QUALITY CONTROL PROCEDURES

I INTRODUCTION

BBL™ CHROMagar® O157 is a selective medium for the isolation, differentiation and presumptive identification of *Escherichia coli* O157:H7.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Streak inoculate with 10^3-10^4 CFUs of *E. coli* ATCC 700728, 35150 and 43895 and 10^4-10^5 CFUs of all other organisms.
   b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
   c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for amount of growth and color formation.
3. Expected Results

Organisms	ATCC™	Recovery	Colony Color
Aeromonas hydrophila	7965	Inhibition (partial to complete)	Colorless to light yellow
**Enterobacter cloacae*	13047	Fair to heavy growth	Blue-green to blue
Enterococcus faecalis	29212	Inhibition (partial to complete)	Blue
**Escherichia coli* O157:H7	700728	Fair to heavy growth	Light mauve to mauve
**Escherichia coli*	25922	Inhibition (partial to complete)	Blue
Escherichia coli	35150	Fair to heavy growth	Light mauve to mauve
Escherichia coli	43895	Fair to heavy growth	Light mauve to mauve
Klebsiella pneumoniae	33495	Inhibition (partial to complete)	Blue
Proteus mirabilis	12453	Inhibition (partial to complete)	Colorless, may have orange-brown precipitate

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under “Product Deterioration.”
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification 7.4 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BBL™ CHROMagar® O157 is a selective medium for the isolation, differentiation and presumptive identification of *Escherichia coli* O157:H7 from human clinical stool specimens.

V SUMMARY AND EXPLANATION

E. coli O157:H7 is the most frequently isolated pathogen from bloody stools.[1] However, absence of bloody diarrhea does not rule out the presence of *E. coli* O157:H7.[2] This serotype causes a broad range of illness from mild non-bloody diarrhea to severe bloody diarrhea (hemolytic colitis), hemolytic uremic syndrome and death.[1] The isolation of *E. coli* O157:H7 exceeds that of some other common enteric pathogens, especially *Shigella* in many areas and age groups. Transmission most often occurs through ingestion of raw or undercooked beef; other foods have also been implicated.[1] In addition, transmission may occur person to person, as well as from recreational water sources.[1]

CHROMagar O157 is intended for the isolation, differentiation and presumptive identification of *E. coli* O157:H7. Due to the chromogenic substrates in the medium, colonies of *E. coli* O157:H7 produce a mauve color, thus allowing presumptive identification from the primary isolation plate and differentiation from other organisms. In samples with low numbers of *E. coli* O157:H7, enrichment methods may be helpful prior to inoculating medium.

VI PRINCIPLES OF THE PROCEDURE

CHROMagar O157 was originally developed by A. Rambach, CHROMagar, Paris, France. BD, under a licensing agreement, has optimized this formulation utilizing proprietary intellectual property used in the manufacturing of the **BBL CHROMagar** O157 prepared plated medium.

Specially selected **Difco™** peptones supply the nutrients. The addition of potassium tellurite, cefixime and cefsulodin reduces the number of bacteria other than *E. coli* O157:H7 that grow on this medium. The chromogen mix consists of artificial substrates (chromogens), which release an insoluble colored compound when hydrolyzed by a specific enzyme. *E. coli* O157:H7 utilizes one of the chromogenic substrates producing mauve colonies. The growth of mauve colonies is considered presumptive for *E. coli* O157:H7 on **BBL CHROMagar** O157. Non-*E. coli* O157:H7 bacteria may utilize other chromogenic substrates resulting in blue to blue-green colored colonies or, if none of the chromogenic substrates are utilized, colonies may appear as their natural color. This facilitates the detection and differentiation of *E. coli* O157:H7 from other organisms.

VII REAGENTS

BBL CHROMagar O157

Approximate Formula* Per Liter Purified Water

Chromopeptone	16.0 g	Cefixime	0.05 mg
Sodium Chloride	7.0 g	Cefsulodin	4.0 mg
Chromogen Mix	0.65 g	Agar	14.0 g
Potassium Tellurite	2.5 mg		

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation. Protect from light during drying. See Storage Instructions.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[3-6] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

As with any selective prepared culture medium, not all organisms (i.e. *E. coli* O157:H7) may grow on the medium due to factors associated with inoculation, recovery or inhibition. The result of this test is not definitive and should be evaluated in coordination with the physiological symptoms of the patient.

Storage Instructions: On receipt, store plates in the dark at 2–8°C in original sleeve wrapping and original cardboard box until time of inoculation. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure of **BBL CHROMagar** O157 to light both before and during incubation, as light may destroy the chromogens. Prepared plates may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII PROCEDURE

Material Provided: BBL CHROMagar O157

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and other laboratory equipment as required.

Specimen Collection and Handling: For human stool use, refer to lab procedures for details on specimen collection and handling procedures.

Test Procedure: Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture.

For clinical specimens, as soon as possible after receipt in the laboratory, inoculate onto a **BBL CHROMagar** O157 plate and streak for isolation. If the specimen is cultured from a swab, roll the swab over a small area of the surface at the edge, then streak from this area with a loop. Incubate plates aerobically at 35 ± 2°C for 18–24 h in an inverted position (agar-side up). Plates are not to be incubated beyond the 24 h time period prior to reading. Interpretation of plate results must be completed within 18–24 h after inoculation of the **BBL CHROMagar** O157 plate.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the clinical user refer to pertinent Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines for appropriate Quality Control practices.

IX RESULTS

After proper incubation, read plates against a white background. Interpretation of plate results must be completed within 18–24 h after inoculation of the **BBL CHROMagar** O157 plate. *E. coli* O157:H7 will produce mauve-colored colonies on **BBL CHROMagar** O157 medium. All mauve colonies should be confirmed biochemically and/or serologically prior to reporting as *E. coli* O157:H7.[1] Gram-positive organisms should be completely inhibited. Gram-negative organisms, other than *E. coli* O157:H7, will either be inhibited or produce colorless, blue, green, blue-green (aqua) or natural color colonies.

X LIMITATIONS OF THE PROCEDURE

BBL CHROMagar O157 does not detect enterohemorrhagic or enteropathogenic serotypes of *E. coli* other than O157:H7, since they may differ biochemically. β-glucuronidase-positive strains of *E. coli* O157:H7 will not be detected on **BBL CHROMagar** O157; however, such strains are rare.

BBL CHROMagar O157 does not differentiate between toxin-producing and non-toxin-producing strains of *E. coli* O157:H7.

Organisms other than *E. coli* O157:H7, such as *Proteus* spp. may grow on this medium; however, they generally produce a different color. If unisolated mauve colonies are observed, isolation can be achieved by subculturing to another **BBL CHROMagar** O157 plate. Rare strains of *E. coli* (biochemically similar to *Shigella*) have been found that produce false positive results on **BBL CHROMagar** O157.

Internal cross reactivity testing has demonstrated that *Salmonella* serotype Heidelberg exhibited mauve colonies when plated on **BBL CHROMagar** O157 medium. As recommended, all mauve colonies should be confirmed by biochemical or serological testing prior to reporting results.

Confirmatory tests are necessary for definitive identification.

Incubation at lower than recommended temperatures may delay detection of positive reactions. If the incubation temperature is below 35 ± 2°C, the plates should be incubated a full 24 h before reporting as negative.

Plates are not to be incubated beyond the 24 h time period prior to reading.

XI PERFORMANCE CHARACTERISTICS

Analytical Testing

An interference study was conducted with substances that may be present in stool or rectal specimens. Fourteen (14) substances were tested which included lubricants, water, soap, laxatives, suppositories, and various hemorrhoidal treatments. None of the substances tested interfered with the performance of the **BBL CHROMagar** O157 medium.

Internal testing of other stool pathogens was conducted in order to determine the potential cross reactivity of these organisms with **BBL CHROMagar** O157. Fifty-nine (59) non-*E. coli* O157:H7 organisms were tested, including selected species from the following genera: *Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, *Aeromonas*, *Campylobacter*, and *Plesiomonas*.

Reproducibility testing was conducted at three different geographical locations (two [2] external clinical sites and one [1] internal) to demonstrate the ability of **BBL CHROMagar** O157 to provide reproducible results with known microorganisms. A blinded panel of *E. coli* O157:H7 strains and non-*E. coli* O157:H7 strains were provided to each site for testing. Each panel was tested in triplicate on three days at each site. For all sites, the results for this study showed 100% reproducible results within each site and across all sites for the entire panel.

Clinical Testing

A clinical study was conducted at an external centralized regional clinical laboratory that routinely tests for *E. coli* O157:H7 in stool specimens. Stool specimens were inoculated onto Sorbitol-MacConkey (SMAC) and **BBL CHROMagar** O157 media and incubated aerobically for 18–24 h at 35°C. Each plate was read by an independent technologist and confirmatory testing (indole and serotyping) was conducted on all suspected colony samples. A total of 3,136 stool specimens were cultured, of which 2,855 specimens provided acceptable results for this study while 281 specimens were determined to be noncompliant to the required testing matrix. The following table shows the breakdown of the results from the study:

	SMAC Result	
CHROMagar Result	**Positive**	**Negative**
Positive	19	5
Negative	3	2828
Totals	22	2833

Positive Percent Agreement: 86.4%

Negative Percent Agreement: 99.8%

XII AVAILABILITY

Cat. No.	**Description**
214984	**BBL™ CHROMagar®** O157 Prepared Plates – Pkg. of 20 plates

XIII REFERENCES

1. Bopp, C.A., F.W. Brenner, P.I. Fields, J.G. Wells, and N.A. Strockbine. 2003. *Escherichia*, *Shigella* and *Salmonella*. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, J.H. Jorgensen and R.H. Yolken (eds.), Manual of clinical microbiology. 8th ed. American Society for Microbiology, Washington DC.
2. CDC MMWR January 26, 2001/50 (RR02):1-69. Diagnosis and management of foodborne illness.
3. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections. 3rd ed., CLSI, Wayne, Pa.
4. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. *17*:53-80.
5. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HIHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
6. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.

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