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# Clinical Evaluation of the AutoMicrobic System Yeast Biochemical Card for Rapid Identification of Medically Important Yeasts

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The AutoMicrobic system Yeast Biochemical Card (Vitek Systems, Inc., subsidiary of McDonnell Douglas, Hazelwood, Mo.), a fully automated and computer-assisted method for identifying seven yeast genera based on 26 biochemical reactions, was compared with the API 20C (Analytab Products, Plainview, N.Y.) yeast identification system, using 253 clinical yeast isolates. There was an 84% correlation between the Yeast Biochemical Card and API 20C systems based on biochemical tests alone and a 96% correlation when morphology was combined with the biochemical profile. Of 22 species examined, 14 were definitively identified within 24 h, using only the biochemical tests; the remaining 8 species were presumptively identified, using biochemical tests within 24 h, with definitive identification being completed in 48 to 72 h when morphological characteristics were ascertained. The Yeast Biochemical Card system was both a facile and, with concomitant morphology, an accurate system for performing yeast identification.

To facilitate biochemical identification of yeast isolates in clinical laboratories, efforts have been directed toward the development of identification systems which are less cumbersome and less time-consuming than traditional methodologies (14, 21). In recent years, a number of rapid methods have been evaluated and found to accomplish reliably the identification of medically important yeasts (2–5, 8, 12, 13, 16, 17, 24). In addition, as another approach to rapid identification, a semiautomated instrument has been used to determine the identification of two yeast genera within 12 h (23).

Continuing developments in the area of automation for microbiology have resulted in the AutoMicrobic system (AMS; Vitek Systems, Inc., subsidiary of McDonnell Douglas, Hazelwood, Mo.), a fully automated system for the detection, enumeration, and identification of urinary tract pathogens (1, 10, 15, 19, 20) and for the biochemical identification of *Enterobacteriaceae* (9). Additional capabilities that recently have been designed for AMS include biochemical identificaton of seven genera of yeasts after in vitro isolation (V. K. Sangar, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, F72, p. 331). This study was undertaken to compare the AMS-Yeast Biochemical Card (YBC) system with the API 20C system (Analytab Products, Plainview, N.Y.) for identification of clinical yeast isolates.

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## MATERIALS AND METHODS

**Organisms.** A total of 253 isolates of yeasts and yeastlike organisms were examined in this study. Of these, 225 yeasts were recent clinical isolates from the mycology laboratory of the UCLA Hospital and Clinics. The remaining 28 less frequently encountered organisms were obtained from D. H. Howard, Department of Microbiology and Immunology, UCLA School of Medicine. The following American Type Culture Collection isolates were used to perform quality control testing on both the API 20C, and AMS-YBC procedures; *Candida stellatoidea* (ATCC 11066), *Cryptococcus laurentii* (ATCC 18803), *Rhodotorula glutinis* (ATCC 32765), and *Trichosporon capitatum* (ATCC 28576).

**API 20C procedure.** All of the tests were performed according to the instructions of the manufacturer. One to three isolated colonies were picked with a sterile wooden stick and suspended in a vial of melted carbohydrate-free basal medium maintained at 50°C. With a sterile pasteur pipette, the suspension was inoculated into each of the 20 cupules of the API strip. One cupule was a growth control containing no carbon source; the other cupules contained the substrates to determine utilization of glucose, glycerol, 2-

keto-D-gluconate, L-arabinose, xylose, adonitol, xylitol, galactose, inositol, sorbitol, methyl-D-glucoside, N-acetyl-D-glucosamine, cellobiose, lactose, maltose, sucrose, trehalose, melezitose, and raffinose. Each inoculated strip was placed in the humidified chamber provided with the strip and incubated at 30°C for 72 h, at which time the reactions were read and recorded.

**Supplemental procedures.** To complete the identification of organisms utilizing the API 20C system, nitrate assimilation, urease production, ascospore production, and microscopic morphology on cornmeal-Tween 80 agar plates were determined by conventional methods (6, 7, 11, 18).

**AMS-YBC procedure.** The instrumentation modules of AMS were described by Aldridge et al. (1). YBC is a disposable plastic card that contains 30 sealed wells of dried media. The 26 biochemical wells contain substrates to determine utilization of glucose, glycerol, 2-keto-D-gluconate, L-arabinose, xylose, adonitol, xylitol, galactose, inositol, sorbitol, methyl-D-glucoside, N-acetyl-D-glucosamine, cellobiose, lactose, maltose, sucrose, trehalose, melezitose, raffinose, dulcitol, palatinose, erythritol, melibiose, nitrate, inhibition by cycloheximide, and urease production. The remaining four wells serve as negative controls for urease, nitrate and 2-keto-D-gluconate, glucose and inositol, and the remaining carbohydrates and cycloheximide. Two lots of YBC (E2110679, D1020180) were utilized during this study.

Well-isolated yeast colonies were selected with a sterile wooden stick from Sabouraud dextrose agar plates (Emmons modification; BBL Microbiology Systems, Cockeysville, Md.) after 48 h of incubation at 30°C and suspended in 1.8 ml of 0.5% NaCl until the density was equivalent to that of a McFarland no. 2 standard. The suspension was blended in a Vortex mixer and transferred to a single-barrel tube injector assembly to which the YBC had been attached. All cards were subsequently inoculated by an evacuation process in the filling module. Once inoculated, the YBC were incubated at 30°C for 22 to 23 h. They were then placed into the reader-incubator module for evaluation. The printout from the data terminal provided a record of all reactions, the most likely identification of the organism accompanied by its probability, and the second best identification with its associated probability. In cases where similar biochemical profiles were generated, the user was instructed to consult morphology for definitive identification.

**Data examination.** Inasmuch as the purpose of this study was to evaluate the reliability of the AMS-YBC system compared with the API 20C system, two criteria were utilized to accept the identification furnished by the AMS-YBC system as a correct response. The most likely identification call from the YBC matched the identification obtained from the API 20C system, and pairs of organisms which yielded similar YBC biochemical profiles could be distinguished, using microscopic morphology characteristics.

## RESULTS

Although identification of 273 yeast isolates was attempted during this study, 20 organisms were eliminated from further evaluation. Five organisms could not be identified by either the API 20C system or the AMS-YBC system and required reference testing for final identification, and 15 of our isolates (2 *Candida humicola*, 2 *Candida lambica*, 9 *Candida lusitaniae*, and 2 *Hansenula anomala*) were not included among the 22 species constituting the current AMS-YBC data base. Of the remaining 253 isolates, 212 (84%) were identified by YBC, using the results of biochemical tests as the only criteria, and 242 (96%) were identified when biochemical tests were used in conjunction with microscopic morphology (Table 1). The pairs of organisms which yielded similar YBC biochemical profiles and required examination of microscopic morphology for differentiation were *Candida guilliermondii-Candida famata* (formerly *Torulopsis candida*; see reference 22), *Trichosporon capitatum-Candida krusei*, *Cryptococcus laurentii-Trichosporon cutaneum*, and *Geotrichum* species-*Trichosporon penicillatum*.

The YBC identification was furnished for the majority of cases at a high level of probability,

TABLE 1. *Clinical comparison of the YBC system with the API 20C system*

| Organism | No. of isolates | Correlation[a] | |
|---|---|---|---|
| | | B | B-M |
| *Candida albicans* | 50 | 49 | 49 |
| *Candida famata (Torulopsis candida)* | 3 | 0 | 2 |
| *Candida (Torulopsis) glabrata* | 22 | 22 | 22 |
| *Candida guilliermondii* | 13 | 0 | 11 |
| *Candida krusei* | 22 | 17 | 21 |
| *Candida lipolytica* | 3 | 2 | 2 |
| *Candida parapsilosis* | 24 | 24 | 24 |
| *Candida pseudotropicalis* | 12 | 12 | 12 |
| *Candida rugosa* | 7 | 5 | 5 |
| *Candida tropicalis* | 28 | 28 | 28 |
| *Candida zeylanoides* | 1 | 1 | 1 |
| *Cryptococcus albidus* | 6 | 6 | 6 |
| *Cryptococcus laurentii* | 1 | 0 | 1 |
| *Cryptococcus luteolus* | 1 | 1 | 1 |
| *Cryptococcus neoformans* | 19 | 19 | 19 |
| *Cryptococcus terreus* | 1 | 1 | 1 |
| *Cryptococcus uniguttulatus* | 2 | 2 | 2 |
| *Geotrichum* species | 3 | 0 | 3 |
| *Rhodotorula pilimanae* | 1 | 1 | 1 |
| *Rhodotorula rubra* | 9 | 9 | 9 |
| *Saccharomyces cerevisiae* | 16 | 13 | 13 |
| *Trichosporon cutaneum* | 9 | 0 | 9 |
| Total | 253 | 212 | 242 |
| Percent correlation | | 84 | 96 |

[a] Number of correct identifications given by the YBC system compared with the API 20C system by using biochemical tests (B) only or by using biochemical tests and morphology (B-M).

TABLE 2. *Probability of identification obtained with YBC*

| Organism | No. of isolates | No. of isolates at following % probability[a] | | | | |
|---|---|---|---|---|---|---|
| | | 50–59 | 60–69 | 70–79 | 80–89 | 90–99 |
| *C. albicans* | 49 | | | | 1 | 48 |
| *C. famata (Torulopsis candida)* | 2 | 2[b] | | | | |
| *C. (Torulopsis) glabrata* | 22 | | | | | 22 |
| *C. guilliermondii* | 11 | 11[b] | | | | |
| *C. krusei* | 21 | 4[b] | | | | 17 |
| *C. lipolytica* | 2 | | | | | 2 |
| *C. parapsilosis* | 24 | | | 4 | 2 | 18 |
| *C. pseudotropicalis* | 12 | | | | | 12 |
| *C. rugosa* | 5 | | | | 1 | 4 |
| *C. tropicalis* | 28 | | 1 | | | 27 |
| *C. zeylanoides* | 1 | | | | | 1 |
| *C. albidus* | 6 | | 1 | | 1 | 4 |
| *C. laurentii* | 1 | 1[b] | | | | |
| *C. luteolus* | 1 | | | | | 1 |
| *C. neoformans* | 19 | | | | 2 | 17 |
| *C. terreus* | 1 | | | | | 1 |
| *C. uniguttulatus* | 2 | | | | | 2 |
| *Geotrichum* species | 3 | 3[b] | | | | |
| *R. pilimanae* | 1 | | | | | 1 |
| *R. rubra* | 9 | | | | | 9 |
| *S. cerevisiae* | 13 | | | 1 | | 12 |
| *T. cutaneum* | 9 | 9[b] | | | | |
| Total | 242 | 30 | 2 | 5 | 7 | 198 |

[a] Percent probability of identification for each isolate as furnished by the AMS computer module upon examination of the biochemical profile.

[b] Microscopic morphology was required to complete the identification of the isolate.

with 82% (198 out of 242) of the isolates being correctly identified at 90% or greater probability (Table 2).

There were a total of 11 misidentifications of yeast with the YBC system (Table 3). For most isolates, a variety of false-negative reactions were observed in the carbohydrate assimilation wells. In some cases, however, false-negative reactions were common to several yeast species. Two of seven isolates of *Candida rugosa* were identified as *Candida krusei* because galactose was not assimilated within 24 h of incubation. Of 16 isolates of *Saccharomyces cerevisiae* examined, 3 which did not assimilate maltose and methyl-D-glucoside were incorrectly identified as *C. krusei*. The biochemical patterns from these two species suggest the possibility that these organisms may represent a cluster of isolates whose reactions warrant fine-tuning of the current computer program.

TABLE 3. *YBC identification discrepancies*

| Yeast | YBC identification | False-negative reaction[a] | Correct identification at 48 h |
|---|---|---|---|
| *C. albicans* | *C. zeylanoides* | AMG, XYL, TRE, XLT, ADO, PAL | No |
| *C. famata (T. candida)* | *C. parapsilosis* | RAF, XLT, DUL, ADO | Yes |
| *C. guilliermondii* | *C. tropicalis* | ARA, RAF, DUL, GLY | No |
| *C. guilliermondii* | *C. parapsilosis* | CEL, RAF, XLT, DUL | Yes |
| *C. krusei* | *C. lipolytica* | ERY[b] | Yes |
| *C. lipolytica* | *C. krusei* | ERY, CYC | Yes |
| *C. rugosa* | *C. krusei* | GAL, SOR | No |
| *C. rugosa* | *C. krusei* | GAL | No |
| *S. cerevisiae* | *C. krusei* | MLT, AMG, RAF | Yes |
| *S. cerevisiae* | *C. krusei* | MLT, AMG, RAF | Yes |
| *S. cerevisiae* | *C. krusei* | GAL, MLT, AMG | Yes |

[a] Abbreviations: AMG, methyl-D-glucoside; XYL, xylose; ARA, L-arabinose; RAF, raffinose; DUL, dulcitol; GLY, glycerol; CEL, cellobiose; XLT, xylitol; ERY, erythritol; CYC, cycloheximide; GAL, galactose; SOR, sorbitol; TRE, trehalose; PAL, palatinose; ADO, adonitol; MLT, maltose.

[b] A false-positive reaction.

To examine the effect of prolonged incubation on identification of yeasts obtained from the YBC system, reactions were recorded at 48 and 72 h for 85% (236 out of 253) of the cards. Of the 11 isolates misidentified at 24 h (Table 3) 7 were correctly identified after 48 h of incubation. However, two isolates of *Trichosporon cutaneum* which had been correctly identified at 24 h were incorrectly identified at 48 h. Incubation for 72 h did not resolve the four remaining discrepancies listed in Table 3. Moreover, five isolates (one *Candida tropicalis*, two *Cryptococcus neoformans*, and two *Rhodotorula rubra*) which previously had been correctly identified were, as a result of prolonged incubation, misidentified by the YBC at 72 h.

## DISCUSSION

The API 20C system, when used in conjunction with tests for microscopic morphology, has been shown to be a reliable method for the identification of medically important yeasts within 72 h (3, 4, 13, 16). In recent months, AMS-YBC has been introduced to provide clinical laboratories with an automated system for examination of biochemical reactions; this system purports to accomplish the task of yeast identification within 24 h. In this clinical trial, the YBC system was shown to possess a high degree of correlation with the API 20C system. Although no auxiliary biochemicals were re-

quired for completing the identification of an unknown yeast isolate with YBC, this system, like API 20C and all other existing yeast identification methodologies, maintained the requirement for microscopic morphology to obtain maximum accuracy. On the basis of biochemical tests alone, the overall agreement between the YBC and the API 20C systems was 84%. When morphology complemented the biochemical profile, there was a 96% correlation between the two systems. For those isolates which required morphology for final identification, reporting was postponed for an additional 24 to 48 h. Although in this study only 30 of 242 isolates (12%) were in this category, concurrent morphology testing with YBC is recommended by the manufacturer, thereby ensuring the availability of essential information when needed.

According to the manufacturer's instructions during this study, an inoculum with a density equivalent to that of a McFarland no. 2 standard was used to inoculate YBC. On those occasions when only one or two yeast colonies are present on the primary plate, subculturing of the organisms may be required, and identification will be delayed. When primary plates contain 5 to 10 well-isolated yeast colonies, achieving the required concentration is possible. However, the requirement for a large inoculum substantially increases the risk of testing a mixed population when several colonies are selected from primary isolation plates. Preliminary experiments now in progress indicate that a lower inoculum can, in some cases, be utilized without compromising the accuracy of the YBC identification system (D.L. Oblack, unpublished data).

Although the manufacturer recommended a 24-h incubation period for YBC, the data from this study showed that over half of the isolates misidentified at 24 h were correctly identified when incubation was extended to 48 h. Unfortunately, extended incubation also resulted in the reversion of correct to incorrect identifications. Based on these data, we feel that users of this system would be advised to adhere to the recommended 24-h incubation period.

A total of 15 strains of infrequently encountered yeasts (*C. humicola, C. lambica, C. lusitaniae,* and *H. anomala*) were isolated during the course of this study. Although these organisms could be identified by the API 20C system, their profiles were absent from the AMS-YBC data base. No doubt, when a sufficient number of these isolates have been collected by the manufacturer, the biochemical profiles will be characterized and added to their AMS-YBC data base.

The introduction of an automated identification system into medical mycology represents a significant departure from traditional practices in this area of diagnostic laboratories. The advantages of the AMS-YBC system include the following: (i) minimal set-up time, (ii) automated inoculation, (iii) ability to perform 26 biochemical tests simultaneously from the same inoculum, (iv) elimination of test interpretation variability, (v) definitive identification within 24 h of 14 of 22 yeast species and presumptive identification of the remaining 8 species, (vi) computer-assisted identification, (vii) reduction of transcription errors through machine recognition of specimen identification numbers, and (viii) easy updating of computer software to reflect changes in the biochemical profiles of isolates and to increase the number of organisms identified. Disadvantages encountered with the use of the AMS-YBC system include the following: (i) a large inoculum is required, (ii) no manual backup is available because reaction thresholds are not visually discernible, (iii) the YBC data base is currently more limited than that of the commercial yeast identification system to which it was compared, (iv) storage space is required for the disposable injector tubes, and (v) the shelf life of YBC is 2 months at 4°C.

When used in conjunction with morphological tests, the AMS-YBC system provides a reliable method for the rapid, automated identification of medically important yeasts. The data from this evaluation indicated that the final identification of yeasts, based on the composite YBC biochemical profile, correlated well with that obtained by using the API 20C system.

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## ADDENDUM

Developments in the AMS-YBC system after this evaluation include replacement of the injector assembly with a transfer tube that requires minimal storage space, and modification of the drying process has resulted in production of cards with a 3-month shelf life at 4°C.

## LITERATURE CITED

1. **Aldridge, C., P. W. Jones, S. Gibson, J. Lanham, M. Meyer, R. Vannest, and R. Charles.** 1977. Automated microbiological detection/identification system. J. Clin. Microbiol. **6**:406–413.
2. **Bowman, P. I., and D. G. Ahearn.** 1975. Evaluation of the Uni-Yeast-Tek kit for the identification of medically important yeasts. J. Clin. Microbiol. **2**:354–358.
3. **Bowman, P. I., and D. G. Ahearn.** 1976. Evaluation of

commercial systems for the identification of clinical yeast isolates. J. Clin. Microbiol. 4:49-53.

4. **Buesching, W. J., K. Kurek, and G. D. Roberts.** 1979. Evaluation of the modified API 20C system for identification of clinically important yeasts. J. Clin. Microbiol. 9:565-569.
5. **Cooper, B. H., J. B. Johnson, and E. S. Thaxton.** 1978. Clinical evaluation of the Uni-Yeast-Tek system for rapid presumptive identification of medically important yeasts. J. Clin. Microbiol. 7:349-355.
6. **Dolan, C. T., and G. D. Roberts.** 1974. Identification procedures, p. 153-201. *In* J. A. Washington II (ed.), Laboratory procedures in clinical microbiology. Little, Brown & Co., Boston.
7. **Haley, L. D., and C. S. Callaway.** 1978. Laboratory methods in medical mycology, 4th ed., p. 111-138. U.S. Department of Health, Education and Welfare, Center for Disease Control, Atlanta, Ga.
8. **Huppert, M., G. Harper, S. H. Sung, and V. Delanerolle.** 1975. Rapid methods for identification of yeasts. J. Clin. Microbiol. 2:21-34.
9. **Isenberg, H. D., T. L. Gavan, P. B. Smith, A. Sonnenwirth, W. Taylor, W. J. Martin, D. Rhoden, and A. Balows.** 1980. Collaborative investigation of the AutoMicrobic System *Enterobacteriaceae* biochemical card. J. Clin. Microbiol. 11:694-702.
10. **Isenberg, H. D., T. L. Gavan, A. Sonnenwirth, W. I. Taylor, and J. A. Washington II.** 1979. Clinical laboratory evaluation of automated microbial detection/identification system in analysis of clinical urine specimens. J. Clin. Microbiol. 10:226-230.
11. **Koneman, E. W., G. D. Roberts, and S. E. Wright.** 1978. Practical laboratory mycology, 2nd ed., p. 103-118. The Williams & Wilkins Co., Baltimore.
12. **Land, G. A., W. H. Fleming III, T. A. Beadles, and J. H. Foxworth.** 1979. Rapid identification of medically important yeasts. Lab. Med. 10:533-541.
13. **Land, G. A., B. A. Harrison, K. L. Hulme, B. H. Cooper, and J. C. Byrd.** 1979. Evaluation of the new API 20C strip for yeast identification against a conventional method. J. Clin. Microbiol. 10:357-364.
14. **Lodder, J. (ed.).** 1970. The yeasts. A taxonomic study, 2nd ed. North-Holland Publishing Company, Amsterdam.
15. **Nicholson, D. P., and J. A. Koepke.** 1979. The Automicrobic System for urines. J. Clin. Microbiol. 10:823-833.
16. **Roberts, G. D., H. S. Wang, and G. E. Hollick.** 1976. Evaluation of the API 20C microtube system for the identification of clinically important yeasts. J. Clin. Microbiol. 3:302-305.
17. **Segal, E., and L. Ajello.** 1976. Evaluation of a new system for the rapid identification of clinically important yeasts. J. Clin. Microbiol. 4:157-159.
18. **Silva-Hutner, M., and B. H. Cooper.** 1974. Medically important yeasts, p. 491-507. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
19. **Smith, P. B., T. L. Gavan, H. D. Isenberg, A. Sonnenwirth, W. I. Taylor, J. A. Washington II, and A. Balows.** 1978. Multi-laboratory evaluation of an automated microbial detection/identification system. J. Clin. Microbiol. 8:657-666.
20. **Sonnenwirth, A. C.** 1977. Preprototype of an automated microbial detection and identification system: a developmental investigation. J. Clin. Microbiol. 6:400-405.
21. **Wickerham, L. J.** 1951. Taxonomy of yeasts. Technical Bulletin no. 1029. U.S. Department of Agriculture, Washington, D.C.
22. **Yarrow, D., and S. A. Meyer.** 1978. Proposal for amendment of the diagnosis of the genus *Candida* Berkhout nom. cons. Int. J. Syst. Bacteriol. 28:611-615.
23. **Yen, J. H. N., and J. A. Smith.** 1978. Use of Autobac 1 for rapid assimilation testing of *Candida* and *Torulopsis* species. J. Clin. Microbiol. 7:118-121.
24. **Zwadyk, P., Jr., R. A. Tarlton, and A. Proctor.** 1977. Evaluation of the API 20C for identification of yeasts. Am. J. Clin. Pathol. 67:269-271.