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# Ability of RapID Yeast Plus System To Identify 304 Clinically Significant Yeasts within 5 Hours

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**The RapID Yeast Plus System (Innovative Diagnostic Systems, Norcross, Ga.) is a qualitative micromethod that uses conventional and chromogenic substrates for the identification of medically important yeasts. The ability of the RapID Yeast Plus system to accurately identify 304 clinical yeast isolates within 5 h was evaluated. The RapID Yeast Plus method correctly identified 286 (94.1%) of strains to the species level without the need for additional tests. A further 12 strains (3.9%) were classified as correct to the genus level or to a low-probability identification with two or more possibilities. In these latter cases, additional tests were required to delineate the correct identification. Organisms in the latter group comprised *Candida parapsilosis* ($n = 1$), *Candida tropicalis* ($n = 1$), *Candida ciferrii* ($n = 1$), *Candida guilliermondii* ($n = 2$), *Candida humicola* ($n = 1$), *Candida kefyr* ($n = 1$), *Cryptococcus neoformans* ($n = 1$), and *Rhodotorula rubra* ($n = 4$). Six strains (2.0%) were misidentified or did not yield codes in the manufacturer's database. These included one *Candida utilis* (identified as *Candida famata*/*Candida guilliermondii*), one *Trichosporon beigelii* (identified as *Cryptococcus neoformans*), one *Candida diddensiae* (identified as *Candida albicans*), one *Candida membranaefaciens* (identified as *Candida parapsilosis*), one *Candida norvegensis* (identified as *Candida zeylanoides*), and one *Candida catenulata* (no code) isolate; the last four strains are not included in the firm's current database. The RapID Yeast Plus system yielded excellent results and may be recommended for use in the routine laboratory for accurate same-day identification of clinically significant yeasts.**

Yeasts and yeast-like fungi are increasingly being implicated in serious systemic infections, especially in debilitated patients with depressed cell-mediated immunity such as those with AIDS. Development of new chemotherapeutic agents active against these fungi makes an accurate identification mandatory. The earlier an identification is made in the laboratory, the sooner effective antifungal therapy may be instituted. Identification is important, because some yeasts and yeast-like fungi differ in their antifungal susceptibilities, and some organisms are associated with specific disease syndromes (13). Conventional identification procedures are time-consuming and are often not within the scope of many routine microbiology laboratories.

The commercially based methodologies commonly used in the United States for the identification of these fungi include API 20C (Analytab Products, Plainview, N.Y.), Uni-Yeast-Tek (Remel, Lenexa, Kans.), the Vitek system (Biomérieux Vitek, Inc., Hazelwood, Mo.), and the Baxter Microscan system (Baxter Microscan, Inc., West Sacramento, Calif.). The API 20C system requires up to 72 h and the Vitek and Uni-Yeast-Tek systems require up to 48 h for reliable identifications. The Microscan system provides identifications in 4 h (1, 2, 4–6, 8–10, 12). Laboratories without facilities for automated systems such as the Vitek and the MicroScan systems require a rapid, cost-effective, reproducible, nonautomated method which provides same-day identifications of yeasts and yeast-like fungi. Rapid and accurate identification of these organisms has the potential to save time, labor, and money, obviating prolonged incubations and the performance of additional tests.

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The RapID Yeast Plus System (Innovative Diagnostic Systems, Norcross, Ga.) is a qualitative micromethod that uses conventional and chromogenic substrates for the identification of medically important yeasts, yeast-like fungi, and similar organisms isolated from human clinical specimens. The purpose of the study described here was to evaluate the capability of the RapID Yeast Plus System to accurately identify 304 clinically isolated yeasts in a timely manner. This is the first study outside of the manufacturer's control to evaluate the RapID Yeast Plus System.

## MATERIALS AND METHODS

**Fungi.** The yeasts studied (Table 1) were all clinical isolates that were maintained at 25°C on Sabouraud glucose agar (SGA) slants (Remel). Strains originated from the Hershey Medical Center and the culture collection maintained at the University of Texas Medical Branch. Throughout the study, purity was consistently verified by Gram staining and colonial morphology. Identification of all strains was by a conventional methodology (13).

**RapID Yeast Plus System.** Cultures were plated onto SGA plates (Remel), and the plates were then incubated for 48 h at 30°C. Sufficient growth from the cultures was suspended in RapID inoculation fluid to yield a visual turbidity approximately equal to that of a no. 3 McFarland turbidity standard, vortexed, and used within 15 min to inoculate the kits. An additional SGA plate was inoculated from the suspension and was incubated at 30°C for 24 to 48 h to validate the colonial and microscopic morphologies of the isolate.

The RapID Yeast Plus System consists of 18 wells containing the following tests: utilization of glucose, maltose, sucrose, trehalose, and raffinose; hydrolysis of fatty acid ester; *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; *p*-nitrophenyl phosphorylcholine; urea; proline β-naphthylamide; histidine β-naphthylamide; and leucylglycyl β-naphthylamide. Panels were inoculated according to the manufacturer's instructions and were incubated at 30°C for 4 to 5 h. The reactions in wells 1 to 6 and 15 were read without the addition of reagents; reactions in wells 7 to 14 were read between 30 and 60 s following the addition of 1 drop of RapID Yeast Plus reagent A, and reactions in wells 16 to 18 were read between 30 and 60 s after the addition of 1 drop of RapID Yeast Plus reagent B. On the basis of the reactions, six-digit microcodes were constructed. These codes were used to search the manufacturer's computer service

TABLE 1. Identification of yeasts and yeast-like fungi by the RapID Yeast Plus System

| Organism (no. of isolates tested) | No. (%) of isolates | | |
|---|---|---|---|
| | Correct to species | Low-probability identification | Misidentification |
| *Candida albicans* (61) | 61 | 0 | 0 |
| *Candida krusei* (14) | 14 | 0 | 0 |
| *Candida lusitaniae* (10) | 10 | 0 | 0 |
| *Candida parapsilosis* (48) | 47 | 1 | 0 |
| *Candida tropicalis* (60) | 59 | 1 | 0 |
| *Candida ciferrii* (3) | 2 | 1 | 0 |
| *Candida guilliermondii* (5) | 3 | 2 | 0 |
| *Candida humicola* (1) | 0 | 1 | 0 |
| *Candida utilis* (2) | 1 | 0 | 1 |
| *Candida kefyr* (3) | 2 | 1 | 0 |
| *Candida stellatoidea* (1) | 1 | 0 | 0 |
| *Candida diddensiae* (1) | 0 | 0 | 1[a] |
| *Candida membranaefaciens* (1) | 0 | 0 | 1[a] |
| *Candida norvegensis* (1) | 0 | 0 | 1[a] |
| *Candida catenulata* (1) | 0 | 0 | 1[a] |
| *Candida lambica* (1) | 1 | 0 | 0 |
| *Cryptococcus neoformans* (8) | 7 | 1 | 0 |
| *Hansenula anomala* (1) | 1 | 0 | 0 |
| *Candida famata* (1) | 1 | 0 | 0 |
| *Saccharomyces cerevisiae* (1) | 1 | 0 | 0 |
| *Trichosporon beigelii* (3) | 2 | 0 | 1 |
| *Candida glabrata* (71) | 71 | 0 | 0 |
| *Rhodotorula glutinis* (1) | 1 | 0 | 0 |
| *Rhodotorula rubra* (5) | 1 | 4 | 0 |
| Total (304) | 286 (94.1) | 12 (3.9) | 6 (2.0) |

[a] Not included in the RapID Yeast Plus database at this time.

database for a species identification. Unfortunately, at the initial phase of the study, a code compendium was not yet available; this is now available and contains, apart from organism identifications, a list of additional tests required to delineate organisms with low-probability identifications (see below). Identifications were classified as (i) correct to the species level, comprising excellent, very good, good, and implicit identifications; (ii) correct to the genus level or low-probability identification with two or more possibilities (in these latter cases, additional tests were required to delineate the correct identification; additional tests required for low-probability identifications are listed in Table 2 and were performed by standard conventional methodology [13]); and (iii) misidentification or no identification.

## RESULTS

The RapID Yeast Plus System is easy to use, with only a few exceptions, and the reactions are distinct and easy to interpret. The identifications made by the system are listed in Table 1. As can be seen, 286 of 304 strains (94.1%) were correctly identified to the species level without additional tests. A further 12 organisms (3.9%) yielded the correct identifications among two or more possibilities, with the correct identification upon performance of additional tests (Table 2). These comprised one *Candida parapsilosis* (*Candida lambica*/*Candida parapsilosis*), one *Candida tropicalis* (*Candida tropicalis*/*Saccharomyces cerevisiae*) one *Candida ciferrii* (*Candida guilliermondii*/*Candida ciferrii*), two *Candida guilliermondii* (*Candida famata*/*Candida guilliermondii*), one *Candida humicola* (*Trichosporon beigelii*/*Candida humicola*), one *Candida kefyr* (*Saccharomyces cerevisiae*/*Candida kefyr*), one *Cryptococcus neoformans* (*Cryptococcus neoformans*/*Pichia* spp.), and four *Rhodotorula rubra* (one *Trichosporon beigelii*/*Rhodotorula rubra*, one *Rhodotorula glutinis*/*Rhodotorula* spp., and two *Rhodotorula* spp.) isolates. The additional tests required were easy to perform and were primarily based on morphology.

Misidentifications occurred for six strains (2.0%). These comprised one *Candida utilis* (identified as *Candida famata*/*Candida guilliermondii*), one *Trichosporon beigelii* (identified as *Cryptococcus neoformans*), one *Candida diddensiae* (identified as *Candida albicans*), one *Candida membranaefaciens* (identified as *Candida parapsilosis*), one *Candida norvegensis* (identified as *Candida zeylanoides*), and one *Candida catenulata* (no code) isolate; the last four strains are not included in the firm's current database (Table 3).

## DISCUSSION

The API 20C yeast identification system was the first commercial method commonly used for yeast identification in the United States. This system is frequently used as one of the main reference methods when evaluations of other commercial systems are performed. Land and coworkers (5), in a 1979 study, found the API 20C system to achieve a 97% correlation with a rapid conventional method when it was used together with morphological criteria. Subsequently, Fenn et al. (4) have found the API 20C system, with improvements in the system's media and database, to yield 99.3% correct identifications, again along with the use of morphological criteria. Use of yeast morphology in conjunction with the API 20C system yields 73.4% correct identifications after 24 h of incubation, with all but 0.5% isolates identified by 48 h (4). The API 20C system possesses promise for the differentiation of some dematiaceous fungi (3). By comparison, the latest updated Vitek yeast biochemical card provides correct identifications of between 89.7 and 97.2% (1, 2, 4, 8). Isolates yielding incorrect or no identification with the Vitek system typically comprise strains that are uncommonly isolated from clinical specimens. Like the API 20C system, the majority of identifications with the Vitek system are available after 24 h of incubation, with 48 h of incubation being required for some slowly growing yeast iso-

TABLE 2. Details of low-probability identifications

| Conventional identification (no. of isolates tested) | RapID Yeast Plus identification | Additional test(s) |
|---|---|---|
| *Candida parapsilosis* (1) | *Candida lambica*/*Candida parapsilosis* | Pellicle in broth, xylose assimilation |
| *Candida tropicalis* (1) | *Candida tropicalis*/*Saccharomyces cerevisiae* | Morphology |
| *Candida ciferrii* (1) | *Candida guilliermondii*/*Candida ciferrii* | Esculin utilization |
| *Candida guilliermondii* (2) | *Candida famata*/*Candida guilliermondii* | Morphology |
| *Candida humicola* (1) | *Trichosporon beigelii*/*Candida humicola* | Morphology |
| *Candida kefyr* (1) | *Saccharomyces cerevisiae*/*Candida kefyr* | Morphology |
| *Cryptococcus neoformans* (1) | *Cryptococcus neoformans*/*Pichia* spp. | Morphology, phenol oxidase, capsule |
| *Rhodotorula rubra* (1) | *Trichosporon beigelii*/*Rhodotorula rubra* | Morphology, pigment |
| *Rhodotorula rubra* (1) | *Rhodotorula glutinis*/*Rhodotorula* spp. | Rapid $KNO_3$ test |
| *Rhodotorula rubra* (2) | *Rhodotorula* spp. | Rapid $KNO_3$ test |

TABLE 3. Misidentifications with the RapID Yeast Plus system

| Conventional identification (no. of isolates tested) | RapID Yeast Plus identification |
|---|---|
| *Candida utilis* (1) | *Candida famata*/*Candida guilliermondii* |
| *Candida diddensiae* (1)[a] | *Candida albicans* |
| *Candida membranaefaciens* (1)[a] | *Candida parapsilosis* |
| *Candida norvegensis* (1)[a] | *Candida zeylanoides* |
| *Candida catenulata* (1)[a] | No identification |
| *Trichsporon beigelii* (1) | *Cryptococcus neoformans* |

[a] Not included in the RapID Yeast Plus database at this time.

lates (1, 2, 4, 8). The accuracy of the MicroScan yeast identification method is reported to be lower than those of the API 20C and Vitek systems (1, 2, 4, 8). Land and coworkers (6) have reported a 92% correlation rate between the MicroScan and API 20C systems for those taxa included in their databases and a correlation of 85% for all yeasts tested. St.-Germain and Beauchesne (12) have described an identification rate of 78% without requiring additional tests and a rate of 96.6% when additional tests are used. Riddle et al. (8) have reported correct identifications of 82%. All of these investigators experienced difficulties with the identification of slowly growing yeasts and yeast-like fungi (6, 8, 12). The inability of the MicroScan system to accurately identify *Cryptococcus neoformans* is of concern. The MicroScan system has the advantage of providing identifications within 4 h (6).

Salkin et al. (9) have reported a correct identification rate of 40% by using the Uni-Yeast-Tek system for isolates in the manufacturer's database. When using the manufacturer's criteria for reliable identifications, the Uni-Yeast-Tek system could not identify many of the common species as well as uncommon species without the use of additional tests. A second disadvantage of this system deals with the need to monitor the growth of yeasts for up to 6 days before a definitive identification can be established (9).

Other methods which hold promise for yeast identification include the Microring YT (Medical Wire and Equipment Co., Victory Gardens, N.J.) (7, 11), YeastIdent (Analytab Products, Inc., Plainview, N.Y.) (9), and Minitek (BBL Microbiology Systems, Cockeysville, Md.) (12) systems. All of these methods possess problems in the accurate identification of clinically important yeasts. The Biolog system (Biolog, Inc., Hayward, Calif.) is used mainly in industrial settings as opposed to clinical laboratories. This system has two shortcomings. First, teleomorphic names are used for the yeasts, with no information provided to help the user correlate these names to the ones used in clinical laboratories. Second, the data management system has problems regarding identifications.

In the current study, the RapID Yeast Plus System yielded within 5 h accurate identifications for 94.1% of the isolates tested. The isolates tested consisted of taxa representing a broad range of commonly and uncommonly encountered yeasts and yeast-like fungi. Only 12 strains (3.9%) required additional tests for accurate identifications. The misidentification rate was only 2.0%. Four of the six incorrectly identified organisms comprised taxa which are rarely isolated from clinical specimens and are not currently included in the RapID Yeast Plus database. The cost per test, including recommended quality control procedures, is comparable to those of the API 20C and Vitek methodologies. A concern regarding all identification systems deals with their ability to exclude taxa not included in their databases so that they do not force-fit unusual yeasts. This results in false-positive identifications. Additionally, in comparison with other commercial methods, the RapID Yeast Plus method requires morphology for occasional strains only. The results of the present study indicate that the RapID Yeast Plus method may be recommended for use in the clinical laboratory for the accurate same-day identification of yeasts, because the system is rapid, accurate, reproducible, and cost-effective.

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