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Comparative Evaluation of Three Commercial Identification Systems Using Common and Rare Bloodstream Yeast Isolates[▽]

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The commercial yeast identification systems API ID32C, Auxacolor, and Vitek were evaluated using 251 molecularly identified bloodstream isolates and 2 reference strains, representing a total of 35 species (6 common and 29 rare). Correct identification rates were higher for common species (Auxacolor, 95%; API ID32C, 94%; Vitek, 92%) than for rare species (Auxacolor, 43%; API ID32C, 56%; Vitek, 64%). All systems performed equally among the former, and Vitek performed best among the latter.

Yeasts are frequent colonizers and opportunistic pathogens, with *Candida* species still constituting the fourth-most-common cause of bloodstream infections in hospitalized patients in the United States (21). Accurate identification of yeast isolates from clinical specimens is vital for the establishment of etiological diagnosis, selection of appropriate antifungal therapy, monitoring of dissemination risk to susceptible patient populations, and epidemiology (14). Currently, yeast identification in the clinical laboratory is based on various commercial systems, utilizing mainly biochemical and occasionally morphological and physiological characteristics (5). However, the performance of these systems should be continually monitored, since it is influenced by dynamic changes in epidemiology, such as the emergence of difficult-to-identify or novel yeast pathogens, progress in taxonomy, and possibly geographical strain variation (6, 15). Comprehensive phenotypic identification combined with molecular characterization should preferentially represent the gold standard for comparison in such evaluation studies, but this has rarely been the case.

In the present study and for the first time in the literature, we aimed to compare three widely available yeast identification systems, API ID32C (BioMeriéux, Marcyl'Etoile, France), Auxacolor (Bio-Rad, Hercules, CA), and Vitek 2 (BioMeriéux) using a large collection of clinically significant isolates definitively identified by sequencing of internal transcribed spacers (ITS) 1 and 2 (18) and the 26S ribosomal DNA gene, variable region D1/D2 (11). The collection was deliberately enriched with rare yeast species to review the systems' strength of identification and test the hypothesis of whether rare species can be misidentified as common, possibly contributing to detection bias toward the common species. This was impelled by the ongoing isolation of rare yeasts in the clinical laboratory, requiring assessment in order to establish accurate identification by commercial systems.

The study included 251 baseline bloodstream isolates and two reference strains of the novel species *Candida bracarensis* (CBS 10154) and *Candida nivariensis* (CBS 9983), representing eight genera (*Candida*, *Cryptococcus*, *Geotrichum*, *Malassezia*, *Rhodotorula*, *Saccharomyces*, *Trichosporon*, and *Zygosaccharomyces*), 6 common species, and 29 rare species (Table 1). Though they are of low incidence, accurate identification of the rare bloodstream isolates was considered in line with the current guidelines (14). All isolates were from immunocompromised, intensive care unit (ICU) or otherwise critically ill patients hospitalized between January 2005 and January 2010 in Greece. The isolates were stored in the UOA/HCPF929 Collection (http://wdcm.nig.ac.jp/CCINFO/CCINFO.xml?929) at −80°C in yeast-peptone-dextrose broth with 10% glycerol (Oxoid, Cambridge, United Kingdom) and before testing were subcultured twice in Sabouraud dextrose agar. Each strain was simultaneously identified with the API ID32C (BioMeriéux), the Auxacolor (Bio-Rad), and the Vitek 2 (BioMeriéux) systems with the new colorimetric card YST (Vitek 2-YST), according to the manufacturers' instructions. The three systems were tested blindly, and whenever identification was not obtained the test was repeated. All isolates were identified by sequencing of the noncoding ribosomal regions internal transcribed spacer (ITS) 1 and 2 and, for rare yeasts, also by sequencing of the 26S ribosomal DNA gene, variable region D1/D2 (10, 11, 17); results were compared with selected (*viz*, Table 1, footnote *e*) GenBank standard sequences (http://www.ncbi.nlm.nih.gov/BLAST/), and identification was concluded when there was ≥99% sequence homology. Identification of *Saccharomyces boulardii* isolates was also confirmed by pulsed-field gel electrophoresis (13).

Each system's results were compared with those of sequence-based identification and were classified as follows: (i) no identification (system failed to identify the isolate), (ii) unequivocal identification (species identical to molecular iden-

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TABLE 1. Identification results obtained with Vitek 2-YST (VIT), API ID32C (API), and Auxacolor (AUX) for 253 yeast isolates in comparison to reference molecular methodology

Reference species identification (n^a)	GenBank accession no. for reference ITS sequence[e]	No. (%) of isolates										
		Identification			Low-discrimination identification		Misidentification			No identification		
		VIT	API	AUX	VIT	API	VIT	API	AUX	VIT	API	AUX
Common species group												
C. albicans (35)	EF567991 EF567992 EF567994	35	34	35	0	1	0	0	0	0	0	0
C. glabrata (31)	EF568002 AY046165	28	29	30	0	0	3	0	1	0	2	0
C. krusei (24)	EF568013 EF568014 EF568015	17	19	21	2	2	5	2	2	0	1	1
C. parapsilosis (45)	AJ635316 EF568031 EF568032	39	42	41	2	0	4	3	4	0	0	0
C. tropicalis (20)	EF568038 EF568041 EF568042	17	18	18	1	1	2	1	2	0	0	0
Cryptococcus neoformans (28)	AJ493559 AB087817 AB087822	27	26	28	0	0	0	2	0	1	0	0
Total, common spp. (183)		163 (89)	168 (92)	173 (95)	5 (3)	4 (2)	14 (8)	8 (4)	9 (5)	1 (1)	3 (2)	1 (1)
Rare species group												
C. bracarensis[b,c,d] (1)	—[f]	0	0	0	0	0	1	0	1	0	1	0
Candida catenulata[b] (1)	AJ853765	0	0	0	0	0	1	1	1	0	0	0
C. dubliniensis (3)	EF567998 EF568000 FM178301	1	0	1	0	0	2	3	2	0	0	0
C. famata (2)	AJ853777 EU569037 EU569039	0	0	0	0	0	2	0	1	0	2	1
Candida. guilliermondii (3)	EF568007 DQ249192 EU568911	2	3	2	1	0	0	0	1	0	0	0
Candida intermedia[b] (1)	EF568011	0	0	0	0	0	1	1	1	0	0	0
Candida kefyr (1)	EF568057 AY046214	0	0	0	0	0	1	1	1	0	0	0
Candida lipolytica (4)	EF568019 EF568020 EF568021	4	4	4	0	0	0	0	0	0	0	0
C. lusitaniae (13)	EF568023 EF568025 EF568048	12	10	12	0	2	1	0	1	0	1	0
C. metapsilosis[b,c,d] (1)	AJ698049	0	0	0	0	0	1	1	1	0	0	0
C. nivariensis[b,c,d] (1)	—	0	0	0	0	0	1	0	1	0	1	0
C. orthopsilosis[b,c,d]	AJ698048	0	0	0	0	0	1	1	1	0	0	0
Candida pelliculosa[b] (2)	DQ249195 EF568036 FM178296	2	2	0	0	0	0	0	0	0	0	2
Candida pulcherrima[b] (2)	AY235809	1	1	0	1	1	0	0	1	0	0	1
Candida rugosa (2)	EF568037	2	2	1	0	0	0	0	0	0	0	1
Candida sphaerica[b] (1)	AY046213	1	1	0	0	0	0	0	0	0	0	1
Geotrichum candidum[d] (2)	AJ853772 AJ279445	0	1	1	0	0	2	0	1	0	1	0
Cryptococcus albidus (2)	AB051039 AB051041 AB051043	1	1	1	0	0	1	0	1	0	1	0
Cryptococcus gattii[b,c,d] (3)	AJ493562 AJ493567 AJ493573 EF568053	0	0	0	0	0	3	3	3	0	0	0
Malassezia furfur[b,c] (8)	AF522059	8	0	0	0	0	0	0	0	0	8	8
M. pachydermatis[b,c] (2)	AF522061	1	0	0	0	0	1	0	0	0	2	2
Rhodotorula glutinis (1)	AF444539 AF522058	1	1	1	0	0	0	0	0	0	0	0

Continued on following page

TABLE 1—*Continued*

Reference species identification (n^a)	GenBank accession no. for reference ITS sequence[e]	No. (%) of isolates										
		Identification			Low-discrimination identification		Misidentification			No identification		
		VIT	API	AUX	VIT	API	VIT	API	AUX	VIT	API	AUX
Rhodotorula lactosa[b,c] (1)	AF444540	0	1	0	0	0	1	0	1	0	0	0
Rhodotorula minuta[b] (1)	AF444579	1	1	1	0	0	0	0	0	0	0	0
Rhodotorula mucilaginosa (2)	AF444649	0	2	2	2	0	0	0	0	0	0	0
Saccharomyces boulardii[b,c,d] (3)	AY240870 AJ632284 AM779768	0	0	0	0	0	3	3	1	0	0	2
S. cerevisiae (2)	AY130311 AY130312 U09327	2	2	2	0	0	0	0	0	0	0	0
Trichosporon asahii (2)	AJ853754 AB018013	2	2	2	0	0	0	0	0	0	0	0
Zygosaccharomyces bisporus[b,d] (2)	AY046192	0	2	0	0	0	2	0	1	0	0	1
Total, rare spp. (70)		41 (59)	36 (51)	30 (43)	4 (6)	3 (4)	25 (36)	14 (20)	21 (30)	0 (0)	17 (24)	19 (27)
Total, all spp. (253)		204 (81)	204 (81)	203 (80)	9 (4)	7 (3)	39 (15)	22 (9)	30 (12)	1 (0)	20 (8)	20 (8)

[a] *n*, no. of isolates tested.
[b] Species not included in the database of Auxacolor.
[c] Species not included in the database of API ID32C.
[d] Species not included in the database of Vitek 2-YST.
[e] To ensure consistency, selected GenBank ITS sequences, also submitted to (http://www.mycologylab.org/DefaultInfo.aspx?Page=ITSDBContributors), were used as a reference.
[f] —, no sequencing performed in this study: type strains *Candida bracarensis* CBS 10154 and *C. nivariensis* CBS 9983 were tested.

tification), (iii) low-discrimination identification (API ID32C or Vitek 2-YST displayed more than one potential species identification, of which one corresponded to molecular identification; Auxacolor does not provide low-discrimination identifications), and (iv) misidentification (a wrong species identification was provided by the system, or testing by API ID32C or Vitek 2-YST showed low-discrimination identification, not including the correct species). Unequivocal and low-discrimination identifications were considered correct identifications.

Out of the 253 isolates, Vitek 2-YST identified correctly 213 (84%), API ID32C 211 (83%), and Auxacolor 203 (80%) strains (Table 1). Among commonly isolated yeasts (*Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, *Candida tropicalis*, and *Cryptococcus neoformans*), the three systems demonstrated comparable performance: Auxacolor correctly identified 94.5% of the isolates, misidentified 5%, and failed to identify 0.5%. This high percentage of correct identifications is similar to those previously published of 81 to 91% (3, 17, 19) and may be due to the test coassessing morphological and biochemical characteristics. The API ID32C system resulted in 94% correct (including 2% low-discrimination identifications), 4% mistaken, and 2% not identified, while the corresponding percentages for Vitek 2-YST were 91% correct (including 3 low-discrimination identifications), 8% mistaken, and 1% with no identification. Comparable correct identification levels for Vitek 2-YST were reported previously, while misidentifications of *C. glabrata*, *C. krusei*, and *C. parapsilosis* were also recorded (1, 2, 7, 10, 12, 16). Notably, the performance of Vitek 2-YST versus API ID32C has not been assessed previously.

With rare yeasts, Vitek 2-YST showed the best performance, with 64% correct identifications (including 6% of low discrimination), while API ID32C showed 56% correct (including 4% of low discrimination) and Auxacolor 43% correct identifications. Notably, only misidentifications (36%) were observed with Vitek 2-YST, while mistaken and no identifications corresponded to 30% and 27% for Auxacolor and 20% and 24% for API ID32C, respectively (Table 1). In interpreting the above results for this whole group of rare yeasts, it should obviously be taken into account that identifiable species differ between systems (Table 1). For example, all systems misidentified the three *Cryptococcus gattii* isolates as *Cryptococcus neoformans* and the three *S. boulardii* isolates as *Saccharomyces cerevisiae*, except for two no-identification results obtained by Auxacolor with the latter (Table 2). Predictably, none of the systems differentiated *C. parapsilosis* from the recently separated *Candida metapsilosis* and *Candida orthopsilosis* and similarly *C. glabrata* from *C. bracarensis* and *C. nivariensis* except for one Auxacolor misidentification result as *Candida inconspicua* and two no-identification results by API ID32C. Overall, isolates within this subgroup of rare yeasts, that is, species not included in each system's database (Table 1), were either misidentified (100%, 44%, and 42% for Vitek 2-YST, API ID32C, and Auxacolor, respectively) or not identified (56% and 55% for API ID32C and Auxacolor, respectively) (data from Table 1).

Concerning the rare species included in each system's repertoire, correct identification rates were increased for all. Vitek 2-YST accurately identified 82% (45/55), API ID32C 75% (39/52), and Auxacolor 74% (29/39) of isolates (data are derived from Table 1). Respective misidentification rates were 18%, 12%, and 21%. Interestingly, among the three *Candida dubliniensis* isolates, only one was not identified as *C. albicans* by Vitek 2-YST and Auxacolor. *Candida*

TABLE 2. Discrepant species identifications of 48 yeast isolates by Vitek 2-YST, API ID32C, or Auxacolor in comparison to reference molecular methodology[a]

Molecular identification (n^b)	Identification by commercial system (n^b)		
	Vitek 2-YST	API ID32C	Auxacolor
Common species group			
C. glabrata (3)	*Candida ciferrii*	No ID	*C. albicans*
	C. dubliniensis	No ID	***C. glabrata***
	C. albicans	***C. glabrata***	***C. glabrata***
C. krusei (5)	*C. guilliermondii*	***C. krusei***	No ID
	C. lusitaniae	No ID	***C. krusei***
	C. glabrata/*C. lipolytica*	*C. lipolytica*	***C. krusei***
	C. famata/*C. guilliermondii*	*C. famata*/*C. guilliermondii*	*C. famata*
	C. famata	***C. krusei***[c]	*C. parapsilosis*
C. parapsilosis (6)	*C. famata*	*Debaryomyces carsonii*	***C. parapsilosis***
	C. famata	*C. albicans*	*C. albicans*
	C. famata	*C. albicans*	*C. albicans*
	C. famata	***C. parapsilosis***	***C. parapsilosis***
	C. parapsilosis	***C. parapsilosis***	*C. tropicalis*
	C. parapsilosis	***C. parapsilosis***	*C. tropicalis*
C. tropicalis (4)	*C. lusitaniae*	***C. tropicalis***	***C. tropicalis***
	C. tropicalis	*C. albicans*	*C. albicans*
	C. parapsilosis	***C. tropicalis***	***C. tropicalis***
	C. tropicalis	***C. tropicalis***	*C. guilliermondii*
C. neoformans (2)	***C. neoformans***	*Cryptococcus albidus*	***C. neoformans***
	C. neoformans	*Cryptococcus albidus*	***C. neoformans***
Rare species group			
C. bracarensis (1)	*C. glabrata*	No ID	*C. glabrata*
C. catenulata (1)	*C. rugosa*	*C. rugosa*	*C. albicans*
C. dubliniensis (3)	***C. dubliniensis***	*C. albicans*	***C. dubliniensis***
	C. albicans	*C. albicans*	*C. albicans*
	C. albicans	*C. albicans*	*C. albicans*
C. famata (2)	*C. lusitaniae*	No ID	*C. guilliermondii*
	C. parapsilosis	No ID	No ID
C. guilliermondii (1)	***C. guilliermondii***[c]	***C. guilliermondii***	*C. famata*
C. intermedia (1)	*C. tropicalis*	*C. tropicalis*	*C. tropicalis*
C. kefyr (1)	*C. albicans*	*C. albicans*	*C. albicans*
C. lusitaniae (1)	*Geotrichum klebahnii*	No ID	*G. candidum*
C. metapsilosis (1)	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*
C. nivariensis (1)	*C. glabrata*	No ID	*C. inconspicua*
C. orthopsilosis (1)	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*
C. pulcherrima (1)	***C. pulcherrima***[c]	***C. pulcherrima***[c]	*C. lusitaniae*
G. candidum (2)	*Trichosporon asahii*	No ID	*T. asahii*
	Geotrichum klebahnii	***G. candidum***	***G. candidum***
C. albidus (1)	*C. lusitaniae*	No ID	*C. lusitaniae*
C. gattii (3)	*C. neoformans*	*C. neoformans*	*C. neoformans*
M. pachydermatis (1)	*M. furfur*	No ID	No ID
R. lactosa (1)	*Candida sake*	***R. lactosa***	*C. albidus*
S. boulardii (3)	*S. cerevisiae*	*S. cerevisiae*	*S. cerevisiae*
	S. cerevisiae	*S. cerevisiae*	No ID
	S. cerevisiae	*S. cerevisiae*	No ID
Z. bisporus (2)	*S. cerevisiae*	***Z. bisporus***	No ID
	C. glabrata	***Z. bisporus***	*C. glabrata*

[a] ID, identification. Correct identifications appear in bold.
[b] *n*, no. of isolates.
[c] Identification representing a low-discrimination result.

famata was not recognized by any system, while Vitek 2-YST presented difficulties with the identification of *Malassezia pachydermatis*. For *Candida lusitaniae*, which was amply represented among rare species, one isolate could not be identified by any system (Table 2).

In order to include a multiplicity of species and since certain species are sporadically isolated, the present study examined small numbers of isolates per species, especially among rare yeasts. Since our collection of 253 strains did not represent isolates obtained consecutively during routine testing, the specificities for species identification provided by the commercial systems could not be assessed, which constitutes a limitation of the present study. However, it was evident that rare species were frequently misidentified as common yeast species (Table 2) (11 out of 25 misidentified rare species by Vitek 2-YST, 8/14 by API ID32C, and 11/21 by Auxacolor). Misidentifications implicating different genera were also observed. Importantly, misidentifications often involved species with known

resistance to antifungals, such as *C. glabrata*, *C. krusei*, and less commonly *C. lusitaniae*, with implications for appropriate patient treatment (14).

Studies which previously evaluated the new Vitek 2-YST card disclosed variable correct identification rates (84% to 99%) (1, 7, 10, 12, 16, 20). This can be attributed to significant differences between studies regarding the examined species and number of tested isolates and possibly to geographic differences. It is important to underline that with only two exceptions (16, 20), all these studies used as the comparator method either other commercial systems, such as API 20C AUX (1, 7) and Vitek 2 in combination with the older fluorometric card (10), or conventional assimilation, fermentation, and morphological tests (12). Two reports compared the Vitek 2-YST card to ITS 1 and 2 sequencing (16, 20). The study by Sanguinetti et al. examined a large number of isolates, and among common yeast species, Vitek 2-YST demonstrated a 98% correct identification rate (16). Unlike the present report, misidentifications and no identifications were rather restricted to common species isolates. However, the spectrum of analyzed species differed between the two studies, since 20 distinct species were tested exclusively by the present report, as opposed to another 9 examined by the other (16). The study by Vijgen et al. examined only common species (67 isolates), and correct identification rates were 91% (20). Finally, an important observation of our study refers to the frequency of species misidentification by Vitek 2-YST (15% overall, 8% among common species, and 36% among rare species); in addition, only one isolate was unidentified by Vitek 2-YST. This finding may represent a disadvantage of that system, because misidentifications generally undermine selection of the correct methodology for strain typing to investigate outbreaks or the sources of nosocomial infections and hinder risk assessment strategies and prevention of infections.

API ID32C was long considered a reference yeast identification method (5). Here it performed poorly with the rare yeasts but demonstrated high correct identification rates with the common species group (56% versus 94%, respectively). Similarly, the application of molecular identification by Latouche et al. disclosed compromised API ID32C performance, where 23% of both common and rare species were not correctly identified (8). A recent report, using ITS 1 and 2 sequencing as the reference identification method, demonstrated that 16.2% of clinical isolates were misidentified by API ID32C (9). Conversely, the poor Auxacolor performance with the rare species group is obviously related to the system's intrinsic limitations.

In conclusion, despite a lack of acceptable yeast cutoff identification criteria, it can be asserted that all three systems performed well in identifying common clinical yeast species, providing correct identification for at least 91% of isolates. The identification of rare species was more challenging, indicating that they require further morphological and physiological testing and sequence-based identification. Yet their definitive identification is important, since their true frequency of isolation during routine testing would remain unknown if they are not accurately identified.

The International Sub-commission on Fungal Barcoding (http://www.dnabarcodes2009.org/meeting_documents/Wednesday/Session%20C/Seminar%20Room%20C%20-%20Wednesday%20-%20Meyer.pdf) and the CLSI (MM18-A) (4) have proposed the ITS region as the default region for species identification. Currently, sequence-based identification represents the gold standard because it generally grants a methodical and dependable approach to fungal identification; it is accurate and yields objective data, provided sequences of ensured quality are selected for comparisons (Table 1). Our data show that the three yeast commercial identification schemes could be inadequately objective due to the changing epidemiology of medically significant yeasts, which may not be included in the systems' databases. Commercial system misidentification reports (Table 2) have clinical consequences in terms of giving incorrect or delayed final reports and constitute an impediment to timely therapeutic intervention. Accurate identification of bloodstream isolates like *S. boulardii* and *Malassezia furfur* to the species level is also of epidemiological significance (14), since it can contribute to the early detection of iatrogenic *S. boulardii* ICU and neonatal ward *M. furfur* outbreaks. In that respect, no identification would be preferable, since it would alert the clinical laboratory and encourage referral of the isolate for molecular identification. Users should be aware of potential rare species misidentifications exhibited by these commercial systems, among which Vitek 2-YST demonstrated the highest rates. Also, in some cases, as in that of *C. lusitaniae* isolates, chromogenic media can facilitate screening for rare species, increasing alertness for likely ambiguous results.

Last, since very few reports have evaluated the performances of widely used commercial systems versus definitive identification by molecular methods, further, multicenter studies would expand and validate our findings, which were obtained by examining isolates from our geographical region for the first time.

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