Optimization of data interpretation criteria for bacterial and yeast identification using MALDI-TOF mass spectrometry.

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ABSTRACT

Identifications (ID) using the MALDI Biotyper (Bruker Daltonics) are made based on a numerical score value (1.0 to 3.0) that is calculated using Bruker's algorithms (Table 1). Identification results are also assigned a consistency category (defined as either A, B, or C) to aide users in interpreting results in terms of species, genus, or no taxonomic consistency (Table 2). We conducted a Fitness-for-Use study with nearly 500 bacterial isolates spanning 104 unique genera representing 352 unique species to re-evaluate MALDI-TOF mass spectrometry (MS) score cutoffs for assigning species-level ID. In addition, we evaluated the suitability of the optimized MALDI-TOF MS score cutoffs for identifying yeasts. DNA sequencing of the first 500bp of the 16S rRNA gene for bacteria, and ITS2 portion of the rRNA region for yeasts was used as reference identification. Analysis of identification scores revealed optimal species-level threshold of ≥1.75. Multiple species above the score threshold were reported in the top 10 results for nearly 30% of isolates. A 0.1 score differential between the top match and additional species to reach single-species-level identifications was optimal to maintain a good compromise between accuracy and No ID rate. Scores, differentials and consistency categories are important data review and quality parameters for MALDI-TOF MS identifications.

Objective

MALDI-TOF MS for routine microbial identification is used by increasing numbers of microbiology laboratories across the globe in Environmental Monitoring (EM) programs from regulated manufacturing settings. As a result, the reference database (or library) is continually expanding with new genera and species to capture the diversity of isolates from EM programs. The purpose of our study is to show the options for interpreting MALDI-TOF MS data, and results of optimizing score cutoffs for reporting species-level identifications for bacteria and yeasts.

FITNESS FOR USE STUDY COHORT

483 isolates were subjected to 16S rDNA Sequencing and MALDI, and spanned 104 unique genera representing 352 unique species, of which 45 were unique closely related species groups.

Fig 1. Isolate distribution by Gram reaction (total=483)

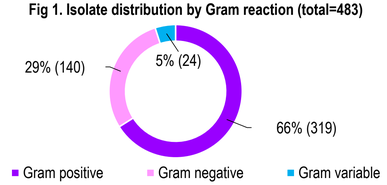

Fig 2. Performance of BacSeq (total=483)

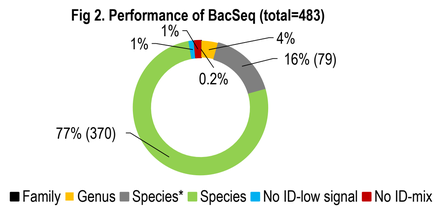

Table 3. Top 10 genera of study cohort

Genus	No. species	No. isolates
Bacillus	44	79
Staphylococcus	29	42
Corynebacterium	18	23
Pseudomonas	14	22
Microbacterium	14	18
Paenibacillus	11	18
Kocuria	8	13
Micrococcus	4	12
Streptococcus	10	12
Acinetobacter	9	10

Analysis cohort for MALDI score optimization
= species + species* by BacSeq
= 449 of 483 isolates

CONCLUSIONS

- Optimized MALDI score threshold of ≥1.75 and a score differential of Δ0.1 between the top match and additional species was a good compromise between accuracy and fall-through rate for species-level ID (Fig 3).
- When the optimized score threshold (≥1.75) and score differential cutoff (Δ0.1) were applied to a yeast cohort (152 isolates) representing 66 unique species and 19 unique genera selected based on their Frequency of Occurrence of testing at Charles River Laboratories, the identification rate was 82% (124/152) to species (score ≥1.75), and 17% (26/152) were not a reliable ID (<1.75).
- When weighted by Frequency of Occurrence, the species-level inaccuracy (or Error rate) stands at about 2.9% for bacterial ID. Species-level errors are mostly due to closely related phylogenetic groups.
- Organizing MALDI results by Consistency Categories (A, B & C) provide useful data review parameters for quality checks.
- This Fitness for Use study, which compared the performance of MALDI directly with the reference identification by Sequencing opened a gold mine for library curation and process workflow improvements (Table 5 and Fig 3). Post library curation, the MALDI error rate will drop resulting in greater accuracy.

BACKGROUND

The MALDI-TOF MS instrument (Bruker Daltonics) generates a spectrum that represents abundant, mostly ribosomal, protein profiles of a microorganism. Peaks from the spectrum of an unknown microorganism are matched with peak lists represented in the library, or reference database. Symmetry of the matching peaks is computed to calculate a final match value, or score, ranging from 0.00 to 3.00. Although Bruker recommends a score between 2.0-3.0 for high confidence species ID, 1.7-1.99 for low confidence ID, and <1.70 as not reliable ID, there is precedent to optimize and validate custom score thresholds based on analysis of data from well-designed Fitness for Use studies (J. Clin. Microbiol. Dec 2012 vol.50 no.12 3845-3852).

In 2009, Charles River\Accugenix® launched its MALDI service (AccuPRO-ID®) backed by Sequencing ID. At that time, the data interpretation criteria was established based on an internal Fitness for Use study, and a score of ≥1.75 was determined to be optimal for assigning species-level ID. Since then until 2016, the MALDI library increased from 3300 to 7900 entries (140% increase) due to library upgrades by both Charles River and Bruker Daltonics. A second Fitness for Use study was designed to reevaluate the appropriateness of the data interpretation criteria to determine if the extensive expansion of our library in both depth and breadth of species entries has effected the accuracy of our identifications.

Table 1. Meaning of MALDI scores (0.00 to 3.00).

Source of spectrum	Score range
Library entry matches itself	3.00
Spectra used to create Library entry	2.50 to 2.80
Customer spectra that generate an ID	1.75 to 2.50
Species threshold (Charles River)	≥1.75
Customer spectra with No match to Library	0.00 to 1.75

Table 2. Meaning of Consistency Categories (A, B or C).

Consistency Category	Description (customized to Charles River score thresholds)
A	Species Consistency: same species above score threshold ≥1.75
B	Genus Consistency: same genus above score threshold ≥1.75
C	No consistency: <1.75 and/or different genera above score threshold ≥1.75

RESULTS

Fig 3. Accuracy and Fall-through rate as a function of MALDI Score Thresholds

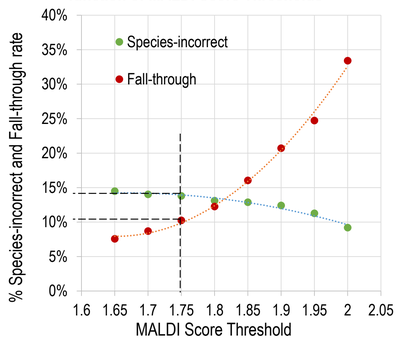

Fig 4. Distribution of results by Consistency Category (n=449)

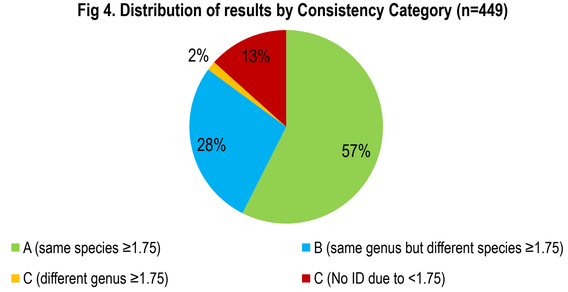

Table 4. Distribution of accuracy of results within Consistency Categories (A, B, and C).

	A ≥1.75	B ≥1.75	C ≥1.75	C <1.75	Overall
Species-Correct	91% (234)	71% (88)	100% (7)	NA	73% (329)
Genus-Correct	9% (24)	29% (36)	0%	NA	13% (60)
Genus-Incorrect	0%	0%	0%	NA	0%
No ID with spectra	NA	NA	NA	77% (46)	10% (46)
No ID due to No spectra	NA	NA	NA	23%	3% (14)
Total	57% (258)	28% (124)	2% (7)	13% (60)	100% (449)

Fig 5. Performance of MALDI weighted by Frequency of Occurrence (n=341 unique species)

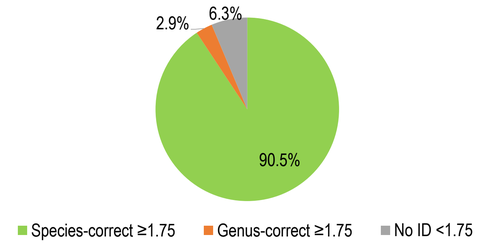

Table 5. Impact of this Fitness for Use study on library curation.

Number of isolates	MALDI library curation scenario due to discrepants
20	Sequencing genus ID when MALDI species ID
24	Single species ≥1.75 (Category A) incorrect to species
36	Multiple species ≥1.75 (Category B) incorrect to species
0	Top 100 species by frequency of occurrence NOT in MALDI library