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Evaluation of Commercial Systems for the Identification of Clinical Yeast Isolates

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The Analytab Products Inc. (API), Micro-Drop (MD), and Uni-Yeast-Tek (UYT) systems for the presumptive identification of common clinical yeast isolates were compared with the oxidation-fermentation (OF) and a conventional procedure. With 229 coded isolates, the identification accuracies were API 94, MD 83, OF 82, and UYT 99%. The API system required the greatest technical ability. The MD materials were prone to malfunction. OF media, if incubated beyond 14 days, gave an accuracy of 87%, but this offered no advantage over the conventional procedure. The UYT system was the easiest to use.

The increased prevalence and incidence of yeast infections has led to a need in the clinical laboratory for simplified methods to identify common medically important yeasts rapidly and accurately. Conventional procedures and schemes for the identification of yeast have been well described by Wickerham (8) and Lodder (5) and modified for selective identification of clinical isolates (1-3, 6). The skilled technical help required for preparation and interpretation of the methods and sufficient time are seldom available in clinical laboratories. Recently, commercially prepared kits have been developed for the identification of medically important yeasts. This study compares and evaluates selected procedures and commercial products for the identification of common clinical yeast isolates.

MATERIALS AND METHODS

Yeast isolates, mostly of clinical origin, were obtained from the Mycology Division of the Center for Disease Control, Atlanta, Ga., Georgia State University and the Centraalbureau voor Schimmelcultues, Delft, Netherlands. Two hundred twenty-nine common clinical isolates and 27 other cultures were identified with conventional media and procedures as adapted from Lodder (5) and Wickerham (8) by Ahearn (3). These procedures included the use of Wickerham assimilation media (Difco), Christensen urea agar, tissue culture medium 199 (BBL) for germ tubes, oxgall and corn meal with Tween-80 agars for morphology, and malt extract agar for the production of ascospores. The identified cultures were streaked on commercially prepared Sabouraud dextrose agar, tested for growth at 30 and 37°C, and provided as coded unknowns for the following.

API 20 clinical yeast system. The API (Analytab Products Inc.) 20 clinical yeast system consisted of a strip of 20 small plastic tubules containing dehydrated assimilation and fermentation media, a vial of solidified agar solution, a plastic incubation chamber, and guidelines for presumptive identifications. The fermentation tubules contained glucose, galactose, maltose, lactose, raffinose, trehalose, and melibiose with bromocresol purple as an indicator. The assimilation tubules tested for utilization of inositol, glucose, galactose, maltose, sucrose, lactose, raffinose, trehalose, melibiose, cellobiose, and growth in the basal medium lacking carbohydrate, and growth in the presence of cycloheximide (Actidione). The agar, provided in a sealed ampule, was melted in boiling water and held in a water bath at 50 to 55°C for at least 10 min before use. The top of the glass ampule was broken off and the agar was inoculated via a 2-mm diameter sterile wooden applicator stick, the end of which had been touched lightly to a single colony of at least 2-mm diameter from the Sabouraud streak plate. The end of the stick was stirred vigorously against the bottom of the ampule to assure a thorough and even suspension of cells in the agar solution. Sufficient cells were added to make the agar solution just visibly denser than the melted uninoculated agar and the lines on the Wickerham card (provided in the system) only slightly less clear than with the uninoculated medium. Each tubule on the strip was inoculated with a Pasteur pipette held at an angle to the strip to minimize entrapment of air in the tubules. The fermentation tubules were filled with the cell-agar suspension to just the lower margin of the portal of the tubule. The assimilation tubules were filled completely so that the agar solution rose just above the plane of the portal. The fermentation tubules were overlayed with vaspar (paraffin and petrolatum 1:1 at 65 to 70°C). If bubbles were produced during the inoculation of the fermentation tubules, the tubules were marked to indicate the position of the bubble after the agar suspension had solidified. The strips were incubated in the moistened plastic chambers at either 30 or 37°C, as predetermined from results of growth on the Sabouraud medium plate. The tests were read at 72 h in accordance with the manufacturer's instructions, but ob-

servations were made daily during 2 to 7 days in an effort to improve accuracy of identification. Morphology and nitrate assimilation were determined by conventional methods (5, 8).

Oxidation-fermentation. The oxidation-fermentation (OF) method of Webb et al. (7) used basal medium containing 0.2% casitone, 0.003% phenol red with 1.0% carbohydrate, and 0.3% agar adjusted to pH 7.4. The carbohydrates included glucose, maltose, sucrose, galactose, lactose, raffinose, cellobiose, trehalose, xylose, inositol, melibiose, and dulcitol. A base medium control and uninoculated carbohydrate media were included as controls. The medium was dispensed in 5-ml amounts into test tubes and inoculated by scraping a generous amount of inoculum off the Sabouraud plate with a wire loop and stabbing it into the medium. Assimilation of the carbohydrates was indicated by the phenol red indicator turning yellow. The tubes were incubated at room temperature, and results were recorded at 14 days or less. In preliminary tests the OF medium was overlaid with petrolatum to evaluate its capacity to detect fermentation.

Micro-Drop assimilation test system. The Micro-Drop assimilation test (MD) system (Clinical Science) included 150-mm diameter plastic petri dishes, bottles containing about 70 ml of sterile basal agar with bromocresol purple, 11 sealed dispensing cartridges that held 2 ml of sterile solutions of basal medium (glucose, galactose, lactose, maltose, cellobiose, sucrose, melibiose, raffinose, trehalose, inositol, and KNO_3), and cartridge needles. A brief instruction pamphlet and a template card for inoculation were included. The assimilation medium was melted, cooled to about 50°C, and inoculated with 5 ml of a saline suspension of cells standardized for turbidity with a no. 5 McFarland standard. After the agar solidified, one drop of each sugar solution was dispensed onto the plate, duplicating the pattern of the template. The plates were incubated at room temperature and observed at 18 to 24 h for color changes indicating utilization of the carbon sources. The nitrate test and delayed sugar reactions were read at 48 h.

Uni-Yeast-Tek system. The Uni-Yeast-Tek (UYT) system (Corning Medical) was composed of a multisectioned dish containing seven carbon assimilation agars (pH 7.0), a central well containing corn meal with Tween-80 for mycelium and chlamydospore production, urea agar, and nitrate assimilation agar, broth containing 0.05% glucose, and 2.6% beef extract (Inolex) for the production of germ tubes, instructions and a wheeled key for the identification of 16 common clinical yeast isolates. The carbohydrates, varying in concentration from 1 to 4%, were sucrose, lactose, maltose, cellobiose, soluble starch, trehalose, raffinose, urea, and nitrate, and a basal medium control prepared in accordance to the formula of Wickerham (8). The corn meal and urea agars serve as positive growth controls. Cells from a single colony on the Sabouraud plate were suspended in 5 ml of sterile-distilled water at about 10^6 cells/ml (about 1+ on a Wickerham card). One drop was used to inoculate each agar through a small portal on the side of the wells via a Pasteur pipette. Positive reactions on the assimilation agars were denoted by growth accompanied by color changes in the indicators: bromocresol purple to yellow for a positive reaction on carbon assimilations, and bromothymol blue to blue-green for a positive reaction on the nitrate medium. Agar reactions, i.e., color changes and growth, were read from 2 to 7 days. The glucose beef extract broth was inoculated by touching the tip of a pipette lightly to the surface of a colony, inserting the pipette into the broth, and incubating the tube at 37 C for 3 to 4 h before examining microscopically. The corn meal well was inoculated with a standard Dalmau cut, and the plate was incubated at 22 to 26°C. The Dalmau cut was examined microscopically daily 3 to 10 days for observation of blastospore, mycelium, chlamydospore, or arthrospore production.

Before testing the above systems with the coded unknowns, all procedures were used repeatedly with more than 50 known isolates to develop technical facility.

RESULTS

The presumptive identifications of common clinical isolates with each system are given in Table 1. For the API system, only 141 of the 229 clinical isolates gave all biochemical results in complete agreement with those obtained by the conventional method; however, the higher number of tests included on the API strip permitted correct presumptive identifications (94% accuracy), even in the presence of false-positive or -negative results. For example, with *Candida tropicalis* only 18 of 34 isolates gave results agreeing with those of the conventional method. With fermentations, three isolates on glucose, four on sucrose, seven on galactose, and eight on maltose gave false-negative results. On assimilation, nine isolates gave false-negative results on cellobiose. Nevertheless, the remaining pattern of biochemical results in conjunction with morphology permitted the correct presumptive identification of all but two isolates. These failed to ferment sucrose or maltose or assimilate cellobiose and were incorrectly identified as *C. parapsilosis*. Growth in the presence of cycloheximide varied in repeat tests with the same isolate for *C. tropicalis* and for several other species. Therefore, results with this test were completely discounted. Presumptive identification for *Candida* species could usually be made within 48 to 72 h with the strips incubated at 37°C. When incubated at 30°C, the assimilation reactions were latent, and gas in the fermentation tubules was sparse. Generally, errors with the API system for candidas were false-negative fermentation results with glucose and maltose and false-negative assimilation tests for cellobiose and raffinose at the recommended reading time of 72 h.

TABLE 1. *Accuracies of the yeast identification systems*

Organism	Systems							
	API		MD		OF		UYT	
	A/T[a]	P[b]	A/T	P	A/T	P	A/T	P
Candida albicans	18/35	33	35/35	33	35/35	33	35/35	33
Candida guilliermondii	7/13	12	4/13	8	13/13	13	13/13	13
Candida krusei	18/18	18	18/18	18	18/18	18	18/18	18
Candida parapsilosis	12/28	28	28/28	28	26/28	28	28/28	28
Candida pseudotropicalis	3/3	3	0/3	3	2/3	3	1/3	3
Candida stellatoidea	2/4	4	4/4	4	4/4	4	4/4	4
Candida tropicalis	18/34	32	13/34	31	7/34	7	31/34	34
Cryptococcus albidus	4/6	5	3/6	6	3/6	3	6/6	6
Cryptococcus laurentii	3/4	3	1/4	3	2/4	2	4/4	4
Cryptococcus neoformans	20/26	23	2/26	21	13/26	18	26/26	26
Cryptococcus terreus	2/2	2	0/2	0	2/2	2	2/2	2
Rhodotorula rubra	2/12	12	4/12	11	4/12	12	12/12	12
Rhodotorula glutinis	1/2	1	0/2	0	0/2	2	2/2	2
Saccharomyces cerevisiae	10/13	13	0/13	0	13/13	13	13/13	13
Torulopsis glabrata	16/17	16	17/17	17	17/17	17	17/17	17
Trichosporon cutaneum	5/12	10	7/12	8	3/12	12	12/12	12

[a] Number of isolates with all biochemicals in agreement with conventional method/number tested.
[b] Number of correct presumptive identifications as compared to conventional method.

Therefore, the accuracy obtained for the identification of *C. albicans* (Table 1) was based on the presence of germ tubes and chlamydospores. A large percentage of cryptococci gave latent assimilation results with only about 50% of the isolates showing completed assimilation spectra by 72 h. Only 70% of the isolates of *Cryptococcus albidus*, *C. laurentii*, and *C. neoformans* utilized inositol within 72 h and only 90% by 7 days. Two isolates of *C. albidus* and six of the isolates of *C. neoformans*, which were correctly identified, took at least 5 days for complete results. Presumptive identification of the isolates of *C. terreus* could not be made in less than 7 days. Two isolates of *C. neoformans* and one of *R. glutinis*, both nonfermenting yeasts, showed false-positive fermentation reactions after 3 days of incubation. Normal acid reaction in the fermentation tubules occurred with a false bubble probably formed by shrinkage of the agar away from the vaspar seal.

The MD system gave the most rapid assimilation results. Sugar reactions were obtained within 24 to 48 h and KNO_3 reactions at 48 h, but only 83% of the identifications were accurate. Frequent false-negative reactions, particularly with cellobiose, raffinose, and nitrate, were obtained. Some strictly oxidative yeasts, mainly isolates of *C. neoformans*, showed assimilation of only glucose. Falsely positive nitrate assimilations were observed with cryptococci and trichosporons. These were due to fading of the yellow acid color of the media. Fading of all dye reactions was common after 48 h, but several reverted to the original color by 48 to 72 h. Unless frequent observations were made it was possible to miss a reaction.

The OF system gave an overall accuracy of 82% when results were recorded at 14 days. Oxidative yeasts, such as the cryptococci, frequently gave only slight growth and often without changes in the indicator. False-negative results were most often observed with cellobiose, inositol, raffinose, and dulcitol. If the tests were kept for 21 days or longer, the accuracy increased to 87%. Petrolatum overlay of the OF media for added detection of fermentation was not thoroughly evaluated, but preliminary studies with six isolates gave accurate results.

The UYT system with an efficiency of 99% gave the highest degree of accuracy. Only two isolates of *C. albicans* were misidentified (also true for the other systems). Only two isolates of *C. tropicalis* and three of *C. pseudotropicalis* gave false-negative assimilations, viz., for cellobiose; but correct presumptive identifications were obtained with the other results. Identifications were usually completed within 4 days, with the exception of most cryptococci which required at least 5 days.

With all systems the identification of *C. albicans* was based mainly on morphology. Three isolates of *C. albicans* did not produce germ tubes and two of these also did not produce chlamydospores. These were from patients who had received extensive polyene therapy. These cultures were mistakenly identified as *C. parapsilosis* on the API, and OF, and as *C. tropicalis* on the UYT. We obtained distinctive mor-

phologies for these strains with prolonged incubation of 7 to 8 h in modified bovine serum.

Twenty-one yeastlike fungi, representing the genera *Hansenula, Rhodotorula, Torulopsis, Cryptococcus, Candida,* and *Geotrichum,* and six isolates of *Prototheca* sp., all of less common occurrence in clinical laboratories, also were screened with the four systems. With the exception of *Prototheca wickerhamii, P. zopfi,* and *Geotrichum candidum,* the systems did not include sufficient tests for presumptive identification to species. The isolates of *Prototheca* generally grew better in the test systems than in the conventional Wickerham broth media. The accuracies of biochemical tests with these 27 cultures as compared to the conventional system were API 89%, MD 74%, OF 85%, and UYT 98%.

DISCUSSION

Reasonably accurate presumptive identifications for common clinical yeast isolates could be achieved with the four test systems, but each required varied degrees of technical skills and interpretation or modification of their prescribed procedures.

The API system required the highest degree of technical proficiency to obtain accurate results. The increased number of tests provided on the strip permitted a correct presumptive identification with some variation in results. The inoculum density was critical, and extreme care was required in reading reactions—always in comparison with a negative control which can become quite dense inasmuch as starved cells are not prescribed for the inoculum. Insufficient inoculum gave false-negative fermentations, whereas too dense inoculum gave rapid fermentations, but false-positive assimilations. Several isolates of *C. albicans* produced gas in the sucrose fermentation tubules at 37°C, but were negative for sucrose fermentation in the conventional test, even with incubation at 37°C. It was difficult in working at a routine pace to fill the fermentation tubules with the agar-inoculum suspension so as not to entrap air, thereby hindering the test interpretation. At first, the extent of any entrapped air spaces was outlined with a marking pen after inoculation was completed. This practice was not entirely satisfactory, as the act of marking occasionally broke the vaspar seal. Finally, we simply indicated a bubble was present on the recording chart. The vaspar was added at a temperature of 70 to 75°C so that it flowed freely from the pipette making a complete seal between the agar and sides of the tubule without entrapping air. Upon cooling there was some contraction of the vaspar and the agar. Therefore, the presence of trapped air, particularly between the vaspar seal and the agar suspension, was best recorded after the strip had completely cooled (at least 20 to 30 min). Since the API strip provided only for fermentations and sugar assimilations, the necessary morphology and nitrate utilization studies were performed with conventional methods. Therefore, identifications could only be accomplished within the time necessary for these test results. The substitution of nitrate assimilation or a nitrate reduction test for the cycloheximide test and improvement of the cellobiose assimilation medium would enhance the value of the strip. A major advantage of the strip is its small size and shelf life of nearly 1 year.

Rapid assimilation results were achieved with the MD system, but it had technical shortcomings which predisposed to error. The kits were inappropriately packaged and frequently arrived with broken components. Mechanical difficulties were encountered with the dispensing cartridges. Different drop sizes, varied times of absorption for the different sugar solutions by the agar, and varied surface areas covered by the solutions were occasional problems. Solutions of inositol and cellobiose frequently crystallized in the cartridge needles. Flaming the needle lightly temporarily alleviated this problem but added the difficulty of increased and uncontrollable flow; also, the rubber stoppers on the dispensing cartridges occasionally became loose. The requirement for frequent observations to record positive reactions between the 18th and 24th h of incubation was sometimes inconvenient but imperative for certain isolates. Alteration of the relative concentrations of components in the cartridge solutions and a redesign of the cartridge would probably resolve these problems. Newby et al. (J. G. Newby, S. Shadomy, and M. Motley, Proc. Abstr. Annu. Meet. Am. Soc. Microbiol., F55, p. 94, 1975) reported that this procedure gave unacceptable results for *Torulopsis, Trichosporon,* and *Cryptococcus.*

The OF system of Webb et al. (7) was proposed initially to obviate the need of specialized yeast media. It was not designed as a rapid method and it did not function as such. The length of time for reactions ranged from 5 to more than 14 days, with a significant number of latent reactions on cellobiose, inositol, and dulcitol. Acid reactions, which were latent, changed from phenol red to orange instead of a clearly positive yellow color. Interpretation of the intermediate color was difficult. Accuracy with this method for *C. neoformans* often re-

quired prolonged incubation. Our analysis of this method is in essential agreement with that of Adams and Cooper (1), but it should be emphasized that the OF method is generally unsatisfactory for recognition of the genus *Cryptococcus*.

Our results with the UYT system are essentially the same as those of an earlier and completely separate examination of this product (4). It was the easiest to use and its biochemical reactions gave the highest correlation with the conventional method. The only discrepancy in biochemical results was with cellobiose assimilation with five isolates. However, many isolates known to be cellobiose latent and which did not utilize this sugar in our earlier examination were now found to be positive. The improvement in agreement with the conventional method was due to an alteration of the UYT cellobiose assimilation medium by the addition of other beta-glucosides. The addition of tests for the definitive identification of *C. neoformans* would make the UYT system more valuable to the clinical laboratory.

The varied techniques and media studied herein all may be used to obtain reasonably accurate identifications. However, in our experience with clinical laboratory personnel unfamiliar with yeasts, neither conventional methods, the OF method, nor rapid presumptive methods give even 90% accuracy. The major problems are mixed yeast cultures, bacterial contamination, inability to recognize a germ tube, and the failure to detect ascospores or pseudohyphae. There is also a general lack of knowledge that numerous species which are distinguished morphologically have similar physiological properties. We have observed an increasing number of morphologically and physiologically altered yeasts from patients receiving extensive antifungal or antileukemic chemotherapy. This can be expected to compound the identification problems. Appropriate background training and practical experience with yeasts are necessary for accuracy in identification, particularly in the use of a presumptive identification system.

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