay, the 16-plex iPLEX assay could offer a higher throughput and a more flexible and cost-effective option for microbiology laboratories. Nevertheless, this study represents only an initial evaluation of the use of this MALDI-TOF MS-based assay for identification of MTBC species and lineages and requires further evaluations with larger collections of MTBC samples.

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**REFERENCES**


