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Evaluation of the MicroScan Rapid Yeast Identification Panel

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The MicroScan Rapid Yeast Identification (RYI) panel is a 4-h microdilution system for identification of clinical yeastlike isolates. Its accuracy was evaluated by using 357 isolates encompassing 11 genera and 30 species. The RYI panel identifications were compared with those obtained by the API 20C system assisted with morphological characterization on cornmeal-Tween 80 agar. The panels were read both visually and with the AutoScan-4, a computer-controlled microplate reader. Both the RYI panel and the API 20C system correctly identified 78% of the strains within 4 and 72 h, respectively, with no additional tests. Supplementary tests recommended by the manufacturers made it possible to identify up to 96.6% (AutoScan-4) and 98.9% (API 20C) of the strains. The accuracy of the RYI panel was 99.5% with common strains and 92.1% with less common strains. The RYI panel misidentified 10 or 12 strains and failed to identify 2 or 3 strains, depending on whether it was read with the AutoScan-4 or visually. Errors occurred with one strain of *Torulopsis glabrata* and the less common yeasts *T. candida*, *Candida lusitaniae*, *C. lambica*, *C. rugosa*, *C. stellatoidea*, *Cryptococcus albidus*, *C. laurentii*, and *C. uniguttulatus*. Overall, the RYI panel appears to be a reliable system for identification of the more common clinical yeast isolates.

The ever-increasing incidence of yeast infections, in particular, those associated with immunocompromised patients (4, 13), and the need to identify yeast isolates accurately and efficiently have led to the development of a variety of manual and automated identification systems. Methods for identifying yeasts in the clinical laboratory may still include some conventional procedures but rely mainly on the use of commercially available micromethod systems employing modified conventional biochemical tests. Among these are the API 20C system, the Flow Laboratories Uni-Yeast-Tek system, the BBL Minitek system, the Vitek Yeast Biochemical Card (2, 5, 8, 9), and others. The basic principle of these systems is carbohydrate assimilation, which requires a minimum incubation period of 24 h for growth. To provide more rapid identifications, two commercial multitest identification systems based on detection of preformed enzymes (1, 3) have been marketed in recent years: the API YeastIdent system and the MicroScan Rapid Yeast Identification (RYI) panel. These systems assess enzyme activities rather than growth, use mostly novel chromogenic substrates, and can identify yeasts within 4 h of inoculation. Biochemical reactions in these systems are used to generate numerical profiles that are compared with a numerical data base to identify organisms.

Reports on the efficacy of the API YeastIdent system have revealed problems related to the limited number of substrates, an insufficient data base, and difficulties in the assessment of results with some of the substrates (7–10). Land et al. have recently reported an overall accuracy of 85% for the MicroScan Yeast Identification system (7). However, the product has since been modified. In this study, we evaluated the accuracy of the latest version of the MicroScan system by comparing the results of automated and visual readings with those of the API 20C system combined with morphological characterization on cornmeal-Tween 80 agar. The API 20C system is the most widely used commercial identification kit for yeasts and has been used repeatedly for evaluation of recent systems (5, 7–10).

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MATERIALS AND METHODS

Organisms. A total of 357 isolates belonging to 30 species (see Table 1) were used in this study. Isolates were obtained from hospital laboratories located mostly in the Montreal metropolitan area in the province of Quebec, Canada. Of these, 77% were recent clinical isolates and the remaining 23% were selected from our culture collection. Overall, 217 were considered common clinical isolates (*Candida albicans*, *C. parapsilosis*, *C. tropicalis*, *Cryptococcus neoformans*, and *Torulopsis glabrata*) and 140 were considered relatively uncommon isolates. Among the 37 isolates of *C. neoformans* in this study, three strains of each serotype, A, B, C, and D, were included. The following organisms were used for quality control and were accurately identified by the API 20C system and the RYI panel: *C. albicans* American MicroScan (AmMS) 225, *C. pseudotropicalis* AmMS 226, *C. tropicalis* AmMS 227, *C. lusitaniae* AmMS 233, *Cryptococcus albidus* AmMS 228, *C. neoformans* AmMS 229, *C. laurentii* AmMS 230, *C. uniguttulatus* AmMS 234, *Torulopsis glabrata* AmMS 231, and *Rhodotorula rubra* AmMS 232. Identifications of clinical isolates with both systems were subsequently compared, and isolates with divergent identifications were checked for purity and retested with both methods. When disagreement persisted, conventional tests were used to provide an accurate identification.

API 20C system. The API 20C system was used as directed by the manufacturer, and results were recorded after the strips had been incubated at 30°C for 72 h. Identifications were made by reference to the API Profile Index, in which as many as five species, in descending order of likelihood, may be listed under each biocode. In our study, the first organism listed under each biocode as an "excellent" or "very good" identification was considered the API 20C identification of the test isolate. Isolates with "good likelihood" and "acceptable identification" comments were submitted to additional tests to obtain a final API 20C identification.

MicroScan system. The RYI panel is a 96-well microdilution plate with 27 dehydrated substrates comprising 13 amino acid β-naphthylamides, nine nitrophenyl-linked carbohydrates, three carbohydrates, urea, and indoxyl phos-

phate (7). It is identical to the yeast identification panel evaluated recently by Land et al. (7), except for the reformulation of four substrates (isoleucine, urea, *N*-acetylgalactosamine, and trehalose) and a complete data base regeneration. Isolates were grown on Sabouraud glucose agar at 30°C for 48 h. Suspensions were prepared and calibrated against the MicroScan turbidity standard. The substrate-containing wells of the RYI panel were inoculated with 50 μl of the suspension, and the panel was incubated aerobically for 4 h at 37°C. The enzyme activities of each isolate were determined by color change in the chromogenic substrates or a pH indicator (7).

After incubation of the RYI microdilution plate, 1 drop of 0.05 N NaOH was added to wells containing nitrophenyl-linked substrates and 1 drop of peptidase reagent was added to wells with β-naphthylamide substrates. After a waiting period of 30 s for color development, the panels were fed to the AutoScan-4 for analysis and, immediately after, read visually by two independent observers with the aid of the MicroScan Microdilution Viewer, all within 5 min. Biochemical test results were converted into nine-digit biocodes and cross-referenced with the computer data bank. A list of corresponding species in decreasing order of probability was produced. Identifications were considered final when the percent probability for a species was ≥85. Below 85%, recommended additional tests were performed to pinpoint the correct species.

Conventional tests. Standard morphologic and physiologic tests were performed whenever additional tests were recommended by the profile indexes of the manufacturers to finalize incomplete identifications. They were also used to arbitrate discrepancies between the API 20C system and the RYI panel. The Dalmau plate technique on cornmeal-Tween 80 agar was used to determine the morphologic characteristics of the strains (4). We consider this test complementary to the API 20C system, and it was therefore performed for all 357 strains (2, 6). Wickerham broth carbohydrate and nitrate utilization, fermentation (12), phenol oxidase, urease, cycloheximide sensitivity, growth at 37 or 42°C, and germ tube tests (4) were also performed when needed.

RESULTS

Both the RYI panel, as interpreted by the AutoScan-4, and the API 20C system identified 78% of 357 strains without additional tests. When additional tests were performed, the RYI panel accurately identified 345 strains, inaccurately identified 10 strains, and did not identify 2 strains, for an overall accuracy of 96.6%. The API 20C system inaccurately identified two strains and failed to identify one strain. The results obtained with the RYI panel, as interpreted by the AutoScan-4, have been broken down by genus and species and are shown in Table 1. Of the 217 common clinical isolates tested, only one strain of *T. glabrata* was incorrectly identified by the AutoScan-4, whereas none were incorrectly identified when the panels were read visually. Among the remaining 140 uncommon clinical isolates, 9 were misidentified and 2 were not identified by the AutoScan-4, for an accuracy of 92.1%. When the panels were read visually by two independent observers, the accuracy was 89.3%, with 12 strains misidentified for each observer and 2 or 3 strains not identified.

An analysis of incorrect or incomplete identifications obtained visually or with the AutoScan-4 is found in Table 2. Six of the 21 strains listed in Table 2 were misidentified by both observers and the AutoScan-4. Another group of 10 strains were misidentified by either the AutoScan-4 or the observers as a consequence of disagreement in the interpretation of results. Throughout the study, difficulties with the interpretation of tests with chromogenic substrates produced discrepancies in profile numbers between the two observers and the AutoScan-4. Identical profile numbers were obtained by both observers and the AutoScan-4 for 44% of the 357 strains. In 25% of the cases, the observers obtained different profiles and the AutoScan-4 profile was in agreement with one of them. For 15% of the identifications, both observers obtained the same profile, which was different from that of the AutoScan-4, and finally, in 16% of the cases, all three results were different. Overall, these differences in the interpretation of the biochemical reactions produced divergent identifications for 14 strains, of which 10 were misidentified and 4 were not identified. With 52 other strains, a significant change in the percent probability of the identification (from ≥85 to <85 or vice versa) was observed without, however, affecting the final outcome. Sixty-four

TABLE 1. Identification of yeastlike organisms by the RYI panel read with the AutoScan-4

Species	No. of isolates			
	Total	Correctly identified	Not identified	Incorrectly identified
Blastoschizomyces capitatus	6	6		
Candida albicans	38	38		
C. guilliermondii	11	11		
C. krusei	14	14		
C. lambica	1			1
C. lipolytica	7	7		
C. lusitaniae	17	15		2
C. parapsilosis	51	51		
C. paratropicalis	3	2	1	
C. pseudotropicalis	6	6		
C. rugosa	2	1		1
C. stellatoidea	3	2		1
C. tropicalis	51	51		
C. zeylanoides	2	2		
Cryptococcus albidus	12	11		1
C. ater	1	1		
C. laurentii	6	5	1	
C. neoformans[a]	37	37		
C. uniguttulatus	6	5		1
Geotrichum sp.	3	3		
Hansenula anomala	3	3		
Prototheca sp.	1	1		
P. wickherhamii	2	2		
Rhodotorula minuta	4	4		
R. rubra	4	4		
Saccharomyces cerevisiae	9	9		
Sporobolomyces salmonicolor	2	2		
Torulopsis candida	3	1		2
T. glabrata	40	39		1
Trichosporon beigelii	12	12		

[a] Isolates consisted of all serotypes.

TABLE 2. Analysis of incorrect or incomplete identifications with the RYI panel

Correct identification (no. of strains)	Incorrect or incomplete identication (no. of strains)			Discrepant test(s)[a]
	Observer 1	Observer 2	AutoScan-4	
Candida lambica (1)	*C. inconspicua*	*C. inconspicua*	*C. inconspicua*	GLY
C. lipolytica (1)	*C. rugosa*			ALA
C. lusitaniae (3)	*C. parapsilosis* (1)	*C. parapsilosis* (1)	*C. parapsilosis* (2)	IDX, SUC2, CELL
		C. guilliermondii (1)		CELL
C. parapsilosis (1)	No identification			PRO
C. paratropicalis (2)	*C. tropicalis* (1)			IDX, (NGAL)
		No identification (1)	No identification (1)	(NAG)
C. rugosa (1)	*C. albicans*	*C. albicans*	*C. albicans*	GGLY, HIS, (NGAL)
C. stellatoidea (1)	*C. albicans*	*C. albicans*	*C. albicans*	(HPR, PRO, GLY)
Cryptococcus albidus (2)	*C. laurentii*	*C. laurentii*		HPR, TYR, GGLY, AARG, HIS, BDF
	C. ater	*C. ater*	*C. ater* (1)	GGLY, HIS, SUC2, BDF, (ILE, PRO)
C. laurentii (2)	*C. neoformans*	*C. neoformans* (1)		BGL, GGAL, (LYAL)
	No identification		No identification (1)	(GLAR)
C. uniguttulatus (1)			*S. salmonicolor*	(GGLY)
Torulopsis candida (2)	*C. guilliermondii* (2)	*C. guilliermondii* (2)	*C. guilliermondii* (2)	BGL, (ILE, TYR, GLAR, STY)
T. glabrata (1)			*C. krusei*	IDX
Trichosporon beigelii (3)	*C. neoformans* (1)			(SUC1)
		C. laurentii (1)		GLPR, NAG
	No identification (1)	No identification (1)		GLPR

[a] HPR, hydroxyproline; ILE, isoleucine; PRO, proline; TYR, tyrosine; GLY, glycine; GGLY, glycylglycine; GLAR, glycylargine; GLPR, glycylproline; AARG, arginylarginine; LYAL, lysylalanine; STY, seryltyrosine; IDX, indoxyl phosphate; HIS, histidine; SUC1 and SUC2, sucrose; BGL, glucopyranoside; BDF, fucopyranoside; CELL, cellobiose; NGAL, galactosaminide; NAG, glucosamine. False-positive tests are in parentheses.

percent of the disagreements involved β-naphthylamide substrates, 22% involved nitrophenyl-linked substrates, and 14% involved other substrates. Among the last group, the AutoScan-4 failed to detect a positive indoxyl phosphate reaction for 21 strains, resulting once in the false identification of a *T. glabrata* strain as *C. krusei*.

DISCUSSION

The MicroScan RYI panel is one of two commercially available systems relying on preformed enzymes for rapid identification of medically important yeastlike organisms. These systems are relatively new on the market. The API YeastIdent system has been the object of several evaluations (8–10), and a first version of the MicroScan system was evaluated recently by Land et al. (7). In the MicroScan panel that we studied, four substrates (isoleucine, urea, *N*-acetylgalactosamine, and trehalose) had been reformulated for ease and consistency of reading and the entire data base had been regenerated for Version 17 software.

The present evaluation shows an overall accuracy of 95.8 or 96.6%, depending on whether the panels were read visually or spectrophotometrically. With the AutoScan-4, 99.5% accuracy was obtained with the more common isolates, compared with 92.1% with the less common ones. The overall correlation with the API 20C system obtained by Land et al. with a previous version of this system was 85%, indicating that this revised panel performs better. Also, in their study, Land et al. pointed out that most of the misidentifications occurred with slowly growing or metabolizing yeasts (7). However, by using these same categories, we found that most (13 of 21) of the misidentifications in Table 2 were associated with rapidly growing organisms. Although 27 (63%) of 43 discrepant test results (Table 2) were false-negative results, the misidentifications appear to correlate better with uncommonly isolated species, indicating a data base-related problem rather than a metabolism-related problem. Furthermore, Land et al. experienced difficulties with the identification of *C. neoformans* serotypes A and D (7). Only 12 of our 37 strains of *C. neoformans* had been serotyped (3 of each serotype, A, B, C, and D), but the remaining 25 were all local strains of the variety neoformans (serotype A or D). All 37 strains were accurately identified, indicating that the data base has been improved in this respect.

Overall, clustering of misidentifications was found for three strains each of *C. lusitaniae* and *Trichosporon beigelii*. There were occasional difficulties with the interpretation of some color reactions, but this did not appear to be a major problem whether the panels were read visually or with the AutoScan-4. However, interpretation difficulties with chromogenic substrates have been described before (8–11) and it can be assumed that use of an automated system consistent in the interpretation of reactions and free of the subjectivity of visual readings is preferable. Furthermore, we noticed at the beginning of our study that many profile numbers were not listed in the MicroScan index manual but were included in the computer data base. We therefore decided to process all profile numbers obtained visually through the data base instead of the index. Finally, it must be kept in mind that, although the RYI panel provides identifications in 4 h, it still requires a 48-h delay after primary isolation to obtain sufficient growth for inoculum preparation.

Until recently, identification of medically important yeasts was based on carbohydrate assimilation tests, as well as morphologic characterization. New systems based on preformed enzyme reactions introduce a basic change in principle to classic methodology and therefore warrant caution before adoption. Most of the substrates used in these systems are unknown to us. We therefore have to become accustomed to new biochemical profiles for the various species encountered. Most important is the fact that morphologic features are no longer used for most of the identifications. This means that many identifications now depend solely on these novel biochemical reactions. We recommend that laboratory workers wishing to use the RYI panel con-

tinue using morphologic characterization on cornmeal-Tween 80 agar to cross-check all of their identifications. This should be done until they have mastered the use of this new system and until additional studies further substantiate its accuracy. Finally, although many may hope for a yeast identification system that eliminates the need to resort to morphologic characterization, it must be emphasized that this expertise remains necessary for the final identification of numerous strains no matter what system is used. Indeed, with morphological observations, errors in identification could have been avoided for 9 of the 16 misidentified strains listed in Table 2 (*C. lambica*, *C. lipolytica*, *C. rugosa*, *C. uniguttulatus*, *T. glabrata*, and 2 strains each of *T. candida* and *T. beigelii*).

In summary, the results of our study indicate that the accuracy of the MicroScan RYI panel compares well with that of the API 20C system in the identification of the more common yeasts. Although a few problems were encountered with the less frequently isolated species, we feel that this system can nevertheless be considered an attractive alternative for routine identification of medically important yeasts in laboratories that already use MicroScan technology.

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