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Update and Evaluation of the AutoMicrobic Yeast Identification System

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The AutoMicrobic system (AMS) Yeast Biochemical Card (Vitek Systems Inc., Hazelwood, Mo.) is a system which has been designed for rapid and automated reporting of yeast identification in the clinical laboratory. Recent improvements have been implemented in the AMS data base to expand and enhance its yeast identification capabilities. These improvements include the addition of seven biotypes, changes in data analysis scheme, and construction of the taxonomic keys. The updated system was compared with the API 20C (Analytab Products, Plainview, N.Y.) yeast identification system and a rapid conventional method, using 1,106 clinical and stock yeast isolates. With these improvements, the AMS Yeast Biochemical Card had a correlation of 98.8% with the API 20C system and 93.4% with the rapid conventional method and significantly increased its capability of identifying *Cryptococcus neoformans* (98%). The most difficult organisms for the system to identify in 22 to 24 h were *Cryptococcus terreus* (58%) and *Cryptococcus uniguttulatus* (73%). The updated AMS not only provided more rapid results which were comparable to the other two systems but gave a substantial savings in set-up and reporting time as well.

As a result of advances in the long-term management of cancer and other immunosuppressed patients, the number of fungal infections due to hitherto clinically uncommon organisms has dramatically increased (3, 5, 15). Additional yeast genera which have now been isolated from clinical specimens include *Kluveromyces*, *Hansenula*, *Debaromyces*, and *Sporobolomyces* (1, 10, 14). Moreover, rarely isolated or new species of more common genera, such as *Candida lusitaniae* and *Candida paratropicalis*, have not only been isolated from clinical specimens but documented as causes of infection as well (2, 6, 8). Because of the natural or acquired antifungal resistance of some of these opportunistic pathogens, the severity of systemic fungal infection, and the intense interest in decreasing the hospital stay of patients, rapid and accurate laboratory identification of these organisms appears to be essential (6, 8).

In response to these needs, several rapid conventional yeast identification tests have been developed to screen for the more common pathogens (10). Additionally, two manual commercial systems, the Uni-Yeast Tek (Flow Laboratories, Inc., Baltimore, Md.) and the API 20C (Analytab Products, Plainview, N.Y.), have been marketed to provide fast and accurate identification of medically important yeasts. There are, however, disadvantages to both conventional and commercial approaches to rapid yeast identification. The newer noncommercial methods, although capable of identification within 24 h, usually require the laboratory to make some of the reagents and to do all of the necessary quality control procedures (10). Noncommercial techniques also lack a data base with which to readily identify some of the more uncommon yeast isolates and rely upon existing taxonomic keys and references to determine the identity of an isolate. The commercial counterparts of these manual systems require no media preparation, offer the advantage of having the bulk of the quality control work already done, and offer extensive data bases capable of classifying many of the less common yeasts, also delineating biovars within a given species. Although these commercial approaches are more rapid than the traditional methods, identification is still not rapid enough to influence management of a patient colonized with a potentially resistant organism, such as *C. lusitaniae* (6, 8).

Toward that end, a yeast identification system (Yeast Biochemical Card [YBC]) has been developed by Vitek Systems, Hazelwood, Mo., for the AutoMicrobic system (AMS), to complement their existing automated identification, susceptibility testing, and reporting system for bacteria. Biochemical data from test isolates are analyzed by comparison with an in situ computer-assisted data base, and a typed report is generated. Oblack et al. reported a 96% correlation of the first generation AMS-YBC with the API 20C system if morphological characteristics were used in conjunction with biochemical data and an 84% correlation with biochemical data alone (13). In a study comparing AMS-YBC with the Uni-Yeast Tek, Hasyn and Buckley found an overall correlation of 84.9% when both morphological and biochemical characteristics were considered (7). The latter authors felt the majority of discrepancies could be attributed to a limited data base and improper weighting of key positive and negative characteristics in establishing taxons. In response to the experiences of these investigators, the data base was expanded and changes were made in the way data were analyzed to determine a specific taxon. The following study was undertaken to determine the efficiency of the AMS-YBC system after these improvements.

MATERIALS AND METHODS

Organisms. All yeasts were recent clinical isolates or isolates maintained in the stock culture collections of Methodist Hospitals of Dallas, Vitek Systems Inc., or the University of Cincinnati Medical Center. Proficiency testing isolates from the College of American Pathologists, Centers for Disease Control, and the New York City Department of Health as well as American Type Culture Collection isolates were used for quality control of all identification systems.

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American Type Culture Collection isolates were as follows: *Cryptococcus laurentii* ATCC 18803, *Candida albicans* ATCC 14053, *Rhodotorula glutinis* ATCC 32765, and *Trichosporon capitatum* ATCC 28576.

RCM. All isolates were initially identified and characterized by a manual noncommercial system consisting of a morphology-phenoloxidase determining medium, a rapid urease test, a swab nitrate test, and a pour plate auxanographic method for determining carbohydrate utilization. Carbohydrate fermentation studies were used when necessary. The details of the preparation and use of these various media have been published elsewhere (10, 11). All of the stock cultures had previously been identified by this method. Many of the isolates were utilized in a comparison study among a rapid conventional method (RCM), the traditional Wickerham method, and the Assimilation slant method, in which all three methods virtually agreed with each other (10, 12). For purposes of this study, identification of isolates by RCM was considered correct.

API 20C yeast system. All tests, including microscopic morphology, were performed according to the directions of the manufacturers. Kits were stored at 4°C until used. Ampoules of API 20C basal medium were melted and allowed to cool to 45°C before inoculation. A 1+ Wickerham suspension was made of the unidentified yeast isolate by picking several colonies with the tip of a sterile applicator stick and emulsifying this material in the cooled basal medium. Each cupule of the dehydrated API 20C strip was filled with the inoculum, with care taken not to run cupule contents together or to transfer contents of one cupule to another. Strips were read at 24, 48, or 72 h, and an octyl biotype number was derived from the strip reactions. An identification was made by comparing the biotype number of the unknown yeast with those in a data base compendium supplied by the manufacturer. For those biotypes not in the compendium, the API computer service was utilized. No further incubation was attempted on isolates remaining unidentified at 72 h.

AMS-YBC yeast system. The automated portion and data management system of the AMS have been previously described (7, 9). The YBC is a disposable plastic card (7.6 by 12.7 cm) divided into 30 wells, each of which provides a separate biochemical test or control. A McFarland 2 or 3 suspension was made by picking several well-isolated yeast colonies with a sterile applicator stick and emulsifying the organisms in 1.8 ml of sterile 0.5% NaCl. The suspension was inoculated into the card via the filling module, incubated at 30°C for 18 to 24 h, and placed into the reading module. The identity of an isolate is expressed as the normalized percent probability of a particular biotype when compared with the in situ data base. In all instances, microscopic morphology was utilized in determining the final identification of these isolates.

Experimental design. This study was conducted in two phases. The first consisted of a direct comparison of both the expanded and enhanced AMS-YBC and API 20C systems with RCM. Two groups of yeasts were tested: metabolically active yeasts, such as *Candida*, *Rhodotorula*, *Torulopsis*, and *Saccharomyces* species, and those yeasts which tend to be less biochemically active, such as *Cryptococcus*, *Trichosporon*, and *Geotrichum* species. In the second phase of the study, varieties of less common perfect and imperfect yeasts were randomized, coded, and sent to Vitek for identification with the updated AMS-YBC system. Upon completion of this phase of the study, the code was broken, and the results were compared.

RESULTS

Both the AMS-YBC and the API 20C achieved a high correlation level with RCM in identifying those yeasts which are quite biochemically active, attaining 96.6 and 97.6% correlations, respectively (Table 1). The lowest AMS correlation within this group was with *Candida lipolytica* (80%), *Candida guilliermondii* (86%), and *Torulopsis candida* (91%) (the latter appears as *Candida famata* in the AMS data base). Four isolates of *Candida krusei* were incorrectly interpreted as no growth by the AMS system, indicating either an inadequate inoculum size which did not utilize the substrate within the testing period or a nonviable inoculum. Incomplete substrate utilization by yeasts in either system may also serve to explain the discrepant AMS-YBC results for *C. guilliermondii* and *T. candida* and why API 20C had a more difficult time with *Saccharomyces cerevisiae* than did the Vitek.

The correlation of YBC and API 20C with RCM for the identification of less biochemically active organisms was 91.4 and 92.8%, respectively (Table 2). With regard to *Cryptococcus* species, YBC encountered little difficulty in identifying *Cryptococcus neoformans*, incorrectly identifying only 3 of 170 isolates (2 as *Cryptococcus albidus* and 1 as *C. guilliermondii*) (Table 2). A false-positive melibiose assimilation in both cases and a false-negative inositol reaction in the latter case led to the incorrect identifications. Difficulty was experienced in correctly identifying other *Cryptococcus* species, however, with *Cryptococcus uniguttulatus* (73%) proving to be the most problematic for the 24-h system. The API 20C system also had trouble in identifying these two organisms as well (Table 2). Both commercial systems identified isolates of *Trichosporin beigelii* fairly readily, with AMS encountering a little more difficulty than API 20C. There were too few examples tested of the other *Trichosporon* species to make an accurate evaluation of the efficiency of either system with these organisms. Although the sample group was again too small to draw any definite

TABLE 1. Identification of selected yeasts (*Candida*, *Torulopsis*, *Saccharomyces*, and *Rhodotorula* species) by the AMS-YBC and API 20C yeast identification systems compared with RCM

Organism[a]	No. of isolates	No. (%) of correct identifications[b]	
		AMS-YBC	API 20C
C. albicans	80	80 (100)	80 (100)
C. parapsilosis	64	61 (95)	63 (98)
C. tropicalis	80	80 (100)	80 (100)
C. krusei	80	76 (95)	78 (98)
C. zeylanoides	8	8 (100)	8 (100)
C. stellatoidea	27	27 (100)	27 (100)
C. guillermondii	22	19 (86)	20 (91)
C. pseudotropicalis	13	12 (92)	12 (92)
C. lipolytica	15	12 (80)	14 (93)
T. glabrata[c]	80	80 (100)	80 (100)
T. candida[c]	11	10 (91)	9 (82)
S. cerevisiae	25	23 (92)	21 (84)
R. glutinis	17	17 (100)	17 (100)
R. rubra	15	14 (93)	15 (100)

[a] Taxon determined by RCM.

[b] In some cases, ancillary morphological and biochemical tests were required by each commercial system before a final identification was made. The numbers in parentheses represent percent agreement with RCM. Total agreement: AMS-YBC, 96.6%; API 20C, 97.6%.

[c] The genus *Torulopsis* has been included under the genus *Candida* in the AMS data base. *T. glabrata* becomes *C. glabrata*, and *T. candida* becomes *C. famata*.

TABLE 2. Identification of selected yeasts (*Cryptococcus*, *Trichosporon*, and *Geotrichum* species) by the AMS-YBC and API 20C yeast identification systems compared with RCM

Organism[a]	No. of isolates	No. (%) of correct identifications[b]	
		AMS-YBC	API 20C
C. neoformans	170	167 (98)	168 (99)
C. albidus	108	100 (93)	97 (90)
C. luteolus	14	14 (100)	14 (100)
C. laurentii	54	47 (87)	52 (96)
C. terreus	19	11 (58)	13 (68)
C. uniguttulatus	11	8 (73)	7 (64)
T. beigelii	41	38 (93)	40 (98)
T. capitatum	13	10 (77)	10 (77)
T. penicillatum	7	6 (86)	5 (71)
Geotrichum sp.	5	3 (60)	4 (80)

[a] Taxon determined by RCM.
[b] In some cases, ancillary morphological and biochemical tests were required by each commercial system before a final identification was made. The numbers in parentheses represent percent agreement with RCM. Total agreement: AMS-YBC, 91.4%; API 20C, 92.8%.

conclusions, the YBC appeared to have difficulty identifying *Geotrichum* isolates, correctly identifying only three of five (60%) of the isolates. When considering the total number of organisms identified during the first phase of the study, YBC had a 98.8% correlation with API 20C (923 YBC of 934 API 20C).

In the randomized study, YBC had an 86.6% correlation with the rapid conventional system, provided the identification of *Kluveromyces fragilis* (a perfect yeast) as *Candida pseudotropicalis* (the imperfect phase of *K. fragilis*) is regarded as a misidentification. If not, then the overall correlation approximates 88%. AMS failed to yield a taxonomic classification in 11 instances and gave an incorrect identification in 4 cases (Table 3). If one considers a data base answer of unidentifiable as neither incorrect nor a misidentification and discounts those isolates, then YBC correlation with RCM was 94.8% (110 of 116 isolates). Among the various *Candida* species identified, one *Candida rugosa* was unidentified, and two isolates which were labeled *Candida krusei* and *Trichosporon capitatum* by RCM and API were identified as *C. famata* and *C. guillermondii*, respectively. The remaining *Candida* species were identified correctly. Of those organisms which were new additions to the updated data base, i.e., the remaining *Cryptococcus* species, *Pichia ohmeri*, *Hansenula anomala*, *Sporobolomyces salmonicolor*, *S. cerevisiae*, and *Prototheca wickerhamii* all were identified correctly.

DISCUSSION

Conventional yeast identification protocols, consisting of a battery of assimilable and fermentable carbohydrates, nitrate reductase, and urease determination in addition to microscopic morphology on vegetable-polysaccharide agars, are the "gold standard" by which all yeast identification methods are measured (10). However, due to the need for more rapid and economic methods they are rarely applicable in today's clinical laboratory. The Vitek AMS-YBC system was developed to provide any clinical laboratory with rapid and accurate reference-level yeast identification capabilities. In the first published studies on the AMS yeast system (7, 13), reports of system accuracy ranged from excellent (96%) to marginal (84.9%). The major criticisms experienced by both groups of investigators were that the data base was limited and certain key biochemical characteristics were improperly weighted when the taxonomic keys were devised. Other minor criticisms concerned the necessity for a large inoculum, increased storage space for injector tubes, and a short shelf-life for the biochemical cards.

TABLE 3. Performance of the AMS-YBC system in the identification of selected clinical and stock isolates in a randomized, blind study

Organism[a]	No. tested	No. correct[b]	Comments
C. albicans	2	1	
C. parapsilosis	1	1	
C. lipolytica	1	1	
C. pseudotropicalis	2	2	
C. tropicalis	3	3	
C. rugosa	3	3	One unidentified[c]
C. krusei and *T. capitatum*	24	22	Two *C. famata* and *C. guilliermondii*[d]
C. stellatoidea	8	8	
C. lusitaniae	6	6	
C. lambica	6	6	
C. humicola	2	2	
T. glabrata[d]	1	1	
T. candida	13	13	
T. mavis[d]	1	0	One *Geotrichum* sp. and *T. penicillatum*
C. neoformans	5	5	
C. laurentii and *T. beigelii*	12	12	
C. terreus	3	3	
C. albidus	5	5	
S. cerevisiae	6	6	
S. exiguus	1	0	One unidentified[c]
Rhodotorula minuta	1	0	One unidentified
Kluveromyces vanudenii	1	0	One unidentified
K. fragilis	1	0	One *C. pseudotropicalis*
K. bulgaricus	1	0	One *C. pseudotropicalis*
Pichia ohmeri	5	5	
Hansenula anomala	3	3	
H. californica	1	0	One *Geotrichum* sp. and *T. penicillatum*[c]
Hanseniaspora uvarum	4	0	Four unidentified
Kloeckera javanica	1	0	One unidentified
Sporobolomyces salmonicolor	1	1	
Endomycopsis sp.	1	0	One unidentified
Pityrosporum orbiculare	1	0	One unidentified
Prototheca wickerhamii	1	1	

[a] Taxon established by RCM.
[b] Total percent correlation with RCM, 94.8.
[c] Unable to match with biochemical patterns of AMS in data base.
[d] The genus *Torulopsis* has been included under the genus *Candida* in the AMS data base. *T. glabrata* becomes *C. glabrata*, and *T. candida* becomes *C. famata*.

The minor criticisms were handled via redesigning the injector system and extending card shelf-life to a year. The necessity for a large inoculum remains, since a large amount of preformed enzyme is necessary for rapid substrate turnover. There is a need for a large inoculum in any enzyme-based identification system, so this minor disadvantage is not just a peculiarity of the AMS-YBC system. On the other hand, growth-based systems offer the advantage of a small (one to two colonies) inoculum but sacrifice rapid positivity in the process.

To correct the major criticisms listed above, the AMS-YBC data base was enhanced by adding multiple biotypes of taxonomic groups already extant in the data base and adding several new taxa with their biotypes as well. Improvements were made in data management by reconstructing the taxonomic keys and restructuring the way data were analyzed by the computer. The overall correlation (sum of Tables 1, 2, and 3) of the updated AMS-YBC data base with the rapid conventional yeast system was 93.4% (1,041 of 1,106), which compared favorably with the API 20C agreement of 96%.

The major difficulty both commercial systems had was in the identification of less biochemically active organisms (Table 2). In earlier evaluations of the API 20C yeast strip (4, 11), problems encountered in identifying these organisms were resolved by extending the incubation period for an additional 24 h. Since we chose to evaluate rapid identification as a function of accuracy, the AMS-YBC cards were not incubated beyond 24 h, and the API 20C strips were not incubated beyond 72 h. Because of those previous evaluations, it is not unreasonable to assume that increasing the incubation time of those isolates would have improved the accuracy of both YBC and API 20C. For example, in identifying cryptococci with either system, disaccharides, such as lactose and melibiose, and sugar alcohols, such as inositol, were often negative. When these aberrant results occurred at 24 h with the YBC system, the computer either labeled the organisms as unidentifiable or placed them within the genus *Candida* or *Trichosporon*. In the latter case, the mucoid or nonfilamentous appearance of the yeasts made any misidentification obvious, and the card should have been incubated further and reread.

If group 2 yeasts were either encapsulated or had extensive mycelial or pseudomycelial growth, resulting mechanical problems hindered identification. Either of these conditions caused clumping of the inoculum, which blocked the filling channels of the biochemical card. To prevent this, a noticeably clumped isolate was suspended in water (the addition of 0.1% Tween 80 improved dispersal of the organism), washed, centrifuged to a pellet, suspended to a no. 2 McFarland standard, and inoculated into the YBC card in the usual way.

The major advantage of the Vitek AMS yeast system was the saving of "hands on" time both in setting up an identification and in generating a final report. Compared with the other two systems, preparation of the inoculum and actual inoculation time were significantly reduced with AMS-YBC. In addition, once in the incubator-reader, data collection and reporting of the YBC were totally automated. In contrast, the RCM and API 20C systems are read manually, the identity of the isolates are located in biotype code books or compendia, and results are reported manually, tasks which occupy a considerable amount of time. The time to final reporting with the AMS automated method was 20 to 24 h, compared with 24 to 48 h for RCM and 48 to 72 h for API 20C (none of the isolates were identifiable at 24 h). Thus, the updated version of the Vitek AMS offers more rapid yeast identification than currently available methods, with an accuracy comparable to both conventional and commercial methods.

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