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Evaluation of a Commercial Medium for Identification of *Candida* Species

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CHROMagar Candida (CHROMagar, France) was evaluated as a medium for the presumptive identification and isolation of yeasts using 1,537 isolates of medically important yeasts, including 970 *Candida albicans*, 165 *Candida parapsilosis*, 131 *Candida glabrata*, 62 *Candida guilliermondii*, 35 *Candida krusei*, 32 *Candida tropicalis*, 31 *Rhodotorula rubra*, 23 *Trichosporon* spp. (17 *Trichosporon beigelii*), 17 *Candida famata*, 16 *Candida pelliculosa*, 10 *Pichia etchelsii*, 10 *Saccharomyces cerevisiae*, 8 *Candida lusitaniae*, 7 *Cryptococcus* spp., and 20 isolates of other *Candida* spp. After 48 h of incubation at 37°C, the sensitivity and specificity were, respectively, 99% and 100% for *Candida albicans*, 93.8% and 99.1% for *Candida tropicalis*, and 100% and 100% for *Candida krusei*. In addition to colony color, other colony characteristics were important for identification of some species, such as rough colonies in *Candida krusei* isolates or the halo around the colonies of *Candida tropicalis*. A great variety of colors was observed among species other than *Candida albicans*, *Candida tropicalis*, and *Candida krusei*. For identification purposes, CHROMagar Candida medium has an accuracy similar to that of germ-tube tests and chlamydospore development tests for *Candida albicans* and to that of the ATB ID32C kit (API, bioMérieux, France) for *Candida tropicalis* and *Candida krusei*.

Candidiasis is a growing problem of great clinical importance, particularly in immunocompromised patients (1, 2), in whom clinical failures or resistance to antifungal therapy have been described (3). *Candida albicans* is the etiological agent usually found in these infections, although species such as *Candida glabrata*, *Candida krusei*, and *Candida tropicalis* are now being isolated more frequently from clinical specimens (4).

Classical methods for the identification of yeasts begin with the isolation of the microorganisms from the clinical specimen. In the case of *Candida albicans*, this identification can take 24 to 48 h, but it takes much longer when other species must be identified. Among the methods used for yeast identification, it is possible to distinguish between those performed after the primary isolation of the microorganism and those that make possible the isolation and presumptive identification of the microorganism directly in the culture medium. The former methods include the germ-tube test, chlamydospore formation, and the fermentation or assimilation of sugars (5). The germ-tube test is a rapid identification method (2–4 h), but it uses potentially dangerous sera and identifies only *Candida albicans* (6). Furthermore, 5% of *Candida albicans* isolates do not produce germ tubes (7), while some *Candida tropicalis* isolates are germ-tube producers (8). These factors complicate the correct interpretation of this test. In the case of methods in which the assimilation or fermentation of sugars is evaluated, complete identification can take 18 to 72 h.

The difficulty in detecting mixed cultures in the same plate with the traditional Sabouraud glucose agar medium as well as the importance of identifying the pathogen quickly has fostered the development of differential media for identification of yeasts. These media are based on the direct detection of specific enzymatic activities by adding certain substrates or fluorochromes to the media. These media include Nickerson (Merck, Germany) (9), Pagano-Levine-Trejo (Difco, USA) (10),

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Costa-de Lourdes Branco (11), and Albicans ID (bioMérieux, France) (12). However, these media are not currently used in most laboratories.

CHROMagar Candida (CHROMagar, France) is a differential medium for the isolation and differentiation of *Candida* spp. It allows for the presumptive identification of *Candida albicans*, *Candida krusei*, and *Candida tropicalis*, differentiating between species that could be in the same plate. Each species, when it grows in this medium, reacts specifically with a chromogenic substrate in such a way that the colonies take on a characteristic appearance according to the species isolated.

In the present study we evaluated this commercial medium as a differential medium for identification of *Candida* spp., using 1,537 isolates of clinically important yeasts.

Materials and Methods

Isolates. A total of 1,537 yeast isolates were screened in the commercial medium. Of these, 345 were fresh isolates from oral specimens obtained directly on the plates supplied, which were used as primary isolation plates. The remaining 1,192 isolates were reference strains or had been obtained from clinical specimens of different origins, received at our laboratory over the past few years. Before screening in the commercial medium, all isolates were subcultured in Sabouraud glucose agar to check their viability. The identity of isolates was always verified by germ tube formation in horse serum (13), chlamydospore production, and morphology in corn meal agar-Tween 80 according to Dalmau's method (5). Assimilation tests were performed using the commercial ATB ID32C galleries (API, bioMérieux, France). The species and number of isolates studied are shown in Table 1.

Commercial Medium. CHROMagar Candida Medium (CHROMagar, France) is a commercial medium that is supplied as a dehydrated powder in preweighed quantities. It was prepared according to manufacturer's instructions.

Table 1: Colony colors observed after 48 h of incubation on CHROMagar Candida plates at 37°C.

Species	No. of isolates	Color (Pantone code)
Candida albicans	970	green, blue-greenish (3258, 3265, 3268, 3278, 3288, 338, 340, 3405, 3155, 320)
Candida parapsilosis	165	white, different shades of pink, violet, purple (435, 4685, 503, 5135, 5175, 5245, 5315)
Candida glabrata	131	pink, violet, purple, white (126, 255, 261, 436, 5135, 5155, 5165, 5315, 520)
Candida guilliermondii	62	pink, violet, purple, white (435, 5135, 5155, 5165, 5175, 520, 5315, 4685)
Candida krusei	35	pink, violet; rough colonies (217, 255, 257, 5025, 503, 5165)
Candida tropicalis	32	blue, blue-violet/greenish; dark halo around the colony (276, 307, 3135, 316, 525, 5405, 549)
Rhodotorula rubra	31	red-orange (159, 179)
Trichosporon spp.	23	mixture of blue-green, white, pink; velvet colonies (305, 325, 5145, 5165)
Candida famata	17	pink, violet, white (434, 436, 5135, 5155, 5165, 5175, 5315)
Candida pelliculosa	16	pink (434, 503, 5175)
Pichia etchelsii	10	white, pink, light violet (3135, 538, 5245, 5315, 435)
Saccharomyces cerevisiae	10	violet, violet-greenish-grayish (261, 276, 436, 450, 4505, 5135, 525, 5635)
Candida lusitaniae	8	light pink, violet (5135, 5155, 5165, 5175)
Cryptococcus spp.	7	light violet, white, blue-greenish (5135, 434, 3145)
Candida lipolytica	4	white, beige (4685)
Candida rugosa	4	violet, light pink (261, 5135, 5165)
Candida sake	4	white, violet, gray-greenish (2695, 5635)
Candida kefyr	3	light pink, white (503, 5165)
Candida holmii	2	light violet (2651)
Candida valida	1	white
Candida zeylanoides	1	blue (307)
Candida silvicola	1	violet (5135)
Total	1,537	

Table 2: Distribution of the colony colors within each yeast species.

Species	Green	Blue	Violet	Pink	White	Others
Candida albicans	960	9	–	–	1	–
Candida parapsilosis	–	–	3	56	104	2
Torulopsis glabrata	–	–	61	64	5	1
Candida guilliermondii	–	–	16	40	5	1
Candida krusei	–	–	1	34	–	–
Candida tropicalis	–	18	14	–	–	–
Rhodotorula rubra	–	–	–	–	–	31
Trichosporon spp.	–	–	–	1	4	18
Candida famata	–	–	6	10	1	–
Candida pelliculosa	–	–	–	15	–	1
Pichia etchelsii	–	–	1	1	8	–
Saccharomyces cerevisiae	–	–	8	–	–	2
Candida lusitaniae	–	–	2	6	–	–
Cryptococcus spp.	–	1	2	–	3	1
Other *Candida* spp.	–	1	7	3	5	4

Utilization and Evaluation of the Medium. The inoculum used came from swabs of clinical specimens or from fresh colonies grown within 24 h at 37°C in Sabouraud glucose agar. These cultures were incubated on the commercial plates supplied at 37°C for 48 h. The colony colors observed were described by comparing them with the Pantone Color Specifier 747XR (Pantone, USA) (14).

To evaluate the utility of the commercial medium as a differential medium, we considered as true-positive results those isolates which, in 48 h, had a characteristic green color for *Candida albicans*, blue-violet-purple (usually with a dark halo around the colony and with different tones in the obverse and reverse of the colony) for *Candida tropicalis*, and pink (with variable intensity) with a rough appearance for *Candida krusei.*

Statistical Analysis. The following values were obtained: sensitivity [true positives x 100/(true positives + false negatives)], specificity [true negatives x 100/(true negatives + false positives)], positive predictive value [true positives x 100/(true positives + false positives)], negative predictive value [true negatives x 100/(true negatives + false negatives)], and efficiency [(true positives + true negatives) x 100/total].

Results

The 345 isolates obtained directly in the CHROMagar Candida plates came from 248 oral specimens. Mixed cultures were detected on 96 of these plates (38.7%); the color difference between colonies was very clear. Of these mixed cultures, 73 (29.4% related to the total of specimens)

Table 3: Utility of CHROMagar Candida plates for primary identification after 48 h of incubation at 37°C.

	Species		
	C. albicans	*C. tropicalis*	*C. krusei*
True-positive results	960	18 (30)[a]	34 (35)[b]
True-negative results	567	1,494 (1,492)[a]	1,502
False-positive results	0	11 (13)	0
False-negative results	10	14 (2)[a]	1 (0)[b]
Percent sensitivity	99	56.3 (93.8)[a]	97.1 (100)[b]
Percent specificity	100	99.3 (99.1)[a]	100
Positive predictive value	100	62.07 (69.8)[a]	100
Negative predictive value	98.3	99.1 (99.9)[a]	99.9 (100)[b]
Percent efficiency	99.4	98.4 (99)[a]	99.9 (100)[b]

[a] Values in parentheses result from considering a wider range of colors, the possible halo around the colony, and/or the differences between the reverse and the obverse of the colony.
[b] Values in parentheses result from considering the violet isolate as if it were dark pink.

contained two species or different colonial morphologies, 16 (6.4%) had three colonial morphologies, and 7 (2.8%) had four or more colonial morphologies. On two plates (0.8%) bacteria grew (very few colonies) and on another one (0.4%) an *Aspergillus* species.

All isolates grew well in this medium. Growth was detectable in 24 h in almost all cases, the color differentiation being very clear. However, colonies detected in the isolates obtained from clinical specimens were small and the color was easier to define after 48 h (the time frame stated by the manufacturer). The color of some colonies changed slightly after more than 48 h: generally, the colonies became darker and, in some cases, the color changed considerably (from green to dark blue-brown-grayish). This effect was not related to the viability of the cells, since the colonies grew well when subcultured. Rather, the change in color seemed to be related to the dehydration of the medium, since it occurred particularly when the plates had a low volume of medium.

The species distribution and the colors observed in the isolates evaluated are shown in Tables 1 and 2. Sensitivity, specificity, positive predictive value, negative predictive value, and efficiency of the commercial plates are shown in Table 3.

Among 970 *Candida albicans* isolates, 960 had a characteristic green color not observed in any other yeast species. Only ten isolates had a different color in the first culture on this medium. Although some isolates of other species were green, their colors were completely different from those seen in *Candida albicans*. For this reason, those colors have been included as "others." One of the ten false-negative results for *Candida albicans* that had grown white in the first culture had the typical green color when subcultured. The other nine had blue-greenish colors more similar to those seen in *Candida tropicalis*. From these results the sensitivity and specificity for *Candida albicans* were, respectively, 99% and 100%, and the positive predictive value 100%.

The 32 *Candida tropicalis* isolates were mainly of bluish color or dark bluish-purple. In general, the obverse of the colonies was bluer than the reverse, which was more purple; in some cases there was a small dark purple halo in the agar around the colonies. The former characteristic was not seen in all the isolates of *Candida tropicalis* but was seen only in this species. On the other hand, only darker blue of the dark blue-violet isolates had this halo (8 of 18); the typical (lighter) blue colonies, which were easy to distinguish as blue, had no halo. In all cases this characteristic halo was observed only around well-separated colonies. Two isolates defined as violet-purple were lighter, with similar tones in obverse and reverse of the colonies, without a halo or any other characteristic that led us to consider them as *Candida tropicalis*. Since *Candida tropicalis* must have a bluish color in this medium, the sensitivity for this species was 56.3%. However, if one takes into account the more typical characteristic of *Candida tropicalis* in this medium, with a color from blue to violet, the sensitivity would be 93.8%. However, the specificity in the first case was 99.3% and in the second case 99.1%. This value is slightly lower in the second case because among the ten isolates of *Saccharomyces cerevisiae*, two had a dark violet-blue color, similar to that observed in many *Candida tropicalis* isolates. These isolates also had a different color in the obverse and reverse of the colony, and although they did not have the halo, they could easily have been mistaken for *Candida tropicalis*. Because of this, they have been considered as false-positive results.

All 35 colonies of *Candida krusei* isolates were rough, had no defined border, tended to spread, and had a pinkish color of varying intensity. These characteristics, taken together, were not observed in any of the other species evaluated. According to these observations and considering that the isolate defined in the table as violet could be included as dark pink, the sensitivity and specificity for *Candida krusei* were both 100%. If the darker isolate is excluded as a true-positive result, the sensitivity would be 97.1%, but the specificity would not change.

The colony colors observed in the 23 *Trichosporon* isolates were highly variable, with different mixed tones (greenish, bluish, or pinkish, all generally light). Even within the same culture and/or colony, the color could change from one zone to another or from obverse to reverse, although in some isolates one color predominated. On the other hand, the rough velvet appearance characteristic of this species made it easy to recognize and distinguish from the other species.

The 31 *Rhodotorula rubra* isolates had the characteristic reddish-orange colony color of this species in any medium. It was not seen in any other species in this study.

The isolates of *Candida parapsilosis*, *Candida famata*, *Candida glabrata*, and *Candida guilliermondii* had various tones from white to dark vio-

let-purple. These colors were difficult to define because they were all similar pastel tones, but are, nevertheless, fairly characteristic and typical of these species. From these species, the easiest to identify was *Candida parapsilosis* because many of its isolates had not only white or light pink tones but also a wrinkled surface, easy to differentiate from the rough *Candida krusei* colonies.

Discussion

Different media for the simultaneous isolation and identification of yeasts from clinical specimens have been described and evaluated (9–12, 15). In most cases the species identified is *Candida albicans*. However, CHROMagar Candida medium allowed for a clear differentiation of different species. The results of this study are in agreement with those described by Odds and Bernaerts (16), with a 100% specificity for *Candida albicans*. This allows for the direct confirmation of the presence of this species in the medium without the need to perform additional tests such as the germ-tube test. The sensitivity value in this study is somewhat lower than that described by these authors, who did not find false-negative results for *Candida albicans*. The false-positive results for *Candida tropicalis* were *Candida albicans* and the two *Saccharomyces cerevisiae* isolates, although they had no halo around the colonies. Therefore, sensitivity and specificity are lower than those described by Odds and Bernaerts (16) for this species. On the other hand, we did not observe, as they described, that some *Pichia* colonies appeared similar to *Candida tropicalis*. When identifying *Candida tropicalis*, it should be taken into account that in many cases there are differences between the obverse and reverse of the colony and/or a halo around the colony. Considering these aspects together, the presumptive identification of *Candida tropicalis* should be easier, meaning the sensitivity of this medium for this species would increase.

Candida krusei is an emerging pathogen in immunocompromised patients and shows a reduced susceptibility to azole antifungal agents (17). Using the commercial medium, all of the *Candida krusei* isolates showed rough colonies without well-defined borders that tended to extend into the agar. Beside this, the colonies had a pink color which, along with the other characteristics, made it easy to identify them as *Candida krusei*.

Trichosporon spp. colonies showed a heterogeneity of colors that could reflect the possible mixture of species (or genera) included in this group. We evaluated 17 isolates identified as *Trichosporon beigelii* (*Trichosporon cutaneum*) by the API ATB ID32C kit, but the remaining six isolates were identified only as *Trichosporon* spp. These six isolates could include some *Blastoschizomyces capitatum* isolates or isolates from other species previously included in the genus *Trichosporon*, but additional identification tests were not performed. However, some authors (12, 18) have observed that *Trichosporon* colonies were highly variable in other culture media, such as Albicans ID plates, and, even within the same colony, the color could change from one zone (bluish) to another (white).

In some clinical specimens, mainly oral swabs from HIV-infected patients and persons without oral candidosis, mixed cultures can be observed. With the commercial plates supplied it is easier to differentiate between colonies from different yeast species (mainly if they are *Candida albicans*, *Candida tropicalis*, or *Candida krusei*). Although in some cases it is not possible to know which species are contained in the agar plate, a mixed culture can be identified, allowing further identification of the different isolates by standard methods. We have evaluated some vaginal specimens from patients with and without vulvovaginal candidosis, and in some of them a mixed culture was observed on the commercial plates while it was not always observed (or observed with more difficulty) when the same specimens were plated onto Sabouraud dextrose agar plates.

When the commercial medium is compared with the germ-tube test or morphology in corn meal agar to determine the identity of one isolate, the former gives more information and is easier to interpret. The criterion used is macroscopic, based on the color and texture of the colonies, as opposed to a microscopic and more subjective criteria. An additional factor for any laboratory is the cost of the identification of isolates in combination with the time required. If identification of a yeast isolate by the germ-tube test cost one unit, the chlamydospores production test and the Albicans ID cost two units, the Rapidec albicans (bioMérieux) three units, the ATB ID32C galleries 23 units, and the Fongiscreen 4H (Sanofi Pasteur, France) 30 units, the cost for the CHROMagar Candida would be 9 units. The cost of isolation work should be added to this calculation in those cases in which it is necessary to have

a previous isolate. For these reasons we consider the CHROMagar Candida plates an effective, easy-to-use medium for the isolation and identification of yeasts from clinical specimens. Its value increases in those specimens in which different mixed species are suspected, such as oral specimens, tested in the present study, or vaginal specimens (data not shown), where *Candida albicans* is occasionally isolated in combination with other species.

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