Comparative evaluation of six chromogenic media for presumptive yeast identification

Alessandra Vecchione,1 Walter Florio,1 Francesco Celandroni,1 Simona Barnini,2 Antonella Lupetti,1 Emilia Ghelardi1

ABSTRACT

Aims The present study was undertaken to evaluate the discrimination ability of six chromogenic media in presumptive yeast identification.

Methods We analysed 108 clinical isolates and reference strains belonging to eight different species: Candida albicans, Candida dubliniensis, Candida tropicalis, Candida krusei, Candida glabrata, Candida parapsilosis, Candida lusitaniae and Trichosporon mucoides.

Results C. albicans, C. tropicalis and C. krusei could be distinguished from one another in all the tested chromogenic media, as predicted by the manufacturers. In addition, C. albicans could be distinguished from C. dubliniensis on BBLCHROMagar Candida, Kima CHROMagar Candida and Brilliance Candida, and C. parapsilosis could be identified on CHROMATIC Candida agar, CHROMGENIC Candida agar, and Brilliance Candida agar.

Conclusions Brilliance Candida provided the widest discrimination ability, being able to discriminate five out of the seven Candida species tested. Interestingly, C. tropicalis and C. krusei could be already distinguished from each other after 24 hours of incubation.

The incidence of invasive fungal infections has increased over the last decades. This is mainly due to the increasing number of immunocompromised hosts, such as patients with lymphoproliferative disorders, chemotherapy-induced neutropaenia and transplant recipients undergoing immunosuppressive therapy.

Candida species have been reported as the fourth most frequent cause of nosocomial bloodstream infection with an overall mortality rate of 39%, being Candida albicans the prevalent. The prolonged administration of azoles has facilitated the emergence of drug-resistant C. albicans strains and, with higher frequency, of other Candida species, such as Candida glabrata and Candida krusei, especially in patients with HIV and patients with cancer.

Yeast identification is clinically relevant since different Candida species may differ in virulence and drug resistance. Conventional methods for yeast identification are based on cultural isolation on Sabouraud dextrose agar (SDA) followed by biochemical assays and/or molecular methods, such as matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry, showing high sensitivity and specificity with monomicrobial cultures. Differently, polymicrobial samples grown on SDA rarely allow macroscopic discrimination of yeast colonies belonging to different species. For all these reasons, rapid presumptive identification of the aetiological agent at species level is of utmost importance in clinical microbiology laboratories.

As an alternative to conventional methods, several chromogenic media have been developed for the isolation and identification of C. albicans and presumptive identification of major pathogenic yeast species in a single step. By reacting with enzymes secreted by yeast cells, that is, hexosaminidase (C. albicans, Candida dubliniensis and Candida tropicalis) and alkaline phosphatase (C. krusei), the chromogenic substrates contained in these media result in colonies with species-specific pigmentation and characteristics. Therefore, the use of such media is particularly suitable to discriminate different yeast species in mixed yeast cultures, as demonstrated by previous studies from our and other research groups. Moreover, chromogenic media represent a valid possibility for rapid identification of the most frequently isolated Candida species for clinical laboratories that do not possess a MALDI-TOF mass spectrometer. Similarly to SDA, chromogenic media contain one or two antibiotics (chloramphenicol and/or gentamicin) to inhibit bacterial growth.

The present study aimed at evaluating the ability of six commercially available chromogenic media to presumptively identify pathogenic yeasts, analysing a broader spectrum of species in comparison with those described by the manufacturers. The performance of these chromogenic media was evaluated on the bases of colony colour and characteristics of the following fungal species: the species selected by the manufacturers (C. albicans, C. krusei, C. tropicalis and, in some media, C. glabrata) and, in addition, C. dubliniensis, Candida parapsilosis, Candida lusitaniae, and the anamorphic fungus Trichosporon mucoides.

A total of 108 yeast isolates were recovered from the yeast collection of the Unità Operativa di Microbiologia Universitaria, Pisa University Hospital (Italy). These strains were identified by ID-YST card system (Vitek System, bioMérieux, Marcy l’Etoile, France) and by MALDI-TOF mass spectrometry (Bruker Daltonics, Bremen, Germany). Forty of these strains were isolated from respiratory tract specimens, 20 from vaginal swabs, 13 from nail swabs, 13 from urine, 10 from wound swabs and 10 from blood cultures. Reference strains included C. albicans ATCC 10231, C. dubliniensis CBS 8500, C. glabrata DSMZ 11226, C. krusei ATCC 6258,
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C. **tropicalis** DSMZ 11953, C. **parapsilosis** ATCC 22019, C. **lusitaniae** DSMZ 70102 and T. **mucoides** ATCC 204094. **Escherichia coli** ATCC 27325 and **Staphylococcus aureus** were used as negative controls.

Chromogenic media used in the present study were the following: CandisSelect 4 (Bio-Rad, Hercules, California, USA), BBL CHROMagar Candida (Becton Dickinson, Franklin Lakes, New Jersey, USA), CHROMATIC Candida (Liofilchem, Roseto degli Abruzzi, Teramo, Italy), CHROMOGENIC Candida agar (Bioline Italiana S.r.l. Mascia Brunelli spa, Milano, Italy), Brilliance Candida agar (Oxoid, Thermo Scientific, Basingstoke, UK) and CHROMagar Candida (Vacutest Kima S.r.l., Arzegrande, Pordenone, Italy). All chromogenic media are conceived to distinctly identify C. **albicans**, C. **krusei** and C. **tropicalis**; CandisSelect 4 also includes C. **glabrata**.

Yeast and bacterial strains were stored at −80°C in Sabouraud broth (BD) and Brain Heart Infusion Broth (BD), respectively, both containing 30% glycerol (vol/vol). Before being tested on chromogenic agar plates, each yeast strain was subcultured on SDA and bacteria on blood agar plates (Columbia Agar with 5% sheep blood, BD). A 0.5 McFarland suspension of yeast or bacteria (−10⁶ CFU yeast/mL, 10⁶ CFU bacteria/mL) was prepared in distilled water. An aliquot (10 μL) of yeast or bacterial suspension, the latter further diluted 1:100, was streaked on chromogenic media to form isolated colonies. Then, plates were incubated at the temperatures indicated by the manufacturers’ instructions, that is, 35°C for all chromogenic media except Brilliance Candida agar (30°C). Plates were examined after 24, 48 and 72 hours in order to establish the optimum growth conditions. All plates were visualised by three independent investigators, regarding colony colour, size, texture, morphology and the presence of colour diffusion into the surrounding area. Colours were described according to the Pantone Colour Formula Guide (https://www.pantone.com/formula-guide).

The study was notified to the local ethical committee, Comitato Etico di Area Vasta Nord-Ovest, University of Pisa, and conducted in full accordance with the principles of the Declaration of Helsinki. Samples were taken as part of the standard patient care and used anonymously. For this type of study, no written informed consent was necessary.

All yeasts, both reference strains and clinical isolates, grew on the six chromogenic media, whereas bacterial strains were completely inhibited, as expected. The definitive colony appearance was obtained after 48 hours incubation for all species but C. **tropicalis** and C. **krusei**, which could be already distinguished at 24 hours incubation in all chromogenic media. No difference in colony appearance was observed when incubation time was extended to 72 hours. Therefore, analysis of colonies was carried out after 24-hour and 48-hour incubation.

A wide variety of morphologies and colony colours were observed, some of which were species specific. Colour, texture and morphology of the colonies observed for each strain on the six media are reported in **table 1**. Some chromogenic media characterised the different strains by colour only, while others characterised them by colour, texture and morphology. Three independent investigators concordantly reported the same colony colour and characteristics for all 108 strains in the six different chromogenic media, showing that colony colours are

| Table 1 Description of colour, texture and morphology of the colonies observed for each strain on the six media* |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Species**     | **N. of isolates** | **CandisSelect 4 (Bio-Rad)** | **BBL CHROMagar Candida (BD)** | **CHROMATIC Candida (Liofilchem)** | **CHROMOGENIC Candida agar (Bioline)** | **CHROMagar Candida (Kima)** | **Brilliance Candida agar (Oxoid)** |
| **Candida albicans** | 25              | Smooth, circular blue grey to pink (2645, 2718) | Smooth, circular pale green (332, 565, 570) | Smooth, circular pale green (3265, 3268) | Smooth, circular pall green (3265, 3268) | Smooth, circular pale green (332, 565, 570) | Smooth, circular dark green (3302, 343, 3435) |
| **Candida dubliniensis** | 12              | Smooth, circular blue grey to pink (2645, 2718) | Smooth, circular dark green (3302, 3308) | Smooth, circular pale green (332, 565, 570) | Smooth, circular bottle green (3265, 3268) | Smooth, circular dark green (3302, 3308) | Smooth, circular dark green (3282, 329) |
| **Candida krusei** | 20              | Flat, rough, irregular green-turquoise, grey periphery (3272, 3275) | Flat, rough, pink with grey periphery (437, 5015, 502) | Flat, rough, irregular red purple with grey periphery (510–513) | Flat, rough, pink with grey periphery (437, 5015, 502) | Flat, rough, pink to brown (482, 4725) | Smooth, circular pink to brown (481, 4725) |
| **Candida tropicalis** | 15              | Smooth, circular pale green-turquoise (3252, 3272) | Smooth, circular dark purple-blue (2735, 274) | Smooth, circular dark green-turquoise (3302, 3308) | Smooth, circular dark blue (2735, 2735, 274) | Smooth, circular dark blue (302, 308) | Smooth, circular pale brown (1205, 1485) |
| **Candida parapsilosis** | 15              | Smooth, circular pale green (565–571) | Smooth, circular pink with grey periphery (3005, 3001) | Smooth, circular pink (4655, 479) | Smooth, circular dark pink with grey periphery (519, 520) | Smooth, circular pink to violet (256, 501) | Smooth, circular beige to light brown (465, 7403) |
| **Candida glabrata** | 12              | Smooth, circular pale green (573) | Smooth, circular pink (256) | Smooth, circular white (warm grey) | Smooth, circular pink to violet (256, 501) | Smooth, circular dark pink with grey periphery (501, 5015) | Smooth, circular beige to light brown (7403) |
| **Candida lusitaniae** | 5              | Smooth, circular pale green (573) | Smooth, circular pink (256) | Smooth, circular white (warm grey) | Smooth, circular pink to violet (256, 501) | Smooth, circular dark pink with grey periphery (501, 5015) | Smooth, circular beige to light brown (7403) |
| **Trichosporon mucoides** | 4              | Rough, small pale purple with dark green halo (2645, 2718) | Rough, small green with green halo (327, 5483) | Rough, small pale green with green halo (333, 334) | Rough, small pale green with green halo (3295, 5305) | Rough, small dark green with dark green halo (333, 334) | Rough, small dark green with dark green halo (312) |

*The codes of Pantone Colour Formula Guide are reported in parentheses.*
Table 2  Number of isolates presumptively identified per species and chromogenic medium after 24 and 48 hours of incubation*  

<table>
<thead>
<tr>
<th>Species</th>
<th>N. of isolates</th>
<th>CandiSelect 4 (Bio-Rad)</th>
<th>BBL CHROMagar Candida (BD)</th>
<th>CHROMATIC Candida (Liofilchem)</th>
<th>CHROMOGENIC Candida agar (Biolife)</th>
<th>CHROMagar Candida (Kima)</th>
<th>Brilliance Candida agar (Oxoid)</th>
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<td>C. glabrata/C. lusitaniae 0/0</td>
</tr>
</tbody>
</table>

*The number of strains presumptively identified after 24 and 48 hours are reported before and after the slash, respectively.  
C. albicans, Candida albicans; C. dubliniensis, Candida dubliniensis; C. glabrata, Candida glabrata; C. krusei, Candida krusei; C. lusitaniae, Candida lusitaniae;  
C. parapsilosis, Candida parapsilosis; C. tropicalis, Candida tropicalis; T. mucoides, Trichosporon mucoides.

easy to distinguish and that this method is highly reproducible. Identification results obtained with the different chromogenic media are reported in table 2.

All C. albicans, C. tropicalis and C. krusei strains appeared on each media as predicted by the manufacturers. As previously mentioned, C. tropicalis and C. krusei could be already distinguished from each other in all the tested chromogenic media after 24-hour incubation (figure 1). This finding is of clinical relevance since anticipating species identification of 24 hours can give timely and useful indications to appropriately streamline empirical antimicrobial therapy. The two species could be distinguished by colour only on Brilliance Candida agar, mainly by texture and morphology on CandiSelect 4, and by colour, texture and morphology on the other chromogenic media. The appearance of colonies of the tested yeast species on the six chromogenic media at 48 hours is shown in figure 2.

After 48 hours of incubation, C. albicans could also be distinguished from C. dubliniensis on the following media: BBL CHROMagar Candida, Kima CHROMagar Candida and Brilliance Candida. C. albicans appeared as pale green colonies and C. dubliniensis as dark green colonies on BBL CHROMagar Candida and Kima CHROMagar Candida, whereas C. albicans appeared as dark green colonies and C. dubliniensis as turquoise colonies on Brilliance Candida. C. dubliniensis and C. albicans

Figure 1  Discrimination at 24 hours of Candida tropicalis (A) and Candida krusei (B) yeast colonies on: (1) BBL CHROMagar Candida (BD); (2) CHROMOGENIC Candida agar (Biolife); (3) CandiSelect 4 (Bio-Rad); (4) CHROMATIC Candida (Liofilchem); (5) CHROMagar Candida (Vacutest Kima); and (6) Brilliance Candida agar (Oxoid).


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Figure 2  Appearance of yeast colonies at 48 hours on: (A) BBL CHROMagar Candida (BD); (B) CHROMOCOLIC Candida agar (Biolife); (C) CandiSelect 4 (Bio-Rad); (D) CHROMagar Candida (Vacutest Kima); (E) CHROMATIC Candida (Liofilchem); and (F) Brilliance Candida agar (Oxoid).

Yeast species were streaked in the following order: (1) Candida albicans; (2) Candida dubliniensis; (3) Candida tropicalis; (4) Trichosporon mucoides; (5) Candida glabrata; (6) Candida krusei; (7) Candida parapsilosis; and (8) Candida lusitaniae.

are very closely related species and share many phenotypic traits, including the ability to produce hyphae and chlamydospores. Epidemiological studies have shown that C. albicans is far more prevalent than C. dubliniensis, the latter representing only 2%–3% of the confirmed cases of candidaemia.

C. parapsilosis and C. glabrata were not visually detectable in any tested media after 24 hours incubation, whereas at 48 hours the two species could be distinguished from each other on three chromogenic media: on CHROMATIC Candida and CHROMOCOLIC Candida agar, where C. parapsilosis produced pale pink and brilliant pink colonies, respectively, and on Brilliance Candida agar, where it appeared as pale brown colonies. C. glabrata, instead, appeared as dark pink colonies with grey periphery on CHROMOCOLIC Candida agar, and beige to light pink on Brilliance Candida agar. C. glabrata appeared as white colonies on CHROMATIC Candida. C. parapsilosis also appeared as white colonies on CHROMOGENIC Candida (Kima), thus being indistinguishable from the other yeasts. C. parapsilosis and C. glabrata were difficult to be distinguished on BBL CHROMagar Candida and CandiSelect 4, both species appearing as pink in the former and green in the latter medium. Although similar in appearance, the antimicrobial susceptibility pattern of C. parapsilosis and C. glabrata, which will be available 24 hours thereafter, can be quite different, as C. glabrata is more frequently resistant to azoles and C. parapsilosis to echinocandins. Therefore, the ability to discriminate macroscopically between these two species can be very useful to provide presumptive species identification, although identification should be confirmed by a validated method. C. parapsilosis is the second/third most common cause of bloodstream infection, especially in preterm newborns.

None of the media allowed discriminating between C. glabrata and C. lusitaniae at any time of incubation. Finally, an emerging opportunistic yeast, T. mucoides, which forms typical colonies also on SDA, could be distinguished after 48 hours of incubation from Candida spp. on all the chromogenic media, where it produced typical small, green, rough colonies. Trichosporon species are the second most common yeast causing fungaemia in patients with haematological malignancies. As invasive trichosporonosis is characterised by resistance to amphotericin and echinocandins, and poor prognosis, its rapid detection in patients not responding to standard antimicrobial therapy might warrant targeted antifungal therapy.

In summary, Brilliance Candida agar allowed to distinguish five species (C. albicans, C. dubliniensis, C. krusei, C. tropicalis and C. parapsilosis), Kima CHROMagar Candida and BBL CHROMagar Candida could discriminate four species (C. albicans, C. dubliniensis, C. tropicalis and C. krusei), on CHROMOGENIC Candida (Liofilchem) and CHROMOCOLIC Candida agar (Biolife) three species could be presumptively identified (C. krusei, C. tropicalis and C. parapsilosis) and with CandiSelect 4 only two species could be discriminated (C. tropicalis and C. krusei). In addition, all the chromogenic media gave some indication of the presence of Trichosporon.

It should be considered that a limit of this and other similar studies is that identification results may vary depending on the species selected for analysis, for example, if C. dubliniensis had not been tested, all chromogenic media would have been able to discriminate C. albicans from the other Candida species. Therefore, it is important to consider the frequency of isolation of different species to evaluate identification error rates that can be expected. This is a well known limit of chromogenic media for presumptive yeast identification, as previously highlighted and discussed by other authors.

In conclusion, the results of the present study show that chromogenic media may allow presumptive identification of
additional yeast species besides those described in the manufacturers’ instructions and provide useful information for future studies aimed at evaluating the ability of chromogenic media to presumptively identify different Candida species from clinical samples.

Take home messages

- Chromogenic media may allow presumptive identification of additional yeast species besides those indicated by the manufacturers’ instructions
- Chromogenic media represent a useful tool to detect mixed fungal infections
- Candida tropicalis and Candida krusei can be distinguished from each other soon after 24 hours of incubation
- The definitive colony appearance of most yeasts is obtained after 48 hours of incubation
- It is important to be able to distinguish Candida parapsilosis from Candida glabrata due to their different antifungal susceptibility profile.

Acknowledgements We wish to thank Silvia Bernini, Antonella Rosellini and Gloria Vitalini for their technical assistance.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

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J Clin Pathol  published online June 29, 2017

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