Original

A Comparison of Methods for Yeast Identification Including CHROMagar Candida, Vitek System YBC and a Traditional Biochemical Method

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Background. CHROMagar Candida (CAC) is a new chromogenic medium for the presumptive identification of clinically-important yeast isolates. A yeast biochemical card (YBC), a part of the Vitek system is an automatic method for the identification of clinically-important yeast isolates. We conducted a comparison of these two methods with a traditional biochemical method in order to choose a rapid and accurate technique for yeast identification.

Methods. All yeast isolates were inoculated onto Sabourand dextrose agar (SDA) and CAC, and incubated at 30 °C for 48 hours. All isolates were simultaneously tested using traditional biochemical methods and the yeast biochemical card from the Vitek system.

Results. We evaluated 235 yeast isolates from clinical specimens, including 89 *Candida albicans*, 47 *Candida tropicalis*, 43 *Candida glabrata*, six *Trichosporon beigelii*, and five *Candida krusei* in addition to 45 isolates of other yeast species. Isolates were presumptively identified on the basis of colony color and appearance on CAC medium. These observations were compared with a traditional biochemical yeast- identification method and also with YBC from the Vitek system. For five commonly-isolated species (*Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei* and *Trichosporon beigelii*), agreement among the CAC medium, YBC method and traditional biochemical method were 98.9% (187/189), 96.3% (182/189), 100% (189/189), respectively.

Conclusions. From our comparison, the CAC medium is a convenient and economic method to identify five commonly-noted yeast species, and the YBC method warrants a greater cost and requires a longer period of time to obtain reliable results.

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Sabouraud dextrose agar (SDA) is the most useful contemporary medium for isolating *Candida albicans* and other yeasts in a clinical laboratory. This medium is reliable and permits the isolation of several different genera, but overall, the colonies cultured on this medium are very similar in appearance and their subsequent identification requires considerable investigative time.[1] Although *Candida albicans* remains

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the most frequently-noted yeast pathogen, various other *Candida* species have exhibited an increasingly important role in nosocomial and community infection, thus it is desirable to identify a greater proportion of *Candida* species from all clinical specimens.[2] The detection of various yeast species and the presumptive identification of such isolated yeasts may be an aid for rapid and appropriate treatment decisions in the light of noted differences in the susceptibility of yeast species to various antifungal agents.[3,4] Some *Candida* species, such as C*andida lusitaniae*, may often be resistant to amphotericin B; C*andida glabrata* is less sensitive than other species to fluconazole and ketoconazole, and *Candida krusei* exhibits innate resistance to fluconazole.[4]

CHROMagar Candida (CAC) is a novel differential culture medium.[5,6] It can be used to elicit the presumptive identification of several commonly-isolated yeast species by way of specific color reactions and resultant colony morphology[7-10] This culture medium can elicit early species-level identification of *Candida* species and, as a consequence, facilitate species-specific treatment decisions.[2] A yeast biochemical card (YBC) constituting a part of the Vitek system contains 32 individual biochemical tests, and is an automated system for yeast identification, although it warrants a greater cost and requires a longer period of time for obtaining reliable results than is the case for the CAC medium. In this study, we compared the efficiency of CAC medium and the Vitek YBC system in order to determine which method is more rapid and economical for clinically important yeast identification.

Methods

Organisms

Two hundred and thirty five isolates of yeast, collected from the Tri-Service General Hospital in Taiwan from March to September 1996, were maintained in Sabouraud dextrose agar at 4 °C. The yeasts were subcultured on SDA for further study. The following strains were used as control strains: *Candida albicans* ATCC 14053, *Candida tropicalis* ATCC 750, *Candida glabrata* ATCC 2001, *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019, *Geotrichum spp* ATCC 34614, *Cryptococcus neoformans* ATCC 14116.

Culture medium

(1) CHROMagar Candida (CHROMagar Company, Paris, France): Peptone 10 g, glucose 20 g, agar 15 g, chloramphenciol 0.5 g, chromogenic mix 2 g, distilled water 1,000 mL. The medium was prepared by stirring and heating to 100 °C and then holding at that temperature for two minutes, the medium subsequently being dispensed into petri dishes. (2) Sabouraud dextrose agar (Becton Dickinson, Cockeysville, Maryland, USA): 65 g powder was added to 1,000 mL of distilled water, autoclaved for 15 minutes and subsequently dispensed into petri dishes. (3) Traditional biochemical method: includes carbohydrate- assimilation test, carbohydrate-fermentation test, nitrate-assimilation test, urease test, Indian ink, ascospore medium and corn meal agar. (4) Yeast biochemical card (YBC, Biomerieux Vitek, Hazelwood, Missouri, USA).

Procedure

All yeast isolates were inoculated in parallel onto SDA and CAC, incubated at 30 °C for 48 hours and subsequently identified by the traditional biochemical method and YBC. Organisms for YBC assay were incubated at 30 °C, for 24 or 48 hours, and read by a Vitek reader (Vitek, Hazelwood, Missouri, USA). Results which showed relative probability ≥ 90% and were different from results from the traditional biochemical method represented "misidentified". Results showing relative probability < 90% represented "unidentified". At the same time, one experienced reader and two inexperienced readers recorded their identification of the 235 isolates by comparing the panel of reference for the CAC plate labelled with the name of the species, the final results of the CAC medium being somewhat dependent upon the skills of the reader, experience being an advantage. The results which showed distinctive color and were different from results from the traditional biochemical method represented

"misidentified". The results showing white color represented "unidentified."

Cost

We evaluated the different method prices for the identification of five commonly-seen germ tube negative yeast species using the three separate identification methods.

Statistical analysis

Colony appearance with the CAC medium was analyzed in terms of sensitivity [the number of true positives/(the number of true positives + the number of false negatives)], specificity [the number of true negatives/(the number of true negatives + the number of false positives)], the predictive value of a positive test [the number of true positives/(the number of true positives + the number of false positives)], and the predictive value of a negative test [the number of true negatives/(the number of true negatives + the number of false negatives)] to determine their likely usefulness in the clinical laboratory setting.

Results

Two hundred and thirty-five yeast isolates from clinical specimens were identified by CAC, YBC and the traditional biochemical test. The results are summarized in Tables 1 and 2. All of the 89 strains of *Candida albicans* revealed green coloration with CAC (sensitivity and specificity = 100%), and for the YBC, both the sensitivity and specificity were 100%. Forty-six of the 47 strains of *Candida tropicalis* exhibited blue colorations with a halo but one strain revealed a white color with CAC. One strain identified as *Candida species* and one strain identified as *Candida guillermondii* exhibited an appearance similar to that of *Candida tropicalis* (sensitivity 97.9% and specificity 98.8% with CAC). One strain identified as *Candida parapsilosis* by YBC demonstrated a sensitivity of 97.9% and a specificity of 100%. Forty-two of the 43 strains of *Candida glabrata* exhibited dark pink coloration except for one strain which produced white colonies on CAC. We found three false positives; all identified as *Toluropsis candida* (sensitivity 97.7% and specificity 98.4% with CAC). One strain was unidentified by YBC and had a sensitivity of 97.7% and a specificity of 100%. Five strains of *C. krusei* appeared to all be flat and revealed a pale pink center with a white peripheries when using the CAC (sensitivity and specificity = 100%). For YBC, all were unidentified, and both sensitivity and specificity values were zero. Six strains of *Trichosporon beigelii* revealed a fuzzy blue-green colony with a white periphery when using the CAC (sensitivity and specificity = 100%). For YBC, one strain was unidentified and had a sensitivity of 83.3% and a specificity of 100%. For the remaining yeasts, we found no species that could be differentiated from each other by colony color and appearance, all exhibiting a white to pink coloration. The degree of concordance between the two inexperienced and the one experienced reader was very high (99%) on the basis of specific colony color for species such as *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Trichosporon beigelii* (data not shown). For the remainder of the yeasts, the appearance of colonies was equivocal and it was difficult to strike good concordance between the three readers.

The cost required to identify the five commonly-noted species with CAC, YBC, and traditional biochemical methods is shown in Table 3. Although the purchase price of the medium required for the CAC is higher than that corresponding to the YBC method and the traditional biochemical method, the identification and labor price of the CAC medium is substantially less expensive than is the case for these other two methods. Overall, for the suite of analyses conducted with the CAC medium, the cost was NT$43 and for the YBC and the traditional biochemical method it was NT$145 and NT$229, respectively.

Discussion

The CAC medium supports the growth of yeasts and some molds while it suppressed the growth of

Table 1. Colony color and morphology of 235 yeast isolates on CHROMagar Candida medium, and results of CHROMagar Candida (CAC), Yeast biochemical card (YBC) and traditional biochemical methods of identification

Species	Morphology and color of colony on CAC	No. of isolates identified by		
		CAC	YBC	Traditional biochemical method
Candida albicans	Green, round, convex	89	89	89
Candida tropicalis	Blue to purple with halo	46	46	47
Candida glabrata	Dark pink center, white edge	42	42	43
Trichosporon beigelii	White center with green blue edge, heaped, fuzzy, dry	6	5	6
Candida krusei	Pale pink center, with white edge, flat, dry	5	0	5
Trichosporon pullalans	Yellow-brown, wrinkle	2	0	2
Geotrichum spp.	Pale pink, fuzzy, dry	1	0	1
Candida parapsilosis	White, convex	0	25	27
Torulopsis candida	White, convex	0	6	7
Cryptococcus neoformans	White, convex	0	0	3
Candida lipolytica	White, convex	0	1	1
Saccharomyces cereviaiae	White, convex	0	0	1
Hansenula anomala	White, convex	0	0	1
Candida guillermondii	White, convex	0	0	1
Candida spp.	White, convex	0	0	1
Misidentified	Color, convex	5	2	0
Unidentified	White, convex	39	19	0
Total		235	235	235

CHROMagar Candida : One *C. guillermondii* and 1 *Candida spp.* were misidentified as *C. tropicalis*, and 3 *T. candida* were misidentified as *C. glabrata*. One *C. tropicalis*, 1 *C. glabrata*, 27 *C. parapsilosis*, 4 *T. candida*, 3 *C. neoformans*, 1 *C. lipolytica*, 1 *S. cereviaiae* and 1 *H. anomala* were all unidentified.
Yeast biochemical card : One C. tropicalis was misidentified as C. parapsilosis and 1 C. parapsilosis was misidentified as C. tropicalis. One C. glabrata, 1 T. beigelii, 5 C. krusei, 2 T. pullalans, 1 Geotrichum spp., 1 C. parapsilosis, 1 T. candida, 3 C. neoformans, 1 S. cereviaiae, 1 H. anomala, 1 C. guillermondii and 1 Candida spp. were all unidentified.

Table 2. Statistical evaluation of CHROMagar Candida (CAC) and Yeast Biochemical Card (YBC) for presumptive identification of five commonly-observed yeast isolates

Species	Sensitivity (%)		Specificity (%)		Positive Predictive value		Negative Predictive value	
	CAC	YBC	CAC	YBC	CAC	YBC	CAC	YBC
Candida albicans	100	100	100	100	100	100	100	100
Candida tropicalis	97.9	97.9	98.8	100	95.8	100	99.5	99.5
Candida glabrata	97.7	97.7	98.4	100	93.3	100	99.5	99.5
Candida krusei	100	0	100	0	100	0	100	0
Trichosporon beigelii	100	83.3	100	100	100	100	100	99.6

bacteria, providing a high selectivity for yeast isolation.[5,7] The medium also is superior to other routine media for the detection of multiple *Candida* species from both clinical and stock cultures.[3] We consider that the CAC medium can be introduced as a standard routine medium when infection by fungus is suspected. In our evaluation of traditional biochemical method, CAC medium, and YBC method with 235 yeast strains, we found that the percentages of reliability for these three methods were 100% (235/235), 81.3% (191/235), 91.1% (214/235), respectively. The traditional biochemical method is still the gold standard. Our results of CAC medium showed the sensitivity for *Candida albicans*, 100%; *Candida trop-*

Table 3. Estimated cost of identification of five commonly-found germ tube negative yeast isolates

	CHROMagar Candida	YBC (VITEK)	Traditional biochemical method
Media	NT $35 (CAC)	NT $15 (SDA)	NT $15 (SDA)
Germ tube	-	NT $2	NT $2
Identification	-	NT $100	NT $168
Labor	NT $8	NT $28	NT $44
Total	NT $43	NT $145	NT $229

YBC = Yeast Biochemical Card; NT = New Taiwan dollars; CAC = CHROMagar Candida; SDA = Sabouraud dextrose agar.

icalis, 97.9%; *Candida glabrata*, 97.7%; *Candida krusei*, 100%; *Trichosporon beigelii*, 100%; respectively. Bernal[9] and Houang[8] also had the same results showed the sensitivity for *Candida albicans*, 99.4 to 100%; *Candida tropicalis*, 95 to 100%; *Candida glabrata*, 95 to 98.9%; *Candida krusei*, 95 to100%; respectively. Willinger[4] and Powell[7] reported the sensitivity for *Candida tropicalis* were 66.7% and 52%. They considered that color variation severely affected the presumptive identification of *Candida tropicalis*. The distinctive green color of *Candida albicans* when cultured on the CAC medium allowed for easy presumptive identification of the yeast species. The specificity of the CAC medium for determining yeast species was excellent, the suggestion being here that additional tests such as the germ-tube test may not be required.[3,7,8] From our experience, 15% (13/89) of *Candida albicans* analysis were germ-tube-negative, the CAC medium clearly being superior in this regard. The unique white center with a green-blue periphery and a fuzzy colony appearance, characteristic of *Trichosporon beigelii* has only previously been reported by Odds and Bernaerts (1994).[5] The typical and characteristic morphological features of this yeast species when identified using the CAC medium were so specific that the CAC would not result in a misidentification of this species as any other clinical-important yeast. Among the many white colonies produced by isolates, only *Candida krusei* colonies could be reliably distinguished from other yeast species by their characteristic dry and flat appearance.[5,8,9] Other species such as *Candida parasilosis*, *Saccharomyces cerevisiae, Hansenula anomala, Candida guilliermondii, Cryptococcus neoformans, Candida lipolytic* and *Candida species* produced white colonies, leading to a high degree of confusion between the different species when using the CAC medium.[1,9,10] From our observations, we suggest that, due to the potential for such uncertainty, these species must be evaluated by traditional biochemical methods.[7] For the 27 white color colonies of *Candida parasilosis*, we just supplemented with corn meal agar and carbohydrate fermentation tests. If positive for pseudohyphae and glucose fermentation, we could differentiate this species from others. This is the advantage of CAC medium. Our results of YBC method showed the sensitivity for *Candida albicans*, 100%; *Candida tropicalis*, 97.9%; *Candida glabrata*, 97.7%; *Candida krusei*, 0%; *Trichosporon beigelii*, 83%, respectively. Bernal[9] and Pfaller[3] also had the same results showed the sensitivity for *Candida albicans*, 100%; *Candida tropicalis*, 100%; *Candida glabrata*, 100%; *Candida krusei*, 100%; respectively. The sensitivity and specificity of the YBC method were closed or greater than the corresponding values for the CAC medium, although the YBC method required additional system equipment including the Vitek equipment, and the method required more time to be completed.

Although the cost of the reagents for the CAC medium is three-fold more than that for Sabouraud dextrose agar, this initial cost may be offset when the cost of secondary biochemical tests and the cost of labor (which is greater for the YBC and traditional biochemical methods) are taken into account.[6,8,9] The reduction in necessary analytical laboratory time when utilizing the CAC medium may allow laboratory managers to cost-effectively rearrange their workloads, allowing medical technologists to complete other tasks more requiring of their particular skills. The cost of using the CAC medium was reduced by using biplates.

Clearly, the speed with which tests are conducted

and isolates identified in the laboratory is of vital importance. Presumptive identification of the previously-prescribed yeast species was not difficult using the CAC medium, and the different-species color differentiation after 48 h was very clear.[4,9] With CAC medium, we estimate that the five species mentioned above can be identified from 24 to 48 hours earlier than is the case with YBC and conventional identification methods, this observation also being noted by others.[2,3,9]

The CAC medium was easy to use, relatively easy to read and interpret, and appeared to be less subjective than either reading germ tubes and/or various biochemical tests.[7] It seems clear that the use of this medium is economical in the overall context of labor and time. Moreover, the cost of using this CAC medium would be more than offset by the decreased need for the use of secondary biochemical tests.

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