Acceptance criteria for identification results of Gram-negative rods by mass spectrometry

Recently, matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) MS has been shown to be a useful and simple method for the rapid identification of micro-organisms isolated in a routine clinical microbiology laboratory (Bizzini et al., 2010; Eigner et al., 2009; Seng et al., 2009; Van Veen et al., 2010). Correct post-analysis interpretation of the MALDI-TOF MS output by the laboratory technician is crucial for implementation of this technique in a laboratory. The output from the MALDI Biotyper, a frequently used MALDI-TOF MS instrument from Bruker, is a log score in the range of 0–3.0, computed by comparison of the peak list from an unknown isolate with the reference main spectra, containing information on mean peak masses, peak intensities and peak frequencies.

To prevent misidentifications, the Bruker acceptance criteria (BAC) for identification of bacteria in the Bruker manual (MALDI Biotyper user manual, version 2.0 SR1, Bruker Daltonik, 2008) are stringent. A result is considered species consistent if all matches with a log score ≥2.0 are of the same species, and if matches with a log score between 2.0 and 1.7 correspond only to other species of the same genus. A genus consistent result is accepted if all log scores ≥1.7 belong to the same genus.

The criteria generally cited in the literature [literature acceptance criteria (LAC)] are as follows: log scores ≥1.7 and ≥2.0, respectively, are the thresholds for a match at the genus and the species level (Bizzini et al., 2010; Marklein et al., 2009; Van Veen et al., 2010). These thresholds were empirically determined. However, most authors do not state how they proceed when more than one species give a match ≥2.0. Marklein et al. (2009) considered the species with the highest log score as correct. In this paper, when referring to LAC, the criteria from Marklein et al. (2009) are meant.

Because we felt that the Marklein et al. (2009) criteria concerning multiple species matches needed validation in our setting, specific additional tests to differentiate between the suggested species if more than one species gave a log score ≥2.0 were systematically performed during a validation phase of the MALDI Biotyper. Based on these results optimized acceptance criteria (OAC) were formulated.

A total of 13 reference strains and 140 clinical strains already identified with routine methods (73 Enterobacteriaceae and 80 non-fermenters) were selected (see Supplementary Table S1 available with the online journal). As our laboratory is connected to a reference centre for cystic fibrosis, a lot of challenging non-fermenters were selected. Bacteria were harvested from tryptic soy agar with 5 % horse blood or from tryptic soy broth with agar incubated at 35 °C. Only the direct transfer method, as described elsewhere (Eigner et al., 2009), was used. Samples were analysed with a Microflex LT MALDI-TOF MS instrument (flexControl version 3.0, MALDI Biotyper version 2.0, MALDI Biotyper automation control version 2.0 and Library version 3.0) in the automated mode using the Wizard software resulting in ranking tables containing the 10 best matching reference main spectra. Different species belonging to one clinical complex as proposed by Murray et al. (2007) were reported as such; no additional tests were performed to distinguish between these different species.

The results of conventional biochemical identification tests and the fatty acid composition of the cell wall of non-fermenters were used as a reference for species designation (Murray et al., 2007). 16S rRNA gene sequencing (Coenye et al., 2002) was performed for the identification of strains difficult to identify biochemically (n=6), and for the resolution of discrepancies between routine and MALDI-TOF MS identification (n=10). recA gene sequencing (Mahenthiralingam et al., 2000) was performed to identify the included Burkholderia species (n=6).

Out of 153 isolates, 92 (60.1 %) were unequivocally identified up to species level with the MALDI Biotyper: identification following BAC or LAC yielded the same result. With the LAC, 41 (26.8 %) additional isolates could be correctly identified up to species level. Three (2.0 %) isolates were misidentified when using the LAC: a Bordetella bronchiseptica isolate was identified as Bordetella pertussis and two Burkholderia multivorans isolates were identified as Burkholderia dolosa (see Table 1 for an example). Although identification of species belonging to the former Burkholderia cepacia complex with MALDI-TOF MS is technically possible (Degand et al., 2008; Miñán et al., 2009; Vanlaere et al., 2008), the Bruker identification software probably needs some fine-tuning in this field. Only two out of three misidentifications were prevented with the additional tests. Eight (5.2 %) isolates were correctly identified at the genus level and for nine (5.9 %) isolates no identification was proposed by MALDI Biotyper when using the LAC. When using the BAC, 1 (0.7 %) Table 1 strain was misidentified, 19 (12.4 %) isolates were identified only at genus level and 41 (26.8 %) isolates could not at all be identified by MALDI Biotyper.

The MALDI Biotyper output of these 153 strains was rigorously reanalysed in an attempt to formulate reliable but less stringent acceptance criteria than those described in the Bruker manual and to prevent misidentifications. We decided to use an additional acceptance criterion, i.e. to accept the first identification when a 0.200 log difference between multiple species is present, besides a log score ≥2.0, as the
Table 1. Example of output interpretation of MALDI Biotyper using different acceptance criteria

Use of BAC and LAC gave the species identity as *B. dolosa*. Use of OAC does not permit the acceptance of the identity as *B. dolosa*; additional tests needed to be performed to exclude/confirm species with a log score below the 2.0 threshold but with a near (difference <0.200 log score) match to the species with the highest (≥2.0) log score – identification was *B. multivorans* after recA sequencing.

<table>
<thead>
<tr>
<th>Rank (quality)</th>
<th>Matched pattern</th>
<th>Score value</th>
<th>Species taken into account by BAC/LAC/OAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (+ +)</td>
<td><em>Burkholderia dolosa</em> DSM 16088 HAM 2.001*</td>
<td>BAC, LAC, OAC</td>
<td></td>
</tr>
<tr>
<td>2 (+)</td>
<td><em>Burkholderia multivorans</em> LMG 14293 HAM 1.896†</td>
<td>OAC</td>
<td></td>
</tr>
<tr>
<td>3 (+)</td>
<td><em>Burkholderia anthina</em> LMG 16670 HAM 1.863†</td>
<td>OAC</td>
<td></td>
</tr>
<tr>
<td>4 (+)</td>
<td><em>Burkholderia gladioli</em> DSM 4285 HAM 1.766†</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>5 (+)</td>
<td><em>Burkholderia vietnamiensis</em> LMG 10929 HAM 1.749†</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>6 (+)</td>
<td><em>Burkholderia cepacia</em> group 18875_1 CHB 1.719†</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>7 (–)</td>
<td><em>Burkholderia ambifaria</em> LMG 11351 HAM 1.678‡</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>8 (–)</td>
<td><em>Burkholderia cenocepa</em> LMG 12614 HAM 1.663‡</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>9 (–)</td>
<td><em>Burkholderia cepacia</em> DSM 11737_DSM 1.653‡</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>10 (–)</td>
<td><em>Burkholderia gladioli</em> Wv22575 CHB 1.583‡</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

*Bruker colour code of score value: log scores ≥2.0 would be indicated in green.
†Bruker colour code of score value: log scores between 2.0 and 1.7 would be indicated in yellow.
‡Bruker colour code of score value: log scores <1.7 would be indicated in red.

correct species identification without additional tests. In case of a difference <0.200, additional tests are best performed even if the second species is below the 2.0 threshold (see Table 1 for an example of prevented misidentification with OAC). In this dataset, when using the OAC, out of 153 isolates, 136 (88.9 %) would have been identified up to species level, 8 (5.2 %) up to genus level and 9 (5.9 %) isolates would not have been identified. All misidentification would have been prevented. The 0.200 log difference was determined from empirical observations based on this dataset.

In conclusion, the proposed OAC are less stringent than the BAC, and more specific than both the BAC and the LAC. They require fewer additional tests for Gram-negative rod identifications than our initial practice. However, it is prudent to perform additional tests if multiple species have close matches. Prospective validation is necessary to investigate if the OAC perform as well under routine conditions for other classes of bacteria and in other laboratories. Although MALDI Biotyper implementation in a routine clinical microbiology laboratory does not require high technical skills, a good knowledge of bacteriology and taxonomy is needed to interpret the output. Introducing the need for a 0.200 log difference between multiple species is a practical tool to prevent misidentifications.

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