

Comparison of Three Commercial Systems for Identification of Yeasts Commonly Isolated in the Clinical Microbiology Laboratory

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We evaluated three commercial systems (RapID Yeast Plus System; Innovative Diagnostic Systems, Norcross, Ga.; API 20C Aux; bioMerieux-Vitek, Hazelwood, Mo.; and Vitek Yeast Biochemical Card, bioMerieux-Vitek) against an auxinographic and microscopic morphologic reference method for the ability to identify yeasts commonly isolated in our clinical microbiology laboratory. Two-hundred one yeast isolates were compared in the study. The RapID Yeast Plus System was significantly better than either API 20C Aux (193 versus 167 correct identifications; $P < 0.0001$) or the Vitek Yeast Biochemical Card (193 versus 173 correct identifications; $P = 0.003$) for obtaining correct identifications to the species level without additional testing. There was no significant difference between results obtained with API 20C Aux and the Vitek Yeast Biochemical Card system ($P = 0.39$). The API 20C Aux system did not correctly identify any of the *Candida krusei* isolates ($n = 23$) without supplemental testing and accounted for the major differences between the API 20C Aux and RapID Yeast Plus systems. Overall, the RapID Yeast Plus System was easy to use and is a good system for the routine identification of clinically relevant yeasts.

Fungal infections, particularly those caused by *Candida* species, are important causes of nosocomial infections in immunocompromised hosts and are being detected more frequently in clinical microbiology laboratories (5). Traditional methods for identifying *Candida* spp. and other yeasts include analysis of microscopic morphologic characteristics and substrate assimilation assays (12). Since traditional methods are tedious and time-consuming to perform in the routine laboratory, numerous commercial systems that can identify these pathogens within 4 to 72 h, depending on the system, have been developed (4, 6, 7, 9, 10). The RapID Yeast Plus System is a micro-method that identifies yeasts in 4 h and has been studied by a number of investigators (3, 9). These studies, however, compared the system only against conventional methods or the API 20C Aux assimilation assay. Side-by-side comparisons of the RapID Yeast Plus System with both the API 20C Aux system and the Vitek Yeast Biochemical Card (YBC) have not been reported to date and are the subject of this study.

MATERIALS AND METHODS

Organisms. Organisms used in this study were clinical isolates obtained from patients at the Hospital of the University of Pennsylvania, a 700-bed tertiary-care center in Philadelphia. A total of 201 yeast isolates were tested and included the following: *Candida albicans* ($n = 45$), *Candida tropicalis* ($n = 39$), *Torulopsis glabrata* ($n = 35$), *Candida parapsilosis* ($n = 27$), *Candida krusei* (23), *Cryptococcus neoformans* ($n = 23$), *Candida lusitanae* ($n = 5$), *Rhodotorula rubrum* ($n = 2$), and *Saccharomyces cerevisiae* ($n = 2$). One hundred two (50.7%) of the isolates were from blood cultures. The remaining strains were isolated from ascitic fluid ($n = 7$), pleural fluid ($n = 7$), urine ($n = 19$), sterile tissues ($n = 11$), cerebrospinal fluid ($n = 20$), sputum ($n = 5$), and other miscellaneous body sites ($n = 30$). *Cryptococcus neoformans* isolates were stored as suspensions in screw-cap vials containing 2 ml of sterile water maintained at room temperature. All other isolates were stored on Sabouraud dextrose agar slants at room tempera-

ture. Isolates to be tested were subcultured at least twice to Remel Sabouraud dextrose agar plates (Emmons formulation). Plates were incubated at 30°C for the time specified by the manufacture for each method.

Isolates were tested by conventional methods and with three commercially available identification systems. Identifications derived from conventional testing served as the reference identifications. Identifications from the commercial systems were considered correct if they were complete (no further testing was required) at an acceptable confidence level and were in agreement with reference identifications. There were no instances in which the results of all three conventional methods disagreed with the reference identification.

Reference identification. Isolates were identified by conventional methods which included microscopic morphology on cornmeal agar and carbohydrate assimilation by the auxinographic plate method (Haley and Standard modification [12]).

RapID Yeast Plus System. The RapID Yeast Plus System (Innovative Diagnostic Systems, Norcross, Ga.; distributed by Remel) is a qualitative micro-method which uses 18 conventional and chromogenic substrates for the rapid identification of clinical yeast isolates. All testing with the RapID Yeast Plus panels was performed according to the manufacturer's instructions. After 4 h of incubation, color reactions were read and scored as positive or negative, generating a six-digit microcode. Identifications were obtained by consulting the RapID Yeast Plus Code Compendium (version 1.95) or Computer Service (version 1.3.9.7). Results were considered correct if the profile was listed as implicit, satisfactory, or adequate and if results were in agreement with reference identifications.

API 20C Aux system. The API 20C Aux system (bioMerieux-Vitek, Hazelwood, Mo.) consists of a plastic strip with 20 cupules which contain dehydrated substrates for assimilation testing. Strips were inoculated and incubated according to the manufacturer's instructions. A profile number based upon the reactions observed was generated at 72 h of incubation for each strip. Identifications were made by reference to the API Analytical Profile Index (version 1.1) or by calling the computerized voice-activated system (version 1.1). Results were considered correct when the profile was listed as excellent, very good, or acceptable and if results were in agreement with the reference identification.

Vitek YBC. The Vitek YBC (bioMerieux-Vitek) is a 30-well plastic card containing 26 conventional tests and four negative controls. All testing with the YBC was performed according to the manufacturer's instructions. Cards were read on the Vitek, and the computer (version 8.1) analyzed the results and produced an identification. Results were considered correct if the reliability was $\geq 85\%$ and the identification agreed with the reference identification.

Statistical analysis. The Cochran Q test was used to determine whether the three tests differed from each other (11). McNemar's paired test was used for comparisons among the different systems (InStat version 2.04; GraphPad Software, San Diego, Calif.).

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TABLE 1. Identification of yeasts by commercial systems

Organism	No. of isolates	System	No. (%) of isolates with the following identification:			
			Correct ^a	Incomplete ^b	Incorrect	Unidentified
<i>C. albicans</i>	45	RapID Yeast Plus	45 (100)	0	0	0
		API 20C Aux	42 (93)	3 (7)	0	0
		Vitek YBC	45 (100)	0	0	0
<i>C. tropicalis</i>	39	RapID Yeast Plus	39 (100)	0	0	0
		API 20C	35 (90)	4 (10)	0	0
		Vitek YBC	34 (87)	2 (5)	3 (8)	0
<i>T. glabrata</i>	35	RapID Yeast Plus	32 (91)	3 (9)	0	0
		API 20C Aux	32 (91)	3 (9)	0	0
		Vitek YBC	34 (97)	0	0	1 (3)
<i>C. parapsilosis</i>	27	RapID Yeast Plus	25 (92)	1 (4)	1 (4)	0
		API 20C	27 (100)	0	0	0
		Vitek YBC	19 (70)	5 (19)	3 (11)	0
<i>C. krusei</i>	23	RapID Yeast Plus	23 (100)	0	0	0
		API 20C Aux	0	23 (100)	0	0
		Vitek YBC	15 (65)	2 (9)	0	6 (26)
<i>Cryptococcus neoformans</i>	23	RapID Yeast Plus	22 (96)	0	1 (4)	0
		API 20C Aux	22 (96)	1 (4)	0	0
		Vitek YBC	19 (83)	1 (4)	3 (13)	0
<i>C. lusitaniae</i>	5	RapID Yeast Plus	4 (80)	1 (20)	0	0
		API 20C Aux	5 (100)	0	0	0
		Vitek YBC	5 (100)	0	0	0
<i>Rhodotorula</i> sp. ^c	2	RapID Yeast Plus	2 (100)	0	0	0
		API 20C Aux	2 (100)	0	0	0
		Vitek YBC	0	0	1 (50)	1 (50)
<i>S. cerevisiae</i>	2	RapID Yeast Plus	1 (50)	1 (50)	0	0
		API 20C Aux	2 (100)	0	0	0
		Vitek YBC	2 (100)	0	0	0
Total	201	RapID Yeast Plus	193 (96)	6 (3)	2 (1)	0
		API 20C Aux	167 (83)	34 (17)	0	0
		Vitek YBC	173 (86)	10 (5)	10 (5)	8 (4)

^a Identification was made to the species level without supplemental testing.

^b Low level of discrimination; the correct identification was listed among two or more choices. Supplemental testing is required.

^c This organism produces a red pigment and was originally reported as *Rhodotorula rubrum*. Both the RapID Yeast Plus and API 20C Aux system identified the organism as *Rhodotorula minuta*, which is also red pigmented. A species identification could not be made by the assimilation test.

RESULTS

Three different commercial systems were tested for the ability to identify nine groups of yeast isolates comprising *Candida* spp., *Cryptococcus neoformans*, *T. glabrata*, *Rhodotorula* sp., and *S. cerevisiae* (Table 1). The ability to correctly identify isolates to the species level differed significantly among the three commercial systems ($Q = 2987$, $df = 2$, $P < 0.001$). Overall, the RapID Yeast Plus System correctly identified 96% of the isolates (193 of 201), compared with 83% for the API 20C Aux system (167 of 201) ($P < 0.0001$) and 86% for the Vitek YBC system (173 of 201) ($P = 0.003$), without the need for supplemental testing. A significant difference between the API 20C Aux and Vitek YBC systems was not observed.

Few isolates were incorrectly identified (i.e., incorrect genus and/or species) by the three systems, and results are summarized in Table 2. Two isolates (*C. parapsilosis* and *Cryptococcus neoformans*) were incorrectly identified by the RapID Yeast Plus System, none were incorrectly identified by the API 20C Aux system, and 10 isolates were incorrectly identified by the Vitek YBC

system. Six of the 10 isolates misidentified by the Vitek YBC system included *C. tropicalis* and *Cryptococcus neoformans*.

The API 20C Aux system had the highest number of isolates that were incompletely identified (i.e., supplemental tests were required for correct identification), as summarized in Table 3. The majority of these isolates (23 of 34) were *C. krusei*, and none were definitively identified without supplemental testing. For the RapID Yeast Plus System, 3 of 35 *T. glabrata* isolates were incompletely identified and accounted for half of the incomplete identifications by this system. For the Vitek YBC system, 5 of 27 *C. parapsilosis* isolates were incompletely identified and accounted for half of the incomplete identifications with this system. Excluding *C. krusei* isolates, there was no significant difference between the three assays with regard to incomplete identifications.

DISCUSSION

Identification of yeasts has become an important activity in clinical microbiology laboratories. Tertiary-care institutions as well as community-based hospitals are providing care to pa-

TABLE 2. Summary of incorrect identifications

System	Total no. of isolates	Organism (no. of isolates)	Incorrect identification
RapID Yeast Plus	2	<i>Cryptococcus neoformans</i> <i>C. parapsilosis</i>	<i>C. albidus</i> or <i>C. tropicalis</i> (overlapping identification) <i>C. lambica</i>
API 20C Aux	0	NA ^a	NA
Vitek YBC	10	<i>C. tropicalis</i> (3) <i>C. parapsilosis</i> <i>C. parapsilosis</i> <i>C. parapsilosis</i> <i>Cryptococcus neoformans</i> (2) <i>Cryptococcus neoformans</i> (1) <i>Rhodotorula</i> sp.	<i>C. lusitaniae</i> <i>C. albicans</i> <i>C. uniguttulatus</i> <i>C. albicans</i> <i>Cryptococcus humicola</i> or <i>Cryptococcus laurentii</i> (overlapping identification) <i>Cryptococcus humicola</i> <i>C. parapsilosis</i>

^a NA, not applicable.

tients that are increasingly at risk for developing nosocomial fungal infections, particularly with non-*C. albicans* species such as *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *T. glabrata* (5). In addition, prophylactic use of antifungal agents is more commonplace by patients with myeloproliferative disorders, transplant patients, and patients with human immunodeficiency virus and may contribute to the emergence of resistant organisms (1, 5). Thus, rapid and accurate identification of common yeasts takes on a more important clinical role than in previous years.

The present study showed that the RapID Yeast Plus System performed better than the other systems in overall terms for the correct identification of yeasts without the need for additional testing, with 96% of 201 isolates being correctly identified. There were few incomplete or incorrectly identified isolates with this system. Our data are also in agreement with results of recent studies of the RapID Yeast Plus System (3, 7).

Problems were observed with the ability of the API 20C Aux system to correctly identify *C. krusei* without the need for supplemental testing. All 23 isolates in our collection required supplemental tests with the API 20C Aux system. This is in contrast to results of previous studies showing the API 20C Aux system to have little difficulty in identifying *C. krusei* (3, 4, 10). In our hands, the Vitek YBC correctly identified 15 of 23

(65%) *C. krusei* isolates without the need for supplemental tests. While none of the isolates were incorrectly identified, six were not identified with the Vitek YBC system. Dooley et al. previously compared the Vitek YBC system with the API 20C Aux system and also found that 9 of 24 isolates were not identified with the Vitek YBC system (1a). An early study by El-Zaatari et al. of the Vitek system, however, did not show any problem with identification of *C. krusei* (2). *C. krusei* is an important pathogen in immunocompromised hosts, and because of innate resistance to fluconazole, delay in identification of this species may have important clinical implications (7).

Excluding the *C. krusei* isolates, all three systems had roughly equivalent rates of incomplete identification. While the numbers were small, the Vitek YBC system incorrectly identified 5% of yeast isolates compared to 1% for the RapID Yeast Plus System and none for the API 20C Aux system. There did not appear to be any pattern to the incorrect identifications with the Vitek YBC system, however. Relatively few isolates of *C. lusitaniae*, *Rhodotorula* sp., and *S. cerevisiae* were tested in this study, and while there were few discrepancies observed with the different identification systems, no conclusions can be drawn about the abilities of these systems to accurately identify these species.

TABLE 3. Summary of incomplete identifications

System	Total no. of isolates	Organism identification (no. of isolates)	Incomplete identification
RapID Yeast Plus	6	<i>T. glabrata</i> (3) <i>C. parapsilosis</i> <i>C. lusitaniae</i> <i>S. cerevisiae</i>	<i>T. glabrata</i> or <i>Blastoschizomyces capitatus</i> <i>C. parapsilosis</i> or <i>Candida guilliermondii</i> or <i>C. lusitaniae</i> <i>C. lusitaniae</i> or <i>C. parapsilosis</i> or <i>Torulopsis candida</i> <i>C. tropicalis</i> or <i>S. cerevisiae</i>
API 20C Aux	34	<i>C. krusei</i> (23) <i>C. albicans</i> <i>C. albicans</i> <i>C. albicans</i> <i>T. glabrata</i> <i>T. glabrata</i> (2) <i>C. tropicalis</i> (3) <i>C. tropicalis</i> <i>Cryptococcus neoformans</i>	<i>Candida lipolytica</i> or <i>C. krusei</i> <i>C. albicans</i> or <i>C. parapsilosis</i> <i>C. albicans</i> or <i>C. tropicalis</i> or <i>Cryptococcus neoformans</i> <i>C. albicans</i> or <i>C. tropicalis</i> or <i>C. lusitaniae</i> <i>Pichia wickerhamii</i> or <i>T. glabrata</i> <i>B. capitatus</i> or <i>C. krusei</i> or <i>T. glabrata</i> <i>C. tropicalis</i> or <i>C. lusitaniae</i> <i>C. lusitaniae</i> or <i>T. candida</i> or <i>C. tropicalis</i> <i>Cryptococcus neoformans</i> or <i>Cryptococcus albidus</i>
Vitek YBC	10	<i>C. tropicalis</i> <i>C. tropicalis</i> <i>C. parapsilosis</i> (5) <i>C. krusei</i> (2) <i>Cryptococcus neoformans</i>	<i>C. tropicalis</i> or <i>C. parapsilosis</i> <i>C. tropicalis</i> or <i>C. albicans</i> <i>C. parapsilosis</i> or <i>C. tropicalis</i> <i>C. krusei</i> or <i>C. lambica</i> <i>Cryptococcus luteolus</i> or <i>Cryptococcus neoformans</i>

Of some concern were the misidentification of three *Cryptococcus neoformans* isolates as saprophytic *Cryptococcus*, *Cryptococcus humicola*, and *Cryptococcus laurentii* with the Vitek YBC system and the misidentification of one isolate with the RapID Yeast Plus System. Saprophytic *Cryptococcus*, *Cryptococcus humicola*, and *Cryptococcus laurentii* would likely be considered clinically insignificant if they were isolated from certain specimens, such as those from the respiratory tract, but *Cryptococcus neoformans* can cause pulmonary disease (8). Certainly, identification of a saprophytic *Cryptococcus* species from a cerebrospinal fluid sample with any system should be questioned and alternative tests such as reaction on birdseed agar (12) should be performed. Previous studies by Kitch et al. (7) and Espinel-Ingroff et al. (3) did not show any problem with the RapID Yeast Plus System in correctly identifying *Cryptococcus neoformans*.

Our study evaluated three systems for identifying commonly isolated yeasts in the clinical laboratory. In an era of cost containment and limited personnel, systems that can accurately identify yeasts without the need for supplementary tests have the ability to improve efficiency in the laboratory as well as provide rapid, clinically relevant information. Choosing a system for routine use in the laboratory will be dependent on all of these factors.

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