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Comparison of RapID Yeast Plus System with API 20C System for Identification of Common, New, and Emerging Yeast Pathogens

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The ability to identify yeast isolates by the new enzymatic RapID Yeast Plus System was compared to the ability to identify yeast isolates by the API 20C system. A total of 447 yeast isolates representing *Blastoschizomyces capitatus*, 17 *Candida* spp., 5 *Cryptococcus* spp., *Geotrichum* spp., 2 *Hanseniaspora* spp., *Hansenula anomala*, *Hansenula wingei*, 3 *Rhodotorula* spp., *Saccharomyces cerevisiae*, *Sporobolomyces salmonicolor*, *Trichosporon beigelii*, and 2 *Prototheca* spp. were evaluated. Also, five quality control strains (*Candida* spp. and *Cryptococcus laurentii*) with well-documented reactivities by the RapID Yeast Plus System were used. Each isolate was evaluated by both methods with a 48-h culture grown at 30°C on Sabouraud dextrose agar (Emmons modification) by following the recommendations of the manufacturers. The RapID Yeast Plus System enzymatic reactions were read after 4 h of incubation, and the API 20C carbohydrate assimilation identification profiles were obtained after 72 h of incubation. There was good (95.7%) agreement between the identifications obtained by the two methods with the eight common *Candida* spp. and with *Cryptococcus neoformans*. The agreement was lower when the emerging *Candida* spp. and other yeast-like pathogens were tested (79.1 and 75.2%, respectively). These preliminary data suggest the potential utility of the RapID Yeast Plus System for use in the clinical laboratory for the rapid identification of common yeast pathogens as well as certain new and emerging species.

Substrate assimilation is based on the development of growth of an organism in the presence of chemically pure substrates, and it is the conventional method used for the identification of yeasts and yeast-like fungi. By using a better basal medium and more carbon compounds than were previously evaluated by other investigators, Wickerham and Burton (11) demonstrated the usefulness of assimilation tests for the classification of yeasts in 1948. These conventional assimilation methods, which were simplified in 1975 (4), remain tedious and time-consuming. The increased incidence of yeast infections among immunocompromised patients demanded even simpler methods of identification, which led to the development of several commercial kits during the mid-1970s. Among the early commercial methods (1, 5, 7, 8), the API 20C Yeast Identification system (bioMérieux Vitek, Inc., Hazelwood, Mo.) was modified to its current version and has been evaluated by several investigators (1, 5). The API 20C system permits the accurate use of 19 assimilation tests for the identification of most clinically important yeasts after 72 h of incubation. The prompt and accurate identification of yeasts is becoming more important because new antifungal agents with different activities against the various species are being developed and the association of common, emerging, and new yeast pathogens with severe infection continues to increase among patients with compromised, cell-mediated immunity and neutropenia.

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A single-substrate, chromogen micromethod has been designed by Innovative Diagnostic Systems, L.P., Norcross, Ga. (RapID Yeast Plus System). The RapID Yeast Plus System is based upon enzymatic reactions of chromogenic substrates involving preformed enzymes and allows the differentiation of yeasts, yeast-like fungi, and similar organisms recovered from human clinical specimens after only 4 h of incubation. The purpose of the present study was to compare the ability of the RapID Yeast Plus System with that of the API 20C System to identify 447 clinical isolates of yeasts.

MATERIALS AND METHODS

Cultures. A set of 447 isolates from two medical centers representing the genera and species listed in Tables 1, 3, and 4 were studied. *Candida albicans* ATCC 14053, *Candida glabrata* ATCC 2001, *Candida* (*Yarrowia*) *lipolytica* ATCC 9773, *Candida kefyr* (*Candida pseudotropicalis*) ATCC 2512, and *Cryptococcus laurentii* ATCC 66036 were tested as quality control isolates. When the expected enzymatic reactivity results were obtained with the control isolates as recommended by the manufacturer, the clinical isolates were tested.

RapID Yeast Plus System. The RapID Yeast Plus System uses a qualitative micromethod with 18 conventional and chromogenic substrates (2): 1% glucose, maltose, sucrose, trehalose, and raffinose (cavities 1 to 5, respectively); 1% fatty acid ester (cavity 6); 0.05% *p*-nitrophenyl-*N*-acetyl-β-D-galactosaminide, *p*-nitrophenyl-α-D-glucoside, *p*-nitrophenyl-β-D-glucoside, *o*-nitrophenyl-β-D-galactoside, *p*-nitrophenyl-α-D-galactoside, *p*-nitrophenyl-β-D-fucoside, *p*-nitrophenyl phosphate, and *p*-nitrophenyl phosphorylcholine (cavities 7 to 14, respectively); 0.3% urea (cavity 15); and 0.01% proline β-naphthylamide, histidine β-naphthylamide, and leucyl-glycine β-naphthylamide (cavities 16, 17, and 18, respectively).

As recommended by the manufacturer, each isolate was subcultured prior to testing to ensure viability and purity. Yeast inoculum suspensions were prepared from 48-h cultures grown on Sabouraud dextrose agar (Emmons modification) plates at 30°C. Briefly, yeast cells were suspended in 2 ml of RapID Yeast Plus Inoculation Fluid to achieve a turbidity which completely obliterated the black lines of the Inoculation Card supplied with the kit. Each yeast suspension was dispensed into a RapID Yeast Plus panel, and the panels were then incubated for 4 h at 30°C. Immediately after the incubation time, RapID Yeast Plus Reagents

TABLE 1. Comparison of the identifications of common yeast pathogens by the RapID Yeast Plus System and the API 20C system[a]

Species (no. tested)	No. (%) of isolates			
	Agreement to species	Agreement with additional tests	Discrepant identification	No code
Candida albicans (33)	33	0	0	0
Candida glabrata (26)	26	0	0	0
Candida guilliermondii (22)	18	1	3	0
Candida krusei (23)	23	0	0	0
Candida lusitaniae (19)	18	1	0	0
Candida parapsilosis (25)	25	0	0	0
Candida tropicalis[b] (46)	42	2	2	0
Cryptococcus neoformans (17)	17	0	0	0
Total (211)	202 (95.7)	4 (1.9)	5 (2.4)	0

[a] For low-probability identifications, additional tests were performed; no code, no identification.
[b] Both sucrose-positive and sucrose-negative strains ($N = 8$) were included.

A and B were added to the designated cavities and color reactions were evaluated by following the manufacturer's directions (2). A six-digit microcode was derived and compared to the codes in the RapID Yeast Plus Code Compendium for the identification of the isolate. All microcodes were also sent to the manufacturer for confirmation.

API 20C system. Molten (50°C) API basal medium ampoules were inoculated with yeast colonies, and the suspension was standardized to a density below 1+ (lines can be clearly distinguished) on a Wickerham card. Each cupule was inoculated, and the trays were incubated for 72 h at 30°C. Cupules showing turbidity significantly heavier than that of the negative control cupule (0 cupule) were considered positive. Identification was made by generating a microcode and using the API 20C Analytical Profile Index or the Voice Response System (for profiles not found in the index). Morphology on cornmeal was also evaluated as determined by the manufacturer.

Analysis of the data. For each isolate, the identifications obtained by the two methods were compared; and each method was evaluated for its ability to identify the isolates (i) to the species level, (ii) to the genus level or when additional tests (low-probability identifications) were required to distinguish between two or more possible species, (iii) for its discrepant identifications, and (iv) for its failure to provide an identification (no codes) (see Tables 1, 3, and 4). When the six-digit microcode provided an identification with a low percentage of probability (<94%), the recommended additional tests described below were performed by conventional methods (10). The percentages of agreement at the species level with additional tests (low-probability identifications) and without and disagreements between the two methods were obtained (see Tables 1, 3, and 4). Some isolates with discrepant identifications between the two methods or for which the RapID Yeast Plus System failed to provide an identification were sent to the Clinical Microbiology (Mycology) Laboratory of the New York University Medical Center (NYU) for confirmation of identification by noncommercial assimilation and fermentation methods, morphological studies, and other tests such as thermotolerance tests and tests for urease and KNO_3 assimilation (11).

RESULTS AND DISCUSSION

In 1979, Land et al. (5) reported a 97% agreement between the identifications obtained by a conventional method and the API 20C yeast identification system when the latter test was used in conjunction with morphological characteristics. During the last two decades, the performance of new commercial methods for yeast identification has often been compared to that of the API 20C system. Because of this, although some (>200) of the isolates evaluated had previously been identified by conventional methods, we reidentified each isolate by the two methods.

Prior evaluations of the RapID Yeast Plus System by the manufacturer (2) have claimed a 95.5% correlation with the API 20C system for the identification of 378 isolates, but the species evaluated were not described. The manufacturer's differential chart (2), however, claims the possible identification of 42 species; we evaluated 36 of these 42 species. As indicated in Table 1, the two methods were in agreement to the species level, without additional tests, for the identification of 202 of the 211 (95.7%) yeasts grouped as common yeast pathogens. This group of species comprised the yeasts most frequently recovered from patients with severe yeast infections (10). Another four (1.9%) common yeasts were identified by the RapID Yeast Plus System with additional tests, e.g., morphological and thermotolerance tests. Among the discrepant identifications (Table 2) between the two methods for isolates in this group, the identification by the API 20C System of three *Candida guilliermondii* and two *Candida tropicalis* (sucrose-negative variety) by the RapID Yeast Plus System were confirmed by conventional methods in the NYU laboratory. Our results are similar to those obtained by other investigators in a prior evaluation of the RapID Yeast Plus System with the same species (3).

As indicated in Table 3, the overall level of agreement be-

TABLE 2. Details of discrepant identifications

Identification by API 20C system or conventional method (no. of isolates)	Identification by RapID Yeast Plus System
Candida ciferrii (2)	*Candida rugosa*, *Trichosporon beigelii*
Candida famata (6)	*Candida parapsilosis*, *Candida zeylanoides*
Candida guilliermondii (3)	*Candida parapsilosis*
Cryptococcus intermedia (2)	*Cryptococcus albidus*
Candida rugosa (1)	*Blastoschizomyces capitatus*
Candida tropicalis (2)	*Candida lambica*, *Candida lusitaniae*
Candida zeylanoides (1)	*Candida lusitaniae*
Cryptococcus albidus (3)	*Hansenula wingei*, *Rhodotorula* spp.
Cryptococcus laurentii (2)	*Cryptococcus albidus*, *Cryptococcus humicolus*
Geotrichum spp. (1)	*Trichosporon beigelii*
Rhodotorula minuta (3)	*Rhodotorula rubra*, *Sporobolomyces salmonicolor*
Rhodotorula rubra (1)	*Rhodotorula* spp.
Trichosporon beigelii (4)	*Cryptococcus humicolus*

TABLE 3. Comparison of the identifications of emerging pathogenic *Candida* spp. by the RapID Yeast Plus System and the API 20C system[a]

Species (no. tested)	No. (%) of isolates			
	Agreement to species	Agreement with additional tests	Discrepant identification	No code
Candida ciferrii (12)	10	0	2	0
Candida famata (9)	0	2	6	1
Candida kefyr (16)	16	0	0	0
Candida lambica (14)	14	0	0	0
Candida lipolytica (17)	16	1	0	0
Candida rugosa (7)	4	2	1	0
Candida zeylanoides (10)	8	1	1	0
Candida spp.[b] (6)	4	0	2	0
Total (91)	72 (79.1)	6 (6.6)	12 (13.2)	1 (1)

[a] For low-probability identifications, additional tests were performed; no code, no identification.
[b] Species tested: *Candida intermedia*, *Candida stellatoidea*, and *Candida utilis.*

tween the two methods for the identification of the yeasts that we grouped as emerging species of *Candida* was 79.1% (72 of the 91 isolates tested). An additional six yeasts (6.6%) were identified by supplementary conventional tests, e.g., thermotolerance tests and the presence of hyphae and of a pellicle in broth. Although the level of agreement between the two methods was low for these *Candida* spp. as a whole (Table 3), the two methods showed good agreement in the identification of *Candida kefyr*, *Candida lambica*, and *Candida lipolytica* strains. Representative strains of these species were reidentified by conventional methods. A previous study demonstrated that the RapID Yeast Plus System identified 94.1% of 304 yeast and yeast-like isolates (3), but those investigators evaluated mostly (264 of the 304) common yeast pathogens. Furthermore, only one to three isolates of each of the new and emerging species of pathogens were included in their study (3).

Table 4 indicates that the level of agreement between the two methods of yeast identification for the fungi grouped as emerging yeast and yeast-like pathogens was similar to the one for the emerging species of *Candida*: 75.2% without additional tests and 83.5% with the aid of additional tests. The additional tests needed were tests for ascospore production (*Hansenula anomala*); thermotolerance tests; nitrate, lactose, and raffinose assimilation tests; capsule formation; and hypha and pigmentation production (*Cryptococcus* spp. and *Rhodotorula* spp.) tests. The major discrepancies between the two methods were observed when evaluating isolates of *Cryptococcus albidus*, *Cryptococcus laurentii*, *Rhodotorula minuta*, and *Trichosporon beigelii* (Table 2). The RapID Yeast Plus method was also unable to identify some isolates of these species as well as isolates of *Blastoschizomyces capitatus* (1 of 9 isolates) and *Hansenula anomala* (2 of 17 isolates). One of the problems was the difficulty in interpreting the color change indicative of positive reactions, especially when reading wells 6 (lipase), 16, 17, and 18 (proline, histidine, and leucyl-glycine β-naphthylamide, respectively). The NYU laboratory confirmed the identifications of two *Rhodotorula rubra* isolates obtained by the RapID Yeast Plus System. These isolates were identified as *Rhodotorula minuta* by the API 20C system. For seven isolates of *Hansenula anomala*, including the two isolates that the RapID Yeast Plus System failed to identify (listed as no code in Table 4), additional tests were performed for confirmation of the identifications. Although conventional methods (10) identified these isolates as probable *Hansenula anomala*, ascospore production was observed in only one of these strains. Nine isolates of *Cryptococcus albidus*, *Cryptococcus laurentii*, and *Trichosporon beigelii* had discrepant identifications by the two methods. The identifications of these isolates by the API 20C system were also confirmed by conventional tests. One site also tested direct inoculation into the RapID Yeast Plus System from the primary isolation medium, inhibitory mold agar. Only 48 of 97 isolates tested (data not shown) were correctly identified. This emphasizes the importance of using the incubation conditions and media specified by the manufacturer.

TABLE 4. Comparison of the identifications of emerging yeast pathogens and yeast-like fungi by the RapID Yeast Plus System[a] and the API 20C system

Species (no. tested)	No. (%) of isolates			
	Agreement to species	Agreement with additional tests	Discrepant identification	No code
Blastoschizomyces capitatus (9)	8	0	0	1
Cryptococcus albidus (12)	5	0	3	4
Cryptococcus laurentii (14)	10	2	2	0
Cryptococcus terreus (4)	3	1	0	0
Cryptococcus uniguttulatus (7)	6	1	0	0
Geotrichum spp. (2)	1	0	1	0
Hanseniaspora spp.[b] (5)	5	0	0	0
Hansenula anomala (17)	14	1	0	2
Hansenula wingei (2)	2	0	0	0
Prototheca spp. (6)	6	0	0	0
Rhodotorula glutinis (1)	1	0	0	0
Rhodotorula minuta (12)	4	3	3	2
Rhodotorula rubra (13)	11	1	1	0
Saccharomyces cerevisiae (17)	16	1	0	0
Sporobolomyces salmonicolor (4)	4	0	0	0
Trichosporon beigelii (20)	13	2	4	1
Total (145)	109 (75.2)	12 (8.3)	14 (9.7)	10 (6.9)

[a] For low-probability identifications, additional tests were performed; no code, no identification.
[b] The RapID Yeast Plus System does not differentiate between the species of this genus. Species tested: the species *Hanseniaspora guilliermondii* and *Hanseniaspora uvarum* and the species *Prototheca wickerhamii* and *Prototheca zopfii.*

In conclusion, although rapid and easy methods are needed for clinical laboratories that have not been able to switch to automated procedures for rapid yeast identification, such as the Vitek and MicroScan systems (6, 9), the RapID Yeast Plus System should be used with caution when identifying the less common yeasts and yeast-like pathogens. However, some of the incorrectly identified isolates belong to the species less frequently isolated from clinical specimens. Our data also suggest that the RapID Yeast Plus method is an accurate, rapid, and cost-effective tool in the clinical laboratory for the identification of common and many of the new and emerging *Candida* spp., *Cryptococcus neoformans*, and certain other yeasts and yeast-like fungi.

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