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CHROMagar Candida, a New Differential Isolation Medium for Presumptive Identification of Clinically Important *Candida* Species

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CHROMagar Candida is a novel, differential culture medium that is claimed to facilitate the isolation and presumptive identification of some clinically important yeast species. We evaluated the use of this medium with 726 yeast isolates, including 82 isolated directly on the medium from clinical material. After 2 days of incubation at 37°C, 285 *C. albicans* isolates gave distinctive green colonies that were not seen with any of 441 other yeast isolates representing 21 different species. A total of 54 *C. tropicalis* isolates also developed distinctive dark blue-gray colonies with a halo of dark brownish purple in the surrounding agar. *C. krusei* isolates ($n = 43$) also formed highly characteristic rough, spreading colonies with pale pink centers and a white edge that was otherwise encountered only rarely with isolates of *C. norvegensis*. *Trichosporon* spp. ($n = 34$) formed small, pale colonies that became larger and characteristically rough with prolonged incubation. Most of the other 310 yeasts studied formed colonies with a color that ranged from white to pink to purple with a brownish tint. The only exceptions were found among isolates identified as *Geotrichum* sp. or *Pichia* sp., some of which formed colonies with a gray to blue color and which in two instances formed a green pigment or a dark halo in the agar. The specificity and sensitivity of the new medium for the presumptive identification of *C. albicans*, *C. krusei*, and *C. tropicalis* exceeded 99% for all three species. A blinded reading test involving four personnel and 57 yeast isolates representing nine clinically important species confirmed that colonial appearance after 48 h of incubation on CHROMagar Candida afforded the correct presumptive recognition of *C. albicans*, *C. tropicalis*, *C. krusei*, and *Trichosporon* spp. None of nine bacterial isolates grew on CHROMagar Candida within 72 h, and bacteria (*Escherichia coli*) grew from only 4 of 104 vaginal, 100 oral, and 99 anorectal swabs. The new medium supported the growth of 19 of 23 dermatophyte fungi tested and 41 of 43 other molds representing a broad range of fungal pathogens and contaminants. In parallel cultures of 348 clinical specimens set up on Sabouraud agar and CHROMagar Candida, both media grew yeasts in the same 78 instances. CHROMagar Candida is recommended as a useful isolation medium capable of the presumptive identification of the yeast species most commonly isolated from clinical material and facilitating recognition of mixed yeast cultures.

The medium most widely used for the isolation of *Candida* and other yeast species from clinical specimens is Sabouraud glucose agar (7), a general-purpose medium that supports the growth of most pathogenic fungi. Sabouraud agar is not a differential medium, and colonies of different pathogenic yeast species grown on this agar cannot be easily distinguished from each other. Careful observers are often able to recognize mixtures of different yeast species when they occur on a single plate, but the absence of any differential indicator property in Sabouraud agar means that there is no guarantee that mixed yeast cultures will be detected. *Candida albicans* is the yeast species most often isolated from clinical material, and most clinical laboratories approach yeast identification by applying rapid tests such as the property of germ tube formation to distinguish *C. albicans* from other species, which require more extensive testing for proper identification. The high overall prevalence of yeast isolations in clinical laboratories has led earlier to the design of at least three isolation media intended to differentiate *C. albicans* from other yeasts on the basis of colony color. However, these media have rarely or never gone into routine laboratory use.

Nickerson's medium (4, 5), which is essentially the same as the commercially available BiGGY agar, relies on the differential reduction of complex bismuth salts to give light- and dark-colored colonies, and its use has been described in several reports (3, 6). Pagano et al. (9) added triphenyl tetrazolium chloride as an indicator to Sabouraud agar; on this medium, *C. albicans* isolates give pale-colored colonies, while other yeast species develop various shades of pink. The medium has been used successfully to reveal mixed yeast species in clinical isolates (12). Costa and de Lourdes Branco (2) devised a phosphomolybdate agar on which *C. albicans* colonies are green and those of other species are blue. At least one other study confirmed the efficacy of this medium (1).

Despite the independent studies confirming the value of these differential isolation media, none of them has achieved wide acceptance for use in the routine isolation of clinically important yeasts. The Pagano-Levin and phosphomolybdate agars are not currently available from commercial sources, and Pagano-Levin medium in practice yields a high rate of both false-positive and false-negative results when used to differentiate species (11). Bismuth-based media do not adequately differentiate yeast species from each other, or from bacteria, since most organisms form colonies with a brown to black color on this substrate (10). Nevertheless, the concept of a reliable routine isolation medium that can facilitate the presumptive differentiation at least of *C. albicans* from other yeasts remains attractive. The present study describes an evaluation of a new

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commercial product, CHROMagar Candida, that can be used for the isolation and presumptive identification of *C. albicans*, *C. krusei*, and *C. tropicalis* and the differentiation of these species from other yeasts on the basis of strongly contrasted colony colors produced by reactions of species-specific enzymes with a proprietary chromogenic substrate. The medium greatly facilitates the detection of specimens containing mixtures of yeast species.

MATERIALS AND METHODS

CHROMagar Candida, a proprietary product, was donated for evaluation by the CHROMagar Company, Paris, France. The medium comprised (per liter) peptone (10 g), glucose (20 g), agar (15 g), chloramphenicol (0.5 g), and "chromogenic mix" (2 g). It was supplied as a white powder in preweighed batches for the preparation of 1,000-ml volumes and was prepared according to the manufacturer's instructions. This entailed stirring the powder into distilled water and heating the mixture to the boiling point, with continuous stirring, to dissolve the powder. The medium, which does not require sterilization by autoclaving, was dispensed in petri dishes after being allowed to cool slightly.

A total of 726 isolates representing 22 yeast species were screened for their abilities to grow and for their colony colors on CHROMagar Candida. (The isolates included five identified as "*Geotrichum* sp.", a taxon with some yeast-like properties but often regarded as a mold rather than a typical yeast species.) Of the 726 isolates tested, 233 had been freshly cultured from clinical samples within the 3 months prior to the study, including 78 that were directly isolated from clinical samples on CHROMagar Candida. The remaining yeasts were selected from the stock collection at the Janssen Research Foundation. The yeasts were maintained and subcultured on Sabouraud glucose agar (Oxoid, Basingstoke, United Kingdom). They were identified according to their morphologies on rice-cream agar inoculated by the Dalmau technique, their formation of germ tubes in serum, and, for isolates that were chlamydospore negative and/or germ tube negative, their assimilation patterns, which were determined with the API ID32C yeast identification panel. Isolates identified as *Geotrichum* sp. or *Trichosporon* sp. were not further identified to the species level.

For tests with CHROMagar Candida, the yeasts were either pipetted onto the agar as 10-μl drops of suspensions containing 4×10^7 cells per ml (8) or streaked out to single colonies from yeast suspensions or clinical samples. (No difference in colony color or form was discernible between those colonies that grew from streak inocula and those that grew from drops of suspensions.) In a preliminary study, 197 cultures on CHROMagar Candida were incubated at 37°C and were examined at 1, 2, and 3 days after inoculation. The extent of growth was scored subjectively as "none," "weak" (small, partially grown colonies), or "positive" (colonies of normal, raised appearance characteristic of yeasts), and colony colors were described with reference to the Pantone Color Formula Guide. The presence or absence of a colored halo in the agar immediately surrounding the colony was also noted, as were colony forms that departed from the most common "smooth, entire" appearance of yeasts. These experiments were carried out twice, with a different manufacturer's lot of CHROMagar Candida used on each occasion. The remaining yeast isolates were grown for 48 h at 37°C on CHROMagar Candida, and colony colors and unusual colony forms were recorded.

For evaluation of the selectivity of CHROMagar Candida, a panel of 9 bacteria and 66 molds was inoculated onto the medium and the plates were incubated at 30 or 37°C; the incubation temperature depended on the species concerned. The panel of molds tested included 18 species of dermatophytes (22 isolates), *Aspergillus* spp. (3 isolates), Mucorales (6 isolates), and dematiaceous molds (5 isolates) as well as a range of opportunistically pathogenic and laboratory contaminant genera represented by species of *Acremonium*, *Chaetomium*, *Cunninghamella*, *Curvularia*, *Fusarium*, *Hendersonula*, *Madurella*, *Paecilomyces*, *Penicillium*, *Phoma*, *Pseudallescheria*, *Scopulariopsis*, *Scytalidium*, *Sporothrix*, *Trichoderma*, *Ustilago*, and *Verticillium*.

To determine how effectively CHROMagar Candida performed as a yeast isolation medium, a total of 348 clinical specimens from 115 patients, comprising mainly vaginal, oral, and anorectal swabs, eight nail clippings, and one sample of skin scrapings, were inoculated in parallel on CHROMagar Candida and Sabouraud glucose agar containing chloramphenicol (50 mg/liter) and gentamicin (20 mg/liter).

To assess the ease with which yeast species could be recognized by their colony colors on CHROMagar Candida, a panel of 57 yeast isolates representing the species *C. albicans* (22 isolates), *C.* (*Torulopsis*) *glabrata* (5 isolates), *C. guilliermondii* (3 isolates), *C. kefyr* (3 isolates), *C. krusei* (5 isolates), *C. lusitaniae* (3 isolates), *C. parapsilosis* (6 isolates), *C. tropicalis* (5 isolates), and *Trichosporon* sp. (5 isolates) was coded randomly and inoculated onto the new medium, and the plates were examined after 24 and 48 h of incubation by four individuals who were not otherwise involved with the evaluation. The four readers separately recorded their presumptive identifications of the yeasts by comparing the growth of the strains coded in a single-blind fashion with that of a panel of reference CHROMagar Candida plates labelled with the name of the species.

To confirm that growth on CHROMagar Candida did not adversely affect the yeasts, all 726 yeasts in the main experiment plus an additional 30 yeast clones grown on CHROMagar Candida were subcultured onto Sabouraud glucose agar and were incubated at 37°C to confirm their viabilities.

Colony appearances on CHROMagar Candida were analyzed in terms of sensitivity (number of true positives/number of true positives + number of false negatives)] and specificity [number of true negatives/(number of true negatives + number of false positives)] to determine their likely usefulness in the clinical laboratory setting.

RESULTS

Appearance of yeast colonies on CHROMagar Candida. All of the yeast isolates tested grew on CHROMagar Candida. After 24 h of incubation at 37°C, the majority of yeasts tested had grown well, forming colonies of 1 to 5 mm in diameter; however, growth and colony color development were inconsistent after 24 h of incubation, and color readings were therefore made only after 48 h of incubation, as specified in the manufacturer's instructions. At this time, 3 of the 24 *Cryptococcus neoformans* isolates tested and 2 of 5 *Geotrichum* sp. isolates had formed only pinpoint colonies, and these isolates were therefore evaluated for colony appearance after an additional 24 h of incubation.

A variety of colony colors was seen, some of which were apparently species specific (Table 1). Colors generally deepened slightly after 72 h of incubation (data not shown), but, except for those for the five slowly growing isolates already mentioned, they were clear and distinguishable after 48 h. The edges of all colored colonies were paler than the colored centers; the descriptions of colors that follow refer only to the

TABLE 1. Growth and colony colors of 726 yeast isolates incubated for 2 days on CHROMagar Candida at 37°C

Species	Total no. of isolates	No. of isolates <3 mo old	Range of colony colors described	Color reference number(s)[a]
Candida albicans	285	117	Green	3258/338
Candida famata	15	12	White, light pink, pink	435/436
Candida (Torulopsis) glabrata	84	44	White, pink, purple	5135/5155
Candida guilliermondii	13	2	Pale pink, purple	435/436
Candida humicola	3	0	Light gray, pink	3/435
Candida inconspicua	3	0	White, pink	435
Candida kefyr	15	0	Pink, purple	435/257
Candida krusei	43	27	Pale pink, purple (rough with spreading, pale edges)	435/5025
Candida lambica	5	0	Pink	5135
Candida lusitaniae	21	4	Pink, grayish purple	5135/5155
Candida norvegensis	5	0	White, pale pink (slightly rough with spreading, pale edges)	435
Candida parapsilosis	74	2	White, pale pink	435
Candida pelliculosa	7	0	Pink, pale purple	434
Candida tropicalis	54	6	Dark blue to blue-gray, with dark halo in agar	548/549
Candida utilis	2	0	Pink, purple	434/5155
Cryptococcus laurentii	1	0	Pink-purple	434
Cryptococcus neoformans	23	1	Gray, pale pink	2/434
Debaryomyces polymorphus	2	0	Pink	434
Geotrichum spp.	5	1	Pale to pink (green in agar in two cases) or purple, fimbriate	5405
Pichia spp.	10	1	Highly variable: pink, gray-purple, purple with green edges, dark agar halo in two strains	
Saccharomyces cerevisiae	22	6	White-purple	435/5155
Trichosporon spp.	34	10	Variable; small, pale, "dirty pink" or "dirty gray-green" (becoming darker and rough on prolonged incubation)	5165/5635-5645

[a] Color reference numbers are from the Pantone color guide. Colonies of yeasts that gave colonies described as "pale to pink to purple" usually had a dirty, brownish color and a narrow, paler edge (Fig. 1).

dominant, central colors. Nearly all isolates of all of the yeast species tested gave colonies with colors described as ranging from white through grayish or brownish pink to grayish or brownish purple after 48 h of incubation at 37°C (Table 1; Fig. 1A and B). However, some species gave different colony colors that were well demarcated from such hues.

All 285 *C. albicans* isolates formed yellow-green to blue-green colonies on CHROMagar Candida (Fig. 1C). This green color was particularly distinctive for the species, and among the other species tested, only two *Geotrichum* isolates formed a similar hue. However, in the case of these *Geotrichum* sp. isolates, the colonies themselves were light pink and of much smaller diameter than those of *C. albicans*, and the green pigmentation was present in the agar beneath the colonies (Fig. 1D), an appearance never seen with *C. albicans*, the colonies themselves of which were green. From these data, the sensitivity and specificity of a green colony color for recognition of *C. albicans* were calculated as 100%.

C. tropicalis isolates ($n = 54$) all developed a distinctive dark blue-gray central color after 48 h of incubation, and their colonies were surrounded by a dark brown to purple halo in the agar surrounding the colony (Fig. 1E). Although a colony color approximately similar to that of *C. tropicalis* was observed with some other species, the dark halo in the agar was seen only with isolates of *C. tropicalis* plus 2 of 12 isolates identified as "*Pichia* sp." among the yeasts tested. The sensitivity and specificity of the dark blue-gray colony color and brown-purple agar halo for *C. tropicalis* were therefore >99%.

The color of *Trichosporon* sp. colonies after 48 h was also distinctive but variable. The isolates formed small colonies with a pale color, subjectively described as "dirty pink" to "dirty gray-green" (Fig. 1F); these colonies became darker and acquired a characteristically rough appearance after 72 h or more of incubation. Isolates identified as *Pichia* spp. on the basis of their API ID32D assimilation patterns also formed conspicuously atypical (but variable) colonies after 48 h of incubation on CHROMagar Candida. (It should be stressed that no ascospore formation has been seen for any of these isolates, so their true identifications remain uncertain). Two *Pichia* sp. isolates gave an appearance indistinguishable from that of *C. tropicalis*, as already mentioned. One formed colonies with a dark purple center and a green edge. The remaining eight isolates gave less remarkable colony colors ranging from brownish pink to grayish purple, without the formation of agar haloes.

All 43 isolates of *C. krusei* tested formed colonies that were typically pale, flat, papillate, and spreading with broad white edges (Fig. 1G). After 48 h, *C. krusei* colonies were easily distinguishable from those of other yeasts that formed smooth, brownish pink to brownish purple colonies on CHROMagar Candida. The characteristic appearance of *C. krusei* was seen only with the five isolates of *C. norvegensis* among the other yeasts tested (Fig. 1H). The specificity and sensitivity of the pale pink, spreading, rough colony form for the presumptive identification of *C. krusei* were therefore >99 and 100%, respectively.

When different yeast species were mixed in a single suspension and plated out on CHROMagar Candida, the distinctions in colony colors and forms were extremely easy to recognize (Fig. 2).

Viabilities of yeasts grown on CHROMagar Candida. All 726 yeast isolates plus 30 additional yeast clones were subcultured onto Sabouraud agar after 48 or 72 h of incubation at 37°C on CHROMagar Candida. All of the isolates grew well in the subcultures, demonstrating that viability was not lost by

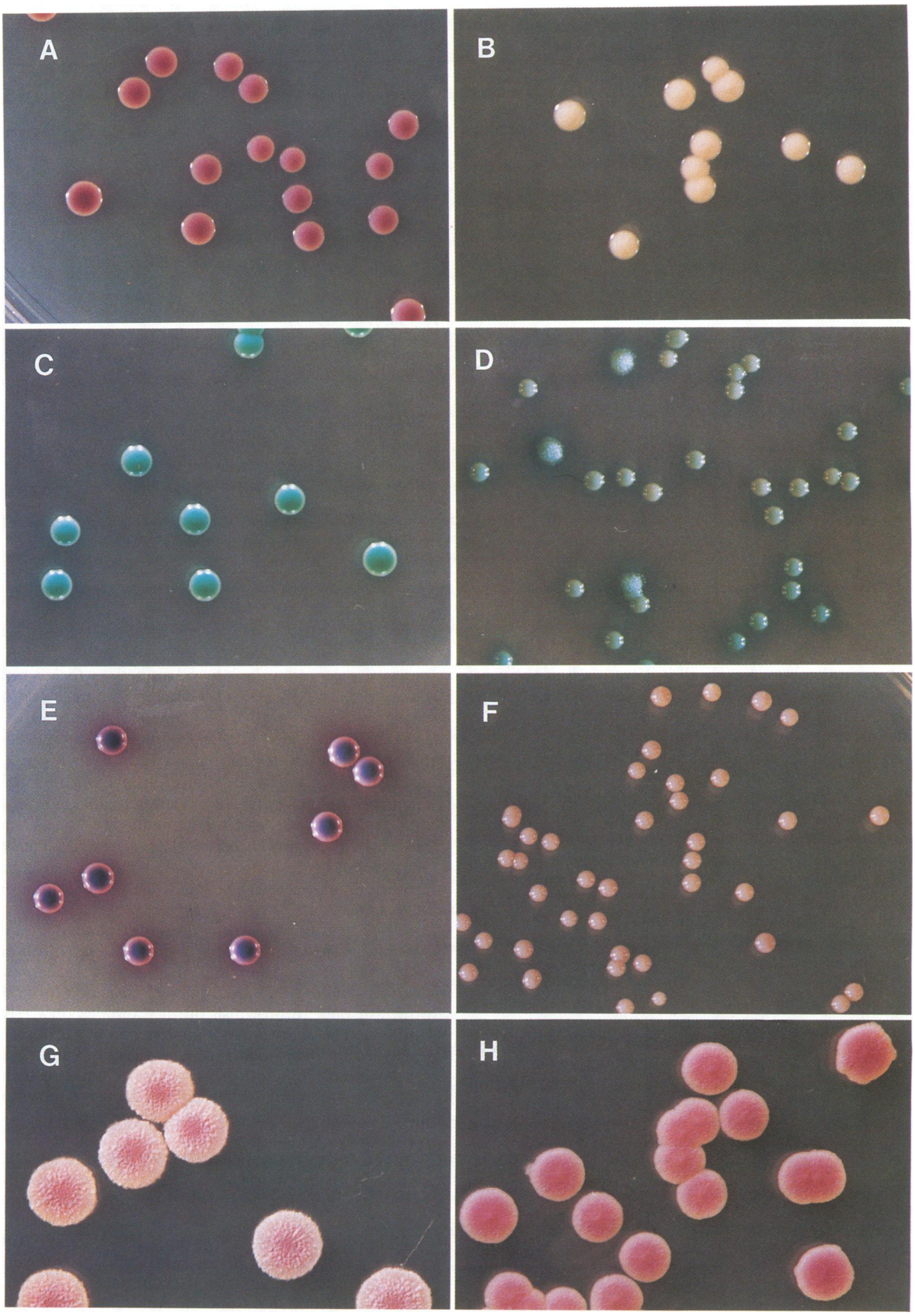
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FIG. 1. (A) Dark pink colonies (paler edges) of *C. glabrata* grown for 48 h on CHROMagar Candida at 37°C. (B) Pale colonies of *C. parapsilosis* grown for 48 h on CHROMagar Candida at 37°C. (C) Green colonies (paler edges) of *C. albicans* grown for 48 h on CHROMagar Candida at 37°C. (D) Colonies of a *Geotrichum* sp. isolate grown for 48 h on CHROMagar Candida at 37°C. Unlike *C. albicans*, this isolate formed small, pale, rough colonies, and the green color is a halo in the agar. (E) Colonies of *C. tropicalis* grown for 48 h on CHROMagar Candida at 37°C. The purple halo in the agar surrounding the dark blue-gray colonies (paler, pinker edges) was observed only with this species. (F) Colonies of a *Trichosporon* sp. isolate grown for 48 h on CHROMagar Candida at 37°C. This isolate formed small, "dirty pink" colonies; other isolates also formed small colonies, but with a pale gray-green hue. (G) Colonies of *C. krusei* grown for 48 h on CHROMagar Candida at 37°C. The large, rough, spreading colonies with broad, pale edges were formed by all isolates tested. (H) Colonies of *C. norvegensis* grown for 48 h on CHROMagar Candida at 37°C. This species was the only 1 of 21 yeasts tested that formed colonies resembling those of *C. krusei* on this medium. Magnifications, ×2.

growth in the presence of the chromophore present in CHROMagar Candida.

Presumptive identification of yeast species by four readers in a single-blind experiment. The 57 yeast isolates coded for single-blind presumptive identification by four independent readers represented nine different yeast species. The four individuals who attempted to identify the isolates from their colonial appearances were asked to record their identifications by reference to single labelled examples of each species. The 22 *C. albicans* isolates were correctly identified by all four observers after 24 and 48 h of incubation. For the other species with distinctive appearances after growth on CHROMagar Candida, all required 48 h of incubation to ensure a correct identification. At this time, all isolates of *C. tropicalis*, *C. krusei*, and *Trichosporon* spp. were correctly identified. None of the other species was correctly identified by all readers at 24 or 48 h, but none was misidentified as *C. albicans*, *C. tropicalis*, *C. krusei*, or *Trichosporon* sp. These observations indicate that the CHROMagar Candida medium serves principally to differentiate *C. albicans*, *C. krusei*, *C. tropicalis*, and *Trichosporon* spp. from other yeast species.

Selectivity of CHROMagar Candida for yeasts and fungi. To determine the selectivity of CHROMagar Candida as an isolation medium for general-purpose use in medical mycological practice, a panel of single isolates representing several bacterial and mold species was inoculated on the new medium. None of the test bacteria, namely, a *Salmonella* sp., *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Morganella morganii*, and *Streptomyces albidus*, had grown after 72 h of incubation on CHROMagar Candida at 37°C. Among 116 vaginal swabs, 113 oral swabs, and 110 anorectal swabs inoculated onto CHROMagar Candida, bacteria grew from just 4 anorectal swabs. In all four instances the bacteria isolated were identified as *E. coli*, and they all gave pink to purple colonies that were recognizable from their sizes and appearances as being typical of bacteria rather than of yeasts.

Tests with 66 mold isolates indicated that CHROMagar Candida supported the growth of most molds. The exceptions included some dermatophytes, 4 of 23 isolates of which failed to grow after 3 weeks of incubation at 25°C, and isolates of a *Verticillium* sp. and a *Phoma* sp., both of which showed no growth after 2 weeks of incubation. The remaining 60 isolates

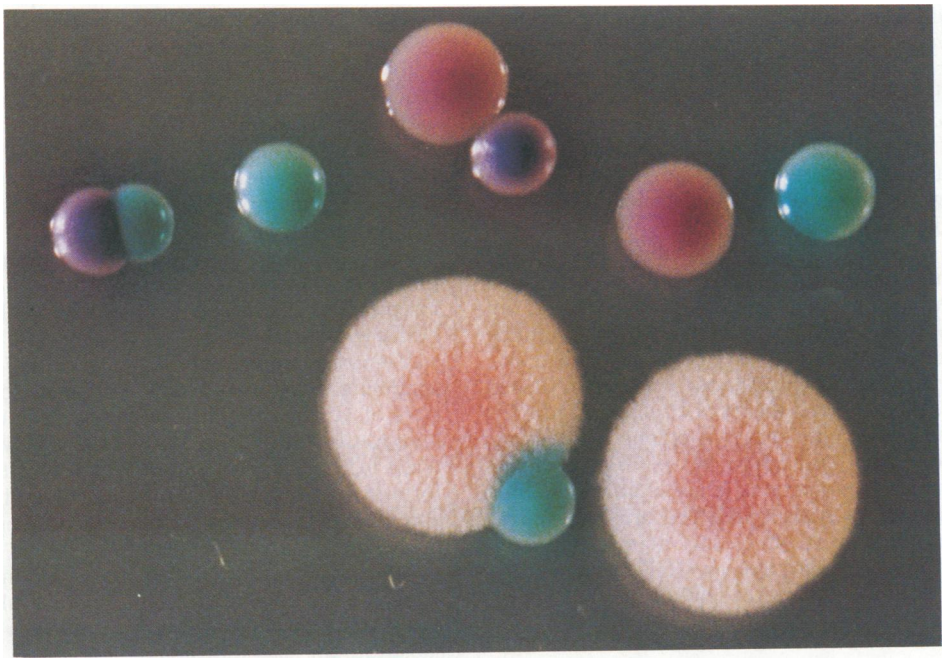

FIG. 2. Colonies plated out from a mixed suspension of four different *Candida* species and incubated for 48 h at 37°C on CHROMagar Candida. All four species can be distinguished by their colony appearances: two *C. glabrata* colonies are pink, two *C. tropicalis* colonies are bluish-purple (the characteristic agar haloes are indistinctly visible under the lighting conditions used), four *C. albicans* colonies are green, and the two large, pale pink, rough colonies are *C. krusei*. Magnification, ×5.5.

gave their normal colonial appearances and colors on CHROMagar Candida except for *Scopulariopsis brevicaulis*, which formed a mold colony with a blue-green color after 48 h. The reverse sides of the mold cultures did not, however, show characteristic appearances; viewed from the reverse they had a black center at the point of inoculation in most cases and an area of blue-green coloration that did not always spread to the edge of the colony.

Batch variation in colony color on CHROMagar Candida. Colony color determination was carried out twice with a panel of 197 yeast isolates inoculated onto two separately prepared batches of CHROMagar Candida. Batch variation in color was minimal; the brownish-pink to brownish-purple color given by most yeasts was judged to be slightly grayer on one batch than the other, but the difference was not great. The characteristic colonies of *C. albicans*, *C. krusei*, and *C. tropicalis* were identical on both batches.

CHROMagar Candida as a yeast isolation medium. The composition of CHROMagar Candida was designed to allow primary isolation of yeasts from clinical material. Its behavior in this respect was studied by culturing a total of 339 oral, anorectal, and vaginal swabs plus nine samples of nail or skin in parallel on CHROMagar Candida and on Sabouraud agar containing chloramphenicol and gentamicin. A total of 78 of the clinical specimens gave positive cultures on both media. There were no instances of specimens that grew on CHROMagar Candida but not on Sabouraud agar or vice versa. The majority of the yeasts isolated were *C. albicans* and were easily recognizable as such by their green color on CHROMagar Candida. As already detailed, *E. coli* grew from four anorectal swabs cultured on CHROMagar Candida. On Sabouraud agar the same four swabs yielded bacterial growth, as did an additional three anorectal swabs. CHROMagar Candida was therefore slightly more selective for yeast isolation than was the Sabouraud agar preparation tested in the present study. Two of the oral swabs that were received yielded mixed cultures that were immediately obvious by the different colony colors on CHROMagar Candida. In one case the mixture contained *C. albicans* plus *C. glabrata*; in the other the mixture contained *C. albicans* plus *C. famata*. From eight nail specimens and one skin sample, *C. albicans* was isolated in one instance, *C. famata* in three, *Trichosporon* sp. in three, *Trichosporon* sp. plus *C. famata* in one, and *Geotrichum* sp. plus *C. famata* in one. In all instances of mixed isolations, the different species cultured were immediately recognizable from their different colony colors.

The prevalence of mixed isolations in the fresh clinical material was not high. This was, however, a sharp contrast to the findings with a batch of 74 specimens received, while the study was under way, as "pure" yeast isolates (not as clinical material) from human immunodeficiency virus-positive patients. The cultures were all plated out on CHROMagar Candida, and 14 of them yielded mixtures of colony colors (in three instances, three obviously different colony colors were represented), all of which proved to be different *Candida* species on subsequent identification. It is unknown whether the mixed yeast specimens represented the original clinical material or whether they arose from cross-contamination after isolation, but the colony appearances on CHROMagar Candida undoubtedly facilitated their recognition as mixed cultures.

DISCUSSION

To be of value for the routine isolation and presumptive differentiation of yeasts, an indicator medium should exhibit several properties. It should support the growth of yeasts but not of bacteria. If the medium also facilitates the growth of fungi other than yeasts, that is not necessarily a disadvantage, since for many clinical samples it is not possible to predict whether a yeast or a mold is likely to be isolated. The differential property of the medium should allow unambiguous presumptive discrimination between the yeast species most commonly encountered in clinical samples, it should facilitate the recognition of specimens containing mixtures of yeast species, and exposure of the fungi to the differential indicator substances should not affect their viabilities for subsequent subculture. CHROMagar Candida appears to fulfill all of these requirements.

Of course, a color-blind person might have difficulties distinguishing the differences in colony appearance between yeast species grown on CHROMagar Candida. Use of the medium by operators afflicted with various types of color-blindness has not been studied. It is not the usual practice of this laboratory to require color-blind personnel to perform microbial identification work since so many of the tests involved require color recognition and discrimination.

The new medium supported the growth of clinically isolated yeasts and most of the molds tested (with exceptions being chiefly among the dermatophytes), but it evidently retards the growth of most bacteria, even those presumed to be present in large numbers in anorectal swabs. The viability of fungi grown on CHROMagar Candida is not affected by exposure to the medium, and the medium affords an extremely high level of discrimination among the most commonly isolated yeast species. The color differential between *C. albicans*, *C. krusei*, and *C. tropicalis* and most other species is much more striking than that seen with previous differential yeast media. The distinctive and unique green color of *C. albicans* isolates, in particular, allows for the confident presumptive identification of this species without resorting to germ tube tests or further laboratory manipulations. The absolute specificity of green colonies for *C. albicans* was evidenced in the present study with 285 isolates of the species. None of the 21 other yeast species tested in the study gave a color that was capable of confusion with that of *C. albicans*, so long as a distinction was made between the color of the colony itself and the green color within the agar that was seen with two *Geotrichum* isolates. This feature alone makes the medium worthy of consideration for routine yeast isolation, since recognition of *C. albicans* removes the majority of clinical yeast isolates from the need for further evaluation.

Moreover, the medium allows for the presumptive distinction of *C. krusei* and *C. tropicalis* from other species of clinical interest. The colony color formed after 48 h by *C. tropicalis* was not unequivocally unique to this species; the blue-gray shade was almost identical to that of two *Pichia* spp. studied. The spreading, rough, white-edged colonies formed by all 43 isolates of *C. krusei* studied were also formed by isolates of *C. norvegensis*. Although *C. norvegensis* is only very rarely encountered in clinical specimens, the fact that the *C. krusei* colony form is not unique to that species reemphasizes the fact that authentic identification of yeast isolates depends on the results of thorough morphologic and physiologic testing.

No colony appearance on an isolation medium can ever serve as a substitute for such proper and full identification tests for yeasts, and CHROMagar Candida is not proposed as a substitute for such thorough identification protocols. The only exception is the case of a colony with the green color and smooth, entire form characteristic of *C. albicans* when such a colony is isolated from a clinical specimen. In such instances the data from the present study suggest that no further steps

are necessary, not even the germ tube test, to confirm the identification of the species. The particular value of the new isolation medium is that it facilitates enormously the recognition of mixtures of yeast species on a single isolation plate, and it offers, for certain yeast species, a strong indication of the likely subsequent identification. In single-blind tests with individuals not involved with the laboratory study, CHROMagar Candida could be used successfully to differentiate isolates of *C. albicans*, *C. tropicalis*, *C. krusei*, and *Trichosporon* spp.

The performance of CHROMagar Candida exactly paralleled that of Sabouraud glucose agar in terms of its ability to support the isolation of yeasts from clinical samples. CHROMagar Candida was slightly superior to the Sabouraud agar used for comparison in terms of its ability to suppress bacterial growth. Its overall superiority has been self-evident in our hands in its ability to reveal mixtures of yeast species present in cultures (although the clinical material directly cultured on CHROMagar Candida happens so far to have yielded only two instances of mixed yeast infections). We have so far experienced several instances of cultures submitted as "pure" yeast isolates, but which were shown to contain mixtures when plated out on CHROMagar Candida.

CHROMagar Candida appears to be a medium well-suited for medical mycological use. It can serve as a primary isolation and differentiation medium for clinical specimens likely to contain yeasts and also as an adjunctive differential medium for the identification of yeasts isolated on other media.

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