



# Comparative evaluation of two matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems for the identification of clinically significant yeasts



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## SUMMARY

**Objectives:** To prospectively evaluate the performance of two matrix-assisted laser desorption/ionization time-of-flight mass spectrometry systems (MALDI-TOF MS) for the identification of clinically significant yeast isolates compared to the VITEK 2 system.

**Methods:** One hundred and eighty-eight consecutive yeast isolates were analyzed by Bruker Biotyper and VITEK MS. The results were compared with the conventional VITEK 2 yeast identification system. Discrepant results were resolved by direct sequencing of rDNA.

**Results:** Accurate identification by VITEK 2, VITEK MS, and Bruker Biotyper MS was 94.1% (177/188), 93.0% (175/188), and 92.6% (174/188), respectively. Three isolates were not identified by VITEK MS, while nine *Candida orthopsilosis* were misidentified as *Candida parapsilosis*, as this species is not present in its database. Eleven isolates were not identified or were wrongly identified by Bruker Biotyper and although another 14 were correctly identified, the score was unreliable at <1.7.

**Conclusion:** The overall accuracy of rapid MALDI-TOF MS systems was essentially comparable to that of the conventional VITEK 2 yeast identification system. However, future expansion of the databases may further improve the outcome and accuracy of identification of yeast species.

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## 1. Introduction

Invasive fungal infections due to opportunistic pathogens cause significant mortality and morbidity, especially in immunocompromised and critically ill patients.<sup>1,2</sup> In the USA, *Candida* species are the fourth most common cause of healthcare-associated blood stream infection, with an associated mortality rate of 39.2%.<sup>3,4</sup> Although *Candida albicans* is the most commonly identified pathogenic yeast species, other non-albicans *Candida* species and species of other genera have also been reported as causative agents of invasive infections.<sup>4</sup> The rapid identification of pathogenic species is helpful to start timely and effective antifungal therapy. This rapid identification will narrow the spectrum of therapeutic options, conceivably prevent treatment with toxic antifungal agents, improve the outcome, and reduce costs.<sup>4</sup> Using conventional methods to identify yeasts takes 2–5 days. These phenotypic

methods may not identify or may misidentify some yeast species.<sup>5</sup> Molecular methods, e.g., 18S rDNA or internal transcribed spacer (ITS) region sequencing, identify yeasts to the species level but are time-consuming, tedious, technically demanding, and not available in many diagnostic microbiology laboratories.<sup>6,7</sup>

Due to the above limitations, recent studies had focused on alternative techniques to rapidly identify yeasts, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).<sup>8–13</sup> The aim of this study was to evaluate the performance of two MALDI-TOF MS systems for the rapid identification of clinically relevant yeast isolates to the species level and to compare the results with the routine conventional methods.

## 2. Materials and methods

### 2.1. Sample collection

A total of 188 clinically relevant fungal isolates were obtained during 1 year of routine laboratory processing of clinical specimens in the Microbiology Unit, Mubarak Al Kabir Hospital, Kuwait. The

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isolates were collected in sequential order without selection criteria. Duplicate isolates from the same patient were excluded. The isolates were obtained from blood culture, bronchoalveolar lavage, cerebrospinal fluid, urine, wound, and high vaginal and endocervical swabs. The isolates were cultured on Sabouraud dextrose agar (SDA, Oxoid, Basingstoke, Hampshire, UK) and incubated for 48–96 h at 30 °C.

## 2.2. Identification by VITEK 2

The identification of the clinical yeast isolates was initially achieved by VITEK 2 system (bioMérieux) using YST ID (Card No. 21343). When necessary, one or more tests were also performed, e.g., morphology on SDA, germ tube test for *Candida* species, and urease assimilation test for *Cryptococcus* species (Becton, Dickinson and Company, Sparks, MD, USA).

## 2.3. Protein extraction

Prior to MALDI-TOF MS analyses, a single colony from a fresh culture was suspended in 1 ml of 70% ethanol, briefly vortexed, and centrifuged at 13 000 g for 2 min. The supernatant was removed, the pellet quickly spun again, and the residual ethanol removed. The pellet was re-suspended in 50 µl of 70% formic acid (Fluka, USA) and 50 µl acetonitrile (Fluka). The sample was vortexed briefly and the mixture centrifuged for 2 min at 3000 g.

## 2.4. MALDI-TOF Bruker MS (Bruker Biotyper; Bruker Daltonics, Bremen, Germany)

One microliter of sample suspension was spotted onto a 96-spot reusable stainless steel target plate (Bruker Daltonics) in duplicate, allowed to evaporate, and dried at 37 °C on a plate warmer; this was then overlaid with 2 µl of matrix solution (Bruker Daltonics) and air-dried. The loaded plate was applied to the instrument in accordance with the manufacturer's instructions and analyzed by Bruker Microflex LT system using FlexControl software (version 3.3). The spectrum of each isolate was compared with those in the database and the identification was provided by score of reliability. Identification was provided with accompanying scores as per the manufacturer's schemes: <1.7 = no reliable identity; ≥1.7 and <2.0 = identity at genus level; ≥2.0 = identity at species level.

## 2.5. MALDI-TOF VITEK MS (VITEK MS; bioMérieux, Marcy l'Etoile, France)

The suspension was tested in duplicate on the MALDI plate. One microliter of the extracted supernatant was transferred onto an individual spot on the 48-well VITEK MS disposable target slide. Each spot was covered with 1 µl ready-to-use VITEK MS HCCH matrix (bioMérieux) and air-dried. The loaded slides were inserted into the VITEK MS machine. The VITEK mass spectrometer was used to generate spectra of the yeast suspension; Biotyper software (version 2.0) was used to analyze the results. Microbial identification was achieved by analyzing the spectra with the VITEK MS database. The peaks from these spectra were compared with the characteristic pattern for the species, genus, or family of the microorganism, leading to identification. The results were evaluated according to a colored index: green means ≥90% identity, yellow 85–89.9% identity, and white <85% identity. All of the identifications to the genus or species level fell into the green zone, with a score of >90% considered reliable. Scores between 85% and 90% were considered acceptable identification. A cut-off of 90% was chosen for the VITEK MS.

## 2.6. Quality control

*Escherichia coli* ATCC 8739 was included as a positive control in each run in both MALDI-TOF MS systems, in accordance with the manufacturer's recommendations. Uninoculated matrix was included in each run as a negative control. For standardization purposes, positive controls were included in each run (*Candida albicans* CBS 5314, *Candida albicans* CBS 1893, and *Candida glabrata* CBS 7904).

## 2.7. DNA isolation and sequencing

DNA was extracted from colonies of yeast isolates and grown on SDA plates, using the QIAamp DNA Kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. PCR and sequencing of the ITS region (including ITS-1, 5.8S rRNA, and ITS-2) and D1/D2 domains of the large subunit (LSU) of rDNA were performed as described previously.<sup>14</sup> The sequences obtained were compared with data available in the GenBank database using nucleotide Basic Local Alignment Search Tool (BLASTn; <http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi>). A threshold of ≥99% sequence identity was used for identification at the species level.

## 2.8. Criteria for final species identification

The final identification was as follows: accurate identification of the species was confirmed if all three methods yielded the same species identity. When discrepant results were observed for the three methods, or when only two of the three methods yielded different identification, molecular identification based on direct DNA sequencing was considered as the gold standard for final species identity.

# 3. Results

## 3.1. Final identification of the isolates

For 163 of 188 (86.7%) isolates, the use of the conventional VITEK 2 method and the two MALDI-TOF systems resulted in accurate identification of 48 *Candida parapsilosis*, 30 *Candida tropicalis*, 27 *C. glabrata*, 27 *C. albicans*, 15 *Candida krusei*, 2 *Candida dubliniensis*, 4 *Candida lusitanae*, 2 *Candida kefyr*, 4 *Geotrichum capitatum*, 2 *Candida intermedia*, 1 *Candida guilliermondii*, and 1 *Trichosporon asahii* (Table 1). For the remaining 25 (13.3%) isolates, the final identification was done via direct DNA sequencing (Table 2). The DNA sequences have been submitted to the European Molecular Biology Laboratory (EMBL) under accession numbers **HG970734** to **HG970744**.

## 3.2. Performance of each identification method

Discrepancies in the errors in the three systems of identification are given in Table 1.

The VITEK 2 system and other conventional tests could accurately identify 177 of 188 (94.0%) isolates. They did not identify one *C. guilliermondii* and misidentified one *Pichia veronae* isolate as *Candida freyschussii* and nine *Candida orthopsilosis* as *C. parapsilosis*.

As shown in Table 1, VITEK MS was able to accurately identify 175 of 188 (93.1%) isolates. It misidentified nine *C. orthopsilosis* as *C. parapsilosis* because it was not present in its database. It failed to identify one *Cryptococcus neoformans*, one *Saccharomyces cerevisiae*, and one *Rhodotorula mucilaginosa* isolate. It misidentified one *P. veronae* isolate as *C. freyschussii*.

The Bruker Biotyper could identify 174 of 188 (92.6%) isolates, but accurate identification with a high score was achieved for only

**Table 1**

List of species and results of identification (ID) using Biotyper and VITEK MS and conventional ID (VITEK 2)

Species (n)	Conventional ID			Biotyper ID			VITEK MS ID		
	Correct	No ID	False	Correct	No ID	False	Correct	No ID	False
<i>Candida parapsilosis</i> (48)	48	0	0	48	0	0	48	0	0
<i>Candida orthopsilosis</i> (9)	0	0	9	0	6 (<1.7)	3	0	0	9
<i>Candida tropicalis</i> (33)	33	0	0	30	3 (<1.7)	0	33	0	0
<i>Candida glabrata</i> (28)	28	0	0	27	1 (<1.7)	0	28	0	0
<i>Candida albicans</i> (27)	27	0	0	27	0	0	27	0	0
<i>Candida krusei</i> (15)	15	0	0	15	0	0	15	0	0
<i>Candida dubliniensis</i> (6)	6	0	0	2	4 (<1.7)	0	6	0	0
<i>Candida famata</i> (1)	1	0	0	0	0	1	1	0	0
<i>Candida lusitanae</i> (4)	4	0	0	4	0	0	4	0	0
<i>Candida kefyr</i> (4)	4	0	0	2	0	2	4	0	0
<i>Geotrichum capitatum</i> (4)	4	0	0	4	0	0	4	0	0
<i>Candida intermedia</i> (2)	2	0	0	2	0	0	2	0	0
<i>Candida guilliermondii</i> (2)	1	1	0	1	0	1	2	0	0
<i>Pichia veronae</i> (1)	0	0	1	0	0	1	0	0	1
<i>Trichosporon asahii</i> (1)	1	0	0	1	0	0	1	0	0
<i>Saccharomyces cerevisiae</i> (1)	1	0	0	0	1	0	0	1	0
<i>Cryptococcus neoformans</i> (1)	1	0	0	0	1	0	0	1	0
<i>Rhodotorula mucilaginosa</i> (1)	1	0	0	0	1	0	0	1	0
Total (188) (%)	177 (94.1)	1	10 (5.3)	163 (86.7)	17 (9.0)	8 (4.3)	175 (93.1)	3 (1.5)	10 (5.3)

**Table 2**

Discrepancies and error in the MALDI-TOF (Bruker MS and VITEK MS) for identification (ID) of yeast

Species (n)	Biotyper ID	VITEK MS ID	Sequence identity (%)	Remarks
<i>Candida orthopsilosis</i> (9)	6 score <1.7; 3 <i>Candida parapsilosis</i>	<i>C. parapsilosis</i>	>99	VITEK MS: not in database
<i>Candida tropicalis</i> (3)	Score <1.7	Correct ID	>99	Biotyper: unacceptable ID
<i>Candida glabrata</i> (1)	Score <1.7	Correct ID	>99	Biotyper: unacceptable ID
<i>Candida dubliniensis</i> (4)	Score <1.7	Correct ID	>99	Biotyper: unacceptable ID
<i>Candida kefyr</i> (2)	<i>Pseudomonas</i> , <i>Staphylococcus</i>	Correct ID	>99	Biotyper: wrong ID
<i>Candida famata</i> (1)	<i>Debaryomyces</i>	Correct ID	>99	Biotyper: wrong ID
<i>Candida guilliermondii</i> (1)	<i>Nocardia spp</i>	Correct ID	>99	Biotyper: wrong ID
<i>Pichia veronae</i> (1)	<i>Pseudomonas</i>	<i>Candida freyschussii</i>	>99	Biotyper: wrong ID
<i>Saccharomyces cerevisiae</i> (1)	1	1	>99	No ID by both systems
<i>Cryptococcus neoformans</i> (1)	1	1	>99	No ID by both system
<i>Rhodotorula mucilaginosa</i> (1)	1	1	>99	No ID by both system

163 of 188 isolates (86.7%) (Table 2). It did not identify one *C. neoformans*, one *S. cerevisiae*, and one *R. mucilaginosa* isolate. One *Candida famata* was misidentified as *Debaryomyces spp*, two *C. kefyr* were misidentified as *Pseudomonas spp* and *Staphylococcus spp*, one *C. guilliermondii* was wrongly identified as *Nocardia spp*, and one *P. veronae* as *Pseudomonas spp*. Three of nine *C. orthopsilosis* were identified as *C. parapsilosis*; the identification of the remaining *C. orthopsilosis* was with an unreliable score of <1.7. Similarly, three *C. tropicalis*, four *C. dubliniensis*, and one *C. glabrata* were also identified correctly, but with an unreliable score of <1.7.

#### 4. Discussion

In this study, correct identification by morphological and conventional tests (VITEK 2) was achieved for 94.1% of the isolates, a finding higher than previously reported: 84% (via API ID 32 C, bioMérieux),<sup>15</sup> 75% (via API ID 32 C or Rapid ID yeast),<sup>11</sup> 91.5% (via API ID 32 C),<sup>16</sup> and 77% (via API).<sup>13</sup> However, our report is concordant with a previous report by Valenza et al., who reported that 93.7% of yeasts can be identified satisfactorily by VITEK 2 with simple additional tests.<sup>17</sup>

MALDI-TOF MS systems have recently been developed and implemented in diagnostic microbiology laboratories for species-specific identification of bacterial and fungal pathogens due to their rapidity, minimum hands-on time, efficacy, and overall cost-effectiveness. The MALDI-TOF MS systems provide objective results for the identification of most yeast species and may also

help to standardize laboratory tests, which are crucial for strengthening the laboratory services in limited-resource areas. However, their optimal use is dependent on the panel of bacterial and yeast reference strains included in their databases. For these reasons, the accuracy of the MALDI-TOF MS systems in the identification of yeasts has varied considerably as compared to the conventional techniques in some studies. For instance, the accuracy of the Bruker Biotyper has been found to vary from 84% to 99%,<sup>12,15,16</sup> while the accuracy of the VITEK MS system has been found to be somewhat lower (84.3–95%)<sup>18–20</sup> compared to the conventional techniques.

In this study, the identification rate was 93.0% for the VITEK MS and 92.6% for the Bruker Biotyper. These findings are slightly lower than those reported in recent studies in which the identification rates for yeast have typically ranged between 97.6% and 98.3%.<sup>8,16,21</sup> Other studies have reported a lower rate of identification of yeasts in the range of 84–89.8% without major errors at the species level via Bruker Biotyper<sup>11,13,19</sup> and 84.3–87.2% via VITEK MS.<sup>18,19</sup>

The VITEK MS failed to identify nine *C. orthopsilosis* isolates, as this species is not included in its database. Some studies have either included very few isolates (e.g., *C. orthopsilosis*) that are not represented in the MALDI-TOF MS database or excluded them while calculating accurate identification.<sup>9,22</sup> If the nine *C. orthopsilosis* isolates are excluded, the accuracy of VITEK MS in the correct identification of yeasts in the present study increases to 97.8%, with only one misidentification.

The Bruker Biotyper could identify 174 of 188 (92.6%) isolates, but accurate identification (score of  $\geq 2.0$ ) was achieved for only 163 of 188 (86.7%) isolates. Six *C. orthopsilosis*, three *C. tropicalis*, four *C. dubliniensis*, and one *C. glabrata* were identified with an unreliable score of  $< 1.7$ . In some studies, repeat testing or extra extraction steps were required for several isolates by Bruker Biotyper to obtain a spectral score of  $> 2.0$ .<sup>9,12,22</sup> We did not find repetition of these steps necessary, as it would involve additional time and resources and would affect routine flow in the clinical microbiology laboratory. The lower scores for some isolates might presumably be because we used SDA, which has been shown previously to perform less optimally compared to brain–heart infusion agar or inhibitory mold agar media, although it is more economical and is most widely used in routine microbiology laboratories.<sup>22,23</sup> Some investigators have argued for lowering the threshold score for the Bruker Biotyper to  $\geq 1.8$  or even to 1.7 without affecting accurate identification.<sup>9,12,22</sup> Our results also show that the cut-off for the Bruker Biotyper can safely be lowered to 1.7 without affecting the accuracy of species-specific identification and this will diminish the need for additional extraction steps during MALDI-TOF MS analyses.

In conclusion, MALDI-TOF MS methods provide a standardized working protocol for the identification of yeasts from clinical specimens. The short turn-around time and expandability of the database demonstrate that this is a suitable first-line test for the identification of yeasts in the routine clinical microbiology laboratory.

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