Accurate differentiation of *Mycobacterium chimaera* from *Mycobacterium intracellulare* by MALDI-TOF MS analysis

Arthur B. Pranada,¹,* Ellen Witt,¹ Michael Bienia,¹ Markus Kostrzewa² and Markus Timke²

**Abstract**

**Purpose.** The increasing number of infections caused by nontuberculous mycobacteria (NTM) has prompted the need for rapid and precise identification methods of these pathogens. Several studies report the applicability of MALDI-TOF mass spectrometry (MS) for identification of NTM. However, some closely related species have very similar spectral mass fingerprints, and until recently, *Mycobacterium chimaera* and *M. intracellulare* could not be separated from each other by MALDI-TOF MS.

**Methodology.** The conventional identification methods used in routine diagnostics have similar limitations. Recently, the differentiation of these two species within the *Mycobacterium avium* complex has become increasingly important due to reports of *M. chimaera* infections related to open heart surgery in Europe and in the USA. In this report, a method for the distinct differentiation of *M. chimaera* and *M. intracellulare* using a more detailed analysis of MALDI-TOF mass spectra is presented.

**Key Findings.** Species-specific peaks could be identified and it was possible to assign all isolates (100 %) from reference strain collections as well as clinical isolates to the correct species.

**Conclusions.** We have developed a model for the accurate identification of *M. chimaera* and *M. intracellulare* by MALDI-TOF MS. This approach has the potential for routine use in microbiology laboratories, as the model itself can be easily implemented into the software of the currently available systems by MALDI-TOF MS manufacturers.

**INTRODUCTION**

Nontuberculous mycobacteria (NTM) can cause severe illness [1], and infections caused by NTM are increasingly recognized as an ‘emerging public health disease’ [2]. As a result, it has been recommended that infective agents are identified to the species level [1]. Within the *Mycobacterium avium* complex (MAC), there are two closely related species that are particularly difficult to differentiate by conventional methods: *M. intracellulare* and *M. chimaera*. *M. intracellulare* was previously described in 1949 as *Nocardioid intracellulare* [3], while *M. chimaera* was once considered to be a genetic variant named MAC-A in the *M. avium* complex. This variant was elevated to species rank in 2004 [4].

The first reported isolates of *M. chimaera* exhibited an unusually high level of virulence, with seven out of 12 isolates being classified as clinically significant in immunocompetent patients [4]. Compared to *M. intracellulare*, which has been associated with severe pulmonary disease, a lower pathogenicity was suggested for *M. chimaera* a few years later [5]. This was supported by Wallace *et al.* [6], who investigated samples taken from US household water and biofilm samples of MAC respiratory disease patients and found *M. intracellulare* to be the dominant species causing respiratory illness (90.7 %) and *M. chimaera* as the dominant species in household water samples (73 %). Similarly, Makovcova *et al.* [7] were able to isolate *M. chimaera* from freshwater reservoirs.

A recent retrospective study investigating the specific composition of MAC reviewed mycobacterial cultures from 448 patients and showed a relevant proportion of *M. chimaera*: 54 % *M. avium*, 18 % *M. intracellulare* and 28 % *M. chimaera* [8]. However, based on diagnostic criteria for NTM lung disease published by the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) [1], it was found that patients with *M. avium* or *M. intracellulare*...
were more likely to have pulmonary infection when compared to patients solely with *M. chimaera*. This finding is consistent with data presented by Schweickert et al. [5] and shows that precise species identification within MAC can help to estimate the clinical significance of the isolates recovered in culture.

Furthermore, recent reports about invasive infections with *M. chimaera* in cardiac surgery from the USA and from several European countries [9–12] have prompted the demand for methods that can accurately identify *M. chimaera*. As the cases described in the aforementioned reports were linked to contaminated heater–cooler devices, the recovery of this organism from clinical and/or environmental samples may also have implications for infection control.

While the identification of *M. avium* is usually reliable using conventional methods, the differentiation of *M. intracellularare* from *M. chimaera* remains a challenge to most laboratories. For example, the GenoType Mycobacterium CM assay (Hain LifeScience) identifies both *M. intracellularare* and *M. chimaera* as *M. intracellularare* [13, 14]; 16S rRNA gene sequencing differs in only 1 bp [4, 6]; the MALDI Biotyper (Bruker Daltonik) groups both species into a complex [15]; and VetMS (bioMérieux) assigns *M. chimaera* to *M. intracellularare* [16]. These findings are consistent with a study from Saleeb and colleagues [17], which includes *M. intracellularare* and *M. chimaera* as one example of closely related organisms which cannot be differentiated by standard matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) methodology.

The aim of the current study was to find species-specific peaks for *M. intracellularare* and *M. chimaera* in MALDI-TOF mass spectra acquired with the default settings of a mass spectrometer. Enhanced analysis of these spectra should allow for the accurate differentiation of these closely related species in routine practice, without any changes in workflow.

Furthermore, in order to allow automated and user-independent differentiation, two software-based approaches were developed and investigated. More specifically, three models were generated by the ClinProTools software (Bruker Daltonik), and two models with a new algorithm based on the MALDI Biotyper PeakShift Prototype [18] were applied.

**METHODS**

**Mycobacterial strains**

For analysis of species-specific peaks, two reference strains of *M. chimaera* (DSM 44623T and DSM 44622) and six strains of *M. intracellularare* (CCUG 27852, CCUG 2800, DSM 43223T, DSM 44151, DSM 44365 and DSM 44655) were investigated.

In addition, clinical isolates (*n=43*) identified by routine methods as the *M. intracellularare/M. chimaera* complex were also included and further analysed by MALDI-TOF MS. These strains originated from samples like sputa and respiratory aspirates submitted to the laboratory for mycobacterial diagnostics during 2013–2015. Six isolates originated from heater–cooler units and two strains originated from inter-laboratory tests.

Mycobacterial culture was performed on solid Löwenstein–Jensen medium (BD or Artelt-Enclit) or in liquid BD BACTEC Mycobacteria Growth Indicator Tubes (MGITs; BD) according to standard procedures.

The routine identification method was conducted using the Hain GenoType CM assay, which identified both species in question as *M. intracellularare*. Definitive identification for all strains was performed by internal transcribed spacer (ITS) sequencing. Furthermore, all strains were analysed by MALDI-TOF MS.

**ITS amplification and sequencing**

For definitive identification, the 16S–23S ribosomal RNA intergenic spacer was amplified using the primers described by Schweickert et al. [5]. After purification, bidirectional DNA sequencing was performed using the BigDye Terminator Cycle Sequencing kit with the amplification primers on an ABI Prism genetic analyser (Applied Biosystems/Thermo Fisher Scientific). ITS sequences of *M. chimaera* DSM 44623T (GenBank accession number: AJ548480 [4]) and of *M. intracellularare* DSM 43223T (GenBank accession number: JQ411532) were used as references.

**MALDI-TOF sample preparation**

Biomass of mycobacteria (about one to three 10 µl inoculation loops from solid media or centrifugation of 1.2 ml of liquid MGIT medium) was collected in 75 % ethanol and stored at −18 °C until processing. The suspension was centrifuged at maximum speed (16060 g) for 2 min. After discarding the supernatant, the pellet was processed according to the inactivated mycobacteria bead preparation method (inMbpm) or the modified, actual protocol MycoEX (Bruker Daltonik). Briefly, after addition of 300 µl deionized water, heat inactivation was performed for 30 min in a boiling water bath or in a thermoblock at a minimum temperature of 95 °C. Ethanol (900 µl) was added and mixed, followed by centrifugation at maximum speed and complete removal of the supernatant. The pelleted biomass was then vortexed with 0.5 mm zirconia/silica beads (Carl Roth) and about 20 µl acetonitrile according to the size of the pellet. An equal volume of formic acid was added and mixed and, after centrifugation, 1 µl supernatant was spotted onto a steel target plate. Dried sample spots were overlaid with 1 µl HCCA matrix solution (α-cyano-4-hydroxycinnamic acid) (Bruker Daltonik) and allowed to dry before MALDI-TOF MS analysis on a microflex LT instrument.

**MALDI-TOF MS analysis**

All samples were spotted eight times on the MALDI target plate and measured three times each. Thus, 24 MALDI-TOF spectra were acquired per sample using the automated functionality of flexControl 3.0 software (Bruker Daltonik). The raw spectra were of high quality and used to generate a main spectrum (MSP) with MALDI Biotyper 4.0 software (Bruker Daltonik) with default settings. A compilation
of Löwenstein–Jensen- and MGIT-grown strains were measured in two different laboratories (data not shown) to demonstrate minimal media and inter-laboratory robustness. The created MSPs were matched against the Mycobacteria Library v.4.0 with 880 references.

**Screening for peaks in MALDI-TOF mass spectra**

For calibration, the spectra were visually screened for peaks common to *M. chimaera* and *M. intracellulare* using flexAnalysis 3.4 software (Bruker Daltonik). In addition, peaks present only in one species and absent in the respective other species were sought. Potential candidate peaks then were confirmed by analysis of the acquired mass spectra of the clinical isolates.

**Software-based analysis of spectra**

Three identification models were created using ClinProTools 3.0 software (Bruker Daltonik). Spectra from eight *M. chimaera* and eight *M. intracellulare* were normalized and recalibrated with standard settings. By using the genetic algorithm (GA) the following models were generated: model 1 was based on the four most discriminating peaks (out of 198), automatically selected by the software, model 2 used four user-defined peaks from the manual screening for characteristic peaks (m/z 6448, 6476, 6904 and 7358) and model 3 was based only on two peaks characteristic for *M. chimaera* (m/z 6448 and 7358).

Two further models were derived from a modified MALDI Biotyper PeakShift Prototype [18]. For this purpose, automated peak-picking at the characteristic masses of *M. chimaera* and *M. intracellulare* was performed and the corresponding spectrum intensities at these masses were determined. Then the ratio of the intensities for *M. chimaera* to the intensities for *M. intracellulare* was calculated. For practical reasons, the logarithm of this ratio was used in the analysis; i.e. this so-called log(IQ) value was calculated by the formula log(IQ)=log[(sum of intensities at *M. chimaera*-specific masses)/(sum of intensities at *M. intracellulare*-specific masses)]. Positive log(IQ) values indicate identification as *M. chimaera* while negative values allude to *M. intracellulare*. This approach was tested with a model based on six characteristic peaks and one with only four peaks.

**RESULTS**

**ITS sequence**

Identification of all eight reference strains was confirmed. Of the isolates, 43 were definitively identified as *M. chimaera* and eight as *M. intracellulare*, totalling 45 *M. chimaera* and 14 *M. intracellulare* strains for analysis (Table 1).

**MALDI Biotyper standard algorithm analysis**

Mycobacteria Library 4.0 includes 33 reference entries leading to identification only as ’*M. chimaera/M. intracellulare* group’. However, for nine *M. chimaera* and 15 *M. intracellulare* references, the species information of the underlying reference strain is given as an additional hint in parentheses, e.g. ’*Mycobacterium chimaera intracellulare* group (M. intracellulare DSM 43223T DSM b)’.

As all strains included in this study were correctly identified to group level, we also evaluated the potential capability of differentiation with the standard algorithm by using the additional information from the hints. For n=56 (94.9 %) strains, the hint was in line with the definitive identification by ITS sequencing and for n=3 (5.1 %) it was not. Log(score) value distance between top species hit and the first hit with correct species hint varied only from 0.006 to 0.157 alluding to the low discriminatory power of this standard method.

**Characteristic species-specific peaks in MALDI-TOF mass spectra**

Visual analysis of the MALDI-TOF mass spectra of the reference strains with flexAnalysis software showed m/z 5660 as a suitable peak for calibration. Furthermore, potential characteristic peaks could be found at m/z 3222, 6448 and 7358 for *M. chimaera* and at m/z 3237, 6476 and 6904 for *M. intracellulare*.

These peaks were confirmed in the mass spectra of the clinical isolates by visual analysis. However, for low intensity signals it was challenging to decide between the presence and absence of a peak. Examples of high and low intensity peaks are shown in Fig. 1.

Complete agreement of this model with the expected presence or absence of the potentially characteristic six peaks was found for 35 strains. Thus, 35 of 59 (59.3 %) strains could be correctly identified by using this approach. The evaluation of the peak patterns (Table 1) revealed that, for a number of the *M. chimaera* strains, the expected peak at m/z 3222 was only of low intensity and furthermore some even showed a peak at m/z 3237, which would have been expected for *M. intracellulare*.

Therefore, the analysis was modified to a four-peak model without the aforementioned two peaks at m/z 3222 and 3237. Results improved with this model to 49 (83.1 %) strains with complete peak pattern agreement (Table 1). In addition, mass spectra of three strains exhibited one essential peak with low intensity, resulting in 52 (88.1 %) reliable species identification results.

**Software-algorithm-based models**

The ClinProTools-calculated GA model with four peaks resulted in a sensitivity for *M. chimaera* identification of 83.1 % for the automatically selected masses (model 1) and of 86.6 % for model 2 with user-defined masses (data not shown). The third GA model with only two masses showed a lower sensitivity for *M. chimaera* (77.1 %). In contrast to sensitivity, specificity was 85.2 % for free-four-peak GA model 1 but 100 % for both GA models with user-defined masses.

For a further software-based approach, log(IQ) values were calculated based on the MALDI Biotyper PeakShift Prototype with six and four species-specific peaks. The results are presented in Table 1 with positive log(IQ) values indicating
identification as *M. chimaera* and negative ones for *M. intracellularare*, respectively. As for each of the 59 strains, at least 20 mass spectra had been acquired, log(IQ) values for a total of 1375 spectra were calculated. For a summarized overview, the resulting identification from the median log(IQ) for the strains was compared to the reference identification. Here, the results from the six-peak model as well as from the four-peak model were in accordance with the expected identification for all strains (100%).

As log(IQ) values close to zero are the result of only minor differences in the characteristic peaks for the two species, and potentially harbour the risk of improper or unreliable identification, more detailed analysis was performed based on all potentially harbour the risk of improper or unreliable identification with a log(IQ) value of +0.004 for an individual spectra of this strain 1T12173955 yielded a negative algebraic sign for the log(IQ) value in accordance with the reference identification (Fig. 2). The misidentification was resolved in the improved four-peak model, which also enhances the distance from log(IQ) value zero (Fig. 2). So, in the six-peak model 1374 (99.9 %) and in the four-peak model 1375 (100 %) log(IQ) calculations resulted in correct identifications.

The influence of mycobacterial culture medium on species assignment was evaluated for MGIT and Löwenstein–Jensen medium. Differences were very minor and never endangered correct species assignment (Table 2).

**DISCUSSION**

The differentiation of closely related mycobacterial species is a common challenge in clinical microbiology laboratories. In our study, we have demonstrated that it is possible to differentiate *M. chimaera* from *M. intracellularare* by MALDI-TOF MS. The distinct identification of these two members of the *M. avium* complex has recently become increasingly relevant as invasive infections due to *M. chimaera* have been reported in patients undergoing open heart surgery [11]. Irrespectively, Griffith *et al.* [19] recently argued for species identification within MAC in

<table>
<thead>
<tr>
<th>Strain*; species confirmed by ITS sequence</th>
<th>Specific peak at m/z†</th>
<th>Frequency of six peak pattern</th>
<th>Frequency of four peak pattern</th>
<th>Model with six peaks log (IQ) value‡</th>
<th>Model with four peaks log (IQ) value‡</th>
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<tr>
<td>3222</td>
<td>3237</td>
<td>6448</td>
<td>6476</td>
<td>6904</td>
<td>7358</td>
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<tr>
<td><em>M. chimaera</em> DSM 44623(T)</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td><em>M. chimaera</em> 1T14099066</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>+</td>
</tr>
<tr>
<td><em>M. chimaera</em> 1T15127668</td>
<td>(+)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td><em>M. chimaera</em> 1T13035919</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td><em>M. chimaera</em> 1T13001113</td>
<td>(+)</td>
<td>(+)</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td><em>M. chimaera</em> 1T13057188</td>
<td>+</td>
<td>(+)</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td><em>M. chimaera</em> 1T13150920</td>
<td>(+)</td>
<td>(+)</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
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<tr>
<td><em>M. chimaera</em> 1T12108273</td>
<td>(+)</td>
<td>(+)</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
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<tr>
<td><em>M. chimaera</em> 1T12192106</td>
<td>(+)</td>
<td>(+)</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td><em>M. chimaera</em> 1T14028123</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td><em>M. intracellularare</em> DSM 43223(T)</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td><em>M. intracellularare</em> 1T13132114</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td><em>M. intracellularare</em> 1T15020999</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td><em>M. intracellularare</em> 1T14093234</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td><em>M. intracellularare</em> 1T12173955</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
</tr>
</tbody>
</table>

Table 1. *M. chimaera* - and *M. intracellularare*-specific masses and calculated log(IQ) values with two different models

*Species with lowest log(IQ) value with four peak model were selected for Table 1, except for the two type strains representing peak pattern groups.
†A low intensity peak is indicated by parentheses.
‡Median log(IQ) value and minimal and maximal values of at least 20 mass spectra.
order to gain further insights into the pathogenic role of this particular mycobacterial species. Besides clinical issues relating to patient management, there are epidemiological questions and aspects of infection control that demand reliable and accurate species identification in microbiology.

MALDI-TOF MS has been introduced in many laboratories as an additional method for microorganism identification and this technology has also been successfully used for the identification of mycobacteria from routine clinical specimens [20–22]. However, examples of closely related NTM which have historically been beyond the limit of resolution of MALDI-TOF MS and the underlying algorithms include *M. abscessus* subspecies, *M. mucogenum* and *M. phocaicum*, as well as *M. chimaera* and *M. intracellulare* [17].

**Identification based on the MALDI Biotyper standard algorithm**

In our study, we were able to confirm the limitations for differentiation of *M. chimaera* from *M. intracellulare* using standard MALDI-TOF procedures. All strains in our study were correctly identified with Mycobacteria Library 4.0 as *M. chimaera* and the analysis based on the underlying references in the database even showed that 56 strains (94.9%) were appropriately classified to species

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**Fig. 1.** Details of MALDI-TOF mass spectral profiles of *M. chimaera*- and *M. intracellulare*-type strains and two mass spectra as examples where absence or presence of specific peaks was difficult to decide manually [categorized as (+)]. Peaks used for species differentiation are indicated by arrows.
level; however, the differences in log(score) values between the two species were low.

The same approach was performed by Rodríguez-Temporal and colleagues [23], and correct identification of 15 \textit{M. intracellulare} and 13 \textit{M. chimaera} strains was reported. They also observed low differences in log(score) values, confirming the limitations in discriminatory power when using standard algorithms.

Identification based on characteristic peaks

Promising results with MALDI-TOF MS were previously published for \textit{M. abscessus} complex differentiation by using the MALDI Biotyper system [24–26]. The approaches were based on characteristic peaks but none of the mentioned masses was confirmed in all studies. However, at least Teng \textit{et al.} [24] and Suzuki \textit{et al.} [26] reported several peaks in common. In addition, two Vitek MS-based studies had no masses in common to differentiate \textit{M. abscessus} subspecies [27, 28]. Interestingly, only two studies included \textit{M. abscessus} subsp. \textit{bolletii} and two masses were identified in both studies to be characteristic, even though Bruker and bioMérieux systems were used [26, 28]. Consequently, MALDI-TOF MS has potential for \textit{M. abscessus} subspecies identification but no consensus model exists at the present time. The fact that common characteristic peaks have been found with Bruker Biotyper and Vitek MS also shows that

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**Table 2.** Comparison of four-peak-based log(IQ) values of mass spectra obtained from liquid and solid media cultivated strains

<table>
<thead>
<tr>
<th>Strain; species confirmed by ITS sequence</th>
<th>Median log(IQ) value</th>
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<tbody>
<tr>
<td></td>
<td>MGIT</td>
</tr>
<tr>
<td>\textit{M. chimaera} 1T12163212</td>
<td>+0.596</td>
</tr>
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<td>\textit{M. chimaera} 1T12105808</td>
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<td>\textit{M. chimaera} 1T12100756</td>
<td>+0.720</td>
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<tr>
<td>\textit{M. intracellulare} DSM 44161</td>
<td>−0.433</td>
</tr>
</tbody>
</table>

**Fig. 2.** Comparison of log(IQ) values of all \textit{M. intracellulare} MALDI-TOF mass spectra and of 12 \textit{M. chimaera} strains with the lowest log(IQ) values of the study set. The four-peak-model-based log(IQ) values (right side) show a clearer differentiation than in the six-peak-based model (left side).
models based on characteristic peaks have the advantage of being independent from the manufacturers of the systems.

In our approach to the differentiation of *M. chimaera* and *M. intracellulare*, we found a promising set of six MS peaks [29]. This was tested in a first visual analysis of the mass spectra. As inter- and intra-laboratory variation of mass spectra is normal and could influence the presence and intensities of masses detected, we decided to tolerate one arguable peak with a lower intensity out of three characteristic masses for the respective species. In this model, reliable species assignment could be achieved for 38 (64.4 %) strains while 21 strains (35.6 %) could not be identified. Hence, further improvements of the peak model were necessary.

Since for two pairs of peaks, namely m/z 3222 and 6448 as well as m/z 3237 and 6476, the peaks might represent double- and single-charged ions of the same molecule, the underlying information would be redundant. The peak at m/z 3222 was present at least with low intensity if the peak at m/z 6448 was definitely present, supporting the one-molecule assumption. The peak at m/z 3237 was detected 12 times although no signal was present at m/z 6476 in MALDI-TOF mass spectra of these ITS-confirmed *M. chimaera* strains (Table 1), indicating mass m/z 3237 as not qualified enough for species differentiation.

Consequently, a four-peak-based model was tested. For such a limited set of peaks, we did not tolerate the complete absence of a peak, nor did we tolerate two questionable peaks with low intensity. These exclusion criteria matched for three *M. chimaera* and four *M. intracellulare* strains resulting in an overall performance of 88.1 % identification rate for the study strains by this visual approach. Therefore, the performance of this model was found to be similar to the standard algorithm described above, albeit with a higher discriminatory power as only species-specific peaks were used.

**Software-algorithm-based models**

ClinProTools software has already been used for differentiation of groups based on mass spectra, e.g. *M. abscessus* sub-species [24] or *Haemophilus influenzae* type b isolates [30]. Here, sensitivity for *M. chimaera* detection was approximately 85 % for ClinProTools-generated GA models. This is in line with the variations of peak intensities visually detected in the mass spectra. Therefore, the log(IQ) algorithm was evaluated, which is a quotient of peak intensities. As the calculation also incorporates the variable intensities of the peaks, it has the potential to work more robustly than just visual evaluation of their presence or absence.

All median log(IQ)-value-based results were in accordance with ITS-sequence-based identification for the six-peak model and the four-peak model. However, three median log(IQ) values of *M. chimaera* strains were below 0.3 with the six-peak model, indicating a probable limitation in discriminatory power. Since a definitive classification is required for every single mass spectrum, it usually would be advisable to define a range around log(IQ) zero to indicate potentially unreliable results. This issue became obsolete when using the four-peak-model-based log(IQ) values (Table 1). In contrast to the six-peak model, the four-peak model showed a clear distance from the transition point at log(IQ)=0 and for all mass spectra the algebraic sign of the calculated log(IQ) value was in line with the reference identification (Fig. 2). Future studies will be needed in order to demonstrate if a transitional log(IQ) value range for probably uncertain classifications has to be defined for safety reasons.

The total number of strains used in this study was somewhat limited; however the strain collection consisted of clinical isolates collected over a period of almost 3 years, as well as isolates from heater–cooler devices. To ensure some robustness, mass spectra of cultures performed in two different laboratories and from two different media (Table 2) were used for construction of this model and its evaluation.

Although in our setting we could show complete agreement with the reference identification, a more comprehensive set of data is most likely needed in order to validate our approach. Some experts might be able to challenge the characteristic peak-based model by applying the model to their own datasets. In this context, van Ingen and colleagues [31] stated that genetic divergence, especially in the MAC, prevents a species assignment based on the analysis of a single genetic target. They proposed naming three clinical MAC strains as 'novel *M. chimaera* variant'. This finding may pose some challenges to our proposed model, especially if the number of clinical isolates tested increases.

In summary, we have developed a model for the accurate identification of *M. chimaera* and *M. intracellulare* by MALDI-TOF MS. To the best of our knowledge, this is the first approach for the differentiation of these two closely related species based on characteristic peaks in mass spectra acquired by standard procedures. This approach has the potential for routine use in microbiology laboratories, as the model itself can be easily implemented into the software of the currently available systems by MALDI-TOF MS manufacturers.

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**Conflicts of interest**

M.K. and M.T. are employed at the mass spectrometry company Bruker Daltonik GmbH. A. B. P. presented his data in symposia organized by Bruker Daltonik GmbH and received speaker fees. The other authors declare no conflicts of interest.

**References**


