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Evaluation of CHROMagar Candida for rapid screening of clinical specimens for *Candida* species

Bewertung von CHROMagar Candida zum Schnellnachweis von *Candida*-Arten aus klinischem Untersuchungsmaterial

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Key words. *Candida*, CHROMagar Candida, isolation, identification.
Schlüsselwörter. *Candida*, CHROMagar Candida, Isolierung, Identifizierung.

Summary. CHROMagar Candida is a new differential culture medium that allows selective isolation of yeasts and simultaneously identifies colonies of *Candida albicans*, *Candida glabrata*, *Candida tropicalis* and *Candida krusei*. We evaluated this medium and compared it with a reference medium, Sabouraud glucose agar, for the presumptive identification of yeast species isolated directly on the medium from 1150 clinical specimens. A total of 731 specimens showed no growth, 299 isolates (70.2%) showed growth to the same extent on both media. Forty mixed cultures were detected on both media. More than one isolate was detected in 30 of the tested specimens on either CHROMagar (26 specimens) or Sabouraud glucose agar (four specimens). We found a sensitivity of 98.8% and a specificity of 100% for *C. albicans*, 66.7% and 99.8% for *C. tropicalis*, 100% and 100% for *C. krusei*, and 98% and 95.7% for *C. glabrata*. Regarding these results, CHROMagar Candida is recommended as a useful isolation medium capable of the presumptive identification of yeasts and better detection of mixed cultures in clinical specimens.

Zusammenfassung. CHROMagar Candida ist ein neues Differentialmedium, das zum Nachweis von Hefen mit gleichzeitiger Identifikation von *Candida albicans, Candida glabrata, Candida tropicalis* und *Candida krusei* dient. Wir evaluierten dieses Medium in Bezug auf Isolation und Identifikation von Hefen aus 1150 klinischem Untersuchungsproben und verglichen es mit dem Referenzmedium Sabouraud-Glucose-Agar. 731 Proben zeigten kein Wachstum, 299 (70,2%) zeigten gleiches Wachstum von Rein- und Mischkulturen auf beiden Medien. 40 Mischkulturen wurden auf beiden Medien beobachtet. In 30 Fällen wurde entweder nur auf CHROMagar (26 Fälle) oder aber auf Sabouraud Glucose Agar (4 Fälle) mehr als ein Isolat nachgewiesen. Sensitivität und Spezifität waren sehr hoch; für *Candida albicans* 98,8 und 100%, für *Candida glabrata* 98 und 95,7%, und für *Candida krusei* jeweils 100%. Bei *Candida tropicalis* zeigte sich eine geringere Sensitivität von nur 66,7%. Die Spezifität mit 99,8% war jedoch ebenfalls sehr hoch. Aufgrund unserer Ergebnisse können wir CHROMagar Candida als nützliches Medium in der Routinediagnostik empfehlen.

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Introduction

The emergence of *Candida* species other than *Candida albicans* as important agents of infection is a concern in several laboratories. The majority of *Candida* infections are caused by *C. albicans, C. glabrata, C. tropicalis and C. krusei.* In addition, clinical samples may contain various yeast species, which may become dominant under selective pressure by an antifungal agent.

The isolation and identification of yeasts in a clinical microbiology laboratory with conventional media is time-consuming and cumbersome as it requires between 4 and 6 days. The use of enzyme-based tests using fluorogenic and chromogenic substrates has been described previously for the

rapid identification of different micro-organisms including yeasts [1–9].

A new differential culture medium so-called 'CHROMagar Candida' allows selective isolation of yeasts and simultaneous identification of *C. albicans, C. glabrata, C. tropicalis and C. krusei.* Odds & Bernaerts [10] found a specificity and sensitivity of this medium for the presumptive identification of *C. albicans, C. tropicalis* and *C. krusei* exceeding 99% for all three species. In addition, Pfaller *et al.* [11] described an overall agreement of 95% in identification on the basis of colony morphology and pigmentation between two observers. Also, San-Millán *et al.* [12] found a sensitivity and specificity of 99% and 100% for *C. albicans*, 93.8% and 99.1% for *C. tropicalis* and 100% for *C. krusei*, whereas Bernal *et al.* [13] described the sensitivity and specificity for these yeasts as being greater than 99%. Freydière *et al.* [14] found a high detection rate combined with 100% specificity.

In medical mycology, Sabouraud glucose agar (SGA) is widely used for the isolation of most fungal pathogens. However, precise identification of yeasts by colony appearance on this medium is impossible, and it is difficult to detect mixed cultures. Ideally, laboratories should be able to simultaneously detect and identify yeasts from mixed cultures. Thus, CHROMagar might be a better alternative than SGA. Therefore, the purpose of the present study was to evaluate the performance of this medium for rapid and non-microscopic presumptive identification of yeast species directly from clinical specimens.

Materials and methods

Yeast cultures

A total of 1150 clinical specimens were investigated in order to determine the effect of CHROMagar on the growth of *Candida* species and to assess the identification in comparison with the routinely used SGA. All specimens were inoculated in parallel onto CHROMagar (Becton Dickinson Europe, France) and SGA (Oxoid, UK). After inoculation, the cultures were incubated in air at 37 °C and were inspected after 48 h. In addition, Sabouraud broth (Biotest, Germany) was inoculated and treated the same way. All yeast isolates observed on CHROMagar were judged by colony morphology and pigmentation according to the manufacturers' instructions as described by Odds & Bernaerts [8]. Colonies on SGA were identified by using the commercial ATB ID32C (API, bioMérieux, France) and by their micromorphology on Rice extract agar (Becton Dickinson Europe). Colonies of uncertain colour on CHROMagar were identified in the same way.

CHROMagar was provided by the manufacturer as ready-to-use agar plates, which were stored at 4 °C and used within 2 weeks.

Statistical analysis

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

Results

Of a total of 1150 clinical specimens, 731 showed no growth on both media, 419 showed either growth on both media or on SGA or CHROMagar only. Growth to the same extent on both media could be observed for 299 samples (70.2%). A total of 260 of these showed growth of one species, 38 growth of two species and two growth of three species, meaning that 40 mixed cultures were detected on both media. More than one isolate was detected in 29 of the tested specimens on either CHROMagar (27 specimens) or SGA (two specimens). In addition, two of the mixed cultures on SGA showed additional growth of *Aspergillus* species, which probably was due to contamination. A total of 90 showed no growth on either SGA (50 specimens) or CHROMagar (40 specimens).

Distribution of the colony colours within each yeast species is shown in Table 1. Presumptive identification of the previously prescribed yeast species was easy; the colour differentiation after 48 h being very clear. Additional characteristics such as rough colonies for *C. krusei* and a halo around the bluish colonies of *C. tropicalis* were also observed.

Sensitivity, specificity, positive predictive value, negative predictive value and efficiency of CHROMagar are shown in Table 2.

Table 3 shows that the majority of the tested fungi easily grows on CHROMagar. This medium supported the growth of yeasts and moulds. A total of 374 isolates were detected on both media, 74 isolates were detected on CHROMagar only and 50 isolates were detected on SGA only.

Table 4 shows that CHROMagar and SGA detected mixtures of yeast species in 40 specimens to the same extent. The total number of mixed samples detected on CHROMagar only was 27, the corresponding number on SGA was much lower, namely two samples.

Table 1. Distribution of the colony colours within each yeast species

Species	Green	Blue	Violet	Pink	White
C. albicans	318	—	—	—	4
C. tropicalis	—	4	1	0	1
C. glabrata	—	—	1	48	—
C. krusei	—	—	—	16	—
C. parapsilosis	—	—	—	3	15
C. guilliermondii	—	—	—	1	—
S. cerevisiae	—	1	2	5	2
C. lusitaniae	—	—	—	2	2
C. sake	—	—	—	—	1
C. humicola	—	—	—	—	1
C. species	—	—	—	4	5
Trichosporon species	—	—	—	—	3
Blastoschizomyces capitatus	—	—	—	1	—
Pichia etchelsii	—	—	—	—	1

Table 2. Usefulness of CHROMagar Candida for primary identification after 48 h of incubation at 37 °C

Species	*C. albicans*	*C. tropicalis*	*C. krusei*	*C. glabrata*
True-positive results	318	4	16	48
True-negative results	120	435	406	357
False-positive results	0	1	0	16
False-negative results	4	2	0	1
Per cent sensitivity	98,8	66,7	100	98
Per cent specificity	100	99,8	100	95,7
Positive predictive value	100	80	100	75
Negative predictive value	96,8	99,5	100	99,7
Per cent efficiency	99,1	99,3	100	91,6

Table 3. Detection of yeast and mould isolates in cultures

Species (number of isolates)	Number of isolates detected on		
	Both media	CHROMagar only	SGA only
C. albicans (350)	275	39	36
C. glabrata (49)	34	15	0
C. tropicalis (6)	4	2	0
C. krusei (16)	15	0	1
C. parapsilosis (18)	9	9	0
S. cerevisiae (9)	5	3	1
C. lusitaniae (4)	2	1	1
C. humicola (1)	1	0	0
C. guilliermondii (1)	1	0	0
C. sake (1)	1	0	0
Candida species (12)	9	2	1
Trichosporon species (3)	2	1	0
Pichia etchelsii (1)	1	0	0
Blastoschizomyces capitatus	1	0	0
Geotrichum species (1)	0	1	0
A. fumigatus (12)	5	0	7
A. flavus (8)	5	0	3
A. niger (5)	5	0	0

Discussion

Our study confirms the results of previous investigations regarding the high accuracy of CHROMagar. We found a presumptive identification of *C. albicans* of 98.8% and of *C. krusei* of 100% but only 66.7% for *C. tropicalis*. *Candida tropicalis* exhibits a bluish colour with a halo around

Table 4. Detection of multiple yeast species using CHROMagar and SGA

Species (number of mixed cultures)	Number of mixed cultures detected on		
	Both media	CHROMagar only	SGA only
C. albicans/*C. glabrata* (24)	10	14	0
C. albicans/*C. parapsilosis* (8)	4	4	0
C. albicans/*C. krusei* (5)	5	0	0
C. albicans/*S. cerevisiae* (3)	2	1	0
C. albicans/*C. sake* (1)	1	0	0
C. albicans/*C.* species (8)	6	2	0
C. albicans/*C. tropicalis* (4)	3	1	0
C. albicans/*Geotrichum* species (2)	0	0	2
C. krusei/*C. glabrata* (1)	1	0	0
C. glabrata/*Trichosporon* species (3)	2	1	0
C. glabrata/*S. cerevisiae* (1)	0	1	0
C. krusei/*C. lusitaniae* (1)	1	0	0
C. krusei/*S. cerevisiae* (1)	1	0	0
Pichia etchelsii/*C. krusei* (1)	1	0	0
C. glabrata/*Blastoschizomyces capitatus* (1)	0	1	0
Geotrichum species/*S. cerevisiae* (1)	1	0	0
C. albicans/*C. glabrata*/*C. tropicalis* (1)	0	1	0
C. albicans/*C. glabrata*/*C. krusei* (2)	2	0	0
C. albicans/*C. tropicalis*/*C. lusitaniae* (1)	0	1	0

the colony, but only four strains out of six showed this characteristic appearance. One violet strain showed a similar appearance and including this would result in a higher sensitivity, i.e. 88.3%. In addition, among *Saccharomyces cerevisiae* strains, one exhibited a bluish and two others a violet colour. Although they did not have the characteristic halo, at least one strain could have been mistaken for *C. tropicalis*. This is the reason why this strain has been considered as a false-positive result. However, San-Millán *et al.* [12] stated that, in general, the obverse of the colonies is bluer than the reverse, which is more purple. Considering these aspects and the fact that a halo should be around the colony, the presumptive identification of *C. tropicalis* should be easier, resulting in an increase in the sensitivity of this medium for this species.

In agreement with Pfaller *et al.* [11], the medium also allowed identification of *C. glabrata* (sensitivity 98%, specificity 92.1%). It has to be emphasized that other *Candida* species such as *C. parapsilosis* (three strains), *C. lusitaniae* (two strains) and four strains of unidentified *Candida* species, which were certainly not *C. glabrata,* also grew as pink smooth colonies on CHROMagar, although the coloration did not seem to be exactly the same as for *C. glabrata*. The same applied for five out of nine strains of *S. cerevisiae*. As it is impossible to judge the right pink coloration without experience, it would seem wise to confirm identification of *C. glabrata* by an additional test, unless the investigator is familiar with the use of CHROMagar.

However, mixed cultures could be detected easily on CHROMagar. Sixty-seven of them could be detected on CHROMagar and 42 on SGA only. The prompt detection of associated yeast species and presumptive identification of the isolated yeasts may be an aid for rapid appropriate treatment decisions in the light of differences in susceptibility of the yeast species to antifungal agents [15]. Some *Candida* species, such as *C. lusitaniae*, are often resistant to amphotericin B; *C. glabrata* is less sensitive than other species to fluconazole and ketoconazole, and *C. krusei* exhibits innate resistance to fluconazole. In particular, the combination of *C. albicans* and *C. glabrata* was detected more often on CHROMagar than on SGA. This was the case in 14 samples in this study. Overlooking this combination would mean overlooking more resistant species. Therapeutic failures could be the consequence. Therefore, CHROMagar not only reduces the time taken to identify certain *Candida* species, but it is also more successful in the detection of mixed cultures. In contrast to CHROMagar, mixed cultures on SGA could be detected with more difficulty or not all. These results are confirmed by Baumgartner *et al.* [16], who compared two chromogenic media, and Bouchara *et al.* [17], who evaluated the routine use of CHROMagar.

In our study, a total of 90 strains showed no growth on either SGA (50 specimens) or CHROMagar (40 specimens). These samples were always characterized by a small number of colony-forming units, this being the reason for the discrepancy in the high number of negative results either on CHROMagar or on SGA. Therefore, both media still seem to isolate fungi with the same efficiency.

Filamentous fungi such as *Aspergillus fumigatus, A. flavus, A. niger* and *Geotrichum* species could easily be detected on CHROMagar. Some of these moulds could only be detected on one medium. This was due to contamination, as could be proved with Sabouraud broth, which was always negative in any doubtful case.

In conclusion, CHROMagar shows sufficient sensitivity to grow the most important yeasts. It can serve as a primary isolation and differentiation medium for clinical species likely to contain yeasts and also as an adjunctive differential medium for the identification of yeasts isolated on other media. A major advantage of CHROMagar is the ability to detect mixed cultures of yeasts in clinical specimens, as has been described by other researchers. Thus, CHROMagar not only facilitates the detection of mixed cultures but also allows a presumptive identification to the species level of isolates within the mixture without any need for additional subcultures. It has to be mentioned that the colony characteristics such as rough colonies in *C. krusei* isolates or the halo around the colonies of *C. tropicalis* were important for the correct identification of isolates. Attention has to be paid when *C. glabrata* is isolated as other *Candida* species may also develop a pinkish colour, which is difficult to judge, especially for the inexperienced.

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