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Evaluation of VITEK 2 and RapID Yeast Plus Systems for Yeast Species Identification: Experience at a Large Clinical Microbiology Laboratory[▽]

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A total of 750 clinical yeast isolates were evaluated by two identification systems, VITEK 2 and RapID Yeast Plus, using sequence analysis of the rRNA gene internal transcribed spacer regions as the reference method. The VITEK 2 and RapID systems correctly identified 737 (98.2%) and 716 (95.5%) isolates, respectively.

Although *Candida* species, including the pathogenic *Candida albicans*, remain the yeast species most commonly encountered in a clinical microbiology laboratory, a variety of other yeasts are recovered from patients with well-documented infections. Accurate identification of these species is clinically important, as certain yeast species are associated with specific diseases (11, 22). In addition, yeast species can differ greatly in their relative virulence levels (22) as well as their susceptibilities to antifungal agents (19). While there is an increasing move toward molecular biology-based diagnostic approaches (1), identification of clinical yeast isolates is still typically performed by biochemical, morphological, and physiological tests (8). These phenotypic systems often produce results that may not be accurate, so their performance must be reassessed to enable users to have a reliable system for yeast identification. In this study, we compared the VITEK 2 (bioMérieux VITEK, Marcy l'Etoile, France) and RapID Yeast Plus (Remel Inc., Lenexa, KS) systems, with sequence analysis of the rRNA gene internal transcribed spacer (ITS) regions used as the reference method, for the identification of medically important yeasts typically found in a large clinical microbiology laboratory.

A total of 750 yeast isolates, representing 24 species of six genera, were studied. Isolates were obtained from clinical samples (oral, vaginal, anorectal, urine, stool, blood, central venous catheter, and respiratory tract specimens) from in- or outpatients from January 2006 through June 2006. *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 2001, *Candida lusitaniae* ATCC 34449, *Candida parapsilosis* ATCC 22019, and *Candida krusei* ATCC 6258 were used as quality control strains. Isolates were grown on Sabouraud dextrose agar (Kima, Padua, Italy) for 48 h at 30°C prior to being tested. Each isolate was simultaneously tested with the VITEK 2 system (version 4.02), using the new colorimetric YST card (BioMérieux), and with the RapID Yeast Plus system (version 1.95), according to the instructions of their respective manufacturers, and the results were compared. In cases of discrepant results, both methods were repeated and the results for the second runs were accepted as the final results. In cases of identification with low discrimination (see below), additional tests (e.g., microscopic morphology on cornmeal-Tween 80 agar or growth at 42 to 45°C) were carried out (3). ITS sequence analysis was performed on all 750 isolates and used as the reference system. Thus, purified genomic DNA was obtained from each yeast isolate, using an EZ1 DNA tissue kit (QIAGEN, Milan, Italy) and a BioRobot EZ1 workstation (QIAGEN) in accordance with the manufacturer's instructions. This included a preliminary step in which yeast colonies were resuspended in 190 μl of buffer G2, 10 μl of lyticase (25 units/μl) was added to each cell sample, and the resulting mixture was incubated at 30°C for 30 min. The extracted DNA was stored at −20°C for further use. The ITS1-5.8S-ITS2 region of the rRNA gene was PCR amplified from a 1:50 dilution of template DNA in a total reaction volume of 50 μl consisting of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.8 mM deoxynucleoside triphosphates (0.2 mM each), 1.2 U of *Taq* DNA polymerase, and 0.5 μM (each) of the fungus-specific universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (21). The following thermal conditions were used: 94°C for 3 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min and 30 s, followed by a final extension step at 72°C for 10 min. Amplicons were purified with a Minielute PCR purification kit (QIAGEN) and sequenced on both strands with primer ITS1 or ITS4 and a BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) on an ABI Prism 3100 genetic analyzer (Applied Biosystems). Species were identified by searching databases using the BLAST sequence analysis tool (http://www.ncbi.nlm.nih.gov/BLAST/). An isolate was assigned to a species if it had ≥99% sequence homology to a sequence entry available in the GenBank database and if the next species showed less than 95% homology over the whole length of the sequence. For each isolate, results from the VITEK 2 and RapID Yeast Plus systems were compared with the data obtained by the reference method and assigned to one of four categories: (i) identified, in which the isolate was correctly identified to the species level or identified with low discrimination and resolved by additional tests; (ii) low discrimination, in which the isolate was

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TABLE 1. Identification results obtained with the VITEK 2 system for 750 isolates

Species (no. tested)	No. (%) of isolates with indicated result			
	Identified	Low discrimination[a]	Misidentification	Unidentified
Candida albicans (258)	252	0	2	4
Candida dubliniensis (14)	14	0	0	0
Candida famata (19)	19	0	0	0
Candida glabrata (160)	159	0	1	0
Candida guilliermondii (4)	4	0	0	0
Candida haemulonii (1)	1	0	0	0
Candida intermedia (1)	1	0	0	0
Candida kefyr (23)	23	0	0	0
Candida krusei (30)	27	1	2	0
Candida lipolytica (4)	3	0	1	0
Candida lusitaniae (14)	14	0	0	0
Candida norvegensis (4)	4	0	0	0
Candida parapsilosis (70)	69	0	1	0
Candida rugosa (4)	4	0	0	0
Candida tropicalis (74)	74	0	0	0
Candida utilis (3)	3	0	0	0
Candida zeylanoides (5)	5	0	0	0
Cryptococcus uniguttulatus (1)	1	0	0	0
Geotrichum capitatum (3)	3	0	0	0
Geotrichum klebahnii (1)	1	0	0	0
Pichia anomala (1)[b]	1	0	0	0
Saccharomyces cerevisiae (50)	48	1	1	0
Trichosporon asahii (5)	5	0	0	0
Trichosporon mucoides (1)	1	0	0	0
Total	737 (98.2)	2 (0.3)	8 (1.0)	4 (0.5)

[a] This category includes isolates identified with low discrimination and not resolved by supplemental tests.
[b] Formerly known as *Hansenula anomala*, this species is included as *Candida pelliculosa* in the VITEK 2 database.

identified with low discrimination and not resolved by additional tests; (iii) misidentification, in which a discrepant result was obtained with regard to the reference method; or (iv) no identification, in which the isolate could be not identified (e.g., unknown profile). Isolates producing misidentifications were further analyzed for species clarification through the use of MicroSeq D2 LSU rDNA fungal sequencing kits (Applied Biosystems), which allowed us to amplify and sequence the D2 expansion segment region of the large-subunit rRNA gene. The procedures for DNA isolation, PCR amplification, and cycle sequencing were the same as those recommended by the manufacturer. The resulting DNA sequences were analyzed using MicroSeq ID analysis software and compared to those in a fungal gene library containing D2 sequence entries from more than 1,000 validated species. Distance scores of 0.00% (100% match) to ≤1.00% (99% match) were used as a guide for identification, and the species giving the closest match was considered the most likely correctly identified (10).

Using ITS sequence analysis as the reference method, 737 isolates out of 750 (98.2%) were correctly identified to the species level by the VITEK 2 system, including those isolates identified with low discrimination but resolved by supplemental tests. Two isolates (0.3%) were identified with low discrimination and not resolved by additional tests, eight isolates (1.0%) were misidentified, and four isolates (0.5%) could not be identified by the VITEK 2 system (Table 1). Conversely, 716 isolates out of 750 (95.5%) were unequivocally identified to the species level by the RapID Yeast Plus system, including those isolates identified with low discrimination but resolved by additional tests. Eighteen isolates (2.4%) were misidentified, and 16 isolates (2.1%) could not be identified by the RapID Yeast Plus system (Table 2).

Among the 24 yeast species evaluated in this study, *C. albicans*, *C. glabrata*, *Candida tropicalis*, *C. parapsilosis*, and *C. krusei* are the species most commonly encountered in a typical clinical laboratory. The isolates belonging to these species were correctly identified at frequencies of approximately 98% and 100% by the VITEK 2 and RapID Yeast Plus systems, respectively. Overall, a slight superiority of the RapID Yeast Plus system over the VITEK 2 system was noted.

The data obtained in this study were similar to those from previous reports (2, 6, 9, 12, 15, 17–18, 20) showing that the VITEK 2 and RapID Yeast Plus systems were comparable to other commercial methods (e.g., API 20C AUX and ID 32C) in their abilities to correctly identify yeast species. The VITEK 2 colorimetric YST card was recently developed to replace the older fluorimetric ID-YST card, yet a recent study conducted by Loïez et al. (15) on 172 clinical yeast isolates showed that 161 (93.6%) and 144 (83.7%) isolates were correctly identified with the ID-YST and YST cards, respectively. These results were in contrast to those from Aubertine et al. (2), which indicated that the new colorimetric methodology consistently performed better than the old fluorimetric system (94.8% and 83.5% correct identifications, respectively). As stated by Loïez et al. (15), the disagreement observed between the two studies, which had in common only 10 taxa of *Candida* spp., might be a result of the geographic origins of the isolates tested. Re-

TABLE 2. Identification results obtained with the RapID Yeast Plus system for 750 isolates

Species (no. tested)	No. (%) of isolates with indicated result			
	Identified	Low discrimination[a]	Misidentification	Unidentified
Candida albicans (258)	258	0	0	0
Candida dubliniensis (14)	0	0	14[b]	0
Candida famata (19)	19	0	0	0
Candida glabrata (160)	160	0	0	0
Candida guilliermondii (4)	3	0	1	0
Candida haemulonii (1)	0	0	0	1[b]
Candida intermedia (1)	0	0	0	1
Candida kefyr (23)	22	0	0	1
Candida krusei (30)	29	0	0	1
Candida lipolytica (4)[c]	3	0	1	0
Candida lusitaniae (14)	14	0	0	0
Candida norvegensis (4)	0	0	0	4[b]
Candida parapsilosis (70)	69	0	1	0
Candida rugosa (4)	4	0	0	0
Candida tropicalis (74)	74	0	0	0
Candida utilis (3)	3	0	0	0
Candida zeylanoides (5)	5	0	0	0
Cryptococcus uniguttulatus (1)	1	0	0	0
Geotrichum capitatum (3)[d]	2	0	0	1
Geotrichum klebahnii (1)	0	0	1[b]	0
Pichia anomala (1)	1	0	0	0
Saccharomyces cerevisiae (50)	49	0	0	1
Trichosporon asahii (5)	0	0	0	5[b]
Trichosporon mucoides (1)	0	0	0	1[b]
Total	716 (95.5)	0	18 (2.4)	16 (2.1)

[a] See Table 1, footnote *a*.
[b] Not included in the RapID database.
[c] Included as *Yarrowia lipolytica* in the RapID database.
[d] Included as *Blastoschizomyces capitatus* in the RapID database.

gardless, further studies should be performed to clarify their results.

In this study, the isolates misidentified or unidentified by both systems did not belong to a single species. The eight isolates misidentified by the VITEK 2 system were distributed among six different species (*C. albicans*, *C. glabrata*, *C. krusei*, *Candida lipolytica*, *C. parapsilosis*, and *Saccharomyces cerevisiae*). Fifteen of the 18 isolates misidentified by the RapID Yeast Plus system belonged to species not included in the database (14 isolates [misidentified as *C. albicans*] to *Candida dubliniensis* and 1 isolate [misidentified as *Candida zeylanoides*] to *Geotrichum klebahnii*). The four isolates not identified by the VITEK 2 system were all *C. albicans*. Conversely, the 16 isolates unidentified by the RapID Yeast Plus system belonged to nine different species, of which four were species not included in the database (*Candida haemulonii*, *Candida norvegensis*, *Trichosporon asahii*, and *Trichosporon mucoides*) and the remaining five were *Candida intermedia*, *Candida kefyr*, *C. krusei*, *Geotrichum capitatum*, and *S. cerevisiae*. Overall, 24 isolates were misidentified by both of the systems. These discrepant identifications are shown in Table 3. It is of interest that, while one isolate of *C. parapsilosis* was misidentified as *Candida famata* by VITEK 2 and as *Candida lambica* by RapID Yeast Plus, both systems erroneously identified one isolate of *C.*

TABLE 3. Discrepant identifications by various methods for 24 yeast isolates studied

No. of misidentified isolates	Result for indicated identification method		
	Reference[a]	VITEK 2	RapID
2	*Candida albicans*	*Stephanoascus ciferrii*	*Candida albicans*
14	*Candida dubliniensis*	*Candida dubliniensis*	*Candida albicans*
1	*Candida glabrata*	*Rhodotorula glutinis*/*Rhodotorula mucilaginosa*	*Candida glabrata*
1	*Candida guilliermondii*	*Candida guilliermondii*	*Candida zeylanoides*
2	*Candida krusei*	*Candida magnoliae*, *Candida lipolytica*	*Candida krusei*
1	*Candida lipolytica*	*Candida krusei*	*Candida krusei*
1	*Candida parapsilosis*	*Candida famata*	*Candida lambica*
1	*Geotrichum klebahnii*	*Geotrichum klebahnii*	*Candida zeylanoides*
1	*Saccharomyces cerevisiae*	*Candida colliculosa*	None

[a] Identifications were confirmed by using MicroSeq D2 LSU rDNA fungal sequencing kits, except for isolates of *Candida dubliniensis* and *Geotrichum klebahnii*, which are not included in the D2 database.

lipolytica as *C. krusei*. In addition, except for isolates of *C. dubliniensis* (14 isolates) and *G. klebahnii* (1 isolate), which are species not included in the MicroSeq D2 database, for the remaining 9 isolates, the results obtained by D2 and ITS sequence analyses were in perfect agreement.

In previous studies (5, 12, 16), the most frequently misidentified species was *C. glabrata*. In this study, only one isolate of *C. glabrata* was misidentified by the VITEK 2 system, for which it gave an excellent identification with two choices (*Rhodotorula glutinis*/*Rhodotorula mucilaginosa*). The percentage of misidentifications by the RapID Yeast Plus system was higher than that for the VITEK 2 system (2.4% and 1.0%, respectively). These misidentifications, representing 75% of all discrepant identifications (18 of 24 isolates), concerned 14 isolates of *C. dubliniensis*, which were correctly identified by the VITEK 2 system. In contrast to the study by Cárdenes-Perera et al. (4) pointing out the need for a greater diagnostic capacity with regard to *C. dubliniensis*, our results confirm those obtained by Graf et al. (9) and Aubertine et al. (2) demonstrating that the VITEK 2 system enables most clinical laboratories to routinely differentiate *C. dubliniensis* from the closely related *C. albicans*.

C. dubliniensis and *G. klebahnii* were not included in the D2 MicroSeq database, which was successfully used in this study to confirm the identities of those isolates with discrepant results between the phenotypic (VITEK 2 and/or RapID) and ITS1/ITS2 sequence-based identification methods (Table 3). Even though the MicroSeq D2 LSU rDNA sequencing kit was applied to few isolates, we chose to compare the D2 and ITS sequences in order to add support to previous evidence that the kit seems to be a reliable and useful system for the identification of medically relevant yeasts in a routine clinical laboratory (10).

Although the cornmeal-Tween 80 (Dalmau) morphology examination was, in our hands, a powerful differentiation method, particularly for instances where four *C. albicans* isolates were unidentified by the VITEK 2 system or one *G. klebahnii* isolate was misidentified as *C. zeylanoides* by the RapID system, the present study demonstrated the usefulness of ITS sequencing for the resolution of discrepant phenotype-based species identifications. It is also potentially a fast and reliable method that can be used for yeast identification in place of the current commercially available phenotypic methods, which sometimes require a series of further tests to confirm the identity of a given yeast species. However, nucleic acid sequencing methods rely upon databases, such as the MicroSeq D2 library mentioned above, which are often limited and not inclusive of all clinically important species that should be recognized (10, 14). In contrast, a GenBank search for sequences of the full (~600-bp) D1/D2 region can be considered a useful tool for the identification of almost all ascomycetous and basidiomycetous yeast species (7, 13).

In conclusion, both the VITEK 2 and the RapID Yeast Plus systems remain rapid and accurate methods for the identification of yeast species seen in the clinical mycology laboratory, some of which are relatively uncommon.

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