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Comparative Evaluation of the Iatron Serological Candida Check Kit and the API 20C Kit for Identification of Medically Important *Candida* Species

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A newly developed commercial serological test (Iatron Laboratories, Inc., Tokyo, Japan) for the rapid identification of medically important species of *Candida* was evaluated against the API 20C (Analytab Products, Plainview, N.Y.) and the standard Wickerham assimilation and fermentation procedures. Our results indicated that the Iatron and the API 20C methods are 95% accurate since both permitted identification of 78 of 82 *Candida* isolates, representing eight medically important species. None of the tests on nine *Cryptococcus*, six *Trichosporon*, three *Geotrichum*, three *Saccharomyces*, and one *Rhodotorula* species yielded false-positive reactions. False-positive serological tests occurred with a species of *Pichia* and *Candida rugosa*. The API 20C procedure correctly identified *C. rugosa* but not the *Pichia* sp. The Iatron method permitted reliable identification of the *Candida* species in 10 min to 5 h, whereas the API 20C procedure required 48 to 72 h. Neither method could properly identify sucrose-negative *Candida tropicalis* or *Candida lusitaniae* isolates. In addition, *Candida albicans* isolates could be serotyped by the Iatron method.

Opportunistic infections by *Candida* spp. and other yeasts are one of the major causes of complications in compromised patients, particularly in those who are immunosuppressed. Proper diagnosis of such infections requires prompt isolation and identification of the etiological agents in clinical materials. Because early administration of antifungal therapy is essential to survival of the superinfected compromised host, speed and accuracy in diagnosis are important. Traditionally, morphological and physiological tests, such as those of Wickerham, are used to reliably identify the *Candida* spp. (1, 13, 14, 21). These procedures are relatively time consuming for routine use, in some cases requiring approximately 1 month to carry out. During the past few years, there has been an increased interest in the development of methods for rapidly and specifically identifying the medically important yeasts. This widespread interest has resulted in the development of several commercial products and their adoption for routine use in the diagnostic laboratory.

The majority of the commercial kits currently available are basically miniaturized carbohydrate assimilation tests in a convenient plate or strip form (3-5, 10, 12). In some kits, additional biochemical tests are also included. In general, miniaturized tests enable assimilation reactions to be read in 3 to 5 days in comparison to a minimum of 5 to 14 days with more conventional methods. The proper interpretation of the results of carbohydrate assimilation tests also requires supportive data on the morphology of the yeast isolate. Generally, the combined data permit identification of a culture in 3 to 6 days.

Tsuchiya et al. (17, 18) successfully produced and applied monospecific agglutinins for identifying the members of the genus *Candida* (17, 18) and proposed a classification scheme for the medically important yeasts on the basis of their antigenic structures (19, 20). These workers also showed that serological tests could be employed for rapidly identifying yeasts. They recommended the use of such reagents in a slide agglutination test. Sweet and Kaufman (15) attempted to utilize Tsuchiya's scheme to identify the medically important *Candida* spp. They produced six factor sera that proved useful in a slide agglutination test. These six sera permitted the specific identification of *Candida guilliermondii*, *Candida krussei*, *Candida parapsilosis*, and *Candida pseudotropicalis* in 3 h, but they could not antigenically delineate *Candida albicans* serotype A from *Candida tropicalis*, or *C. albicans* serotype B from *Candida stellatoidea*. Morphological tests were needed to make these distinctions. The separation of *C. albicans* type A from *C. tropicalis* was achieved by recognizing the ability of *C. albicans* to form

germ tubes in serum, whereas *C. stellatoidea* was distinguished from *C. albicans* type B by its predominantly filamentous growth on a nutritionally deficient medium. Sweet and Kaufman (15), using a combination of serological and morphological procedures, were able to rapidly identify the clinically important *Candida* spp. within 24 h.

Fukazawa et al. (Y. Fukazawa, T. Tsuchiya, H. Hatano, and T. Nishikawa, World Congr. of the Int. Soc. Trop. Dermatol., 2nd, abstr. no. 69, 1969) and Kanno and Suzuki (11) developed a practical identification procedure using biochemical procedures, along with selected reference and monospecific sera for identifying the medically important *Candida* spp., including the serotypes of *C. albicans*. Their reports on the combined use of rapid biochemical tests and factor sera resulted in the production of a commercial kit called Candida Check by Iatron Laboratories, Inc., Tokyo, Japan. This kit is widely used in many clinical laboratories in Japan and recently became available to us. The availability of the Iatron kit in our laboratories offered a unique opportunity to evaluate and compare it with the API 20C kit (Analytab Products, Plainview, N.Y.) and the conventional modified Wickerham technique (1, 21) for rapidly and accurately identifying the medically important species of *Candida*.

MATERIALS AND METHODS

Cultures. One hundred and twenty isolates belonging to the genera *Candida, Cryptococcus, Geotrichum, Pichia, Rhodotorula, Saccharomyces,* and *Trichosporon* were selected for this study. The majority of the isolates either had been received as diagnostic specimens for identification by the Yeast Reference Laboratory of the Mycology Division, Center for Disease Control (CDC), or were stock cultures maintained in the Mycology Division's culture collection. Of the 120 isolates, 12 were given to us as unknowns by D. G. Ahearn for identification by the Iatron Candida Check and the API 20C kits.

Candida Check identification system. This system is designed to identify eight medically important species of *Candida*. The kit consists of 10 factor sera, sucrose disks, and sucrose strips. The 10 factor sera were produced from rabbit immunoglobulin G serum fractions by the method described by Fukazawa et al. (6). Adsorptions were performed by the methods of Tsuchiya et al. (17, 18).

Procedure for serological identification by Candida Check kit. All cultures were subcultured on Sabouraud dextrose agar containing 2.0% dextrose, 0.5% yeast extract, 1.0% peptone (Difco Laboratories, Detroit, Mich.), and 1.5% agar and were incubated at 27°C for 48 h. They were then checked for purity and suspended in saline to give a concentration of 2×10^8 cells/ml. For slide agglutination tests, slides (50 by 75 mm) marked with 12 circles were used. Approximately 0.05 ml of each of the 10 factor sera and control physiological saline was added respectively to the circles on the slides, and approximately 0.05 ml of a cell suspension was then added to each of them. The reactants were mixed on a platform rotary shaker (A. H. Thomas Co., Philadelphia, Pa.) at 125 ±25 rpm/min for 2 to 3 min. The agglutination reaction with each factor serum was recorded, and the unknown isolate was identified on the basis of the agglutination patterns developed after being tested with all the factor sera (Table 1). An agglutination reaction of at least 1+ was considered positive. Such a reaction with factor 1 serum was prerequisite for a culture to be tested further with the Candida Check kit. Although most of the clinical isolates were *Candida* spp., any culture whose antigen reacted with factor 1 was further examined by morphological tests to assure its being a *Candida* sp. and not any of four yeasts belonging to the genera *Hansenula, Kloeckera, Pichia,* and *Saccharomyces* which could also react with factor 1. None of the species of *Cryptococcus, Rhodotorula, Trichosporon,* or *Geotrichum* reacted with factor 1.

Sucrose disk test. Those isolates that showed positive reactions with factor sera 4, 5, and 6 were tested further for their ability to rapidly utilize sucrose. The rapid "fermentation" of sucrose was recommended by the manufacturer to differentiate *C. albicans* type A from *C. tropicalis.* The sucrose disk test is not carried out in a reduced environment, nor is the production of carbon dioxide measured. Consequently, a positive test indicates rapid utilization of sucrose, not necessarily fermentation. The sucrose disk was dropped into a small test tube containing 0.5 ml of sterile distilled water. The sucrose solution was inoculated with 2 to 3 loopfuls of growth from the culture, and the tube was incubated at 37°C for 2 to 4 h. *C. tropicalis* utilizes sucrose within this short incubation period, causing a color change of the medium to yellow. In contrast, *C. albicans* serotype A does not utilize sucrose and fails to produce any color change.

Sucrose strip test. The sucrose strip test was designed to permit the rapid differentiation of *C. albicans* serotype B from *C. stellatoidea.* Those isolates that reacted with factor sera 1, 4, and 5, and occasionally with 13b but not with 6, were tested further by this procedure. A 0.2-ml amount of distilled water was added to a small sterile test tube and inoculated with 1 loopful of growth from the culture. A sucrose test strip was inserted into the tube until it was completely coated with the suspension. The inoculated strip was then incubated at 37°C for 4 to 8 h. Within this incubation period *C. stellatoidea* isolates caused the strip to change from orange to pink, whereas isolates of *C. albicans* serotype B did not do so. An orange or yellow strip denoted a negative reaction.

API 20C. The 120 isolates were tested and identified according to the manufacturer's instructions by using 19 assimilation tests. An identification was made on the basis of results of the assimilation tests and morphology of the isolates.

Conventional biochemical and morphological studies. Cultures were examined for their morphological characteristics after growth on cornmeal-Tween 80 agar incubated at 25°C for 2 to 3 days in the dark.

TABLE 1. *Agglutination and biochemical patterns for differentiating medically important Candida spp.*

Candida spp.	Monospecific antibody reactions										Sucrose disk reaction	Sucrose strip reaction
	1	4	5	6	8	9	11	13	13b	34		
C. albicans type A	+	+	+	+	−	−	−	−	−[a]	−	−	NR[b]
C. tropicalis	+	+	+	+	−	−	−	−	−	−	+	NR
C. albicans type B	+	+	+	−	−	−	−	−	+[a]	−	NR	Yellow or orange
C. stellatoidea	+	+	+	−	−	−	−	−	−	−	NR	Pink
C. guilliermondii	+	+	−	−	−	+[a]	−	−	−	−		
C. parapsilosis	+	−	−[a]	−	−	−	−	+	+	−		
C. krusei	+	−	−[a]	−	−	−	+	−	−	−		
C. pseudotropicalis	+	−	−	−	+	−	−	−	−	−		
C. glabrata	+	+	−[a]	+	−	−	−	−	−	+		

[a] Variable.
[b] NR, Not required.

The biochemical studies were performed as described by Ahearn (1).

RESULTS

Results of the identification of the 120 isolates by the three systems are shown in Table 2 and described below. Since the Candida Check system was designed to identify only eight medically important species of *Candida*, isolates belonging to the species of other genera could not be identified by that system. Results shown in Table 2 are divided into two groups: (i) identifications achieved by the three systems with respect to the eight medically important *Candida* spp.; and (ii) identification of the remaining isolates belonging to the genera *Cryptococcus*, *Rhodotorula*, *Geotrichum*, *Saccharomyces*, *Pichia*, *Trichosporon* and the species of *Candida* other than those shown in (i).

With respect to the identification of the eight medically important species of *Candida*, 78 (95%) of the 82 isolates were identified correctly with the Candida Check system and the API 20C kit. Four atypical isolates of *C. tropicalis*, demonstrating negative reactions for sucrose fermentation by the conventional method, could not be identified correctly either by the Candida Check or the API 20C kit. These atypical *C. tropicalis* isolates were identified as *C. albicans* serotype A by the Candida Check system and as *Candida* sp. by the API 20C kit. Their assimilation profiles could not be accounted for in the API profile index (Table 2).

In the second part of Table 2, isolates belonging to species of the genera *Cryptococcus*, *Geotrichum*, *Rhodotorula*, and *Trichosporon* were identified by the Candida Check system as "not *Candida* sp." since the kit was not designed to identify species of genera other than *Candida*.

TABLE 2. *Comparison of results on 110 yeast isolates identified by the Candida Check, the API 20C, and conventional methods*

Species tested	No. of isolates identified by conventional tests	No. of isolates identified by Candida Check	No. of isolates identified by API 20C
Eight medically important *Candida* species			
C. albicans	17	17	17
Type A	10	10	Cannot distinguish
Type B	7	7	Serotypes
C. tropicalis	15	11	11
C. stellatoidea	4	4	4
C. guilliermondii	8	8	8
C. parapsilosis	17	17	17
C. krusei	10	10	10
C. pseudotropicalis	5	5	5
C. glabrata	6	6	6
Other yeasts[a]			
C. lusitaniae	1	0[b]	0[c]
C. rugosa	1	0[b]	1
Candida sp.	3	3	3
S. cerevisiae	3	3[d]	3
Pichia sp.	1	0[b]	0
Cr. neoformans	5	5[d]	5
Cr. laurentii	2	2[d]	2
Cr. albidus	1	1[d]	1
Cr. terreus	1	1[d]	1
Rh. rubra	1	1[d]	1
Tr. beigelli	5	5[d]	5
Trichosporon sp.	1	1[d]	1
Ge. penicillatum	3	3[d]	3

[a] Abbreviations: *C.*, *Candida*; *S.*, *Saccharomyces*; *Cr.*, *Cryptococcus*; *Rh.*, *Rhodotorula*; *Tr.*, *Trichosporon*; *Ge.*, *Geotrichum*.
[b] False positive.
[c] Incomplete identification.
[d] Identified as non-*Candida* sp.

Twenty-five (89%) of the 28 isolates yielded correct results in agreement with those obtained by the conventional method. Three isolates, one of each of the three species of *Candida lusitaniae*, *Candida rugosa*, and *Pichia* sp., were misidentified. *C. lusitaniae* keyed out as *C. albicans* serotype A, and *C. rugosa* was identified as an atypical *C. guilliermondii* with factor serum 9 negative. The *Pichia* sp. was identified as a *Candida* sp. since it reacted with factor sera 1 and 4 and also showed weak reactions with factor sera 5, 6, 13, and 13b. This pattern, however, did not fit the description for any of the medically important *Candida* spp. studied. This false-positive result could possibly have been avoided had the yeast morphology been more carefully studied.

The API 20C system correctly identified 26 (93%) of the 28 isolates. The *C. lusitaniae* and *Pichia* sp. isolates each gave assimilation profiles that could not be accounted for in the profile index. By their morphology, these isolates were identified as *Candida* sp. (Table 2).

When the results of the two sections in Table 2 were considered together, it was evident that 94% of the isolates had been identified correctly by the Iatron Candida Check system, and 95% of the isolates were identified accurately by the API 20C. The sensitivity and specificity of each method were calculated by the method of Galen and Gambino (7). When the conventional Wickerham method was considered 100% sensitive, the percent sensitivity obtained for the API and Candida Check methods was 95%. When the conventional method was considered 100% specific, the Candida Check and API 20C systems were found to be 90 and 93% specific, respectively.

Because of the problems encountered in identifying *C. lusitaniae* and the sucrose-negative atypical *C. tropicalis* isolates, additional isolates of these species were obtained from D. G. Ahearn to ascertain their assimilation profiles with the API 20C system and also to determine their reactivity and agglutination pattern with the Candida Check system.

We included Centraalbureau voor Schimmelcultures (CBS) type culture 4413 in our studies with five isolates of *C. lusitaniae* and six isolates of sucrose-negative *C. tropicalis*. The *C. lusitaniae* isolates were all identified as *C. albicans* type A by the Candida Check system. With the API 20C kit, these five *C. lusitaniae* isolates yielded an assimilation profile identical to that of *C. tropicalis* (profile 6 556 371), assimilating glucose, glycerol, 2-keto-D-gluconate, xylose, adonitol, galactose, sorbitol, methyl-D-glucoside, *N*-acetyl-D-glucosamine, cellobiose, maltose, saccharose, trehalose, and melezitose. The morphology of the five isolates varied considerably from that of *C. tropicalis*, especially the blastospores which were bent and curved. Of the six sucrose fermentation-negative *C. tropicalis*, five were identified as *C. albicans* type A, and one was identified as *C. tropicalis* by the Candida Check system. By the API 20C system, two of the six isolates were properly identified as *C. tropicalis* (profile 6 556 371). The profiles of the remaining four isolates could not be properly identified with the API profile index (two with profile 2 552 150 and two with profile 2 552 140).

DISCUSSION

Studies with 82 yeast isolates, representing eight medically important species, revealed that both the Iatron Candida Check and API 20C methods permitted the accurate identification of 95% of the isolates that had been identified by the conventional Wickerham method. The Iatron method generally permitted reliable identification of the *Candida* species in 10 min. In those cases where supplementary biochemical tests were necessary, identification required up to 5 h. The API procedure required 48 to 72 h to identify the same fungi. The results noted with the Candida Check procedure are consistent with those of Taguchi et al. (16) and Kanno and Suzuki (11), who independently reported the serological test kit to have an accuracy of 96 and 89%, respectively. Both groups of workers noted that spontaneous agglutination posed difficulties in identifying some isolates. In the present study, 8 (10%) of the 82 *Candida* spp. isolates studied showed spontaneous agglutination reactions. These isolates were accurately identified, however, by the slide agglutination test, after heating the culture suspension at 100°C for 2 h and removing the large clumps. Antigenic diversity proved no problem if one strictly followed the agglutination patterns provided with the kit. Certain yeasts such as *Hansenula* spp., *Kloeckera* spp., *Pichia* spp., and *Saccharomyces* spp. may also react with factor serum 1. These yeasts are not as common as the *Candida* spp. in clinical materials. Should they be encountered, however, their differentiation from *Candida* spp. by further morphological studies is necessary to avoid misleading serological reactions such as were noted with the *Pichia* sp. in this study (Table 2).

One of the major problems with both kits was their failure to properly identify the sucrose-nonfermenting *C. tropicalis* isolates (2). Of six such atypical isolates studied, only one was correctly identified by the Candida Check method, and two were correctly identified by the API

20C kit. The serological method identified the remaining five isolates as *C. albicans* type A, and the API 20C kit identified four as *Candida* sp. since their assimilation profiles could not be accounted for in the API 20C profile index.

Studies with yeast species other than the eight important *Candida* species revealed that the Candida Check and the API 20C methods were 89 and 93% specific, respectively (Table 2). Our API 20C test results are similar to those obtained by Buesching et al. (5) and Land et al. (12). One of the *Candida* sp. which could not be properly identified with the API kit was *C. lusitaniae*. Holzschu et al. (9) reported *C. lusitaniae* as an opportunistic yeast pathogen in a patient with acute myelogenous leukemia. Four isolates from this patient were identified by conventional methods in the clinical laboratory as *C. parapsilosis*. It is interesting to note that with the API 20C kit all of the five isolates of *C. lusitaniae*, including the type culture, gave an assimilation profile similar to *C. tropicalis* rather than that of *C. parapsilosis*. The assimilation pattern of the *C. lusitaniae* isolates was identical to that of two of the six sucrose-nonfermenting *C. tropicalis* isolates. Antigenically, *C. lusitaniae* showed an antigenic pattern identical to that of *C. albicans* serotype A. Furthermore, our studies on the proton magnetic resonance spectrum (9) of the cell wall mannan from the type culture (CBS 4413) gave basically the same value as that obtained with cell wall mannan from *C. albicans* serotype A, although the intensity of each of the five peaks was different.

Preliminary studies with *C. albicans* type A, atypical sucrose-negative *C. tropicalis* (variant 446), and *C. lusitaniae* (CBS 4413) antigens and rabbit antisera indicate that these yeasts share antigens 1, 4, 5, and 6. Further adsorption studies indicate that antigenically the atypical *C. tropicalis* isolates are closely related to one another, and that *C. albicans* and *C. lusitaniae* are also so related, with *C. albicans* being more complex.

It is apparent from our data that any yeast isolate which is identified as *C. albicans* serotype A by the Candida Check method must be studied further to distinguish it from the sucrose-negative *C. tropicalis* or *C. lusitaniae* which may be similarly antigenically characterized. It is also clear that with the present API data base, the two species mentioned above cannot be differentiated. Undoubtedly, the API data base must continue to expand so that yeast species such as those discussed above can be accurately identified. The advantage of using the Candida Check system is that it offers speedy and accurate identification of 95% of the isolates belonging to eight medically important *Candida* spp. Thus, those yeasts which are the most frequent causes of candidiasis will be reliably identified. The data base of the Candida Check system, like that of the API 20C kit, must be expanded to enable the accurate recognition of the atypical isolates of *C. tropicalis* and other opportunistic yeasts.

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