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Chromogenic Agar Media in the Clinical, Food, and Environmental Testing Arenas, Part I

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Abstract

Chromogenic agar media have become useful tools in the laboratory for the rapid recovery and identification of specific microbial species or the isolation of bacteria that can be identified at the genus level. A wide variety of chromogenic media are currently available and target the pathogenic microorganisms that are important in both clinical and environmental testing laboratories. The greatest utility of chromogenic agar in the clinical microbiology laboratory revolves about the recovery of methicillin-resistant *Staphylcoccus aureus* and gram-negative urinary pathogens. The greatest utility of the chromogenic agar in the environmental laboratory revolves about the recovery of gram-negative pathogens, such as *Escherichia coli* O157 and *Salmonella* spp. from water, foods, and the environment. In Part I of this two-part article, the development of chromogenic agar medium and its practical utility for the detection and identification of *Candida* spp. and the *Enterobacteriaceae* are discussed.

Introduction

The classical approach to differentiating and identifying bacterial colonies growing on an agar medium involved a phenotypic array of individual observable characteristics and simple biochemical tests. Colonies were described by these characteristics and then subjected to tests that were relatively rapid, taking less than 30 minutes to perform or requiring overnight incubation in individual biochemical broth tubes to assess utilization. The "pigmentation" of the growing colony (1) was one of the first important observable clues to use in the process of identifying a bacterial colony, in addition to colony size, shape, edge properties, presence and amount of surface polysaccharide, and the selective medium upon which the colony was growing.

Colony color expression in the absence of selective pressure can be misleading, as different genera of microorganisms can produce very similar color hues. For example the pale yellow hues of *Micrococcus luteus* and *Staphylococcus aureus*, the purple hues of *Serratia* spp., the various shades of green and red for the *Pseudomonas* spp., and the natural sulfide-black color of *Salmonella enterica* serovar Typhimurium have long been used as a guide for preliminary identification. Virtually all bacterial colonies display of varying shades of white, off-white, or ivory, the distinctions of which can be confounding even to an experienced clinical microbiologist.

The commercial introduction of chromogenic agar media (CAM) occurred in the 1990s. The basic scientific principles underlying this product relied on the use of selective media, a proprietary carbohydrate substrate-chromogen complex, and targeted bacterial uptake. Essentially, the carbohydrate substrate complex is enzymatically hydrolyzed by the microorganism, and the residual chromogen accumulates in the bacterial cells, leading to a pigmented colony by which the selected microorganism can be named at the genus or species level within a statistically reliable confidence interval.

Objective

The aim of this review article is to describe succinctly the various uses and applications of CAM in clinical, food, and environmental testing laboratories as useful, efficient, and accurate agar medium tools for the isolation of specific microorganisms. Secondly, among the results and conclusions expressed

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by the majority of investigators in the articles cited below is the consistent idea that the traditional microbiological approach to growth, isolation and identification of bacteria can be simplified and achieved more rapidly with the use of CAM than with conventional microbiological agar media.

Media

The basal composition of CAM consists of proprietary mixtures of high concentrations of carbohydrate and peptone and selective concentrations of agents such as NaCl or classes of antibiotics active against gram-positive or gram-negative bacteria. The medium formulations can be reviewed on-line in the manufacturer's product inserts.

Biochemistry

Bromine and chlorine form halogenated compounds with indoxyl in single-, double- or triple-substituted positions. Bromoindoxyl, chloroindoxyl, or bromochloroindoxyl and other derivatives are the common chromogens bound to carbohydrate substrates used in CAM. The carbohydrate substrate-chromogen complex is metabolized by the bacterial isolate via enzymatic hydrolysis, the carbohydrate is utilized, and the halogenated indoxyl compounds remain to emit their color. Two or more carbohydrate substrate-chromogens may be incorporated. Enzymatic hydrolysis is dependent on a number of bacterial enzymes, including β-galactosidase, β-glucosidase, β-glucuronidase, α-glucosidase, α-galactosidase, α-mannosidase, *N*-acetyl-β-glucosidase, *N*-acetyl-β-galactosidase, sulfatase, esterase, lipase, peptidase, and phosphatase.

The Uses for Chromogenic Agar Media and Types of Products Available

CAM have been used in all phases of microbiological investigation, including clinical isolation and identification and food, water, and environmental testing. There are many varieties of CAM offered by several manufacturers. A brief summary of the manufacturers and their products is shown in Table 1. This review article will focus on a few of the research reports in which CAM have assisted in the scientific expansion of knowledge in the clinical and non-clinical arenas. The manufacturer of the CAM and the location where the medium was manufactured will be stated (if described in the research report) in order to aid the reader in distinguishing between the products, the study objectives, and the results obtained by the various investigators.

Use of Chromogenic Agar Media to Identify *Candida* spp. in Clinical Specimens

The first report of the use of a CAM to identify clinically relevant yeasts involved CHROMagar Candida (CC) (CHROMagar, Paris, France); the authors, Odds and Bernaerts (2), obtained greater than 99% sensitivity and specificity of the new medium for the presumptive identification of *Candida albicans*, *Candida krusei*, and *Candida tropicalis* when evaluated against growth of the same isolates on Sabouraud's Dextrose Agar (SDA). Seven hundred twenty-six isolates representing 33 strains of *Candida* spp. were used, including stock strains and clinical specimens obtained from vaginal, oral, ano-rectal, nail, and skin sites. Distinct colors were detected for *C. albicans, C. tropicalis, Candida norvegensis, C. krusei, Trichosporon* spp. and *Geotrichum* spp. The authors also showed that the isolates could be subcultured from the CC agar medium back to the SDA medium without loss of viability. Using CC agar medium, Beighton et al. (3) showed that *Candida* spp. could be identified in patients' dental specimens, including oral rinses from denture wearers, patients with dry-mouth syndrome, and HIV-infected patients; palate swabs; and dental-root caries lesions. Pfaller et al. (4) showed the utility of the CC medium on stock yeast isolates and the recovery of yeast from patients' stool specimens and argued that the simultaneous identification of different species of *Candida* would facilitate improved appropriate anti-fungal therapy for the patient. The first comparative study among these CAM was reported in 1996 by Baumgartner et al. (5) in France using CC, Albicans ID (BioMérieux, Marcy l'Etoile, France), and Sabouraud-Chloramphenicol agar plate (Diagnostics Pasteur, Marnes la Coquette, France). The authors showed that both CAM had higher detection rates for 615 isolates of *Candida* spp. from 951 clinical specimens than the Sabouraud-Chloramphenicol agar medium formulation. The authors noted that yeast colonies were smaller on Albicans ID after 24 h of incubation but that the medium far surpassed CC in identification of *C. albicans* at 24 h; however, this distinction was minimized after 48 h of incubation. The CC medium performed better than Albicans ID medium after 72 h of incubation for overall number of yeasts isolated.

Candida dubliniensis was identified by Kirkpatrick et al. (6) in a separate report as having a darker-green colony appearance on primary CC agar medium, distinguishable from the light-green appearance of *C. albicans*. *C. dubliniensis* is associated with oral candidiasis in HIV-infected patients but can be confused phenotypically as an atypical-appearing *C. albicans* using traditional growth approaches. Fifty-one dark-green isolates were subjected to extensive phenotypic and genotypic assays for confirmation as *C. dubliniensis*. Twenty-three were confirmed as *C. dubliniensis*, and the other 28 were

Table 1. Manufacturers' chromogenic agar media[a]

Pathogen or target organism	Application							
	BD BBL[b]	BL Rainbow[c]	BM chromID[d]	BR Select[e]	Rambach CHROMagar[f]	Hardy HardyChrom[g]	Oxoid Brilliance[h]	Remel Spectra[i]
Clinical								
Acinetobacter					■			
Candida	■		■	■	■	■	■	
Clostridium difficile			■					
Escherichia coli O157	■				■			
Extended spectrum beta-lactamases (ESBL)					■	■	■	
CTX-M ESBL					■			
Carbapenemase (KPC)					■	■		
Methacillin-resistant *Staphylococcus aureus* (MRSA)	■		■	■	■	■	■	■
Urine pathogens	■		■		■	■	■	■
Urine pathogens (biplate)	■					■		■
Pseudomonas			■		■			
Salmonella	■		■		■	■	■	
Salmonella-Shigella						■		
Shiga-toxigenic *E. coli* (STEC)					■			
S. aureus	■		■	■	■	■		
Streptococcus B			■		■			
Vibrio			■					
Vancomycin-resistant enterococci (VRE)			■		■	■	■	■
Industrial								
Bacillus cereus							▲	
Cronobacter sakazakii			▲			▲	▲	
CampyCount							▲	▲
E. coli	▲				▲			
E. coli O157	▲	▲	▲		▲	▲		
E. coli coliform ECC	▲		▲		▲	▲	▲	
Enterococci	▲							
Listeria	▲		▲		▲	▲	▲	
Pseudomonas					▲			
Salmonella	▲	▲	▲		▲	▲	▲	
S. aureus	▲				▲	▲	▲	
Shigella-Aeromonas		▲						
Shiga toxin *E. coli*					▲			
Vibrio					▲			

[a] ■, Clinical applications; ▲, food, water and environmental testing applications.
[b] BBL is the trademark of Becton, Dickinson and Company, Sparks, MD.
[c] Rainbow is the trademark of Biolog, Inc., Hayward, CA, (BL).
[d] chromID is the trademark of bioMérieux Corporation, Marcy l'Etoile, FR.
[e] Select is the trademark of Bio-Rad Laboratories, Hercules, CA.
[f] CHROMagar is a trademark of Dr. A. Rambach, Paris, FR.
[g] HardyChrom is the trademark of Hardy Diagnostics, Santa Maria, CA.
[h] Brilliance is the trademark of Oxoid Diagnostics, Cambridge, UK.
[i] Spectra is the trademark of Remel Diagnostics, Lenexa, KS.

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confirmed as *C. albicans*. In 2001, Jabra-Rizk et al. (7) published a comparison study assessing the difference between the original formulation of CC medium and a re-formulation by BBL, also called CC (Becton Dickinson BBL, Cockeysville, MD). Approximately 90 stock yeast isolates and 522 clinical specimens were examined, from which 173 yeast species were recovered. The CAM were equivalent in performance as defined by examination of colony growth rates and size, but there appeared to be little difference in color development to distinguish yeast species by colonial growth at 24 h. Plates were re-incubated for an additional 24 h, at which point the authors reported that colonies growing on the BBL re-formulation were larger and deeper in color intensity. Willinger et al. (8) reported upon the results of their study comparing a re-formulation of Candida ID (BioMérieux) to CC. They used colony morphology and pigmentation as comparative criteria. Four hundred twenty-four yeast isolates were recovered from 596 clinical specimens. At 24 h of incubation, the sensitivity of Candida ID was 96.8% compared to a sensitivity of 49.6% with CC. After 48 h, the statistical sensitivity gap closed to 99.7 and 98.9%. The authors indicated that the color intensity was more easily distinguishable at 24 h with Candida ID than with CC.

Hospenthal et al. (9) addressed a practical issue for mycology laboratories with part-time staffing schedules. They examined color development in the reading of mycology cultures growing on CC (DRG International, Mountainside, NJ.) daily for 7 days of incubation. They determined that color intensity did not change for the majority of *Candida* spp. The authors stated that *C. albicans* and *C. krusei* were easily distinguishable after 24 h of incubation but that incubation, examination, and interpretation of colonies growing on the medium could be extended out to 7 days with reliability.

Direct detection of *Candida* spp. was reported by Horvath et al. (10) in an analytical study showing that CC could be used for direct isolation of yeast from blood cultures. Fifty strains of *Candida* spp. were inoculated into blood cultures including: *C. albicans* (*n* = 12), *Candida tropicalis* (*n* = 12), *C. glabrata* (*n* = 9), *C. krusei* (*n* = 5), *Candida lusitaniae* (*n* = 3), *Candida parapsilosis* (*n* = 3), *Candida guilliermondii* (*n* = 2), *Candida kefyr* (*n* = 2), *Candida firmetaria* (*n* = 1),and *Candida rugosa* (*n* = 1). Each strain was used as its own control by growth on SDA and subculture to CC. Plates were compared for visual color disparities, but there were none. Murray et al. (11) showed that the CC medium (Becton Dickinson, Sparks, MD) could be used for the direct isolation of yeast from throat, urine, and genital specimens. In addition, they obtained 69 "signal-positive" clinical blood culture specimens that were shown to be "smear positive" for the presence of yeast and were subcultured onto CC and SDA. All yeast isolates recovered from blood grew on both medium types. Tan and Peterson (12) similarly showed that CC (BD Diagnostics, Sparks, MD) could be used for direct susceptibility testing of yeasts isolated from blood cultures. Ninety-five stock and clinical specimens were seeded into blood culture bottles monitored on an automated blood culture instrument. An aliquot of signal-positive blood culture medium was used to produce a confluent lawn of bacteria on CC agar medium plates, and a 25-µg disc of fluconazole was affixed. Zones of inhibition were read and compared to both standardized disc diffusion and minimal inhibitory concentration (MIC) methods specifically for the determination of fluconazole resistance. The results for direct susceptibility obtained with CC compared favorably to standardized disc diffusion and MIC methods. The authors concluded that direct susceptibility testing with CAM had the potential to provide rapid and clinically useful information but stopped short of recommending routine use due to difficulties in the comparison of interpretation zones and MICs observed with *C. glabrata* and *C. paropsilosis*.

Use of CAM to Identify *Enterobacteriaceae* spp. in Clinical Specimens

CHROMagar Orientation (CO) (Becton Dickinson, Heidelberg, Germany), was designed to differentiate the aerobic gram-negative bacilli and the two major *Enterococcus* spp. from clinical specimens. In an article published in 1996 by Merlino et al. (13), over 1,400 gram-negative bacilli and 74 enterococci were tested. Previously well-characterized and identified microbial isolates were tested for their expected color development on CO, and positive percentages of agreement were calculated. The authors concluded that the medium was suitable for use in the clinical microbiology laboratory for primary isolation and differentiation of the most commonly encountered gram-negative bacilli but that less frequently isolated gram-negative bacilli and *Enterococcus* spp. required further investigation.

In an article by Hengstler et al. (14), 658 urine specimens were evaluated for colony color development on CO () and the results were compared to a traditional rapid indole spot test or conventional commercial biochemical identification panels. Growth of gram-negative bacilli and *Enterococcus* spp. on CO was shown to outperform similar growth on Columbia agar with 5% sheep blood, MacConkey agar, and CPS ID2 medium. It was noted that recovery of the *Proteus-Morganella-Providentia* group of microorganisms was not as robust on CO as on traditional agar media. All cultures were compared at $\geq 10^5$ and $\leq 10^5$ CFU/ml concentration breakpoints.

BBL CO medium (Becton Dickinson) was compared with a standard two-plate setup for urine cultures by D'Souza et al. (15). Over 1,000 consecutive urine specimens were sampled with a 1-µl calibrated loop, followed by inoculation onto tryptic soy agar with 5% sheep blood, MacConkey agar, and CO medium. There were 250 positive cultures, 199 of which were an exact match on all media. However, there were an additional 51 discrepant culture results. Forty of the 51 discrepant results consisted of pure isolate culture results; 28 of these results were correctly identified by the CO agar medium, and 12 of the results were correctly identified by conventional media. The remaining 11 mixed-isolate culture results yielded discrepant findings, but the majority of the correct isolate identifications were obtained by use of the CO compared to the conventional media. Additionally, automated susceptibility test results were compared between the microorganisms picked from the traditional media and the CO medium. There were 0.4% major errors and 2% minor errors

detected. The authors calculated cost savings and labor efficiencies with CO medium compared to the conventional media and concluded that the use of CO in the clinical microbiology laboratory was faster than the conventional methods, decreased overall labor output related to interpretation, and resulted in an overall cost saving by reducing individual reagent costs.

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