MALDI-TOF MS for species identification in single laboratories or laboratory networks

Guidelines for the validation of identifications of taxonomically defined species using MALDI-TOF MS in such laboratories have been published in Germany. Here, Jörg Rau outlines the basics of what you need to know.

"MALDI-TOF MS systems generate mass spectra of biomolecules (eg, proteins) of unknown samples"

IN FOOD MICROBIOLOGY or veterinary diagnostics, MALDI-TOF MS already has a central role in many laboratories. Further applications, such as the identification of animal species in meat or cheese by means of user-made databases, were also developed by labs interested in food control. In general, MALDI-TOF MS is operated with several goals: as a qualitative method for non-directed identification (screening), for the identification of concrete microbial parameters, or as a confirmation test eg, for relevant bacterial pathogens.

MALDI-TOF MS systems generate mass spectra of biomolecules (eg, proteins) of unknown samples. These mass spectra are compared with reference spectral patterns organised in databases. Through a computer-aided ranking of the best match with the reference patterns, and the application of empirical decision rules, the sample is assigned a taxonomic level, like species or genus, and thus identified. A well sorted, fitting and maintained database is vital for reliable and successful identification.

For such previously non-standardised solutions and technologies, method validations are particularly relevant for laboratories. Validations are intended to prove the usability of the methods for the respective application. Therefore, in 2021, a German expert group published guidelines for the validation of identifications of taxonomically defined species (eg, bacteria, fungi or animal species) using MALDI-TOF MS in single laboratories or laboratory networks.[1,2]

In this article, we introduce a selection of ideas and criteria and briefly summarise them from the user's perspective.

Three categories of validations have been discussed in the guidelines:

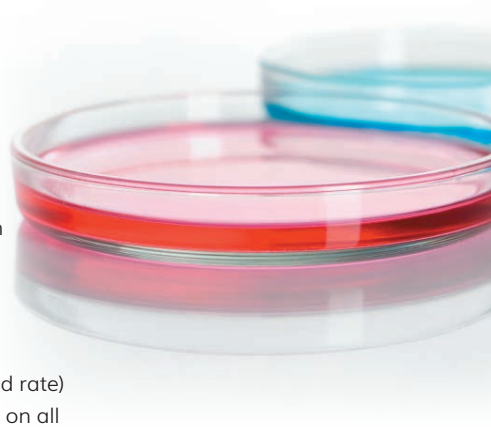
1) Non-directed identification (screening) checks whether the intended target parameters are correctly analysed in the large taxonomic range
2) The validation of a targeted identification aiming at a single, concrete identification decision (target parameters; eg, a microorganism species, an animal species, or a fungal genus)
3) MALDI-TOF MS is also used as a confirmation method, eg, for food-relevant species like *Listeria monocytogenes* or *Staphylococcus (S.) aureus* and others. Validation of a confirmatory method focuses on a single, concrete identification decision target parameter. In the case of a confirmation workflow, a clear statement should be possible for the parameter, even in the negative case. This requires special specifications for the validation. The confirmation workflow will be presented elsewhere.

Here, we show details of the guideline for the two cases. However, we should mention general parameters first, because MALDI-TOF MS has a few special features that are not relevant for other assays such as ELISA or PCR. To ensure mass accuracy, regular mass calibration of the system with a suitable standard is imperative. Formal validation checks a concrete version of the reference database used (commercial, in-house, or mixed). The validation focuses on a target parameter (eg, *S. aureus*) in the in-house laboratory environment/your world of samples (eg, for governmental food control, or for mastitis specimens in the case of milk testing). The in-use database shall be documented for every experiment. For the validation of evolving databases, ie, libraries that change gradually due to adjustments of the reference entries in the content (eg, through new versions), a permanent collection of individual spectra (test datasets) of reliably named and well-documented microbial isolates or other sample materials is helpful. The analysis programs used for the respective experiments, the evaluation method and the decision rules are part of the methodology and thus the documentation. The variants of sample preparation and extraction are accounted for as a robustness test.

An important aspect of the validation concepts presented in the guidelines is that they are based on the evaluation of spectra of well-defined isolates or materials. Only a part of the used spectra collection must be generated in the in-house laboratory. Therefore, active exchange of spectra between users and effective cooperation facilitates the validation. For instance, interested MALDI-users are looking for partners, database entries, validation spectra, or available field isolates in the catalogue of the open and non-commercial MALDI-User Platform *MALDI-UP*, which is maintained by the Chemical and Veterinary Analysis Agency Stuttgart, Germany.[3]

Validation criteria proposal for non-directed identification (screening) and validation of a targeted identifying MALDI-TOF MS method

Bacteria and fungi are usually cultivated before MALDI-TOF MS analysis. In the case of animal or plant samples, little or no-processed – in particular, pure products – are suitable for direct species identification by MALDI-TOF MS.



Targeted identification rules for single laboratories or laboratory networks

For the validation of a single workflow for targeted identification of concrete parameters, only successful identification decisions of the MALDI-TOF MS system are considered for the calculation. Thus, the experimental review shows the proportion of materials/isolates of the target parameter (accurately detected rate) correctly identified by the method, based on all identified samples. Expected target values have been defined as thus:

a) Value for the right-positive rate (inclusivity) should be ≥ 95.0 percent
b) The right-negative rate (exclusivity) should be ≥ 99.0 percent
c) The two values for the false-negative rate and the false-positive rate should be ≤ 1.0 percent.

Verification of right-positive rate and false-negative rate is carried out on a sufficiently large sample number of isolates or materials that are known to be positive with regard to the target parameter. The selection should include isolates and materials from the sample types/matrices relevant in laboratory practice (eg, 'S. *aureus* from food' or 'Veterinary bacteriology samples').

A right-positive rate below 95 percent results in limitations in the interpretability of targeted identification. In these cases, for example, it may be specified that in addition to MALDI-TOF MS identification, further analysis by another suitable method is necessary. If a broad selection of species (eg, field strains) or subspecies are available, it makes sense to include them in the selection of different variants of the target parameter (eg, for microorganism serotypes, biotypes, morphological variants, animal sources). Since MALDI-TOF MS is a spectrometric phenotypic method, validation using spectra from reference strains alone is not always »

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sufficient to account for the variance of current spectral phenotype occurring in the intended test samples.

Recommended minimum sample sizes focusing on targeted identification

For inclusivity testing, the number of independent, evaluated spectra of the target parameter should be n≥ 20, of which a minimum of 20 percent own spectra (20 percent of n). Spectra exchange with other institutes is permitted and desired as many single laboratories do not have a broad biobank, but could benefit from laboratory networks and their MALDI-TOF MS systems.

For exclusivity testing, the number of independent spectra of the non-target parameter should be m≥ 30; and above 20 percent own spectra (in-house).

The guidelines show two simple examples of targeted identification. Details can be found in the publication mentioned.

Targeted identification of:

a) *Staphylococcus aureus* (Gram-positive bacterium) with 51 spectra for inclusivity and 171 spectra for exclusivity used for validation of MALDI-TOF MS identification
b) Muscle meat of horse-like animals (genus *Equus*) with 35 spectra *Equus* (genus) for inclusivity and 1053 spectra for 265 different animal species for exclusivity.

Further examples that were generated according to the concept of the guidelines can be found on MALDI-UP.[2]

Validation of a non-directed identification (screening)

In the case of validation of a workflow for non-directed identification (screening), only successful identification decisions of the MALDI-TOF MS system are considered. Thus, the experimental review shows the proportion of materials/isolates of the target parameter (accurately detected rate) correctly identified by the method, based on all identified samples.

Here, we introduce another parameter to cover all samples: The correct classification rate (CCR) is a simple additional measure that indicates the sum of the correctly classified results (correct-positive and correct-negative) over the sum of measurement results. The CCR demonstrates the general coverage of the MALDI-TOF MS system for the intended application. For non-directed identification, the CCR is thus the comprehensive key figure and we will provide target values and examples below.

"In the case of validation of a workflow for non-directed identification (screening), only successful identification decisions of the MALDI-TOF MS system are considered"

Recommended minimum sample sizes focusing on non-directed identification (screening)

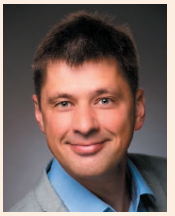
For screening workflow validations, a minimum sample of n + m ≥ 500 is sufficient, of which a minimum of 20 percent own spectra (20 percent of n) should be included, and a CCR of ≥ 95 percent should be achieved for successful validation.

The guidelines show an example of non-directed identification with more than 500 samples: A study has been performed for screening of muscle meat of animals with 1088 spectra (of which 863/1088 with ID result, 861/863 in accordance with expectation) and a correct classification rate > 99 percent. This example allows implementation of the MALDI-TOF-MS method for the animal species identification of meat in a single lab according to the criteria discussed. Therefore, an independent new application based on an in-house database can easily be introduced in an accredited governmental state lab.

Deviating from the minimum size (500), a second example of veterinary microbial isolates is also shown: A set of microbiological samples from animals with 375 spectra (of which 362/375 with ID result, 359/362 in accordance with expectation) and a correct classification rate > 99 percent. The laboratory must explain the reason for limited sample numbers and the future work on additional samples which could be added to the initial validation study.

Further aspects are highlighted in the German guidelines, eg, the usability of MALDI-TOF MS systems in a laboratory network could be challenged in a proficiency test in which identical samples are examined by several laboratories during the same period. Further details can be found in the publication of the guidelines (currently available in German; English in preparation).

The validation options presented in the guidelines can be applied perfectly to dynamic MALDI-TOF systems, as they allow evaluation of commercial versioning and/or in-house database extensions, but also applications outside the field of microbiology with a reasonable amount of effort. We have already been able to implement this on many practical examples in the network of the accredited laboratories of the CVUA's in Baden-Württemberg.



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