Tropical Biomedicine 28(2): 269–274 (2011)

Identification of local clinical *Candida* isolates using CHROMagar Candida™ as a primary identification method for various *Candida* species

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Received 17 August 2010; received in revised form 16 December 2010; accepted 18 January 2011

Abstract. The objective of our study was to study the effectiveness of CHROMagar Candida™ as the primary identification method for various clinical *Candida* isolates, other than the three suggested species by the manufacturer. We studied 34 clinical isolates which were isolated from patients in a local teaching hospital and 7 ATCC strains. These strains were first cultured in Sabouraud dextrose broth (SDB) for 36 hours at 35ºC, then on CHROMagar plates at 30ºC, 35ºC and 37ºC. The sensitivity of this agar to identify *Candida albicans*, *Candida dubliniensis*, *Candida tropicalis*, *Candida glabrata*, *Candida rugosa*, *Candida krusei* and *Candida parapsilosis* ranged between 25 and 100% at 30ºC, 14% and 100% at 35ºC, 56% and 100% at 37ºC. The specificity of this agar was 100% at 30ºC, between 97% and 100% at 35ºC, 92% and 100% at 37ºC. The efficiency of this agar ranged between 88 and 100% at 30ºC, 83% and 100% at 35ºC, 88% and 100% at 37ºC. Each species also gave rise to a variety of colony colours ranging from pink to green to blue of different colony characteristics. Therefore, the chromogenic agar was found to be useful in our study for identifying clinical *Candida* isolates.

INTRODUCTION

Over the last decade, the common etiology of many fungal infections especially among immunocompromised patients was known to be *Candida* species. Diagnosis was simply based on whether it was *Candida albicans* or non-*albicans Candida* species which indicated the appropriate treatment for these fungal infections. On the other hand, it was reported that apart from *C. albicans*, other species that can cause infections in humans are *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida guilliermondii*, *Candida glabrata*, *Candida kefyr*, *Candida lusitaniae*, *Candida rugosa*, *Candida viswanathii* and *Candida stellatoidea* (Basetti *et al.*, 2007; Arora, 2004). They are present as normal microbiota in the human body, i.e. on skin, mouth, large intestines, urinary and reproductive systems (Tortora *et al.*, 2007). They can cause diseases in humans when the physiological balance of the body is upset or the host's defence is in a compromised state. With the overuse of antibacterial agents, immunosuppressive agents, cytotoxins, irradiation and steroids, this new category of systemic mycoses has emerged. These patients have been deprived of immune resistance by the body's normal flora, thus develop opportunistic mycoses, for example candidiasis, aspergillosis, mucormycosis and others (Block & Beale, 2004). The conventional yeast identification method in a clinical laboratory is based on Sabouraud dextrose medium, which requires about 24 to 72 hours for the cultures to grow depending on the species. The identification of *C. albicans* is usually based on the formation of germ tube in human or animal serum at 37ºC for 3 hours (Schoofs *et al.*, 1997). Following

plate cultures, Gram staining and carbohydrate assimilation tests were usually done to verify the species further which can be time consuming as they require at least 3 working days to obtain the results.

A new presumptive identification medium for *C. albicans*, *C. tropicalis* and *C. krusei* known as CHROMagar Candida™ was recently developed. This medium contains chromopeptone, glucose, chromogen mix, chloramphenicol and agar. There were many reports that evaluated the use of this medium for these *Candida* species (Beighton *et al*., 1995; Bouchara *et al*., 1996; Willinger & Manafi, 1998). We studied the effectiveness of CHROMagar Candida™ to identify various clinical *Candida* isolates obtained from a hospital in Kuala Lumpur, apart from the three species used for CHROMagar identification. Colony colours at various incubation temperatures and time periods were observed. We also investigated the variation of colours formed by several different *Candida* species than the three species recommended by the manufacturer. This primary identification of clinical *Candida* isolates could give an indication on the most appropriate antifungal drug for the treatment of candidiasis as some species are inherently resistant to some antifungal drugs. Colony characteristics of the strains on potato dextrose agar (PDA) were also observed and compared with the colonies formed on CHROMagar Candida.

MATERIALS AND METHOD

Candida isolates

The *Candida* isolates were obtained from patients with *Candida* infections from a local public hospital and were cultured on Sabouraud's dextrose agar (Difco, USA). A total of 34 clinical isolates were studied and 7 American Type Culture Collection (ATCC) strains were used in this study. All these strains were first subcultured in Sabouraud's dextrose agar (Difco, USA) at 35ºC for 36 hours to check the viability of the fungi.

Culture conditions

The CHROMagar Candida™ was prepared according to the instructions given by the manufacturer (Becton Dickinson, USA). A loopful of an isolate was streaked on a CHROMagar plate and incubated at 35ºC. The growth was checked every 24 hours until 72 hours of incubation and the colour of the colonies were noted by a single observer. The colony characteristics were also studied for each isolate.

Incubation temperatures

As a comparative study, the steps were repeated for incubation at different temperatures. One set of the strains was incubated at 30ºC and another set was incubated at 37ºC (following body temperature). All cultures were observed for their colony colours after 24 hours, 48 hours and 72 hours of incubation.

Potato dextrose agar medium

Potato dextrose agar (PDA) media were prepared according to the instruction by the manufacturer (Difco, USA) and poured into 9 cm Petri dishes. Clinical *Candida* isolates and reference strains were streaked on the agar individually and the plates were incubated at 35ºC. Observation of the colony colour and characteristics was done 48 hours after incubation.

Biochemical identification

Biochemical identification of all the strains was performed with RapID™ Yeast Plus System biochemical test kits (Remel, USA). It was used as a gold standard for the identification of *Candida* species.

Analysis

To study the effectiveness of the CHROMagar Candida™ in species identification, the results of the CHROMagar were compared with the biochemical tests. The following analyses were used to obtain the sensitivity, specificity and efficiency of the CHROMagar Candida™.

Sensitivity = True positives/[True positives + False negatives] x 100%

Specificity = True negatives/[True negatives + False positives] x 100%

Efficiency = [True positives + True negatives]/ Total x 100%

RESULTS

The results are summarized in Table 1 and 2. Table 1 shows true positive results of the colony colours on CHROMagar Candida™ and Table 2 shows the characteristics of the colonies for each species. All the reference strains used from ATCC were identified with molecular identification at NCBI for confirmation. The colony characteristics and colour on CHROMagar were compared to these reference strains. The colony characteristics of strains belonging to the same species appeared to be the same from our observation. Colonies of *C. albicans* seemed to have an apple green colour, *Candida dubliniensis* had dark green appearance and *C. rugosa* were green. Although *C. krusei* and *C. glabrata* appeared to have the same shade (lavender or pink), but the colonies of *C. krusei* had velvety texture whereas colonies of *C. glabrata* seemed to have glossy texture. However, one *C. albicans* strain gave rise to greyish pink and one with cream coloured colonies, compared to the ATCC strain and other *C. albicans* strains which were apple green. Colonies of *C. parapsilosis* exhibited a variety of colours, from cream to pink to metallic blue to dark green. Isolates of *C. rugosa* appeared light green on CHROMagar at 30°C, dark green at 35°C and 37°C, but one isolate appeared as metallic blue at all three temperatures. The identity of all the isolates was confirmed with biochemical tests. We found that this metallic blue colony was *Candida tropicalis*.

Table 1. Effectiveness of CHROMagar Candida™ as a primary identification medium after 48 hours of incubation at 30ºC, 35ºC and 37ºC, as compared with biochemical test kits (RapID™ Yeast Plus System, Remel USA)

Species (n)	*C. albicans* (7)			*C. dubliniensis* (4)			*C. tropicalis* (5)			*C. glabrata* (3)			*C. rugosa* (9)			*C. krusei* (6)			*C. parapsilosis* (7)		
Temperature (ºC)	30	35	37	30	35	37	30	35	37	30	35	37	30	35	37	30	35	37	30	35	37
True positive	5	5	5	1	4	4	5	5	5	2	3	3	7	9	5	6	6	6	2	1	5
True negative	34	34	34	37	37	34	36	36	36	38	38	37	32	32	31	35	35	33	34	33	32
False positive	0	0	0	0	0	3	0	0	0	0	0	1	0	0	1	0	0	2	0	1	2
False negative	2	2	2	3	0	0	0	0	0	1	0	0	2	0	4	0	0	0	5	6	2
Sensitivity (%)	71	71	71	25	100	100	100	100	100	67	100	100	78	100	56	100	100	100	29	14	71
Specificity (%)	100	100	100	100	100	92	100	100	100	100	100	97	100	100	97	100	100	94	100	97	94
Efficiency (%)	95	95	95	93	100	93	100	100	100	98	100	98	95	100	88	100	100	100	88	83	90

Table 2. Colony characteristics of various *Candida* species on CHROMagar Candida™

Candida species	Colour	Size (cm)	Form	Elevation	Margin
C. albicans ATCC 14053	Apple green	1 - 2	Circular	Convex	Entire
C. dubliniensis ATCC MYA178	Dark green	0.5	Punctiform	Raised	Entire
C. rugosa ATCC 10571	Green	3	Circular	Raised	Entire
C. glabrata ATCC 2001	Lavender/ Dark pink	3	Punctiform	Convex	Entire
C. krusei ATCC 6258	Pink velvet	Irregular	Irregular	Raised	Filiform
C. tropicalis ATCC 750	Metallic blue	2	Circular	Umbonate	Entire
C. parapsilosis ATCC 22019	Pink/ Light pink	1 - 2	Punctiform	Convex	Entire

This selective and differential medium was most effective in identifying *C. glabrata* which appeared as glossy lavender, *C. krusei* which appeared as pink velvet and *C. tropicalis* which appeared as metallic blue with a halo surrounding the colonies. However, the temperature and incubation period were also important in determining the identity of the clinical strains. From our observation, incubation at 30ºC for 24 hours showed lighter coloured colonies or just cream colonies as seen with all the species studied except for *C. krusei* which appeared as pink velvet at all temperatures studied. An extension to the incubation period at this temperature still did not give clear results as there was still a mixture of cream coloured colonies or lighter shade of the colony colour as what it is supposed to be. When these clinical isolates were incubated at 35ºC, the 48-hour cultures seemed to give clearer colony colours than the cultures incubated at 24 hours or 72 hours. This was the recommended temperature and period (35ºC, 48 hours) by the manufacturer.

Colony characteristics were also studied to see the patterns of growth. Mixed cultures of clinical *Candida* species showed no variation in colour and appearance on normal agar medium (Odds & Bernaerts, 1994). However, all strains of *C. dubliniensis*, *C. glabrata*, *C. krusei* and *C. tropicalis* showed the same colony characteristics as the ATCC strains respectively. In the case of two *C. albicans* that exhibited cream coloured colonies, the colony characteristics were the same as the other *C. albicans* strains and the ATCC strain. As for *C. parapsilosis* one colony showed the same characteristics as *C. glabrata* (clinical and ATCC strain), whereas colonies of another strain of *C. parapsilosis* showed same characteristics as *C. tropicalis* (clinical and ATCC). Two species of *C. rugosa* showed the same colony characterictics as *C. krusei* and *C. tropicalis*.

DISCUSSION

Sensitivity of CHROMagar was 100% for *C. dubliniensis* (35ºC and 37ºC), *C. tropicalis* (30ºC, 35ºC and 37ºC), *C. glabrata* (35ºC and 37ºC), *C. rugosa* (35ºC), and *C. krusei* (30ºC, 35ºC and 37ºC). It showed the least sensitivity of 14% for *C. parapsilosis* at 35ºC. Specificity of this agar was 100% for *C. albicans* (30ºC, 35ºC and 37ºC), *C. dubliniensis* (30ºC and 35ºC), *C. tropicalis* (30ºC, 35ºC and 37ºC), *C. glabrata* (30ºC and 35ºC), *C. rugosa* (30ºC and 35ºC), *C. krusei* (30ºC and 35ºC) and *C. parapsilosis* (30ºC). It had 92% specificity for *C. dubliniensis* at 37°C. Efficiency of CHROMagar was 100% for *C. dubliniensis* (35ºC), *C. tropicalis* (30ºC, 35ºC and 37ºC), *C. glabrata* (35ºC), *C. krusei* (30ºC, 35ºC and 37ºC) and *C. rugosa* (35ºC). However, this agar showed only 83% sensitivity for *C. parapsilosis* at 35ºC. Overall, it is suggestive to use CHROMagar not only for *C. albicans*, *C. tropicalis* or *C. krusei* as recommended by the manufacturer, but also for *C. dubliniensis*, *C. glabrata and C. rugosa* at 35ºC, as recommended by the manufacturer. Our results also confirmed the results of previous investigations on the effectiveness of using CHROMagar to identify clinical *Candida* species from various sources (Beighton *et al*., 1995; Bouchara *et al*., 1996; Willinger & Manafi, 1998). Oral isolates of yeasts from dental samples were identified using this medium (Beighton *et al*., 1995). They found distinguishable colony colours for *C. albicans* and *C. tropicalis*. They reported that *C. glabrata* colony colours may vary from purple to pale pink after 48 hours of incubation and may be confused with *C. parapsilosis*. However, they concluded that CHROMagar Candida medium was useful for the identification of oral yeast flora and recognise the presence of mixed yeast populations. In a study conducted with 6150 clinical samples, 77.5% was identified as *C. albicans*, 10.6% as *C. glabrata*, 3.8% as *C. tropicalis*, 2.7% as *C. krusei*, 2.7% as *Saccharomyces cerevisiae*, 2.3% as *C. kefyr* and the rest were either *Malassezia furfur* or mixed cultures using CHROMagar Candida (Bouchara *et al*., 1996). Willinger & Manafi (1998) evaluated the CHROMagar Candida medium for the identification of 1150 clinical isolates of yeasts. They found that 67 mixed cultures were detected using this medium, the sensitivity was 98.8% for *C. albicans*, 98%

for *C. glabrata*, 100% for *C. krusei* and 66.7% for *C. tropicalis*.

When we compared these results with the *Candida* cultures on potato dextrose agar (PDA), we found no difference in the colony colours for all the seven species studied as they all appeared cream. The other characteristics such as size, margin and elevation of the colonies varied, depending on the species. These colony characteristics were the same as that observed using CHROMagar Candida (Table 2). Generally all *Candida* colonies on agar start appearing as cream coloured colonies and gradually become darker. This was also observed by Schoofs *et al.* (1997) among *C. albicans* and *C. dubliniensis* isolated from HIV infected patients. Although it was reported that *C. albicans* can be mistaken for *C. dubliniensis*, but in our study the green colours for *C. albicans*, *C. rugosa* and *C. parapsilosis* were distinctive, which was also found by Kirkpatrick *et al.* (1998) and Sullivan *et al.* (1999). There was also distinctive identification for *C. tropicalis* and *C. krusei* especially in their colour and texture. Although *C. glabrata* and *C. parapsilosis* could be misidentified, we found that *C. glabrata* was glossy in texture and had a darker pink shade, i.e. lavender than *C. parapsilosis*. This observation was also reported by Odds & Davidson (2000). From our observation, we found that it is not necessary to have experienced laboratory personnel to identify clinical *Candida* species using CHROMagar if one has correct details of colony colours and characteristics for each *Candida* species. Thus, we would highly recommend the use of CHROMagar medium as a routine laboratory procedure to identify clinical *Candida* species directly from the patient. It can be used as rapid confirmation of species as an aid for effective treatment for patients with candidiasis.

CHROMagar Candida™ was found effective in our study as a primary identification method for various clinical *Candida* species. Since many other methods are time consuming, using CHROMagar was not only fast and easy but it was also cost effective comparatively. The significant use of this medium is to obtain the results of the culture fast enough to provide the most appropriate treatment for patients with candidiasis, as there are a few species which are resistant to certain antifungal drugs. This would avoid the usage of broad-spectrum antifungal drugs which could lead to acquired resistance among these *Candida* species.

Acknowledgments. The authors would like to thank University Putra Malaysia for funding this research under the RUGS Research Grant.

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