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New Chromogenic Agar Medium for the Identification of *Candida* spp.

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A new chromogenic agar medium (*Candida* diagnostic agar [CDA]) for differentiation of *Candida* spp. is described. This medium is based on Sabouraud dextrose agar (Oxoid CM41) and contains (per liter) 40.0 g of glucose, 10.0 g of mycological peptone, and 15.0 g of agar along with a novel chromogenic glucosaminidase substrate, ammonium 4-{2-[4-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)-3-methoxyphenyl]-vinyl}-1-(propan-3-yl-oate)-quinolium bromide (0.32 g liter^{-1}). The glucosaminidase substrate in CDA was hydrolyzed by *Candida albicans* and *Candida dubliniensis*, yielding white colonies with deep-red spots on a yellow transparent background after 24 to 48 h of incubation at 37°C. Colonies of *Candida tropicalis* and *Candida kefyr* were uniformly pink, and colonies of other *Candida* spp., including *Candida glabrata* and *Candida parapsilosis*, were white. CDA was evaluated by using 115 test strains of *Candida* spp. and other clinically important yeasts and was compared with two commercially available chromogenic agars (Candida ID agar [bioMerieux] and CHROMagar Candida [CHROMagar Company Ltd.]). On all three agars, colonies of *C. albicans* were not distinguished from colonies of *C. dubliniensis*. However, for the group containing *C. albicans* plus *C. dubliniensis*, both the sensitivity and the specificity of detection when CDA was used were 100%, compared with values of 97.6 and 100%, respectively, with CHROMagar Candida and 100 and 96.8%, respectively, with Candida ID agar. In addition, for the group containing *C. tropicalis* plus *C. kefyr*, the sensitivity and specificity of detection when CDA was used were also 100%, compared with 72.7 and 98.1%, respectively, with CHROMagar Candida. Candida ID agar did not differentiate *C. tropicalis* and *C. kefyr* strains but did differentiate members of a broader group (*C. tropicalis*, *C. kefyr*, *Candida lusitaniae* plus *Candida guilliermondii*); the sensitivity and specificity of detection for members of this group were 94.7 and 93.8%, respectively. In addition to the increased sensitivity and/or specificity of *Candida* detection when CDA was used, differentiation of colony types on CDA (red spotted, pink, or no color) was unambiguous and did not require precise assessment of colony color.

Infections due to *Candida* spp. and other fungi have increased dramatically in recent years and are of particular importance because of the rising number of immunocompromised patients (6). *Candida albicans* accounts for approximately 90% of *Candida* spp. isolated from yeast-infected patients; however, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, and *Candida krusei* are of increasing significance as they tend to be more resistant to antifungal agents (8, 10, 17).

Currently, two chromogenic agars are widely used in clinical mycology laboratories for presumptive detection and identification of *Candida* spp., particularly *C. albicans*. These are Candida ID agar, a product of bioMerieux (3, 15), and CHROMagar Candida, produced by CHROMagar Company Ltd. (3, 16, 18, 24). Candida ID agar, which superseded Albicans ID2 medium (3, 7, 14, 20), is based on a chromogenic indolyl glucosaminide substrate which is hydrolyzed by *C. albicans* to give a turquoise or blue insoluble product. *C. tropicalis*, *Candida lusitaniae*, and *Candida guilliermondii* appear pink on this agar, and other species of *Candida* are white. CHROMagar Candida also uses a chromogenic β-glucosaminidase substrate, which is metabolized to give green colonies of *C. albicans*, steel blue colonies of *C. tropicalis*, and fuzzy rose-colored colonies of *C. krusei*.

In the present study, a number of novel chromogenic glucosaminide substrates (1) were evaluated for their usefulness in differentiating *Candida* spp. in agar media. This led to development of a new agar medium containing the substrate ammonium 4-{2-[4-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)-3-methoxyphenyl]-vinyl}-1-(propan-3-yl-oate)-quinolium bromide (VLPA-GlcNAc) (Fig. 1). This medium was optimized for sensitivity to *C. albicans*, *C. tropicalis*, and *Candida kefyr* and was tested with a wide range of yeasts and some molds. The efficacy of the new medium was compared to the efficacies of the two commercially available chromogenic agars described above.

MATERIALS AND METHODS

Cultures. The test fungi were obtained from the National Collection of Pathogenic Fungi, Public Health Laboratory, Bristol, United Kingdom; the American Type Culture Collection, Manassas, Va.; and the Medical Mycology Department, St. John's Institute of Dermatology, St. Thomas' Hospital, London, United Kingdom The clinical test strains were identified at St. Thomas' Hospital by using conventional tests, including colony morphology, germ tube production, chlamydospore production, growth at 45°C (*C. dubliniensis* is unable to grow at this temperature), and API 20C tests (bioMerieux, Basingstoke, Hampshire, United Kingdom). In the following list of strains, the strains whose designations begin with NCPF and ATCC were obtained from the National Collection of Pathogenic Fungi and the American Type Culture Collection, respectively. All other

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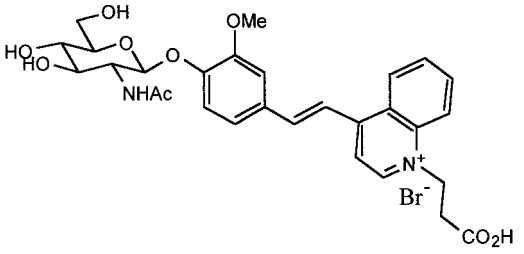

FIG. 1. Chemical structure of the chromogenic substrate VLPA-GlcNAc.

strains were clinical isolates from the St. Thomas' Hospital collection. The strains used were *C. albicans* NCPF3189, NCPF3351, EQ2096, EQ3325, EQ3491, EQ2824, EQ2814, EQ2950, 628, EK6271, EM6285, M207, EM628, M924, Q444, Q390, EQ3676, EQ3842, Q390, EQ3311, EQ3991, EQ4055, EQ3943, Q252, EQ3392, EQ3553, EQ3845, JP43, EQ740, EQ602, EQ813, EM627, EQ248, EM1951, ATCC 18804, EQ597, EL2026, EK5915, EM8100, EM628, and M20; *C. dubliniensis* NCPF3108, NCPF3949, and UK NEQAS159; *C. glabrata* UK NEQAS5097, UK NEQAS4020, L999, Q258, 6971, and EQ2423; *C. guilliermondii* 7668, EL4835, EQ2423, and UK NEQAS4447; *C. kefyr* 22197; *C. krusei* UK NEQAS2936, ATCC 44507, ATCC 6258, and NCPF3321; *C. lusitaniae* P37, EM269, EP1898, and UK NEQAS4620; *C. parapsilosis* EQ2197, Q225, Q258, EQ2970, EQ2987, ATCC 22019, EQ3243, EM1128, EK568, EK6021, EL7825, and EQ248; *Candida pelliculosa* EL6892 and EP2144;*Candida rugosa* EK5365; *C. tropicalis* UK NEQAS2233, Q258, EQ2832, ST4-97, EL8047, EM5579, NCPF3290, NCPF3097, EL804, and UK NEQAS3025; *Blastoschizomyces capitatus* PMC2558 and EQ161; *Cryptococcus neoformans* A strains NCPF3210, NCPF3168, NCPF3226, and NCPF3088; *Cryptococcus neoformans* B strains NCPF3252 and NCPF3003; *Cryptococcus neoformans* C strains NCPF3021 and NCPF3308; *Cryptococcus neoformans* D strains B3501, NCPF3186, NCPF3250, and NCPF3287; *Hansenula anomala* STHA; *Malassezia pachydermatis* CBS1879, EP2026, and N955; *Saccharomyces cerevisiae* STSC; *Trichosporon beigelii* UK NEQAS4521 and EQ162; *Trichosporon capitatum* M98 and L1502; *Trichosporon inkin* 4286 and ATCC 18020; *Trichosporon mucoides* 7972 and ATCC 90046; and *Trichosporon ovoides* ATCC 90040. All strains were grown in nutrient broth (CM4; Oxoid, Basingstoke, United Kingdom) and stored after addition of glycerol (final concentration, 25% [vol/vol]) at −70°C.

Chromogenic substrates. The chromogenic substrates usedwere closely related to the phenols described by Aamlid et al. (1) and were obtained from PPR Diagnostics Ltd., London, United Kingdom. They were 2-{2-[4-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)-3-methoxyphenyl]-vinyl}-3-methyl-benzothiazolium iodide (VBzTM-GlcNAc), 4-{2-[4-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)-3-methoxyphenyl]-vinyl}-1-ethyl-quinolinium iodide (VLE-GlcNAc), 4-{2-[4-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)-3-methoxyphenyl]-vinyl}-1-methyl-quinolinium iodide (VLM-GlcNAc), VLPA-GlcNAc (Fig. 1), 2-{2-[4-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)-3-methoxyphenyl]-vinyl}-1-ethyl-quinolinium iodide (VQE-GlcNAc), 2-{2-[4-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)-3-methoxyphenyl]-vinyl}-1-methyl-quinolinium iodide, and 5-[4-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)-3-methoxyphenylmethylene]-2-thioxothiazolidin-4-one-3-acetic acid (VRA-GlcNAc). In water, most of the substrates had comparatively low solubilities (<2.0 mM); the only exception was VLPA-GlcNAc, whose solubility was >20 mM.

Chromogenic media. Sabouraud dextrose agar (SDA) (Oxoid) was the preferred basal medium to which novel glucosaminide substrates were added; different batches of this agar and substrates were used in a 2-year period. The SDA (CM41; Oxoid) contained (per liter) 10.0 g of mycological peptone, 40.0 g of glucose, and 15.0 g of agar (Bacteriological No. 1). SDA plus a chromogenic glucosaminide substrate (0.32 g liter^{-1}) was quickly heated to the boiling point in glass bottles over a Bunsen burner and tripod and then removed from the heat. After it cooled to about 55°C, the medium was poured into 90-mm petri dishes. After the medium set, the plates were surface dried for 20 min at 37°C and used immediately or stored at 4°C for 6 weeks. The pH of agar plates was 5.6 ± 0.2, as determined with a flat pH electrode (Gelplas combination electrode; Merck Ltd., Poole, United Kingdom).

In the initial experiments, the new chromogenic substrates (at concentrations of 0.35 and 0.7 mM) were also tested in a variety of basal media (obtained from Oxoid, except where indicated otherwise). These media included (i) media formulated for the growth of fungi, including SDA, Sabouraud maltose agar, malt extract agar, potato dextrose agar, corn meal agar, and rice extract agar (with and without Tween 80; Becton Dickinson and Co., Cowley, Oxfordshire, United Kingdom); and (ii) media usually used for bacterial growth, including nutrient agar (Oxoid; Merck, Darmstadt, Germany), Lab-lemco agar, cystine-lactose-electrolyte-deficient medium (CLED), modified CLED (with and without lactose), standard plate count agar, and yeast extract agar. The substrates were added to the basal agar in each of the following ways: as a powder before boiling as described above; as a powder before autoclaving; and as a filter-sterilized solution after the agar was autoclaved and cooled to 55°C.

In addition, VLPA-GlcNAc hydrolysis was compared by using SDA obtained from a number of suppliers, including MAST Group Ltd. (DM200D; Bootle, Merseyside, United Kingdom), Lab M (Lab009; Bury, United Kingdom), Difco (SDA 210950 and modified SDA; purchased from Becton Dickinson and Co.), BBL (SDA 4311584 and SDA Emmons 4311589; purchased from Becton Dickinson and Co.), Oxoid (CM41), bioMerieux, and Merck (Sabouraud agar containing 2% glucose and Sabouraud agar containing 4% glucose). VLPA-GlcNAc was boiled with each test agar (as described above) before plates were poured.

Optimization of the VLPA-GlcNAc medium. During development of the medium, the effects of chromogenic substrate concentration, incubation temperature, and pH on the development of colony color were investigated. In addition, the effects of including the following groups of compounds in the medium were determined: inducers of yeast morphogenesis, including *N*-acetyl-D-glucosamine (0.25 to 5 g liter^{-1}) (5, 22) and glucose (0.5 to 100 g liter^{-1} (11, 19); an inducer of germ tube production, hemin (10 to 50 mg liter^{-1}) (4); an inducer of chlamydospore production, Tween 80 (2 to 10 g liter^{-1}); sugars assimilated by *C. albicans*, including trehalose (2 to 20 g liter^{-1}), raffinose (1 to 30 g liter^{-1}), and sucrose (2 to 50 g liter^{-1}); and cell wall-permeabilizing agents, including *n*-octylglucoside (0.003 to 0.09%, wt/vol), sodium dodecyl sulfate (0.005 to 0.08%, wt/vol), dithiothreitol (0.005 to 0.1%, wt/vol), and 2-mercaptoethanol (0.05 to 1%, vol/vol) (13).

Inoculation of media. Test yeasts and fungi were streaked onto chromogenic media by using sterile plastic inoculating loops. Inocula were grown on SDA (Oxoid) plates for 24 to 48 h at 37°C or, as indicated below, for 48 or 72 h at 30°C.

Comparison of the new *Candida* agar with commercially available media. A total of 125 test strains (98 *Candida* strains and 27 non-*Candida* strains) were used. The media evaluated were (i) Candida diagnostic agar (CDA) developed in this study and containing VLPA-GlcNAc, (ii) Candida ID agar ready-poured plates (code 4354093; bioMerieux), and (iii) CHROMagar Candida ready-poured plates (code 43591; Becton Dickinson and Co.). Candida ID agar and CHROMagar Candida plates were stored at 4°C in the dark for a maximum of 7 days. CDA plates were similarly stored but for up to 6 weeks. Test strains were grown on SDA (Oxoid) and streaked onto plates of CDA, Candida ID agar, and CHROMagar Candida, as described above. Plates were incubated at 37°C and observed to determine colony coloration and morphology at 24, 48, and 72 h. In accordance with the manufacturer's instructions, Candida ID agar plates were incubated in the dark. Experiments were replicated in those cases in which strains gave atypical results. In all cases, the results of repeated tests were the same as the results of the original tests.

RESULTS AND DISCUSSION

Hydrolysis of chromogenic glucosaminide substrates by *Candida*. Initially, a panel of six *Candida* strains (*C. albicans* NCPF3153 and NCPF3189, *C. kefyr* 22197, *C. krusei* NCPF3321, and *C. tropicalis* NCPF3290 and NCPF3097) were tested for the ability to hydrolyze the chromogenic substrates in agar media. For these six species, dramatic differences in colony coloration were observed depending on the medium-substrate combination used.

The most intense coloration of *C. albicans* colonies was observed on corn meal agar (substrate concentration, 0.7 mM) at an incubation temperature of 37°C; the colonies appeared pink-orange (VBzTM-GlcNAc), pink (VLE-GlcNAc), brown (VLM-GlcNAc and VLPA-GlcNAc), orange-brown (VQE-GlcNAc), or orange (VRA-GlcNAc) after 48 h. However, although the colony colors were intense, the colored chromophores also diffused into the agar surrounding the colonies. Thus, the most effective substrate-medium combination was

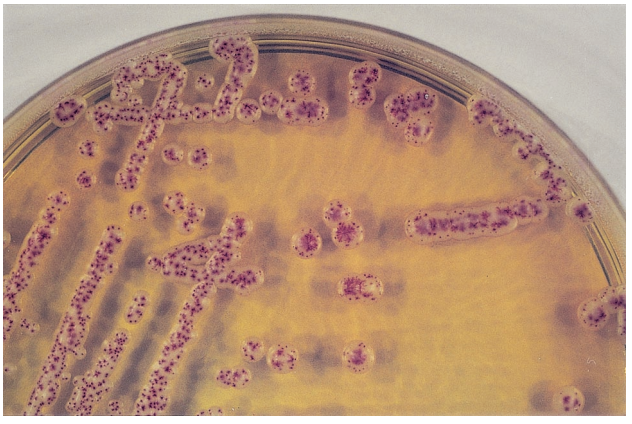

FIG. 2. Colonies of *C. albicans* on CDA, showing deep-red spots after 24 h of incubation at 37°C.

VLPA-GlcNAc in SDA, in which, unusually, colonies of *C. albicans* appeared white with deep-red spots (Fig. 2) while the background agar remained yellow.

Colonies of *C. krusei* were white for all substrate-medium combinations, and colonies of *C. tropicalis* were generally very poorly colored; however, on SDA containing VLPA-GlcNAc, they were pink at 24 h and dark pink at 48 h. *C. kefyr* was also dark pink on this medium at 24 h. Thus, the most promising medium for the differentiation of *Candida* spp. appeared to be VLPA-GlcNAc in SDA. The structure of VLPA-GlcNAc is shown in Fig. 1.

SDA preparations from several different companies were used in combination with VLPA-GlcNAc to determine whether the use of different medium formulations affected colony coloration. Deep-red multispotted colonies of *C. albicans* were observed with SDA formulations from Oxoid and MAST, but the latter medium resulted in slightly smaller colonies. The SDA formulations from Lab M, Difco, and BBL gave *C. albicans* colonies which were orange with red spots, and there was also some diffusion of the orange color into the surrounding agar; in addition, red spots were also evident in colonies of *C. tropicalis* and *C. kefyr*. When the SDA formulation from bioMerieux was used, *C. albicans* and *C. tropicalis* gave unspotted yellow colonies, although *C. kefyr* colonies were white with deep-red spots. The Oxoid SDA formulation was therefore chosen for use in the new medium as clearly defined spotted colonies were produced by *C. albicans* and uniformly pink colonies were produced by *C. tropicalis* and *C. kefyr*.

Optimization of the chromogenic medium for detection of *Candida* spp. A series of experiments were undertaken to optimize the medium by maximizing *C. albicans*, *C. tropicalis*, and *C. kefyr* color production.

(i) Substrate concentration. Increasing the concentration of VLPA-GlcNAc (bromide form; 0.1 to 1.0 mM) increased the number of spots in *C. albicans* colonies and the pink color of *C. tropicalis* and *C. kefyr* colonies. Maximal coloration for all three species was achieved with concentrations of ≥0.5 mM.

(ii) Incubation time and temperature. *Candida* species are grown routinely at 37°C or occasionally 30°C. When test plates were incubated at 30°C, it was noted that the spots in colonies of *C. albicans* tended to merge together to give colonies a ringed appearance at 48 h. At 25°C, growth of *Candida* spp. was too slow (3 to 4 days) for a routine clinical agar, although spots were formed. At 42°C, growth of the three strains of *C. dubliniensis* was inhibited, but spot development in *C. albicans* was greatly reduced. Thus, the most suitable incubation temperature appeared to be 37°C, with plates observed at 24 and 48 h.

(iii) Effect of heat on the substrate. The substrate decomposed when it was autoclaved in SDA, giving a brown agar. However, no color change was observed when the substrate was boiled in SDA (see Materials and Methods).

(iv) pH. Maximal spot formation in *C. albicans* colonies was observed when SDA with a pH between 5.0 and 7.5 was used; the pH of unmodified SDA medium (pH 5.6) is within this range.

(v) Additions to the medium. Addition to SDA of cell wall-permeabilizing agents, metabolizable sugars, and inducers of yeast morphogenesis, germ tube production, and chlamydospore production (see Materials and Methods) failed to enhance the color reactions of *C. albicans* or *C. tropicalis* colonies.

The results suggested that the most appropriate formulation for a differential *Candida* medium was simply SDA (Oxoid) plus VLPA-GlcNAc (0.55 mM or 0.3223 g liter^{-1}). This agar was designated *Candida* diagnostic agar (CDA), and plates of this medium were prepared by briefly boiling the constituents prior to pouring.

Colony coloration on CDA. Ninety-eight *Candida* strains and 28 non-*Candida* strains were streak plated onto CDA and incubated at 37°C. The plates were observed for coloration for 48 h or for 72 h if there was any indication of a color change at 48 h. Among the *Candida* spp., deep-red-spotted colonies of *C. albicans* were observed (Table 1 and Fig. 2) after 24 h of incubation, and the spots increased in size and number when plates were incubated for 48 h. The pattern of spots varied from many small (diameter, <1 mm) pinprick spots per colony to a smaller number of large spider-like spots (diameter, 2 to 4 mm). *C. dubliniensis* strains also gave spotted colonies; however, strains of this species could be differentiated by their much poorer growth if plates were incubated at 42°C. Colonies of most *C. tropicalis* strains were pale pink at 24 h, and maximum coloration was observed at 48 h; however, maximum coloration of strains EL804, 258, El8047, and EM5579 required 72 h of incubation. The *C. kefyr* strain gave pink colonies at 24 h. Colonies of other *Candida* species were white. Except for *Trichosporon* spp., other yeasts (*S. cerevisiae, M. pachydermatis, B. capitatus*, *H. anomala*, and *Cryptococcus* spp.) also gave all-white colonies. Colonies of 7 the 10 *Trichosporon* strains, representing five species (see Materials and Methods), had some spots. However, colonies of these strains were readily differentiated from those of *C. albicans* and *C. dubliniensis* by their folded-lace appearance.

Comparison of CDA with two commercially available media. The color reactions of 115 yeast strains on CDA, Candida ID agar, and CHROMagar Candida are given in Table 1. The color reactions of nine additional *Trichosporon* strains are described separately below. On CDA, colonies of all *C. albicans* and *C. dubliniensis* strains were distinguished by being white with deep-red spots. Colonies of all *C. tropicalis* and *C. kefyr*

TABLE 1. Comparison of three test agars for detection and differentiation of *Candida* spp.[a]

Yeast	Strain(s)[b]	Colony color on:		
		CDA	Candida ID agar	CHROMagar Candida
Candida albicans	39 of 41 strains	Red spotted	Blue	Green
	M207	Red spotted	Blue	Blue, purple, and turquoise[c]
	JP43, EM628	Red spotted	Blue	Blue and turquoise (24 h), Green (48 h)[c]
C. dubliniensis	3 of 3 strains	Red spotted	Blue	Green
C. glabrata	5 of 6 strains	White	White	Purple
	Q258	White	Pink	Pale lilac (24 h), White (48 h)
C. guilliermondii	4 of 4 strains	White	Pink	Purple
C. kefyr	One strain	Pink	Lilac	Blue
C. krusei	3 of 4 strains	White	White	Pink
	ATCC 44507	White	White	Purple and white[c]
C. lusitaniae	3 of 4 strains	White	Pink	Purple and white[c]
	UK NEQAS4620	White	Pink	Pink
C. parapsilosis	9 of 12 strains	White	White	Purple and white[c]
	EK6021, EL7825	White	White	Pink
	EQ568	White	Pink	Purple (24 h), gray (48 h)
C. pelliculosa	EL6892	White	Pink	Purple
	EP2144	White	Pink	Pink
C. rugosa	EK5365	White	White	Purple
C. tropicalis	6 of 10 strains	Pink	Pink	Blue
	UK NEQAS2233	Pink	Pink	Purple (24 h), dark blue (48 h)
	EQ2832, EM5579,	Pink	Pink	Blue and purple[c]
	NCPF3097	Pink	Pink	Purple
Blastoschizomyces capitatus	2 of 2 strains	White	White	Pink
Cryptococcus neoformans	9 of 12 strains	White	White	Purple
	NCPF3021	White	Blue and white[c]	Blue
	B3501	White	Pink	Purple
	NCPF3250	White	Pink	Purple
Hansenula anomala	STHA	White	White	Purple
Malassezia pachydermatis	3 of 3 strains	White	NG[d]	NG
Saccharomyces cerevisiae	STSC	White	White	Purple

[a] Plates were observed after 24 and 48 h of incubation at 37°C; differences in color that were observed after these incubation times are indicated.
[b] When strains gave the same pattern of colony coloration on the three test agars, the strains are not indicated individually; e.g., for *C. albicans* 39 of 41 test strains gave colonies which were red spotted, blue, and green on CDA, Candida ID agar, and CHROMagar, Candida, respectively.
[c] Different colony colors in different regions of the same plate.
[d] NG, no growth.

strains were uniformly pink, and colonies of all other yeast strains listed in Table 1 were white.

In addition to differentiation of *C. albicans* plus *C. dubliniensis* strains, the chromogenic agars tested also differentiated additional groups of strains. These groups were *C. tropicalis* plus *C. kefyr* strains for CDA and CHROMagar Candida and *C. tropicalis* plus *C. kefyr*, *C. lusitaniae*, and *C. guilliermondii* strains for Candida ID agar. The sensitivity (Table 2) and specificity for differentiation of these secondary groups of strains were, however, much greater (both 100%) for CDA than for Candida ID agar (94.7 and 93.8%, respectively) and CHROMagar Candida (72.7 and 98.1%, respectively). CHROMagar Candida has also been reported to differentiate *C. krusei*; the present study included only four test strains of this species, and three gave the predicted (pink) color reaction. *C. albicans* was clearly distinguishable from other known pathogenic microorganisms in mixed cultures

Candida ID agar is reported to give blue colonies of *C. albicans*, pink colonies of *C. tropicalis*, *C. kefyr*, *C. lusitania*, and *C. guilliermondii*, and white colonies of all other *Candida* spp.

TABLE 2. Sensitivity of CDA, Candida ID agar, and CHROMagar Candida for detection of *Candida* spp.[a]

Sensitivity	% Sensitivity or specificity of:		
	CDA	Candida ID agar	CHROMagar Candida
C. albicans plus *C. dubliniensis*	100	100	97.6[b]
C. tropicalis plus *C. kefyr*	100	NA[c]	72.7[d]
C. tropicalis plus *C. kefyr, C. lusitaniae*, and *C. guilliermondii*	NA	94.7	NA
C. krusei	NA	NA	75.0

[a] Sensitivity was determined by dividing the number of strains of the target species giving the predicted color reaction by the total number of test strains of the target species.
[b] The sensitivity was 92.7% for plates incubated for 24 h.
[c] NA, not applicable.
[d] For CHROMagar Candida the color reaction of *C. kefyr* is not given by the manufacturer; however, the test strains used gave the same color reaction (blue) as that predicted for *C. tropicalis*. The sensitivity was 63.4% for plates incubated for 24 h.

In the present study, all *C. albicans* test strains gave turquoise-blue colonies, as claimed, although *C. dubliniensis* strains gave a similar colony color and *Cryptococcus neoformans* strain NCPF3021 gave some blue colonies. Among the *C. tropicalis-C. kefyr-C. lusitaniae-C. guilliermondii* group, the *C. kefyr* strain gave lilac colonies, although colonies of the other test strains were pink, as predicted; however, colonies of some strains of *C. glabrata* (one of seven colonies), *C. parapsilosis* (3 of 12 colonies), *C. pelliculosa* (two of two colonies), and *Cryptococcus neoformans* (2 of 12 colonies) were also pink.

CHROMagar Candida is reported to give green colonies of *C. albicans* and steel blue colonies of *C. tropicalis*. In this study, most *C. albicans* strains gave green colonies after 48 h of incubation; the exception was strain M207, which gave blue-turquoise colonies. However, two additional strains (JP43 and EM628) gave blue-turquoise colonies at 24 h. Colonies of the three *C. dubliniensis* strains were green. Only 7 of 10 *C. tropicalis* strains gave the predicted steel blue colony color. CHROMagar Candida is also reported to detect *C. krusei* colonies by their fuzzy rose color. Three of the four test strains gave pink colonies after 48 h of incubation, but after only 24 h of incubation the colony colors of two of these strains varied from pink to purple. The remaining *C. krusei* strain gave purple or white colonies even after 48 h of incubation. In addition, the usefulness of colony color in identification of *C. krusei* appears to be limited as several other yeasts gave pink colonies on CHROMagar Candida, including *C. lusitaniae* UK NEQAS4620, *C. parapsilosis* EK6021 and EL7825, and *B. capitatus* EQ161 and PMC2558. Many other strains gave purple colonies (Table 1), which might also be confused with *C. krusei* colonies.

In addition to the strains listed in Table 1, nine *Trichosporon* strains (representing five species) were also streaked on plates of the three test agars. Except for both strains of *T. capitatum* and one of the two test strains of *T. mucoides*, the colony colors were similar to those of *C. albicans* on all three media. However, as noted above, *Trichosporon* strains are readily differentiated from *C. albicans* by their colony morphology (2). Thus, the similarity of colony colors does not appear to affect the usefulness of the chromogenic agars for *Candida* identification.

All of the chromogenic media tested (CHROMagar Candida, Candida ID agar, and CDA) appeared to be useful in presumptive identification of *Candida* spp. from clinical specimens, although there was variation in the range of species differentiated and in the sensitivity and specificity for target groups. All of the test media had a high sensitivity for *C. albicans* detection but failed to distinguish strains of this species from *C. dubliniensis*. This was not unexpected as the newly recognized species *C. dubliniensis* is known to be closely related to *C. albicans* and was formerly referred to as atypical *C. albicans*. It is most commonly isolated from immunosuppressed patients and intravenous drug users who are not infected with human immunodeficiency virus, but it represents only a small proportion of the total *Candida* isolations in clinical laboratories. *C. albicans* may be differentiated from *C. dubliniensis* by PCR methods (12) or by its poorer growth at high incubation temperatures (9). The manufacturers claim that on CHROMagar Candida, *C. dubliniensis* appears as dark green colonies whereas colonies of *C. albicans* are light green. Tintelnot et al. (23) found that 56% of *C. dubliniensis* test strains gave dark green colonies, but it is generally agreed that this feature alone is not sufficient for differentiation of *C. dubliniensis* (21).

The 100% sensitivity and 100% specificity of CDA for detection of the *C.albicans-C. dubliniensis* and *C. tropicalis-C. kefyr* groups provide a significant advantage over Candida ID agar and CHROMagar Candida. A further advantage is that for strains of other important *Candida* spp. and non-*Candida* yeast strains, colony color was consistent (white). In contrast, for example, colonies of *C. glabrata* and *C. parapsilosis* strains were white or pink on Candida ID agar and purple, white and purple, pink, or gray on CHROMagar Candida (Table 1). In addition, a general difficulty in the use of CHROMagar Candida was that colonies of all of the test strains were various shades of blue, turquoise, purple, or pink. In contrast, on CDA colonies were strikingly red spotted, uniformly pink, or white. Colony colors on Candida ID agar (mostly turquoise, pink, or white) were also relatively easy to differentiate; however, a disadvantage of this agar is that plates must be incubated in the dark.

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