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Performance and Cost Analysis of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Routine Identification of Yeast[▽]

Neelam Dhiman, Leslie Hall, Sherri L. Wohlfiel, Seanne P. Buckwalter, and Nancy L. Wengenack*

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota 55905

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Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry was compared to phenotypic testing for yeast identification. MALDI-TOF mass spectrometry yielded 96.3% and 84.5% accurate species level identifications (spectral scores, ≥1.8) for 138 common and 103 archived strains of yeast. MALDI-TOF mass spectrometry is accurate, rapid (5.1 min of hands-on time/identification), and cost-effective ($0.50/sample) for yeast identification in the clinical laboratory.

Candida is the fourth leading cause of nosocomial bloodstream infections in the United States (22). *Candida albicans* is the major species causing morbidity and mortality, but other, less common opportunistic yeasts, including non-*albicans Candida*, *Cryptococcus*, *Pichia*, *Rhodotorula*, *Trichosporon*, and *Saccharomyces* species have also been seen in immunocompromised settings (4, 15, 31). Accurate and rapid identification of yeasts is critical for treatment due to species-specific susceptibility patterns. Phenotypic identification can be time- and labor-intensive and can at times yield erroneous identifications (6, 20, 24, 30). Rapid latex agglutination assays are available, but for only a limited number of species (12, 23). Molecular assays are often accurate but can be expensive and complex.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has recently been described as a rapid, reliable, and cost-effective alternative for bacterial, mycobacterial, and fungal identification (1, 10, 19, 21, 27, 29). The technique relies on the generation of microorganism "protein fingerprints" that are compared to reference spectra in a well-characterized library (9).

In this study, we assessed the performance of the MALDI Biotyper 2.0 Microflex LT spectrometer (Bruker Daltonics, Inc., Billerica, MA) for the identification of common and uncommon yeasts (n = 261). A 1-month, blinded, prospective study included 145 freshly collected yeast isolates encountered in the routine laboratory workflow on various selective fungal isolation media, including Mycosel agar, brain heart infusion (BHI), Sabouraud's (SAB) agar, and inhibitory mold agar (IMA) (Becton Dickinson, Sparks, MD) (17). *C. albicans* was identified by germ tube formation, and *Candida glabrata* was identified by the rapid-assimilation-of-trehalose (RAT) test (17). Both tests have inherent limitations, including false-positive germ tube formation by non-*albicans* species (e.g., *Candida dubliniensis*) and the need to carefully control the inoculum size (11, 16). Round, variably-sized yeasts resembling *Cryptococcus* were identified at the species level using urea and pigment production methods, as described previously (14). All other yeasts were identified using the API 20C AUX yeast identification system (bioMérieux, Hazelwood, MO) and microscopic morphology on cornmeal agar with Tween 80 (17). Additionally, 116 strains (IMA subcultures at 30°C) from an archived collection of less common yeasts that were previously identified using API 20C and D2 sequencing were identified using MALDI-TOF MS (13).

MALDI-TOF MS was performed on 2 or 3 yeast colonies picked using a 1-μl inoculating loop into 1 ml of 70% ethanol (Sigma-Aldrich, St. Louis, MO). The suspension was pelleted, dried, and reconstituted in 50 μl of 70% formic acid (Sigma-Aldrich) and 50 μl of acetonitrile (Sigma-Aldrich). Two microliters of supernatant was applied in duplicate to a 24-spot BigAnchor steel plate (Bruker Daltonics, Inc.) and dried. Two microliters of MALDI matrix (α-cyano-4-hydroxycinnamic acid [HCCA; Bruker Daltonics, Inc.]) was applied to the spot and dried. Each run included a negative extraction control, bacterial standard, and two QC organisms (*C. albicans* ATCC 201148 and *Candida tropicalis* ATCC 13803). Mass spectrometry was performed with the MALDI Biotyper 2.0 Microflex LT system, using the manufacturer's settings. Briefly, ions in a mass range of 2 to 20 kDa were generated with a 337-nm nitrogen laser and were captured in positive linear mode using 240 laser shots. Captured spectra were analyzed using Flex control 3.0 and MALDI Biotyper automation control software. The generated spectrum of biomarkers for each sample was then compared with 3,740 spectra of well-characterized organisms in the Bruker library (Reference Library version 3.0). Failures were repeated using the routine methods and MALDI-TOF MS. Discordant results were resolved using sequence analyses of the D2 region of the 28S ribosomal target as previously described (13).

Of the 261 isolates, 20 were excluded, as they were not present in the MALDI Biotyper database (Tables 1 and 2). In the prospective study, the MALDI Biotyper correctly identified 92.0% and 96.3% of isolates at the species level using scores of ≥2.0 and ≥1.8, respectively (Table 1). Nine discordant results were resolved in favor of the MALDI Biotyper using D2 sequencing. The MALDI Biotyper performed better

* Corresponding author. Mailing address: Mayo Clinic, Hilton 860A, 200 First Street SW, Rochester, MN 55905. Phone: (507) 284-3021. Fax: (507) 284-9859. E-mail: wengenack.nancy@mayo.edu.

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TABLE 1. Yeast identification using a MALDI Biotyper for common isolates in the prospective study

Phenotypic identification (no. of isolates tested)	No. of Isolates with Bruker score[b] of:				Discordant identification (no. of isolates)[a]
	<1.7	1.7–1.799	1.8–1.999	≥2.0	
Aureobasidium/*Hormonema* sp. (3)	2	1			
C. albicans (81)			2	79	*C. dubliniensis* (2)
C. glabrata (16)				16	
C. guilliermondii (4)	1			3	
C. kefyr (2)				2	
C. krusei/*C. inconspicua* (4)				4	*C. krusei* (4)
C. lusitaniae (3)				3	
C. parapsilosis (11)			2	9	
C. pelliculosa (1)				1	
Candida spp. (3)				1	*C. glabrata* (1)
				2	*C. lipolytica* (2)
C. tropicalis (5)			1	4	
Cr. neoformans (1)	1				
Pseudozyma sp. (1)			1		
S. cerevisiae (3)				3	
Total[c] (138) (%)	4 (2.9)	1 (0.7)	6 (4.3)	127 (92.0)	

[a] All discordant results were resolved using D2 sequencing.

[b] Results from two replicates and repeat testing. The numbers of isolates (n = 11) that resolved on repeat testing at the species level were as follows: for *C. albicans*, 6; for *C. krusei*, 1; for *C. pelliculosa*, 1; for *C. lusitaniae*, 2; and for *C. tropicalis*, 1.

[c] The numbers of isolates (n = 7) excluded from the study, as the isolates were not present in the Bruker library, were as follows: for *C. naganishii*, 1; for *Cryptococcus* sp. (not *C. neoformans*), 3; for *C. sojae*, 1; and for *Rhodotorula minuta*, 2.

TABLE 2. Yeast identification using a MALDI Biotyper for uncommon isolates in the retrospective study

Organism (no. of isolates tested)	No. (%) of isolates with Bruker score[a] of:			
	<1.7	1.7–1.799	1.8–1.999	≥2.0
Blastoschizomyces capitatus (1)	1			
C. albicans (4)	1			3
C. colliculosa (3)	3			
C. dubliniensis (4)				4
C. famata (4)				4
C. glabrata (1)				1
C. guilliermondii (6)		1		5
C. inconspicua (3)	2			1
C. kefyr (1)				1
C. krusei (10)				10
C. lambica (2)			1	1
C. lipolytica (3)				3
C. lusitaniae (3)				3
C. nivariensis (2)				2
C. norvegensis (1)				1
C. orthopsilosis (1)				1
C. parapsilosis (8)	1			7
C. pararugosa (1)				1
C. pelliculosa (4)				4
C. tropicalis (7)				7
C. utilis (2)				2
C. zeylanoides (1)				1
Cr. albidus (1)	1			
Cr. laurentii (2)			2	
Cr. neoformans (6)	2	2		2
Lodderomyces elongisporus (1)				1
P. anomala (1)				1
Rothia mucilaginosa (3)				3
S. cerevisiae (11)				11
T. asahii (3)	1	1		1
T. mucoides (3)				3
Total[b] (103)	12 (11.6)	4 (3.9)	3 (2.9)	84 (81.6)

[a] Results from two replicates and repeat testing. The numbers of isolates (n = 6) that resolved on repeat testing were as follows: *C. albicans*, 1; *C. dubliniensis*, 1; *C. lambica*, 1; *C. lipolytica*, 2; and *C. nivariensis*, 1.

[b] The numbers of isolates (n = 13) excluded from the study, as the isolates were not present in the Bruker library, were as follows: *C. bracarensis*, 1; *C. sphaerica*, 1; *C. viswanathii*, 1; *Cr. bhutanensis*, 1; *Cr. gattii*, 4; *Geotrichum klebahnii*, 1; *Prototheca zopfii*, 1; *Kloeckera apis*, 1; *Nakaseomyces delphensis*, 1; and *P. salicaria*, 1.

than routine methods for identification of *Candida krusei* (n = 4 isolates), where biochemical methods could not distinguish between *C. krusei* and *Candida inconspicua*. *Candida dubliniensis* (n = 2 isolates) was also correctly identified by the MALDI Biotyper (score, ≥1.8), while it was misidentified as *C. albicans* with the use of the traditional methods, likely due to the close phylogenetic relationship between these two species (3, 18).

Our results are comparable with those obtained in previously published studies, where Marklein et al. achieved 92.5% and Stevenson et al. reported 87.1% species level identification using a Bruker score of ≥2.0 (19, 27). Further, by lowering the score threshold to ≥1.8, Stevenson et al. (27) were able to increase the species level identification to 99%. In our study, the score of ≥1.8 improved accurate identifications at the species level from 92.0% to 96.3%. Since spectral scores between 1.8 and 2.0 consistently gave accurate identification to the species level with no misidentifications, a threshold of ≥1.8 for species identification may be appropriate in a clinical laboratory. Repeat MALDI testing was required to obtain a spectral score of ≥2.0 for 11 isolates, and possible explanations may include the presence of mixed cultures or bacterial contamination in direct specimens. Further, different medium types and culture times may also influence the spectral quality as previously reported (2, 28). In our experience, culture on BHI agar and IMA routinely provided robust spectra, SAB agar was also satisfactory but a bit less robust, and, as expected, Mycosel agar failed to support the growth of some strains. Little difference was noted due to incubation time, but slight loss of spectral quality was noted between day 4 and day 8 for some isolates. However, our ability to identify yeasts from patient specimens cultured on different media for various amounts of time (1 to 8 days) without the need for subculture is important, as it reflects the routine diagnostic laboratory workflow. Further, by lowering the score to ≥1.8, repeat testing would be eliminated. Unreliable identifications were obtained for *Aureobasidium*/*Hormonema* sp. (n = 2 isolates), *Candida guilliermondii* (n = 1 isolate), and *Cryptococcus neoformans* (n = 1 isolate) (Table 1). The study by Stevenson et al. also indicated difficulty with *C. guilliermondii* and *Cr. neoformans*, correctly identifying 66% and 50% of isolates, respectively (27). This may be due to inherent properties of isolates that result in poor-quality spectra or insufficient database entries to allow robust spectral matches.

In the retrospective study, 81.6% and 84.5% of the isolates were identified at the species level using scores of ≥2.0 and ≥1.8, respectively (Table 2). The isolates not identified reliably (*Blastoschizomyces capitatus*, *Candida colliculosa*, *C. inconspicua*, *Cr. albidus*, and *Trichosporon asahii*) are rare and often require molecular methods for identification at the species level (3, 6, 8, 26, 32). *Cr. neoformans* also had lower identification scores, which may be attributable to its thick capsule, which makes extraction and solubilization of proteins difficult. Species not currently in the MALDI Biotyper database, specifically *Cryptococcus gattii*, an emerging human pathogen in the United States (7), need entries for improved performance.

During this study, all isolates were assayed in duplicate.

TABLE 3. Time and cost estimates per isolate for routine identification versus those for identification using the MALDI Biotyper

Method	Hands-on time per specimen (min)	Total turnaround time per specimen (h)	Cost of materials per specimen ($)
API 20C AUX yeast identification system	6.5	48–72	6.86
Germ tube	4.4	2.5	0.80
Rapid assimilation of trehalose	6.4	1.5–19.5[a]	0.95
Sequencing	8[b]	3.5[b]	20.02
MALDI Biotyper	5.1	0.64	0.50

[a] Subculture to Sabouraud's agar may be required to obtain adequate growth to perform the test.

[b] For practical purposes, laboratories often batch specimens rather than sequencing a single isolate, so the total hands-on time and turn-around time are longer if the specimens are batched.

Overall, 6.8% and 1.4% isolates required a second spot for resolution using cutoffs of ≥2.0 and ≥1.8, respectively. Therefore, it might be more efficient to perform all testing using single spot in a clinical laboratory, and a fraction of specimens with borderline spectral scores can be repeated from saved extracts.

Finally, we performed a cost analysis to determine whether the MALDI Biotyper is economically competitive for the diagnostic laboratory. We calculated a reagent cost of $0.50 and an average hands-on-time of 5.1 min per isolate for identification (Table 3). These values are lower than those for conventional and other molecular testing and are in agreement with previous studies (5, 25). The cost of the instrument and software ($150,000) is comparable to that for DNA-sequencing platforms. The same system can be used for bacterial, fungal, and mycobacterial identification and may replace sequencing of unusual isolates in some laboratories, providing a cost-effective, consistent platform and streamlining workflow. Also, MALDI-TOF MS can be used to eliminate many supplementary biochemical tests and the associated quality control testing.

In conclusion, MALDI-TOF MS is an economical, rapid, and reliable method of yeast identification that has the potential to revolutionize clinical diagnostics. Advances in the technology may offer future promise for direct testing of specimens and determination of drug resistance and other genotypic markers.

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