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Evaluation of CHROMagar Orientation for Differentiation and Presumptive Identification of Gram-Negative Bacilli and *Enterococcus* Species

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A new chromogenic plate medium, CHROMagar Orientation, was evaluated for use in the differentiation and presumptive identification of gram-negative bacilli and *Enterococcus* species by a multipoint inoculation (replicator) technique. In this study, 1,404 gram-negative bacilli and 74 enterococcal isolates were tested on CHROMagar Orientation. Six control American Type Culture Collection strains were also included with the testing to ensure quality control of the media. Of the *Escherichia coli* isolates ($n = 588$) tested, 99.3% produced a pink-to-red color. Only in four isolates that were *O*-nitrophenyl-β-D-galactopyranoside (ONPG) negative did this result differ. *Proteus mirabilis* and *P. vulgaris* were well differentiated on this medium. *P. mirabilis* ($n = 184$) produced a clear colony with diffusible brown pigment around the periphery. By contrast, 15 of 16 *P. vulgaris* isolates produced bluish-green colonies with a slight brown background. All *Aeromonas hydrophila* isolates ($n = 26$) tested produced clear to pink colonies at 35 to 37°C. This colony color changed to blue after 2 to 3 h of incubation at room temperature. *A. hydrophila* exhibited stronger color and better growth at 30°C. *Serratia marcescens* ($n = 29$) demonstrated an aqua blue color that deepened to a darker blue when exposed to room temperature. All enterococcal isolates ($n = 74$) resulted in a blue color and gave pinpoint colonies on purity subcultures at 35 to 37°C after 18 h of incubation. Similarity in color resulted in failure to discriminate accurately between *Klebsiella*, *Enterobacter*, and *Citrobacter* species. However, these species could be readily differentiated from other members of the family *Enterobacteriaceae*. *Pseudomonas aeruginosa* ($n = 151$) was easily differentiated from members of the *Enterobacteriaceae* but was less easily distinguishable from other gram-negative nonmembers of the *Enterobacteriaceae*. The medium was found to facilitate easy visual detection of mixed bacterial isolates in culture. When used in a replicator system, it easily detected mixed growths of organisms which may have otherwise led to false antibiotic susceptibility results. These mixed growths were not obvious on the routine susceptibility testing medium (Isosensitest).

Infections caused by gram-negative bacteria and *Enterococcus* spp. continue to be a major problem for hospitalized patients (15, 17). Increasing resistance of these organisms to conventional broad-spectrum therapy has extended patient hospital stay and placed additional demands on infection control teams to isolate patients and institute appropriate infection control measures (1, 2, 17). Rapid identification of the infecting organism provides useful information to the clinician for appropriate antibiotic choice prior to organism susceptibilities being available and can alert infection control teams to potential outbreaks (15, 22).

Since 1905 the most widely used medium in the clinical laboratory for the isolation and differentiation of coliform organisms and enteric pathogens has been the MacConkey plate (13, 15). MacConkey agar differentiates gram-negative bacteria by determining lactose utilization with a neutral red indicator. Red or pink to colorless colonies are produced depending on the ability of the isolate to ferment lactose. Careful observers are often able to recognize mixtures of different gram-negative bacteria when they occur on a single plate, but the absence of any differential genus-specific indicator property in the MacConkey agar means that there is no guarantee that mixed coliform cultures are always detected. CHROMagar Orientation is one of a number of new chromogenic agars (5, 9, 10, 18, 19, 21). It claims to facilitate and expedite the identification of some gram-negative bacteria and some gram-positive bacteria on the basis of different contrasted colony colors produced by reactions of genus- or species-specific enzymes with a proprietary chromogenic substrate.

The present study aims to establish a color catalog and to describe bacterial colony morphology on CHROMagar Orientation to presumptively identify commonly isolated gram-negative bacteria and *Enterococcus* spp. from hospitalized patients at the Concord Repatriation General Hospital.

(This paper was presented in part at the Annual Scientific Meeting of the Australian Society for Microbiology, Canberra, Australian Capital Territory [15a].)

MATERIALS AND METHODS

CHROMagar Orientation, a proprietary product, was provided for evaluation by the CHROMagar Company, Paris, France, and imported to Australia by DUTEC Diagnostics (a division of DUTEC Pty. Ltd.). The medium (15 g of agar per liter; 16 grams each of peptone, meat extract, and yeast extract per liter; special chromogenic mix, pH 7) was supplied as a white powder in preweighed batches for the preparation of 250-ml or liter volumes. The medium was prepared according to the manufacturer's instructions. Powdered CHROMagar Orientation is added to distilled water and dissolved by slow rotation; when dissolved the medium is boiled under continuous stirring. The medium can also be prepared with a boiling water bath or with flowing steam in an unpressurized autoclave (100°C). After boiling, the medium is swirled gently and cooled to 45°C. Then, 25 ml of the medium was dispensed into sterile petri dishes (85 mm),

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allowed to solidify, and then stored at 4°C in a dark container and used within 1 month. The medium does not require sterilization by autoclaving. Plates should be well dried (plate lids kept ajar) for up to 2 h preferably in the dark before use, and incubation at 35–37°C should be in air in the dark. Plates are read after 16 to 24 h of incubation as instructed by the manufacturer.

Bacterial strains. A total of 1,478 isolates, comprising 1,404 gram-negative bacilli and 74 enterococcal isolates, were screened for their abilities to grow and for their colony colors on CHROMagar Orientation. The isolates used in the trial were obtained from routine clinical specimens from hospitalized patients at the Concord Repatriation General Hospital. Twenty-three strains of *Aeromonas hydrophila* tested were donated by the University of Technology, Sydney; these comprised 15 environmental strains and 8 clinical isolates.

Control strains *Enterobacter cloacae* ATCC 13047; *Escherichia coli* ATCC 35218; *Enterobacter faecalis* ATCC 29212; *Proteus vulgaris* ATCC 6380; *Pseudomonas aeruginosa* ATCC 9721; and *Salmonella typhimurium* ATCC 13311 were used to continuously quality control the media and assess color stability. That is, at least three control American Type Culture Collection (ATCC) strains were tested for each test run, and all six ATCC control strains were tested for each new batch reconstituted.

Control mixtures were made by emulsifying isolates of different ATCC strains (one or more isolated colonies) into 5 ml of trypticase soy broth (no. 11768; BBL, Cockeysville, Md.) incubated 4 h at 35 to 37°C.

Bacterial identification. All isolates from members of the family *Enterobacteriaceae* were identified by a modified Mast replicator identification (Mast International Ltd., Merseyside, United Kingdom) method (11). This is an agar-based system in which biochemical substrates are incorporated into the agar and substrate utilization or enzyme reactions are manifested by color change. Each agar plate can be challenged with a number of different isolates by using a multipoint inoculation technique (replicator). The medium was reconstituted, and reactions were interpreted according to the manufacturer's instructions. Any questionable results were confirmed with an API 20E reference profile system (Biomérieux, Marcy-l'Etoile, France), with tests performed as instructed by the manufacturer. Nonfermenters were identified with an API 20NE reference profile system (Biomérieux systems). The enterococcal isolates in this survey were initially tested for esculin hydrolysis or Lancefield antigen grouping (Oxoid, Unipath Limited, Basingstoke, Hampshire, England). Isolates that were esculin positive or group D were identified by using an API Strep reference profile system (Biomérieux systems). *Salmonella* spp. were further sent to a reference laboratory, the Institute of Clinical Pathology and Medical Research (ICPMR)–Westmead Hospital, for confirmation of identification.

The oxidase test was performed with oxidase sticks (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) by following the instruction of the manufacturer. The indole spot test was performed as described by Vracko and Sherris (25). A confirmatory direct test was performed by washing pure cultures with sterile saline, placing a small amount of suspension in a test tube, and adding Kovacs reagent.

Multipoint inoculation technique. All isolates were emulsified in phosphate-buffered saline (pH 7.4) (BR14a, Dulbecco A tablets; Oxoid), and the suspension was standardized to contain 10^8 CFU/ml. A multipoint inoculation technique first described by Steers et al. (23) was used to inoculate the bacterial suspension onto the agar. In this case, Mast multipoint inoculator (Mast systems) was used to apply a maximum of 36 organisms with an inoculum of 10^4 CFU each to a CHROMagar Orientation plate and a MacConkey plate (Oxoid, CM7) to check organism viability. Inoculated plates were incubated in air at 35 to 37°C for 16 to 24 h in the dark prior to reading as instructed by the manufacturer.

Color estimation. Control organisms and representative isolate colors were compared with a Pantone international printing color chart. Color estimations were conducted independently by four of the authors, following which Pantone color reference numbers were agreed on independently. If discrepancies occurred, a further description was added for that species and more than one Pantone color reference number was given.

RESULTS

Appearance of isolates on CHROMagar Orientation. All organisms tested grew on CHROMagar Orientation plates and MacConkey agar incubated at 35 to 37°C for 16 to 24 h. There was a consistent color reaction observed for some species or genus, with discrepancies summarized in Table 1. Figure 1 demonstrates representative color reactions for selected organisms.

Of the total 1,478 isolates tested, 60% were correctly presumptively identified on CHROMagar Orientation by color and morphology alone (Table 1). The accuracy of identification increased to 98% when simple extra tests were performed in conjunction with or directly upon the chromogenic medium (Table 2).

Escherichia coli ($n = 592$) was the predominant isolate in the study. Of the *E. coli* strains tested, 99.3% produced a pink to red colony (Fig. 1, no. 1). Only four strains failed to produce this color, all four being *o*-nitrophenyl-β-D-galactopyranoside (ONPG) negative. *Proteus mirabilis* isolates ($n = 184$) produced clear colonies with diffusible brown pigment around the periphery of each colony. *Proteus vulgaris* strains ($n = 15$) demonstrated bluish-green colonies with a slight brown background (Fig. 1, no. 2). One isolate of *P. vulgaris*, however, demonstrated a reaction typical of *P. mirabilis. Morganella morganii* ($n = 29$) also exhibited clear colonies with a slight brown background which were difficult to differentiate from other *Proteus* spp. (Fig. 1, no. 3 and 12). *Pseudomonas aeruginosa* isolates ($n = 151$) resulted in characteristic transparent yellow to green colonies. Indeed this appearance proved useful in the identification of this organism. However, two strains of *Pseudomonas fluorescens* included in the study also displayed a transparent to yellow colony that was not easily distinguishable from *P. aeruginosa*.

Citrobacter spp., *Enterobacter* spp., and *Klebsiella* spp. regularly demonstrated a metallic blue-violet colony with or without a purple to pink halo (Fig. 1, no. 4, 5, and 6). Of *C. freundii* isolates, 67% produced a violet to purple colony with a pink border which made these strains easier to identify. *Serratia marcescens* strains ($n = 29$) appeared aqua blue in color. This color deepened to a darker blue (Fig. 1, no. 14) when exposed after 1 h during reading at room temperature. *Serratia liquefaciens* isolates ($n = 6$) produced the same color as *S. marcescens* but did not deepen in color when exposed to room temperature. Of 26 strains of *Aeromonas hydrophila* tested, all produced initially a clear or pink color after incubation at 35 to 37°C for 16 to 24 h, changing to a blue color (Fig. 1, no. 8) after 2 to 3 h at room temperature. Superior growth and deeper color were observed with this genus at 30°C. Three other strains presumptively identified as *Aeromonas sobria* produced a pink to red color at 35 to 37°C after 16 to 24 h of incubation; however, these three isolates retained their original color even after 7 days of incubation at room temperature (data not presented). All *Enterococcus faecalis* ($n = 68$) and *Enterococcus faecium* ($n = 6$) isolates produced a blue color (Fig. 1, no. 17) and grew as pinpoint isolated colonies (on purity subculture) at 35 to 37°C after 16 to 24 h of incubation.

Shigella boydii, *Salmonella* spp., *Stenotrophomonas maltophilia*, *Alcaligenes denitrificans*, and *Acinetobacter calcoaceticus* resulted in white to clear undifferentiated colonies. Single isolates of *Yersinia enterocolitica*, *Plesiomonas shigelloides*, and *Shigella sonnei* tested demonstrated blue, transparent pink, and pink-red colors, respectively (Fig. 1, no. 11, 13, and 9).

CHROMagar Orientation supports the growth of staphylococci, similar to MacConkey agar. The two species tested in this study, *Staphylococcus aureus* and *Staphylococcus epidermidis*, both grew as clear to white undifferentiated colonies.

This chromogenic medium also allowed easy visual detection of mixed cultures (Fig. 2) both from purity subcultures or when used as a primary medium in direct specimen plating.

Viabilities, color stability, and batch variations of organisms on CHROMagar Orientation. The viability of the organism was not lost during testing on CHROMagar. All control and test organisms grew equally well on CHROMagar Orientation and on the MacConkey agar. In the dark, color was stable; however, when exposed to light the color intensified, deepened, and in some cases diffused. Batch variation was minimal. Control strains showed no variations with each new lot tested.

Pantone color reference determination. The Pantone printers' color chart was used because of its universal color accep-

TABLE 1. Pigment reaction and presumptive identification of gram-negative and -positive bacteria on CHROMagar Orientation

Organism	Total no. tested	No. (%) with described color	Description of pigment	Pantone color chart reference no.[a]	No. of isolates		
					With correct presumptive identification[b]	Correctly identified with additional tests[c]	Not identified by CHROMagar Orientation[d]
Acinetobacter calcoaceticus	16	16	White nontransparent	Not referenced[e]			16
Aeromonas hydrophila	26	26	Initially clear to pink at 37°C changing to blue with clear edges at RT[f]	217C 271C	26		
Alcaligenes denitrificans	1	1	Clear	Not referenced			1
Citrobacter diversus	7	7	Light bluish green with diffused edges	280C		7	
Citrobacter freundii	15	10 (67)	Violet purple interior with pinkish border	271C		15	
		5 (33)	Metallic blue with strong purple pink halo (diffused)	280C/281C/286C			
Enterobacter aerogenes	15	15	Metallic blue (may have diffused edges)	281C		15	
Enterobacter agglomerans	2	2	Metallic blue	281C		2	
Enterobacter cloacae	52	44 (85)	Metallic bluish purple with strong purple-to-pink halo around periphery				
		8 (15)	Metallic bluish purple with no halo	281C			
Enterobacter sakazaki	1	1	Metallic blue with strong purple halo	281C/204C		1	
Enterococcus faecalis	68	68	Diffuse blue pinlike within agar (<24 h)	300C			68
Enterococcus faecium	6	6	Diffuse blue pinlike within agar (<24 h)	300C			6
Enterococcus spp.	74	74	Diffuse blue pinlike within agar (<24 h)	300C	74		
Escherichia coli	592	588 (99.3)	Pinkish to red	189C/192C	588		
		4 (0.7)	Clear	Not referenced		4	
Klebsiella oxytoca	18	18	Metallic blue with no pink halo	286C		18	
Klebsiella pneumoniae	228	169 (74)	Metallic blue with slight pink halo around periphery	280C		228	
		59 (26)	Metallic blue with no pink halo	280C			
Morganella morganii	26	29	Clear transparent—slight brownish background	4060U		29	
Plesiomonas shigelloides	1	1	Transparent pink	2050C			1
Proteus mirabilis	184	184	Clear with diffusible brown	468U		184	
Proteus vulgaris	16	15 (94)	Bluish green	341C	15		
		1 (6)	Clear with diffusible brown	468U		1	
Pseudomonas aeruginosa	151	151	Transparent, yellow to green-serated edges, diffused	5845C	151		
Pseudomonas fluorescens	2	2	Transparent, yellow	Not referenced			2
Salmonella							
Group B	2	2	Clear	Not referenced			2
paratyphi	1	1	Clear	Not referenced			1
potsdam	1	1	Clear	Not referenced			1
typhimurium	1	1	Clear	Not referenced			1
Serratia liquefaciens	6	6	Light aqua blue—may darken to navy blue with pigment production at RT	296C	6		
Serratia marcescens	29	29		296C/320C	29		
Shigella boydii	1	1	Clear-pale grey	Not referenced			
Shigella sonnei	1	1	Pink	189C/192C		1	
Stenotrophomonas maltophilia	5	5	Clear	Not referenced			5
Yersinia enterocolitica		1	Diffused blue at 30°C	300C			1

[a] Pantone Color Guide 747XR 1988–1989.
[b] Presumptive identification based on color and morphology on CHROMagar Orientation only.
[c] Identification with additional (one to three) rapid tests as described in Table 2.
[d] Could be identified only with a commercial kit or a larger number of tube tests.
[e] Clear and transparent colonies that could not be referenced by the color guide.
[f] RT, room temperature (~24°C).

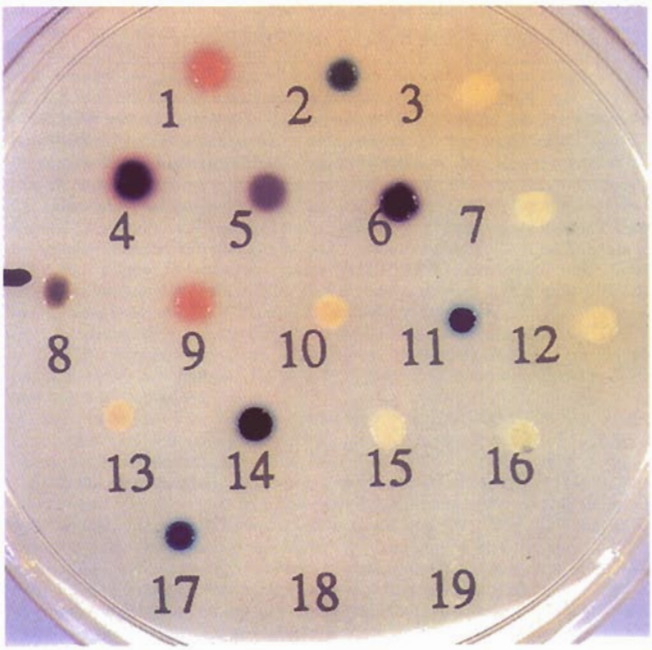

FIG. 1. Representative chromogenic reactions of selected organisms on CHROMagar. 1, *E. coli*; 2, *P. vulgaris*; 3, *P. mirabilis*; 4, *E. clocae*; 5, *C. diversus*; 6, *Klebsiella pneumoniae*; 7, *Salmonella typhimurium*; 8, *Aeromonas hydrophila*; 9, *Shigella sonnei*; 10, *P. aeruginosa*; 11, *Y. enterocolitica*; 12, *M. morganii*; 13, *Plesiomonas shigelloides*; 14, *Serratia marcescens*; 15, *A. calcoaceticus*; 16, *Stenotrophomonas maltophilia*; 17, *Enterococcus faecalis*.

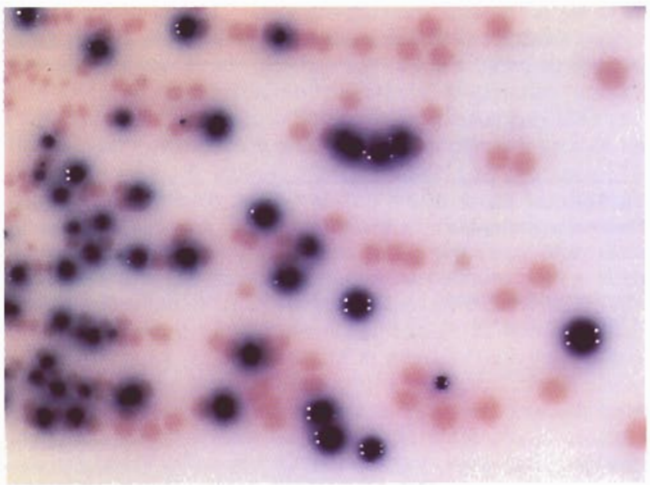

FIG. 2. Colonies plated from a mixed suspension, showing two different gram-negative isolates on CHROMagar. The two species can be distinguished by their color appearance. Pink-red, *E. coli*; blue, *Klebsiella pneumoniae*.

tance. The color colony reference was determined by two of the researchers and later confirmed by two other researchers. Where discrepancies occurred additional reference profile numbers were included (Table 1). Clear, white, and transparent colonies were not referenced because the color chart was not considered suitable for these colonies.

DISCUSSION

CHROMagar Orientation proved applicable for the differentiation and presumptive identification of certain bacterial genera and species (Table 1) on the basis of color and morphology alone. The ability of the agar to allow for simple added tests increased the overall accuracy of identification from 60 to 98% (Tables 1 and 2). Results compared favorably with those obtained by a proven biochemical identification method (11). In comparison with other *E. coli* strains tested, 99.3% gave good correlation with the chromogenic agar. The remaining 0.7% were ONPG-negative strains and were not differentiated by this medium and remained colorless. However, these isolates could be easily differentiated with the use of a spot indole test as described by Vracko and Sherris (25). One *E. coli* strain which was nutritionally dependent had no problems growing on the agar and gave the correct chromogenic reaction. It is probable that the peptone and yeast extract in the formulation may have provided additional enrichment for the organism to grow. This makes CHROMagar Orientation an attractive primary screening medium, considering that the majority of urinary tract infections are caused by *E. coli* and that this microbe is responsible for most nosocomial and community-acquired urinary tract infections (7, 8). On the basis of the enzyme substrate color characteristic reaction alone, the agar may be employed for the specific detection of *E. coli* from water, food, and biological fluids (12). The only other species tested in this

TABLE 2. Analysis of colored bacterial strains needing extra biochemical tests for correct identification to species level

Species	No. of strains	Color	Pantone color code	Type and key test reaction[a]
Escherichia coli	4	Clear	NR[b]	ONPG−ve,IND+ve
Shigella sonnei	1	Pink-red	189C-192C	IND−ve, serology
Klebsiella pneumoniae	228	Blue with or without halo	280C	LDC+ve,ODC−ve,IND−ve
Klebsiella oxytoca	18	Blue with or without halo	286C	LDC+ve,ODC−ve,IND+ve
Enterobacter cloacae	52	Blue with or without halo	281C/204C	LDC−ve,ODC+ve,IND−ve
Enterobacter agglomerans	2	Blue with or without halo	281C	LDC−ve,ODC−ve,IND+/−*
Enterobacter aerogenes	15	Blue with or without halo	281C	LDC+ve,ODC+ve,IND−ve
Enterobacter sakazaki	1	Blue with or without halo	281C/204C	LDC−ve,ODC−ve,IND−ve,SORB−ve
Citrobacter freundii	15	Blue with or without halo	271C/280C/281C	LDC−ve,ODC−ve,IND−ve
Citrobacter diversus	7	Blue with or without halo	280C	LDC−ve,ODC+ve,IND+ve
Proteus mirabilis	184	Diffused brown	468U	IND−ve
Morganella morganii	29	Diffused brown	4060U	IND+ve
Plesiomonas shigelloides	1	Transparent pink	2050C	OX+ve,IND+,MAN−ve
Pseudomonas aeruginosa	151	Transparent yellow-green	5845C	OX+ve

[a] Key reaction codes: IND, spot indole test; ONPG, *o*-nitrophenyl-β-D-galactopyranoside; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; MAN, mannitol fermentation; SORB, sorbitol fermentation; OX, cytocrome oxidase test; +ve >90%; −ve, <10%; +/−, 50%; *, variable; serology, latex agglutination sera.

[b] NR, not referenced.

study with a similar pink to red color was *Shigella sonnei*. The frequency of isolation of this microorganisms in the routine laboratory is very low; however, it will make CHROMagar Orientation less useful as a screening agar for *Shigella* infections.

Klebsiella spp., *Enterobacter* spp., and *Citrobacter* spp. gave a common color reaction. Other biochemical data were needed to differentiate them to a species level (Table 2).

Proteus mirabilis and *Proteus vulgaris* are common bacterial isolates in patients with indwelling urinary catheters (3, 12). CHROMagar Orientation gave good differentiation between these two species without further biochemical testing proving necessary. *P. mirabilis* and *M. morganii* were differentiated by the use of an indole spot test.

The chromogenic agar supported the growth of different *Pseudomonas* spp. Apart from the pyocyanin production of *Pseudomonas aeruginosa*, it was otherwise difficult to distinguish chromogenically among different species of pseudomonas. Our findings suggest that the medium should not be used to try to distinguish among different nonfermentative organisms. Clear colonies need to be identified further by other means.

Compared to results with the MacConkey agar, *Aeromonas hydrophila* isolates were distinct from *Pseudomonas* spp. on CHROMagar Orientation and were easily differentiated by color. von Grarvenitz (24) suggests that some strains of *Aeromonas* spp. are biochemically more active at 22°C than at 37°C. As a result we further compared growth of *Aeromonas* spp. on CHROMagar Orientation, and we found that growth and color were better at 30°C than at 37°C (data not presented).

Enterococcal isolates were chromogenically distinct on CHROMagar Orientation. The two most common encountered species, *E. faecalis* and *E. faecium*, gave similar color reactions and were indistinguishable at the species level. In a recent study enterococci represented the third most common cause of hospital-acquired bacteremia (6, 22). Detection and presumptive identification of enterococci at the genus level is important for appropriate treatment of certain infections, especially with the emergence of enterococci particularly of vancomycin-resistant strains, as important nosocomial pathogens (4, 6, 16).

In this study, CHROMagar Orientation medium permitted a good discrimination of some of the common species of bacteria when in mixed culture in clinical specimens after direct plating from urines, biliary fluids, blood cultures, and wound swabs. No interference in color development was found from mucus or other cellular material during primary direct plating. The medium also prevented the spreading of *Proteus* spp. and mucoid *E. coli* and *K. pneumoniae* strains which often mask underlying growth. Further evaluation of this medium as a direct isolation medium is required before comprehensive statements as to its suitability can be made. Nevertheless, preliminary work is encouraging, and the medium is well suited for use in a multipoint inoculation system. Colors of colonies are stable in the dark, but changes were observed when they were exposed to light for a period of time. Colony color became more diffusible and color toning deepened, altering the originally noted color. Manufacturer's instructions should be adhered to for color reproducibility and accuracy. This means CHROMagar Orientation plates should be taken out of the dark environment just prior to initial reading and then left at room temperature to detect any color changes occurring with time (e.g., *Aeromonas hydrophila* initially showing a pink color and changing to blue as described in Table 1.).

CHROMagar orientation has the added advantage over MacConkey agar of having substrates which allow greater differentiation of gram-negative bacilli and *Enterococcus* spp. in culture. The distinct color exhibited by some species, together with a few extra tests (Table 2), allows visual presumptive identification to be made of some isolates. The major disadvantages of this medium are as follows: (i) it is a light-sensitive medium, and the manufacturer's strict guidelines on preparation, incubation and reading should be followed; (ii) as pointed out by Odds and Bernaerts (18), use of this medium by laboratory staff afflicted with various types of color blindness may provide difficulties in distinguishing differences in color and colony appearance; and (iii) no colony color charts are currently available for this medium; therefore there may be variability among different laboratory in-house color charts.

In summary, CHROMagar Orientation appears to be a medium well suited for the bacteriological laboratory. It can serve as a primary isolation and differentiation medium for clinical isolates from various specimen sites. Introduced in a replicator multipoint inoculation identification and antibiotic susceptibility testing system, it helps in the (i) detection, presumptive identification, and differentiation of some gram-negative bacilli, especially when questionable biochemical data are obtained, and (ii) detection of mixed bacterial cultures, so that identifications and antibiotic sensitivities are more accurately reported. The "chromogenic mix" of substrates in the agar appear to be enzymatically both genus and/or species specific for some commonly encountered gram-negative bacilli and enterococcal species. Less frequently encountered species of *Enterobacteriaceae* and of *Enterococcus* spp. need to be further investigated on this new medium before an accurate account of their color reaction can be made. CHROMagar Orientation is useful in the interpretation of culture results without complete identification of the microorganism. With this in mind, we still emphasize that species identification and selection of differential characteristics on a primary medium are only a screening procedure and can never serve as a substitute for proper and full identification tests (18).

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