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Interlaboratory Comparison of Sample Preparation Methods, Database Expansions, and Cutoff Values for Identification of Yeasts by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Using a Yeast Test Panel

Anneloes Vlek,[a,b] Anna Kolecka,[c] Kantarawee Khayhan,[c,d] Bart Theelen,[c] Marizeth Groenewald,[c] Edwin Boel,[b] Multicenter Study Group, Teun Boekhout[c,e,f]

Department of Medical Microbiology, Diakonessenhuis Utrecht, Utrecht, the Netherlands[a]; Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, the Netherlands[b]; CBS Fungal Biodiversity Centre (CBS-KNAW), Utrecht, the Netherlands[c]; Department of Microbiology and Parasitology, Faculty of Medical Sciences, University of Phayao, Phayao, Thailand[d]; Department of Dermatology, Shanghai Key Laboratory of Molecular Medical Mycology, Institute of Dermatology and Medical Mycology, Changzheng Hospital, Second Military Medical University, Shanghai, China[e]; State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China[f]

An interlaboratory study using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) to determine the identification of clinically important yeasts ($n = 35$) was performed at 11 clinical centers, one company, and one reference center using the Bruker Daltonics MALDI Biotyper system. The optimal cutoff for the MALDI-TOF MS score was investigated using receiver operating characteristic (ROC) curve analyses. The percentages of correct identifications were compared for different sample preparation methods and different databases. Logistic regression analysis was performed to analyze the association between the number of spectra in the database and the percentage of strains that were correctly identified. A total of 5,460 MALDI-TOF MS results were obtained. Using all results, the area under the ROC curve was 0.95 (95% confidence interval [CI], 0.94 to 0.96). With a sensitivity of 0.84 and a specificity of 0.97, a cutoff value of 1.7 was considered optimal. The overall percentage of correct identifications (formic acid-ethanol extraction method, score ≥ 1.7) was 61.5% when the commercial Bruker Daltonics database (BDAL) was used, and it increased to 86.8% by using an extended BDAL supplemented with a Centraalbureau voor Schimmelcultures (CBS)-KNAW Fungal Biodiversity Centre in-house database (BDAL+CBS in-house). A greater number of main spectra (MSP) in the database was associated with a higher percentage of correct identifications (odds ratio [OR], 1.10; 95% CI, 1.05 to 1.15; $P < 0.01$). The results from the direct transfer method ranged from 0% to 82.9% correct identifications, with the results of the top four centers ranging from 71.4% to 82.9% correct identifications. This study supports the use of a cutoff value of 1.7 for the identification of yeasts using MALDI-TOF MS. The inclusion of enough isolates of the same species in the database can enhance the proportion of correctly identified strains. Further optimization of the preparation methods, especially of the direct transfer method, may contribute to improved diagnosis of yeast-related infections.

Fast and reliable identification of causative agents of fungal infections is important, as this contributes to the choice of appropriate antifungal treatment in order to provide the best possible management of patients. Conventional procedures for the identification of pathogenic microorganisms in clinical microbiology laboratories are rapidly being replaced by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)-based methods. In comparison with other identification methods, such as sequence analysis of the D1/D2 domains of the large subunit ribosomal DNA (rDNA) and the internal transcribed spacer (ITS) 1 and 2 regions of the rDNA, MALDI-TOF MS is able to provide accurate identifications of microorganisms with a short turnaround time (1, 2). No major errors, such as genus-level misidentifications, have been reported in many MALDI-TOF MS-based studies on yeasts and filamentous fungi (3–11). Most yeasts can easily be processed and correctly identified; even sibling species that cannot be distinguished with common biochemical methods can be discriminated with MALDI-TOF MS (12, 13).

Currently, the MALDI-TOF MS approach is commercialized by a number of manufacturers: MALDI Biotyper (Bruker Daltonics, Germany), Vitek MS (bioMérieux, France), Axima (Shimadzu)-Saramis, and Andromas (Andromas, France) (14–20). Some studies have directly or indirectly compared the preparation methods currently used, but these studies were done by a single laboratory only (2, 3, 18). Cassagne et al. (2) compared four sample preparation methods and concluded that the formic acid-ethanol extraction method is preferred for use in clinical laboratories. The two other studies (3, 18) compared two MALDI-TOF MS systems (Bruker and Andromas) using the procedures recommended by the respective manufacturers. Both concluded that the

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Address correspondence to Teun Boekhout, t.boekhout@cbs.knaw.nl.
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platforms performed equally well for yeast identification and outperformed traditional identification methods.

In previous studies, the sensitivity and specificity of the identification results of yeasts are variable and difficult to compare between laboratories, as they used different sample preparation methods. The percentages of correct identifications ranged from 16% (21) and 21.3% (2) with direct transfer to >90% with formic acid-ethanol extraction (22–24).

Here, we present results from a multicenter European interlaboratory study in which the performance of the MALDI Biotyper (Bruker) was investigated regarding the identification of yeasts in a clinical setting, comparing three methods of sample preparation, and using the Bruker commercial database and an expanded database (Bruker Daltonics database plus the Centraalbureau voor Schimmelcultures in-house [BDAL+CBS in-house]). Moreover, the optimal cutoff values and the number of entries in the database with respect to the number of correct identifications were evaluated.

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MATERIALS AND METHODS

Strains and sample preparation for MALDI-TOF MS analysis. Eleven clinical centers, comprising eight academic and three general hospitals, one company (Bruker Daltonics, Bremen, Germany), and one reference center (CBS-KNAW) participated in this study. A blinded heterogeneous set of 35 reference strains was selected from the CBS-KNAW yeast collection, comprising common and rare yeast human pathogens as well as strains phylogenetically related to pathogenic species but known to be nonpathogenic (see Table S1 in the supplemental material). Twenty-two out of 35 strains were ascomycetous yeasts and 13 were basidiomycetous yeasts. The identifications of all strains were verified by sequence analyses of internal transcribed spacers (ITS) 1 and 2 and the D1/D2 regions of large subunit ribosomal DNA (rDNA) (see Table S1) (12, 25). All strains were different from those present in the commercial (BDAL) database, but in an extended (BDAL+CBS in-house) database reference, the MSP of seven out of the 35 send strains were present (see Table S1). As a control, the set also included a species that had no reference mass spectra in either database (see Table S1). The strains were cultured on Sabouraud's dextrose agar (SDA) plates and incubated for 48 h at 30°C and prepared for shipment on SDA agar slants to the participating laboratories. As reported by the participating laboratories (see Table S2 in the supplemental material), the growth of strains for MALDI testing was usually sufficient after 24 h.

Sample preparation was carried out according to the Bruker protocols using three methods: (i) direct transfer (DT), (ii) extended direct transfer (eDT), and (iii) ethanol (EtOH)-formic acid (FA) extraction. In the DT method, a thin smear of biological material was placed onto a target plate, which was immediately overlaid with 1 μl of alpha-cyano-4-hydroxycinnamic acid matrix solution (HCCA) (Bruker Daltonics, Germany), prepared according to the protocol of the manufacturer. In the eDT method, the biomass was treated with 1 μl of 70% FA on the target plate prior to the HCCA matrix overlay. In the EtOH-FA method, one or two loops of yeast biomass (1 μl volume, sterile inoculation loop) was used for the crude protein extraction, as described previously (16, 17). One microliter of the crude protein extract was spotted onto the target plate, and after air-drying, it was overlaid with 1 μl of HCCA matrix solution. For all methods, each tested strain was spotted in duplicate.

Commercial and extended databases for MALDI-TOF MS analysis. Two versions of the standard commercially available Bruker Daltonics database (BDAL) were used in this study (see Table S2 in the supplemental material). The first version (version 3.1.2.0) included 3,995 main spectra (MSP) and was used by six labs, and the second version (version 3.2.1.1), with 4,110 MSP, was used by seven labs. The yeast panel BDAL with 4,110 MSP contained an additional 20 MSP of 18 species belonging to 11 genera (see Table S3 in the supplemental material). Four hundred forty-seven yeast strains representing 104 species were selected from the CBS-KNAW collection to create an extended database, namely, BDAL supplemented with a CBS in-house database (BDAL+CBS in-house), consisting of 510 reference MSP. Ethanol extracts of those yeast strains cultivated on SDA were sent to Bruker Daltonics (Bremen, Germany) to create MSP according to the manufacturer's standard operating procedures. Each of 510 MSP was generated from 22 to 24 individual spectra using the MALDI Biotyper automated FlexControl software version 3.0 (Bruker Daltonics). Additionally, in the CBS in-house database, the MSP of some *Cryptococcus neoformans* strains were included from growth on media that reduced capsule formation, namely, SDA plus 0.5 M NaCl. The CBS in-house database was made available to all participating laboratories.

The MALDI-TOF MS identification results were automatically classified using the log-score values generated by the MALDI Biotyper software (Bruker Daltonics, Germany), performed according to the manufacturer's instructions. Each laboratory received six Microsoft Office Excel files with premade tables to report the data of the MALDI-TOF MS-based identification results, comprising the 1st best-matched organism identity and score value for spots 1 and 2, per three methods and per two databases. When the final overview of the participating laboratories was made, each laboratory was marked blindly with a numeric code, and the raw data were distributed to all participants.

Data analyses. A receiver operating characteristic (ROC) curve was plotted in order to investigate different MALDI-TOF MS score cutoff values. This analysis was performed using all data and repeated using only the results from the full extraction method and extended database analysis. Based on the sensitivities and specificities for different cutoff values (i.e., 2.0, 1.9, 1.8, 1.7, and 1.6), the most optimal cutoff value was determined.

Based on the optimal cutoff value, the percentages of correct identification were compared between those from the commercial (BDAL) and extended (BDAL+CBS in-house) databases, both for the overall results and those per participating laboratory. Using the results from the EtOH-FA-extraction (Ex) protocol, the association between the number of MSP per strain included in the extended (BDAL+CBS in-house) database and the proportion of correct identifications of strains were analyzed. The percentages of correct identifications were also compared between the three different sample preparation methods using either one spot or duplicate spots.

The results for continuous variables are expressed as the mean with standard deviation (SD) or as the median with interquartile range (IQR) when not normally distributed, and for categorical variables, they were expressed as percentages with the absolute number. The summary data were calculated using Student's *t* test (normal distribution) or Mann-Whitney U test (skewed distribution) for continuous variables and by chi-square analysis for categorical variables. Logistic regression analysis was used to calculate the association between correct MALDI-TOF MS identification and the number of MSP included in the database. Statistical analyses were performed with SPSS version 21.0 (Windows, Chicago, IL, USA).

RESULTS

Determination of an interpretative cutoff value for yeast species identification by MALDI-TOF MS. In total, 5,460 MALDI-TOF MS results were available for analysis in principle (13 laboratories, 35 strains, 2 databases, 3 extraction methods, and 2 spots; Table 1). Using all 5,460 results, the area under the ROC curve (AUC) was

TABLE 1 Interpretation of MALDI-TOF MS results

Conclusions made	Identification result(s)	No. of identifications
Results leading to final and correct identification	Correct identification (species level) with score of ≥2.0	1,550
	Correct identification (species level) with score of 1.7–2.0	993
	Correct identification of species complex with score of ≥1.7	17
Results not leading to final and correct identification	Score of <1.7, correct identification (species level)	450
	Not reliable identification with score of <1.7	545
	No peaks found	755
	Correct identification of species complex with score of <1.7	33
	Incorrect identification (species or genus level) with score of 1.7–2.0	14
	Incorrect identification (species or genus level) with score of ≥2.0	6
Not performed or missing value	No growth	109
	Not performed	680
	Correct identification (species level), but species not available in database	27[a]
	Incorrect identification (species level) with score of ≥1.7, species not available in database	8
	Incorrect identification (species level) with score of <1.7, species not available in database	273

[a] Most likely the use of the wrong database.

0.95 (95% confidence interval [CI], 0.94 to 0.96). The sensitivity values ranged from 0.50 (with 2.0 cutoff) to 0.89 (with 1.6 cutoff), and the specificities were between 0.89 (with 1.6 cutoff) and 0.99 (with 2.0 cutoff) (Table 2). A cutoff value of 1.7 showed a sensitivity of 0.84 and a specificity of 0.97 and was considered the most optimal cutoff value. When the analysis was repeated using only the results from the extended (BDAL+CBS in-house) database and the full extraction method ($n = 910$), as these gave the most consistent and high scores in all labs, a cutoff of 1.7 led to 90.6% of the results showing scores above the cutoff value, of which 99.3% were correctly identified and 0.7% were incorrect (0.5% errors at the species level and 0.2% errors at the genus level), resulting in a sensitivity of 0.97 and a specificity of 0.90 (AUC, 0.98; 95% CI, 0.96 to 1.00). Errors at the species level were with a *Candida viswanathii* strain that was identified as *Candida tropicalis*, a *Cryptococcus gattii* strain identified as *Cryptococcus curvatus*, and a *Kodamaea ohmeri* strain identified as *Candida guilliermondii* (2 times). One time, a *Cryptococcus neoformans* strain was misidentified at the genus level as *C. guilliermondii*.

The cutoff of 1.7 was used for all further analyses, for which MALDI-TOF MS results were categorized as correct identification, no correct identification, no peaks found, or not performed/missing (Table 1).

MALDI-TOF MS identification results obtained with commercial and extended databases. From all 35 strains tested, the species least often correctly identified when the MALDI-TOF MS EtOH-FA-Ex protocol and extended (BDAL+CBS in-house) database were used were *Malassezia pachydermatis* (correct identification, 53.8%), *C. viswanathii* (correct identification, 69.2%), *C. curvatus* (correct identification, 69.2%), and *Cryptococcus adeliensis* (correct identification, 69.2%). For *Cryptococcus amylolentus*, a close relative of *C. neoformans* and *C. gattii* that is not represented in the commercial (BDAL) or extended (BDAL+CBS in-house) databases, only nonreliable identifications (NRIs) were achieved, with scores of <1.6, indicating that the MALDI-TOF MS Biotyper system did not provide false-positive identifications with scores of >1.7 for this species.

TABLE 2 Sensitivities and specificities according to different MALDI-TOF MS cutoff values using all MALDI-TOF MS results ($n = 5,460$)

Cutoff value	Sensitivity	Specificity
1.6	0.89	0.89
1.7	0.84	0.97
1.8	0.75	0.98
1.9	0.64	0.99
2.0	0.50	0.99

When analyzing the results generated with the commercial (BDAL) and extended (BDAL+CBS in-house) databases, the highest number of correct and reliable identifications was observed when the extended (BDAL+CBS in-house) database was applied, independent of the sample preparation procedure used. The overall percentage of correct identification using the commercial (BDAL) database was 61.5%, versus 86.8% with the extended (BDAL+CBS in-house) database (full extraction method). Although the proportion of correct identifications differed between laboratories, all laboratories showed an increase in correct identifications using the extended (BDAL+CBS in-house) database compared to that with the commercial (BDAL) database (Table 3).

In addition, the association between the number of MSP of strains in the extended (BDAL+CBS in-house) database and the results of MALDI-TOF MS identification was evaluated. Strains that were correctly identified by MALDI-TOF MS were represented by a median of 11 (IQR, 5 to 31) strains in the database, compared to a median of 2 strains (IQR, 1 to 6) for those not correctly identified ($P < 0.01$). The number of MSP in the database was significantly associated with a correct MALDI-TOF MS identification (odds ratio [OR], 1.10; 95% CI, 1.05 to 1.15; $P < 0.01$).

MALDI-TOF MS identification results obtained with different sample preparations. The proportions of correct identifications with the use of the extended database were 39.8%, 68.1%, and 86.8% when using three sample preparation methods,

TABLE 3 Percentage of correct MALDI-TOF MS identifications to species level per laboratory using the commercial (BDAL) versus the extended (BDAL+CBS in-house) database

Database used	Identification	% correct (absolute no. of tested strains) MALDI-TOF MS identifications for laboratory:												
		1	2	3	4	5	6	7	8	9	10	11	12	13	Total
BDAL	Correct ID[a]	71.4 (25)	74.3 (26)	71.4 (25)	0.0 (0)	65.7 (23)	65.7 (23)	85.7 (30)	51.4 (18)	65.7 (23)	45.7 (16)	71.4 (25)	71.4 (25)	60.0 (21)	61.5 (280)
	Not performed	0.0 (0)	2.9 (1)	0.0 (0)	100.0 (35)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	2.9 (1)	0.0 (0)	0.0 (0)	0.0 (0)	10.3 (47)
BDAL+CBS in-house	Correct ID	91.4 (32)	91.4 (32)	94.3 (33)	88.6 (31)	82.9 (29)	91.4 (32)	94.3 (33)	62.9 (22)	94.3 (33)	71.4 (25)	88.6 (31)	94.3 (33)	82.9 (29)	86.8 (395)
	Not performed	0.0 (0)	2.9 (1)	0.0 (0)	2.9 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	2.9 (1)	0.0 (0)	0.0 (0)	17.1 (6)	2.0 (9)

[a] ID, identification.

namely, DT, eDT, and EtOH-FA, respectively. However, large differences in performance occurred between the laboratories, especially for DT, with only four laboratories delivering high rates of correct identification (71.4% to 82.9%) using this method (Table 4). When only those species known to be most often identified from clinical samples (i.e., *Candida albicans* and *Candida glabrata*) were considered for the analysis, DT, eDT, and EtOH-FA led to correct identification in 46.2%, 75.4%, and 98.5% of the strains, respectively.

When the direct or extended direct transfer method was performed on two spots in the first step, in 52.1% and 24.0% of the strains, respectively, no identification is available, which requires proceeding with the EtOH-FA method. When the EtOH-FA method is performed using duplicates, a correct identification will be available in 88.6% of strains, leaving 11.4% without MALDI-TOF MS identification (Table 5).

DISCUSSION

In this multicenter study, we evaluated the usefulness and performance of the MALDI-TOF MS Biotyper for identifying yeast isolates in clinical laboratories across Europe using the commercial Bruker database (BDAL) and an expanded (BDAL+CBS in-house) database that included an additional 510 MSP of yeast species. As it is preferred to avoid the laborious full extraction procedure in a clinical lab if direct transfer or extended transfer delivers the same results, we also compared different sample preparation methods.

Based on this study, the effect of applying a lower cutoff score was evaluated, and a cutoff value of 1.7 was considered appropriate for routine yeast identification in clinical laboratories. The majority of misidentifications were associated with scores of <1.7 (nonreliable identification [NRI]), regardless of the sample preparation method and database used. This is in agreement with previous studies that reported an increase in the number of accurate identifications at the species level when lowering the score threshold to a value of 1.7 or 1.8 (6, 12, 26–28). These studies showed 88.7% (27) and 96% correct identification rates with a score of >1.7 (21), compared to 97.4% (29) and 97.9% (30) with a score of >1.8. The Bruker MALDI Biotyper system did not generate erroneous fungal identifications (10, 31).

This study showed the usefulness of database expansion for reliably identifying emerging rare pathogenic yeast species. Also, the identification of commonly found yeasts improved by adding MSP, most likely because a higher number of reference MSP per species increased the biological diversity of the database. This is in agreement with recent studies that showed that creating an in-house library of previously accurately identified reference strains (e.g., determined by rDNA sequencing) improved fast and reliable identification of strains (2, 4, 8, 9, 13, 18, 29). Some recent single-laboratory-based studies reported that the simultaneous use of commercial and in-house databases allowed rapid and correct identification of yeast isolates confirmed by molecular identification (8–10). The results of Kolecka et al. (9) indicated that a self-established database for *Malassezia* species improved the identification capability of MALDI-TOF MS for diagnosing skin pathogens. An in-house database of arthroconidial yeasts (e.g., *Trichosporon*, *Geotrichum*, and *Saprochaete* spp.) improved the number of correct identifications at the species level (8). Taj-Aldeen et al. (32) showed 100% correct identifications by MALDI-TOF MS of 68 *C. albicans* isolates and 133 isolates of other *Can-*

TABLE 5 Percentage of correct identification per extraction method using 1 or 2 duplicates (based on the extended database [BDAL+CBS in-house], strains not performed, or no growth excluded)

Extraction method[a]	% correct identifications by no. of spots, selection[b]:			
	1, worst case	1, best case	1, mean	2
DT	29.9	47.9	38.9	47.9
eDT	58.2	76.0	67.1	76.0
EtOH-FA	80.7	88.6	84.7	88.6

[a] DT, direct transfer; eDT, extended direct transfer; EtOH-FA, ethanol-formic acid extraction.
[b] Worst-case scenario, the single spot that was selected is the worse of the 2 results generated; best-case scenario, the single spot that was selected is the better of the 2 results generated.

dida spp. and uncommon yeasts from bloodstream infections, with scores of >1.9 using the extended (BDAL+CBS in-house) database used in the present study. De Carolis et al. (10) suggested that the improvement of sample processing by combined DT and EtOH-FA methods led to improved identifications of yeasts when the same method was used for the in-house database construction. In that study, a correct identification rate of 95.5% was obtained at the species level, and 4.5% were not identified with a cutoff value of 2.0 during the first run (10).

From a clinical perspective, it is important to know which method is preferred to obtain a reliable identification and whether one or more spots have to be used per tested strain. Our data show that when the DT, eDT, or EtOH-FA method is performed on two spots, the correct identification rates are 47.9%, 76%, and 88.6%, respectively. We also calculated that when only one spot per strain is available for identification, the correct identification rates are lower, namely, 38.9%, 67.1%, and 84.7%, by the DT, eDT, and EtOH-FA methods, respectively. The most recent evaluation of an eDT method for routine yeast identification by Gorton et al. (28) suggested that lowering the cutoff value from 2.0 to 1.9 allowed successful identification to the species level for 90% of isolates, with 7.4% of the isolates identified to the genus level only, while 2.6% remained unidentified after the first attempt. Dhiman et al. (27) suggested that when using only a single spot for the EtOH-FA method, only 6.8% or 1.4% of yeast isolates would require additional testing, depending on the cutoff values selected (>2 or >1.8, respectively). Stevenson et al. (29) applied yeast strains in quadruplicate and showed that 89.6% and 97.4% of the isolates were correctly identified on the first run and within the first two spots, respectively. They recommend to use duplicates to retest strains if their spectral scores were <1.8. Our data indicated preliminary yet promising application of the DT method for routine yeast identification, as appealing results were delivered by four laboratories using this method. Obviously, further optimization of this method is needed before it can be applied in all laboratories. Optimization may need to focus especially on the amount of cells used on the target plate, which may also differ between groups of yeasts.

Previous studies reported that discordant yeast identifications were observed mainly at the species level and only when comparing results from conventional biochemical methods with those obtained by MALDI-TOF MS (12, 14, 27). Discordant results require confirmation by molecular identification methods, e.g., sequencing of ribosomal DNA, to resolve problematic identifications. This is also true for obtaining a conclusive identification of

TABLE 4 Percentage of correct MALDI-TOF MS identifications per laboratory using the different extraction methods

Extraction method[a]	Identification	% correct (absolute no. of tested strains) MALDI-TOF MS identifications for laboratory:												
		1	2	3	4	5	6	7	8	9	10	11	12	13	Total
DT	Correct ID[b]	22.9 (8)	82.9 (29)	40.0 (14)	0.0 (0)	71.4 (25)	11.4 (4)	11.4 (4)	71.4 (25)	62.9 (22)	0.0 (0)	5.7 (2)	82.9 (29)	54.3 (19)	39.8 (181)
	Not performed	0.0 (0)	2.9 (1)	0.0 (0)	100.0 (35)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (35)	0.0 (0)	0.0 (0)	17.1 (6)	16.9 (77)
eDT	Correct ID	91.4 (32)	94.3 (32)	65.7 (23)	0.0 (0)	77.1 (27)	82.9 (29)	77.1 (27)	45.7 (16)	65.7 (23)	71.4 (25)	54.3 (19)	91.4 (32)	68.6 (24)	68.1 (310)
	Not performed	0.0 (0)	2.9 (1)	0.0 (0)	100.00 (35)	0.0 (0)	0.0 (0)	14.3 (5)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	17.1 (6)	10.3 (47)
EtOH-FA	Correct ID	91.4 (32)	91.4 (32)	94.3 (33)	88.6 (31)	82.9 (29)	91.4 (32)	94.3 (33)	62.9 (22)	94.3 (33)	71.4 (25)	88.6 (31)	94.3 (33)	82.9 (29)	86.8 (395)
	Not performed	0.0 (0)	2.9 (1)	0.0 (0)	2.9 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	2.9 (1)	0.0 (0)	0.0 (0)	17.1 (6)	2.0 (9)

[a] DT, direct transfer; eDT, extended direct transfer; EtOH-FA, ethanol-formic acid extraction.
[b] ID, identification.

strains that were identified with score values of <1.7 that did not allow a reliable identification or that were not identified (i.e., no peaks found). To our knowledge, no study has reported misidentification of yeast isolates with high MALDI-TOF MS scores. Cassagne et al. (2) reported that *C. neoformans* isolates were not identified using the DT and eDT methods, while identification succeeded when the EtOH-FA method was applied. In this study, one laboratory failed to correctly identify *C. curvatus* CBS 570^T using the eDT method. The strain was misidentified as the nonrelated *Cryptococcus uniguttulatus*, with scores of 2.258 in both databases, and we speculate that this may be caused by a mix-up of the isolate samples.

In conclusion, this multicenter study indicates that MALDI-TOF MS is a reliable system for identifying commonly occurring clinically relevant and more rare emerging yeast pathogens. Lowering the cutoff value to 1.7 is suggested for routine yeast identification at the species level. Differences in the methods used for sample preparation and the choice of databases have a significant impact on the accuracy of identifications in routine laboratory practice. In the future, further studies are needed to optimize the DT method, as this will greatly benefit routine practice in diagnostic (clinical) microbiology laboratories.

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The Multicenter Study Group comprised the following collaborators: Arjan Jansz, Department of Medical Microbiology, PAMM Laboratories, Veldhoven, the Netherlands; Bart de Koning, Department of Medical Microbiology, PAMM Laboratories, Veldhoven, the Netherlands; Dirk Faro, Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, the Netherlands; Julio García-Rodríguez, Servicio de Microbiología, Hospital Materno-Infantil, Hospital Universitario La Paz, Madrid, Spain; Greetje A. Kampinga, Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands; Markus Kostrzewa, Bioanalytical Development, Bruker Daltonics GmbH, Bremen, Germany; Ed J. Kuijper, Department of Medical Microbiology, Center of Infectious Diseases, University Medical Center of Leiden, Leiden, the Netherlands; Johannes G. Kusters, Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, the Netherlands; Cornelia Lass-Flörl, Division of Hygiene and Medical Microbiology, Innsbruck Medical University (MUI), Innsbruck, Austria; Jacques F. Meis, Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, the Netherlands, and Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, the Netherlands; Anna Mertens, Laboratory for Microbiology, ZNA Middelheim, Antwerp, Belgium; Niels Peterse, Medical Microbiology and Immunology Laboratory, Rijnstate Hospital, Velp, the Netherlands; Antonio Rezusta, IIS Aragón, Department of Microbiology, University Hospital Miguel Servet, Universidad de Zaragoza, Zaragoza, Spain; Bruno van Herendael, Laboratory Clinical Biology, Microbiology, University Hospital of Antwerp, Antwerp, Belgium; Sebastian van Marm, Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, the Netherlands; and Herman F. Wunderink, Department of Medical Microbiology, Center of Infectious Diseases, University Medical Center of Leiden, Leiden, the Netherlands.

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