Use of CHROMagar Candida for genital specimens in the diagnostic laboratory

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Abstract

***Objective*—To evaluate CHROMagar Candida (CA), a new yeast differential medium, for yeast isolation in a clinical laboratory for the routine examination of high vaginal swabs.**

***Methods*—Results of high vaginal swab cultures processed in a standard manner on plates containing equal halves of Sabouraud dextrose agar (SDA) and CA were compared. Non-*Candida albicans* yeast isolates were further speciated with API 20C AUX or API 32C. To assess the ease of use of CA, laboratory staff lacking in experience of the medium were asked to identify 23 unlabelled yeast cultures on CA by referring to six labelled reference plates.**

***Results*—Of the 1784 swab cultures processed, yeasts were isolated from 373 SDA and 368 CA. Of the 78 non-albicans isolates further speciated, CA identified correctly all cultures of *C krusei* and *C tropicalis*, and 82% of *C glabrata*. All the 38 inexperienced laboratory staff achieved 100% accuracy for *C albicans* and over 90% for *C krusei* and *C tropicalis*.**

***Conclusions*—CA is a satisfactory isolation medium for genital specimens, allowing immediate and correct identification of the commonly encountered yeasts and easy recognition of mixed cultures.**

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CHROMagar Candida (CA) is a new differential medium for the presumptive identification of some clinically important yeast species. Odds and Bernaerts carried out an extensive evaluation of its ability to support the growth and production of characteristic colonial features of different yeast isolates.[1] They concluded that the medium is capable of the presumptive identification of several commonly isolated yeast species and its accuracy for *Candida albicans* means that further identification is not necessary. We assessed the use of CA as a yeast isolation medium in a clinical laboratory for the routine examination of vaginal specimens, including attention to its ease of use by inexperienced laboratory staff.

Materials and methods

CHROMagar Candida (CHROMagar Company, Paris, France) is composed of (per litre) peptone (10 g), glucose (20 g), agar (15 g), chloramphenicol (0.5 g) and "chromogenic mix" (2 g). Details of the last component, which is responsible for the differential colour reactions, are not released by the manufacturer. An excellent account of the appearance of different yeast species with colour photographs has been published by Odds and Bernaerts.[1] The appearance of colonies of four clinically important species, C *albicans*, *C glabrata*, *C tropicalis*, and C *krusei* is shown in figure 1. High vaginal swab specimens received for routine examination by the clinical laboratory at the Prince of Wales Hospital in Hong Kong were used for the study. Each swab was plated onto a whole blood agar plate and a second plate with equal halves of Sabouraud's dextrose agar (SDA) and CA. By rotating the swab, a third of the surface of the swab tip was used to inoculate each medium before spreading with a loop, and before a smear was made and the swab immersed in Feinberg's medium. The blood agar plate was incubated under hypercapnic (5% CO_2) conditions and examined after 24 and 48 hours for significant pathogens other than yeasts (not discussed

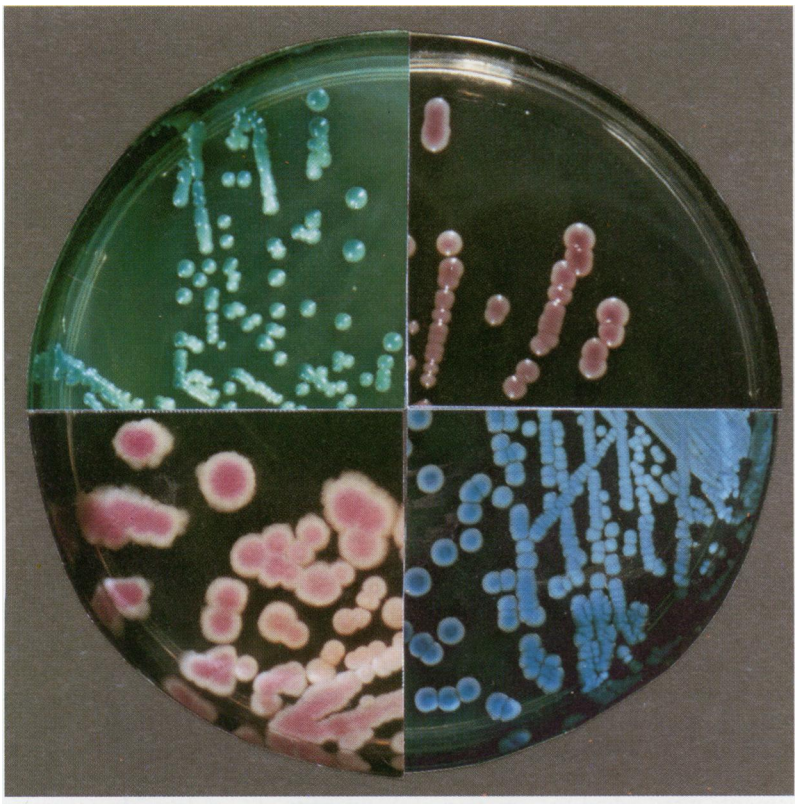

Figure 1 Appearance of some clinically important yeast species on CHROMagar after 48 hours of incubation. Clockwise from 12 o'clock: C glabrata, C tropicalis, C krusei, C albicans.

Table 1 Identification of yeast isolates on CHROMagar by laboratory staff inexperienced in the use of the medium

	Percentage of staff who identified correctly strains A to E of the given species*				
Species	*A*	*B*	*C*	*D*	*E*
C glabrata	71	89	100	97	95
C tropicalis	95	100	97	87	
C krusei	97	95	76	97	
C parapsilosis	97	100			
C albicans	100	100	100		
T beigelii	82	82			
Others†	13	66	13		

*A–E, strains of the same yeast species used for identification.
†Others, including *C lusitaniae* (one), *C humicola* (one), and *C pelliculosa* (one).

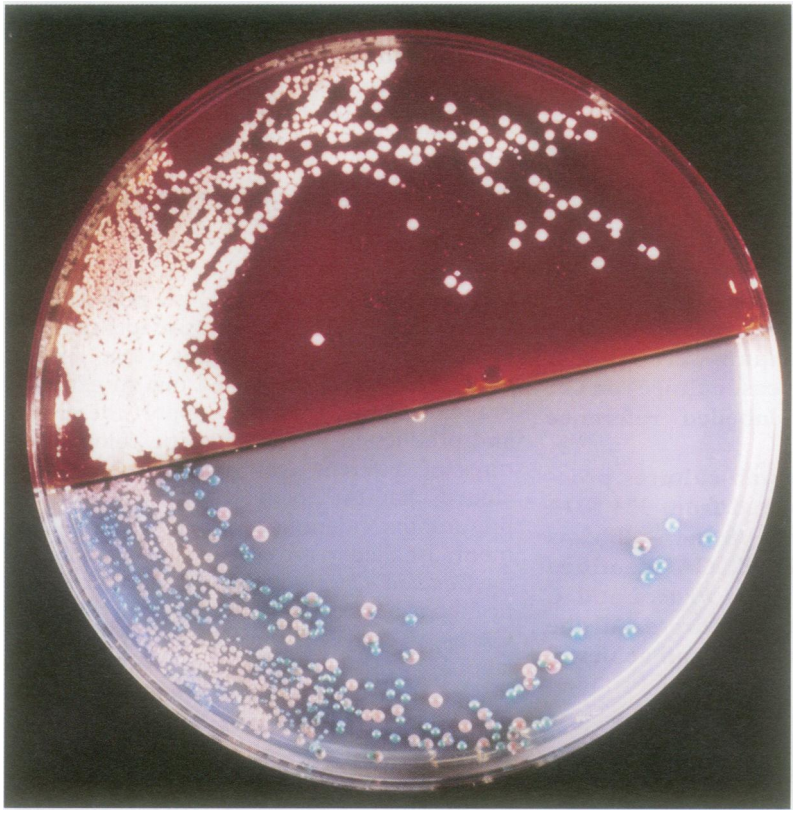

Figure 2 A Petri dish showing the 48 hour culture of a high vaginal swab. Half of the plate contained blood agar showing mixed cultures and the other half CHROMagar showing a mixed growth of C albicans (green colonies) and C glabrata (pale mauve colonies).

here). After 48 hours of incubation, the presence of yeasts was sought on both halves of the yeast plate and semiquantified. Isolates on the CA were identified according to the manufacturer's instructions and as described by Odds and Bernaerts.[1] Isolates from the SDA that were not germ-tube positive, and those not typical of *C albicans* on CA, were further identified by API 20C AUX or API 32C. The appearance of *C albicans* on CA is so reliably characteristic that no further identification is deemed necessary.[1]

To assess the ease of use of CA by inexperienced staff, medical laboratory technicians who had not previously seen cultures of yeasts on CA were asked to take part anonymously in a single blind exercise. A reference panel of 48 hour old pure cultures on CA of each of the following species was displayed: *C albicans, C tropicalis, C krusei, C glabrata, C parapsilosis,* and *Trichosporon beigelii.* Participants were asked to identify a set of pure 48 hour cultures of "unknown" yeast species by comparing them with the reference panel. They were given no training on the reported capabilities of the medium, nor instructions on what colony characteristics to look for.

Results

The study was carried out for seven weeks during January and February 1996 and for eight weeks between July and mid-September 1996. After 48 hours of incubation, yeast growth was easy to observe on both CA and SDA plates. Of the 1784 high vaginal swab specimens examined, yeasts were present in 21% (on 373 SDA plates and 368 CA plates, $p > 0.5$), with mixed growth observed on five CA plates. Growth on one medium only was observed for 18 specimens on SDA and 13 specimens on CA. Of these, 27 showed scanty growth only (fewer than five colonies). There were 102 germ-tube negative isolates from SDA, 78 of which were available for identification by API. Of these, 65 were *C glabrata,* two *C krusei,* one *C albicans,* five *C tropicalis,* four *C parapsilosis,* and one *C lusitaniae.* Of these, the *C albicans, C tropicalis,* and *C krusei* were all initially identified as such by their colonial morphology on CA by experienced staff. Glossy pink-purple colonies with a pale edge were usually *C glabrata,* but 18% of *C glabrata* colonies had a different appearance.

In the single blind assessment for the ease of use of CA, the 38 inexperienced members of staff who took part correctly identified all the strains of *C albicans,* 95% of the *C tropicalis,* and 91% of the *C krusei* (table 1). For the latter species, those not identified correctly were classified as "unknown", not misidentified as belonging to another species.

Discussion

Consistent with findings of others,[2] the majority of yeast isolates from high vaginal swabs were *C albicans* (74%) followed by *C glabrata* (21%). In addition to *C albicans,* high specificity and sensitivity (> 99%) in identification of *C tropicalis* and *C krusei* on CA have been reported.[1 3-5] In our studies, trained staff achieved 100% correct identification of isolates from clinical specimens, and untrained staff > 90% in the single-blind assessment with no guidelines on what characteristics to look for. Isolates of *C glabrata,* the second most common yeast isolated in our survey and present in 4% of high vaginal swabs, presented more difficulties. The colonial colour varied from white or yellow through to different hues of pink or brown, though most common were glossy pink-purple colonies with a paler edge. Eighteen per cent of *C glabrata* isolates (confirmed by API) did not present enough constant or unique definitive colonial features on CA to allow identification with certainty. Also, in our assessment exercise, other species with glossy purple colonies—for example, *C lusitaniae,* were wrongly identified as *C glabrata* by inexperienced staff. These difficulties with *C glabrata* have been noted by others,[1 3] who felt that further methods were required for confirmation of the identity of *C glabrata.* In contrast, Pfaller *et al*[4] reported satisfactory identification by CA as they found nine of the 10 stock

isolates of *C glabrata* all showed dark pink colonies with pale edges. Other colonial appearances have been noted in our study and others. In our study, no clinical correlation was made with the laboratory findings. Species other than *C albicans* accounted for almost 27% of all yeast isolates from high vaginal swabs in our patient population, and 86% of these cultures yielded a moderate to heavy growth.

The clinical role of non-albicans yeasts in infections of the vagina and other sites has been recognised,[6] and recent reports indicate that they tend to be more resistant to commonly used antifungal therapies such as fluconazole.[7 8] Nevertheless, because of resources required to identify *Candida* spp other than *C albicans,* we routinely report the presence of germ-tube negative yeasts in high vaginal swabs as "yeast other than *C albicans*". Further identification is carried out only in exceptional circumstances, when accurate identification of species is indicated for overall clinical management.

In conclusion, the results of our prospective study on the use of CA for genital specimens such as high vaginal swabs demonstrate that CA has several advantages over SDA. The former offers a reliable and rapid identification of *C albicans,* the most common yeast found in high vaginal swabs, and allows presumptive identification of *C tropicalis* and *C krusei.* The coloured yeast colonies allow easy recognition of mixed cultures. The cost of CA is considerably more than SDA, but this may be offset by savings in both staff time and turnaround time as no germ-tube test is required. The use of split Petri dishes half with blood agar and the other half with CA provides a convenient and economical method for the primary culture of high vaginal swabs (fig 2). Our single blind trial results indicate that, with the exception of *C glabrata,* presumptive identification of key yeast species in high vaginal swabs on CA is not difficult to learn.

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