Evaluation of the API 20 C Microtube System for the Identification of Clinically Important Yeasts

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The API 20 C microtube system containing 20 biochemical tests for the identification of yeasts was compared with conventional tests used in the Mayo Clinic mycology laboratory. Three hundred isolates of clinically important yeasts were studied, and agreement of the carbohydrate fermentation and assimilation tests between the systems was good. The API 20 C represents a useful, commercially available screening method for the identification of yeasts; however, it is not a complete system and must be used in conjunction with microscopic morphological features and, when appropriate, with other biochemical tests.

During the past few years there has been increased interest in methods used for the identification of yeasts seen in clinical laboratories. Approximately 80% of all clinical specimens submitted to the mycology laboratory at the Mayo Clinic contain yeasts, most of which belong to the genera *Candida*, *Torulopsis*, or *Cryptococcus*. Several schema have been proposed for the identification of yeasts (1-7); however, until recently none was commercially available. In this study we evaluated a commercial microtube system that contained 20 standard biochemical tests for the identification of yeasts.

MATERIALS AND METHODS

Three hundred isolates of yeasts and yeastlike organisms recovered from clinical specimens were used in this study (see Table 1). The identification of each was made using methods described previously by Dolan and Roberts (4) and Roberts (Hum. Pathol., in press), which are based on microscopic morphology, germ-tube formation, growth on cornmeal agar, urease production, nitrate reduction, temperature tolerance, pigment production on niger seed agar, and carbohydrate fermentation and assimilation. Each was coded and the identity was unknown until the data were examined. In all instances the API 20 C system (Analytab Products, Inc., Plainview, N.Y.) was compared with the conventional carbohydrate fermentation and assimilation methods used in this laboratory.

API 20 C method. All tests were performed according to the manufacturer's instructions. Ampules containing the API 20 C basal medium were placed in a boiling-water bath until the medium became liquefied. Each was removed and kept at 50 to 52 C until inoculation. The inoculum was prepared by lightly touching a single yeast colony with a sterile

applicator stick and then grinding the applicator against the bottom of an ampule containing the basal medium. The resulting suspension was stirred and adjusted to the correct optical density so that it was just visibly (<1+) denser than uninoculated medium when held against a Wickerham card. With a Pasteur pipette, the suspension was inoculated into each of the microtubes containing 20 different biochemical tests. In the first eight tubes, which were used to detect fermentation of glucose, galactose, maltose, sucrose, lactose, raffinose, trehalose, and melibiose, only the tube sections were filled. The remaining 12 tubes, used to detect resistance to cycloheximide and assimilation of the carbohydrates inositol, glucose, galactose, maltose, sucrose, lactose, raffinose, trehalose, melibiose, and cellobiose, were filled completely with the suspension. In addition, one well contained the basal medium used for a growth control. An overlay of Vaspar (vaseline and paraffin 1:1, vol/vol) was placed over the fermentation tubes, and all air bubbles present were circled so there would be no confusion with gas bubbles produced during fermentation. The strips containing the microtubes were placed in a tray containing 5 ml of water and were incubated at 30 C for 72 h. Readings were made after 24, 48, and 72 h of incubation. Gas production was used as the criterion for fermentation, and turbidity greater than that of the growth control was used as an indication of assimilation. Cycloheximide resistance was based on the presence of growth after 24 h. The identification of organisms was based on the differential charts enclosed with the commercial product.

Conventional method. Conventional fermentation tests were performed as described previously (4). Tubes were read after 24 and 48 h and again after 10 days for evidence of gas production, which indicated fermentation of the carbohydrate substrate.

The method used in the Mayo Clinic mycology laboratory is an auxanographic determination of

carbohydrate assimilation as described by Roberts (Hum. Pathol., in press). A suspension of yeast equivalent to a McFarland standard no. 4 was used to flood the surface of each plate containing yeast nitrogen base (Difco) and 0.4% bromocresol purple (Difco). Carbohydrate disks (Difco) 6 mm in diameter were placed evenly apart on the agar surface. Only the cellobiose disks were prepared in our laboratory by soaking 6-mm blank disks in 20% cellobiose. Cultures were incubated 24 to 48 h and examined for a color change from purple to yellow around the disks, which indicated assimilation of the carbohydrates. The same carbohydrates were used as described for the API 20 C method.

Cycloheximide resistance was determined by the presence of growth on Mycosel agar (BBL) after 48 h of incubation at 30 C.

RESULTS

A comparison of the number of correctly identified organisms by both methods is presented in Table 1. The API 20 C system correctly identified 95% of the isolates compared with 98.7% by the conventional method. The API system identified 94.2, 94.2, and 96.1% of the members of the genera *Candida*, *Cryptococcus*, and *Torulopsis*, respectively. All other genera tested were identified correctly. The conventional method correctly identified all organisms except for four isolates of *C. tropicalis*.

The optimal time required by both methods for the definitive identification of the organisms tested was 72 h; however, it was possible to identify greater than 75% of the *T. glabrata* isolates after 48 h by either method.

Agreement of the carbohydrate fermentation tests between the two methods was high, as shown in Table 2; however, the conventional method detected 35 more tests for trehalose fermentation, of which 27 isolates were *C. albicans* and 8 were *T. glabrata*; 6 more galactose tests were positive for *C. parapsilosis*.

The API system yielded 15 more false-negative galactose fermentation tests for *C. tropicalis*, 10 more false-negative maltose tests for 9 isolates of *C. tropicalis* and 1 of *C. stellatoidea*, and 11 false-positive sucrose tests for *C. albicans*.

Both methods yielded two false-negative glucose tests for *C. krusei*, four false-negative sucrose tests for *C. tropicalis*, and one false-positive galactose test for *C. stellatoidea*. Melibiose was not fermented by any organism tested.

In most instances detection time for fermentation by the API system was 72 h; the conventional method required at least 6 days.

Agreement of the carbohydrate assimilation tests between the two methods was high, as shown in Table 3. The conventional method yielded 15 more positive cellobiose tests for *C. tropicalis*, 5 more positive lactose tests for *C. albidus/albidus*, 1 more positive raffinose test for *C. neoformans*, and 1 more positive melibiose test for *C. guilliermondii*.

The API system gave the following false-positive tests: three raffinose tests for *C. terreus*; two galactose tests for *C. krusei*; two lactose tests for *C. albidus/diffluens*; and one maltose test for *C. krusei*.

TABLE 1. Comparison of correct identification of yeasts by API 20 C and conventional methods

Organism	No. of strains tested	API 20 C (No. correctly identified)			Conventional (No. correctly identified)		
		24 h	48 h	72 h	24 h	48 h	72 h
<i>Candida albicans</i>	42	4	33	5	10	29	3
<i>C. guilliermondii</i>	4	0	2	1	0	1	3
<i>C. krusei</i>	16	0	14	0	9	5	2
<i>C. parapsilosis</i>	33	0	19	14	0	17	16
<i>C. pseudotropicalis</i>	7	0	7	0	0	2	5
<i>C. stellatoidea</i>	2	0	1	0	0	1	1
<i>C. tropicalis</i>	35	1	16	14	0	12	19
<i>Cryptococcus albidus/albidus</i>	6	0	2	3	0	3	3
<i>C. albidus/diffluens</i>	6	0	1	3	0	5	1
<i>C. laurentii</i>	4	0	3	1	0	4	0
<i>C. luteolus</i>	2	0	0	1	1	1	0
<i>C. neoformans</i>	48	0	13	35	0	33	15
<i>C. terreus</i>	3	0	0	3	0	0	3
<i>Geotrichum candidum</i> ^a	2	0	2	0	2	0	0
<i>Rhodotorula</i> sp.	3	0	2	1	0	1	2
<i>Saccharomyces cerevisiae</i>	4	0	4	0	0	2	2
<i>Torulopsis glabrata</i>	78	11	51	13	47	28	3
<i>Trichosporon cutaneum</i>	5	2	3	0	0	4	1

^a Although *G. candidum* is not a yeast, it often has microscopic features similar to yeasts.

TABLE 2. Comparison of carbohydrate fermentation tests in API 20 C and conventional methods

Carbohydrate	No. positive by API 20 C			No. positive by conventional		
	24 h	48 h	72 h	24 h	48 h	>72 h
Glucose	167	26	14	152	49	18
Galactose	9	18	22	1	9	34
Maltose	16	41	13	30	37	14
Sucrose	42	8	7	36	9	4
Lactose	3	4	0	5	1	1
Raffinose	2	4	1	0	3	6
Trehalose	6	76	19	81	30	33
Melibiose	0	0	0	0	0	0

TABLE 3. Comparison of carbohydrate assimilation tests in API 20 C and conventional methods

Carbohydrate	No. positive by API 20 C			No. positive by conventional		
	24 h	48 h	72 h	24 h	48 h	72 h
Inositol	22	38	10	54	11	6
Glucose	289	10	1	298	2	0
Galactose	155	33	10	177	9	4
Maltose	182	13	3	191	3	2
Sucrose	184	13	1	192	3	0
Lactose	16	9	2	16	0	3
Raffinose	23	54	10	59	19	5
Trehalose	94	132	43	176	82	11
Melibiose	6	3	5	7	5	0
Cellobiose	20	26	39	35	38	21

Both methods yielded the following false-negative reactions: four sucrose tests for *C. tropicalis*; one raffinose test for *C. luteolus*; one melibiose test for *C. luteolus*; one cellobiose test each for *C. terreus* and *C. luteolus*; and one inositol test each for *T. cutaneum*, *C. albidus/albidus*, *C. albidus/diffuens*, and *C. terreus*. There also were three false-negative trehalose tests for *T. glabrata* with the API system and two with the conventional method. There were eight false-negative cellobiose tests for *C. neoformans* with the conventional method and four with the API system.

The cryptococci required 72 h of incubation before assimilation could be detected for inositol, raffinose, melibiose, and cellobiose. In most other instances, assimilation of carbohydrates by the other genera was detectable within 24 h; however, trehalose tests required 72 h of incubation.

Table 4 lists organisms that were incorrectly identified by both methods and the reasons for misidentification.

Agreement of the cycloheximide susceptibility tests between the two methods was high. The conventional method gave two false-positive tests for *C. tropicalis* and four false-negative tests for three isolates of *C. guilliermondii* and one of *T. cutaneum*. Both methods had four false-positive tests for two isolates of *Geotri-*

chum candidum and one each of *C. parapsilosis* and *C. luteolus*. In addition, there were two false-positive tests for *C. krusei* with the API system and one with the conventional method.

A "carry-over" phenomenon where growth was observed in the zero-growth control microtube occurred with the following isolates: *C. albicans* (15), *C. tropicalis* (24), *C. parapsilosis* (4), *Saccharomyces cerevisiae* (4), *C. pseudotropicalis* (3), *C. krusei* (2), and *T. glabrata* (1), yielding an overall rate of 18%.

DISCUSSION

The API 20 C system provided correct identification for 95% of the organisms tested compared with 98.7% by the conventional method.

Agreement of the carbohydrate fermentation and assimilation tests between the two methods was high; however, there were several instances in which differences were noted for individual organisms (see Results section). Most individual differences that occurred were for reactions that are described as "variable" by most sources. There were several false-positive and false-negative tests given by both methods, but in all instances except those listed in Table 4, a correct identification could be made because all other reactions for the organisms were characteristic.

In general, the API system was equivalent to

TABLE 4. Analysis of incorrectly identified organisms by API 20 C system

Organism	No.	Explanation for incorrect identification
<i>Candida guilliermondii</i>	1	Melibiose not assimilated after 72 h
<i>C. krusei</i>	2	Glucose not fermented after 72 h; false-positive galactose assimilation; resistant to cycloheximide after 24 h
<i>C. stellatoidea</i>	1	Maltose not fermented after 72 h; false-positive galactose fermentation after 72 h; susceptible to cycloheximide after 24 h
<i>C. tropicalis</i> ^a	4	Sucrose not assimilated after 72 h; galactose, maltose, and sucrose not fermented after 72 h
<i>Cryptococcus albidus/albidus</i>	1	Inositol not assimilated after 72 h
<i>C. albidus/diffluens</i>	2	False-positive assimilation of lactose
<i>C. luteolus</i>	1	Raffinose, melibiose, and cellobiose not assimilated after 72 h
<i>Torulopsis glabrata</i>	3	Trehalose not assimilated or fermented after 72 h

^a Incorrectly identified by API and conventional methods.

the conventional method in its ability and time required to detect carbohydrate assimilation; however, it had a definite advantage of detecting carbohydrate fermentation within 72 h compared with 6 days for the conventional method. The "carry-over" phenomenon should present little or no problem when the carbohydrate assimilation tests are read if the reader compares the tests with the zero-growth control tube.

The manufacturer's instructions state that the API strips should be incubated at 30 C and that reactions be read after 24, 48, and 72 h. We are in agreement and recommend that all strips be kept for 72 h so that the cryptococci will be detected.

The identification of yeasts based entirely on carbohydrate fermentation and assimilation reactions is not reliable. For example, several of the cryptococci and *T. cutaneum* present patterns that are similar, and they cannot be differentiated by fermentation and assimilation tests. False-negative and false-positive tests encountered allow for misidentification; i.e., *C. neoformans* and *C. luteolus* cannot be differentiated if a false-negative melibiose assimilation test occurs. Failure to include appropriate carbohydrates when testing also allows for misidentification; i.e., *C. neoformans* and *C. uniguttulatus* have assimilation patterns that differ only in their ability to utilize dulcitol. In addition, some of the cryptococci differ in their ability to utilize KNO₃. It is necessary that microscopic morphological features, growth on cornmeal agar, germ-tube formation, capsule formation, urease production, temperature tolerance, pigment production on niger seed agar, and nitrate reduction be used, when appropriate, to supplement an identification made by any test system employing carbohydrate fermentation and assimilation tests. We feel that

the melibiose and raffinose fermentation tests and cycloheximide resistance test should be deleted from the API strip since they play no helpful role in the identification of clinically important yeasts. We recommend that they be replaced with tests for urease activity, nitrate reduction, and dulcitol assimilation.

In conclusion, we feel that the API 20 C represents a commercially available system useful for detection of carbohydrate fermentation and assimilation by yeasts. It is not a complete system, however, and must be used in conjunction with microscopic morphological features and, when appropriate, with other biochemical tests. It is a good screening method, which should expand the capability of even small laboratories to identify yeasts. The system requires little storage space, is stable for prolonged periods at 4 C, and does not require any media preparation by the laboratory.

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