Evaluation of the AutoMicrobic System for Identification of Yeasts

JOHN J. HASYN^{1†} AND HELEN R. BUCKLEY^{2,3*}

Department of Microbiology and Immunology, Temple University School of Medicine,^{2*} Temple University Hospital,³ and Department of Medical Technology, College of Allied Health Professions,¹ Philadelphia, Pennsylvania 19140

Received 4 November 1981/Accepted 21 July 1982

The AutoMicrobic system (Vitek Systems, Hazelwood, Mo.) for identification of yeasts was evaluated by testing 352 freshly isolated, germ tube-negative yeasts from clinical laboratories. Of these isolates, 84.9% were correctly identified. The system correctly identified 86.5% of the *Candida* isolates and 74.3% of the *Cryptococcus* isolates.

There has been an increased prevalence in recent years of opportunistic yeast infections, especially in transplant and oncology patients, resulting in serious and often fatal outcomes (1, 2, 7, 9, 17). This increase has made it imperative for clinical laboratories to identify yeasts to (i) establish an exact diagnosis, (ii) enhance understanding of the clinical significance of the various yeast species in compromised hosts, and (iii) enable accurate interpretation of mycoserological tests (6, 16). However, conventional identification procedures (13) based on the methods of Wickerham (19) for assaying assimilation and fermentation are time-consuming and often complex for routine clinical use. Recently, manual commercial systems such as Uni-Yeast-Tek (UYT) (3, 5) and API 20C (4, 12) have been developed for the identification of medically important yeasts. Such systems offer convenience, ease of storage, and reliable identifications. Even though the commercial systems are somewhat faster than the traditional methods (72 to 96 h until final identification), they still require a fairly long incubation period before results are available.

Within the past decade, interest in the development and use of automated instrumentation in clinical microbiology laboratories has increased. One recently developed, automated, computerized system is the AutoMicrobic system (AMS; Vitek Systems, Inc., Hazelwood, Mo.). Originally marketed for automated detection, enumeration, and identification of organisms in urine (11, 14), identification of members of the *Enterobacteriaceae* (8, 10) and nonfermenters, and drug susceptibility testing for gram-negative bacteria, the AMS has been expanded to identify

clinical yeast isolates (15). The Vitek Yeast Biochemical Card (YBC) is a plastic disposable card containing various assimilation substrates for the identification of 27 species of medically important yeasts in 22 to 24 h.

The purpose of this study was to determine whether the AMS-YBC system provided a rapid, reliable, and accurate method for the identification of germ tube-negative yeasts isolated in a diagnostic microbiology laboratory.

(Preliminary results of this study have been presented [J. J. Hasyn and H. R. Buckley, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, C185, p. 293]).

MATERIALS AND METHODS

Yeasts. A total of 379 germ tube-negative yeasts isolated from clinical specimens were evaluated in this study (see Table 1). Of these yeasts, 352 were fresh clinical isolates representing 22 species (see Table 1). Germ tube-positive isolates were not included in the study because they are considered to be *Candida albicans*; hence, no further identification is necessary (18).

Identification methods. Primary identification was made by the UYT system in accordance with the instructions of the manufacturer. The UYT system provides tests for carbohydrate assimilation, urease, and nitrate assimilation and a cornmeal-agar medium in which morphological examinations can be performed. Most yeasts can be identified within 72 h with the UYT system; however, some require up to 7 days for identification.

Conventional tests (14) were performed on isolates that were questionably identified by the UYT system and on isolates whose UYT system identifications differed from the AMS-YBC system identifications.

Description and use of the YBC in the AMS. The AMS consists of six modules: the diluent dispenser, the filling module, the sealer module, the reader-incubator, and the computer control module with data terminal.

† Present address: Microbiology Laboratory, Philadelphia College of Osteopathic Medicine and Hospital, Philadelphia, PA 19131.

FIG. 1. The YBC showing the various components used for identification of yeasts. The following tests are shown: 1, carbohydrate base control (negative control); 2, galactose; 3, lactose; 4, sucrose; 5, maltose; 6, cellobiose; 7, methyl-D-glucoside; 8, xylose; 9, arabinose; 10, trehalose; 11, melezitose; 12, raffinose; 13, *N*-acetyl-D-glucosamine; 14, xylitol; 15, dulcitol; 16, adonitol; 17, palatinose; 18, glycerol; 19, sorbitol; 20, erythritol; 21, melibiose; 22, cycloheximide; 23, carbohydrate base control (negative control); 24, glucose; 25, inositol; 26, nitrate base control (negative control); 27, nitrate; 28, 2-keto-D-gluconate; 29, urea base control (negative control); and 30, urea.

The AMS-YBC system incorporates a series of conventional and nonconventional substrates in a plastic, disposable card consisting of 30 microcuvettes containing dehydrated media (Fig. 1). Testing was carried out in accordance with the instructions of the manufacturer. During the 1-year period (February 1980 to February 1981) over which this study was conducted, several lots of the YBC were used. The results presented here are those obtained with the final product version.

As recommended by Vitek, four yeasts were tested with each new lot of cards: *Trichosporon capitatum* (ATCC 28576), *Cryptococcus laurentii* (ATCC 18803), *Rhodotorula glutinis* (ATCC 32765), and *C. albicans* (ATCC 14053). A YBC inoculated only with saline served as a negative control.

The 27 yeasts in the AMS-YBC database were kindly supplied by Vitek. The following yeasts are included in the database: *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. glabrata*, *C. famata*, *C. guilliermondii*, *C. pseudotropicalis*, *C. stellatoidea*, *C. rugosa*, *C. lipolytica*, *C. zeylanoides*, *Cryptococcus neoformans*, *C. laurentii*, *C. albidus*, *C.*

luteolus, *C. uniguttulatus*, *C. terreus*, *Saccharomyces cerevisiae*, *Geotrichum* sp., *Trichosporon pullulans*, *T. cutaneum*, *T. capitatum*, *T. penicillatum*, *Rhodotorula glutinis*, *R. pilimanae*, and *R. rubra*.

RESULTS

The accuracy of identification of 352 clinical yeast isolates by the AMS-YBC system is shown in Table 1. The AMS-YBC system correctly identified 299 of the 352 isolates. An agreement of 90% or greater was seen for 9 of 22 species: *C. famata*, *C. glabrata*, *C. guilliermondii*, *C. lipolytica*, *C. pseudotropicalis*, *C. rugosa*, *C. albidus*, *C. uniguttulatus*, and *R. rubra*. An agreement of >75% was seen for the following species: *C. albicans*, *C. krusei*, *C. parapsilosis*, *C. stellatoidea*, *C. tropicalis*, *C. neoformans*, *S. cerevisiae*, *T. capitatum*, and *T. beigelii*. Less or no agreement was seen for the remaining four species studied. Thus, of all of the clinical yeast isolates studied, 84.9% were correctly identified by the AMS-YBC system.

Each laboratory sets criteria for adequacy of identification before issuing a final report; Vitek has provided such a guide for the AMS-YBC yeast identification system. The final report given by the AMS-YBC system gives both the species identification and the probability that the organisms selected fit the biochemical growth pattern determined by the AMS computer. Of the 267 isolates that were correctly identified by

TABLE 1. Accuracy of identification by the AMS-YBC system

Final identification	Total no. of isolates	No. (%) of isolates correctly identified by AMS-YBC ^a
<i>C. albicans</i>	49	42 (85.7)
<i>C. famata</i>	3	3 (100)
<i>C. glabrata</i>	71	70 (98.6)
<i>C. guilliermondii</i>	2	2 (100)
<i>C. krusei</i>	24	20 (83.3)
<i>C. lipolytica</i>	3	3 (100)
<i>C. parapsilosis</i>	36	32 (88.8)
<i>C. pseudotropicalis</i>	5	5 (100)
<i>C. rugosa</i>	1	1 (100)
<i>C. stellatoidea</i>	12	9 (75)
<i>C. tropicalis</i>	76	57 (75)
<i>C. albidus</i>	1	1 (100)
<i>C. laurentii</i>	5	2 (40)
<i>C. luteolus</i>	1	0 (0)
<i>C. neoformans</i>	25	21 (84)
<i>C. terreus</i>	1	0 (0)
<i>C. uniguttulatus</i>	2	2 (100)
<i>Geotrichum</i> spp.	2	1 (50)
<i>R. rubra</i>	3	3 (100)
<i>S. cerevisiae</i>	19	16 (84.2)
<i>T. capitatum</i>	4	3 (75)
<i>T. beigelii</i>	7	6 (85.7)

^a Percentage of total correctly identified, 84.9%.

the AMS-YBC system, 82.4% showed a probability of >0.9 , whereas of the 38 isolates that were misidentified, only 26.3% showed a probability as high.

Some variability was observed in the final identification by the AMS-YBC system of the four recommended quality control yeasts. Subsequently, we evaluated the 27 standards used in the Vitek database. Each yeast in the database was tested four times, using the same yeast suspension for each species. On three occasions the AMS-YBC system provided an incorrect identification: *C. krusei* was misidentified as *T. capitatum* in one trial, *C. laurentii* was misidentified as a *C. krusei*-*T. capitatum* (1:1) culture in one trial, and *C. glabrata* was misidentified as *T. penicillatum* in one trial.

DISCUSSION

The capabilities of the AMS have been further expanded to provide rapid identification of medically important yeasts. Included are biochemical tests for carbohydrate assimilation, urea hydrolysis, resistance to cycloheximide, and nitrate reduction, which provide final identification of 19 yeast species and the presumptive identification of 8 species within 22 to 24 h after primary isolation. Many of the labor-consuming activities of conventional and manual commercial identification systems are reduced or eliminated in an automated, computerized system. The AMS-YBC system offers the following advantages: minimal time to set up tests, automated inoculation of all substrates with the same suspension of each isolate, objective interpretation of biochemical reactions with computer-assisted identification, and ability to update the database and the programming with only minor changes in the software.

The AMS-YBC system correctly identified 84.9% of the germ tube-negative clinical isolates (Table 1). Only 86.5% of the *Candida* isolates and 74.3% of the *Cryptococcus* isolates were correctly identified. Of the 84.9% isolates correctly identified, 89.3% were correctly identified solely by biochemical tests, whereas the remaining 10.7% also required microscopic morphological examinations, as recommended by the manufacturer.

Incorrect identifications of both clinical isolates and Vitek quality control organisms were caused, in part, by incorrect biochemical test results. For certain organisms, upon repeat AMS-YBC system trials, there was a lack of reproducibility of final identification because of a fluctuation of certain biochemical tests. Biochemical test errors were particularly evident for *C. albicans*, *C. parapsilosis*, and *C. tropicalis*, accounting for 30 of the 53 misidentifications encountered in the study. Misidentifications by

biochemical testing were caused by variable carbohydrate (arabinose, glycerol, melzitose, and xylitol) assimilation results and by variable resistance to cycloheximide. As an example, there were six instances in which *C. tropicalis* was misidentified as *C. albicans*. There was a fluctuation between these two identifications with repeat AMS-YBC system trials, owing to a variability seen for xylitol assimilation and resistance to cycloheximide. According to the AMS database, only 5% of the *C. tropicalis* strains tested assimilate xylitol and are resistant to cycloheximide. We noted that for some of the misidentifications, multiple false-negative reports of utilization of biochemical substrates were responsible. This caused 9 of 35 *Cryptococcus* isolates to be misidentified. In particular, 4 of a total of 25 *C. neoformans* isolates that were misidentified showed a tendency for yielding multiple false-negative test results.

As a result of the above problems, it appears that deficiencies exist in the AMS yeast computer program and database. It appears that, in some instances, there was an improper weighting of the results of the tests offered by the AMS-YBC system. Especially evident were cases in which positive test reactions are given more weight than are certain key negative test reactions.

Experience with the other capabilities of the AMS indicates that there is more variability for results with the AMS-YBC system. Unlike the other test cards available for use with the AMS (i.e., Urine Identification Card, *Enterobacteriaceae*-plus Biochemical Card), in which the growth wells or biochemical tests are monitored throughout a specific test cycle by the optical system, the YBC is given only a final reading in the AMS reader-incubator module after incubation at 30°C. Therefore, random variability of tests, probability of final identification, and final identification itself, as observed in this study, may be partially attributed to the one-reading method of the AMS-YBC system. Inoculum size was also found to be an important consideration. The achievement of a McFarland no. 2 standard may cause problems, since in some instances an inoculum this large cannot be feasibly obtained from a primary isolation plate. Identification would be delayed, and there would also be the increased risk of mixed cultures with the use of several colonies. Another disadvantage of the AMS-YBC system is the necessity of a manual back-up system should the AMS reader-incubator module break down, since the YBC biochemical profiles cannot be visibly interpreted.

Although use of the YBC with the automated, computerized AMS eliminates many of the labor-consuming activities of the conventional and UYT systems and decreases the time required

for biochemical identification of medically important yeasts, we failed to achieve a high degree of accurate yeast identification. The AMS-YBC system itself is not a complete identification system, since microscopic morphology studies are required for confirmation of the identification of several groups of organisms.

ACKNOWLEDGMENTS

For supplying clinical isolates, we thank K. R. Cundy, Temple University Hospital; J. Campos, Children's Hospital of Philadelphia; D. Jungkind, Thomas Jefferson Hospital; Alan Evangelista, Cooper Medical Center; and J. McKittrick, Hospital of the University of Pennsylvania. We thank G. Harvey for typing the manuscript.

LITERATURE CITED

1. Bodey, G. P. 1974. Microbiologic aspects in patients with leukemia. *Hum. Pathol.* 5:687-698.
2. Bodey, G. P., and V. Rodriguez. 1975. Infections in cancer patients on a protected environment-prophylactic antibiotic program. *Am. J. Med.* 59:497-504.
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. Bueschling, 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. Dee, T. H., and M. W. Rytel. 1977. Detection of candida serum precipitins by counterimmunoelectrophoresis: an adjunct in determining significant candidiasis. *J. Clin. Microbiol.* 5:453-457.
7. Hart, P. D., E. B. Russell, Jr., and J. S. Remington. 1969. The compromised host and infection. II. Deep fungal infection. *J. Infect. Dis.* 120:169-191.
8. Hasyn, J. J., K. R. Cundy, C. C. Dietz, and W. Wong. 1981. Clinical laboratory evaluation of the AutoMicrobic system for rapid identification of *Enterobacteriaceae*. *J. Clin. Microbiol.* 13:491-497.
9. Hersh, E. M., G. P. Bodey, B. A. Nies, and E. J. Freireich. 1965. Causes of death in acute leukemia. A ten-year study of 414 patients from 1954-1963. *J. Am. Med. Assoc.* 193:99-103.
10. 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.
11. 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.
12. 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.
13. Lodder, J. (ed.). 1970. The yeasts. A taxonomic study, 2nd ed. North-Holland Publishing Co., Amsterdam.
14. Nicholson, D. P., and J. A. Koepke. 1979. The Automicrobic System for urines. *J. Clin. Microbiol.* 10:823-833.
15. Oblack, D. L., J. C. Rhodes, and W. J. Martin. 1981. Clinical evaluation of the AutoMicrobic system yeast biochemical card for rapid identification of medically important yeasts. *J. Clin. Microbiol.* 13:351-355.
16. Prevost, E., and R. Newell. 1978. Commercial cryptococcal latex kit: clinical evaluation in a medical center hospital. *J. Clin. Microbiol.* 8:529-533.
17. Rifkind, D., T. L. Marchloro, S. A. Schneek, and R. B. Hill. 1967. Systemic fungal infections complicating renal transplantation and immunosuppressive therapy. Clinical, microbiologic, neurologic and pathologic features. *Am. J. Med.* 43:28-38.
18. Silva-Hutner, M., and B. H. Cooper. 1980. Yeasts of medical importance, p. 562-576. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), *Manual of clinical microbiology*, 3rd ed. American Society for Microbiology, Washington, D.C.
19. Wickerham, L. J., and K. A. Burton. 1958. Carbon assimilation tests for the classification of yeasts. *J. Bacteriol.* 56:363-371.