



BD BBL™ CHROMagar™ O157

U.S. Patent No. 6,165,743



*See footnote below

INTENDED USE

BBL™ CHROMagar O157 is a selective medium for the isolation, differentiation and presumptive identification of *Escherichia coli* O157:H7 from clinical, food, veterinary and environmental sources.

BBL CHROMagar O157 has been validated by the AOAC™-Research Institute under the Performance Tested MethodsSM Program for the analysis of raw ground beef and unpasteurized apple cider when using FDA BAM, USDA FSIS and ISO methods.¹⁻³

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

E. coli O157:H7 is the most frequently isolated pathogen from bloody stools.⁴⁻⁶ However, absence of bloody diarrhea does not rule out the presence of *E. coli* O157:H7.⁷ This serotype causes a broad range of illness from mild non-bloody diarrhea to severe bloody diarrhea (hemolytic colitis), hemolytic uremic syndrome and death.⁴⁻⁶ 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.⁴⁻⁶ In addition, transmission may occur person to person, as well as from recreational water sources.⁴⁻⁶

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.

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.

*PRODUCER-SUPPLIED SAMPLES OF THIS TEST KIT MODEL WERE INDEPENDENTLY EVALUATED BY THE AOAC RESEARCH INSTITUTE AND WERE FOUND TO PERFORM TO THE PRODUCER'S SPECIFICATIONS AS STATED IN THE TEST KIT'S DESCRIPTIVE INSERT. THE PRODUCER CERTIFIES THIS KIT CONFORMS IN ALL RESPECTS TO THE SPECIFICATIONS ORIGINALLY EVALUATED BY THE AOAC RESEARCH INSTITUTE AS DETAILED IN *Performance Tested MethodsSM* CERTIFICATE NUMBER 090501.

REAGENTS

BD CHROMagar O157 Medium

Approximate Formula* Per Liter of Purified Water

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

pH: 7,1 ± 0,2

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

PRECAUTIONS

IVD . For professional use only.

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 AND SHELF LIFE**.

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

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. Standard precautions⁸⁻¹¹ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

Pathogenic microorganisms, including *E. coli* O157, may be present in food samples. Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures.

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

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. Do not open until ready to use. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C. **Minimize exposure to light before and during incubation, as light may destroy the chromogens.**

USER QUALITY CONTROL

Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions (for details, see **GENERAL INSTRUCTIONS FOR USE** document). The test strains mentioned in the Table below are recommended. Incubate aerobically for 18 to 24 hours at 35 ± 2° C in the dark.

Strains	Growth Results
<i>Escherichia coli</i> O157:H7 ATCC™ 700728 (=NCTC 12900)	Fair to excellent growth. Colonies grey violet to rose-violet (= mauve)
<i>Escherichia coli</i> ATCC 25922	Inhibition partial to complete; colonies blue-green; may be surrounded by a blue-green halo
<i>Enterobacter cloacae</i> ATCC 13047	Growth: blue-green to blue colonies
Uninoculated	Colorless to light beige, transparent

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.

PROCEDURE

Materials Provided

BD CHROMagar O157 Medium (90 mm **Stacker™** plates). Microbiologically controlled.

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

Specimen Types

For clinical use, refer to lab procedures for details on specimen collection and handling procedures. This medium is used for the isolation of *Escherichia coli* O157:H7 from stool specimens or rectal swabs of patients suspected to be infected with this agent.

For agrifood testing, follow appropriate standard methods for details on sample preparation and processing according to sample type and geographic location.

See also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**.

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. Alternatively, plates may be inoculated from preenrichments. Incubate plates aerobically at $35 \pm 2^\circ\text{C}$ for 18-24 h in an inverted position (agar-side up). Other media such as **BD MacConkey II Agar** may also be inoculated to provide detection of other enteric pathogens.

For food samples, consult appropriate references and follow applicable standard methods. Inoculate incubated enrichment broth or screened food sample particle onto **BBL CHROMagar O157** and streak for isolation. Incubate plates aerobically at $35 \pm 2^\circ\text{C}$ for 18-24 h in an inverted position (agar-side up).

Results

After proper incubation, read plates against a white background. *E. coli* O157:H7 will produce mauve-colored colonies on **BBL CHROMagar O157** medium. All mauve colonies must be confirmed biochemically and/or serologically prior to reporting as *E. coli* O157:H7.^{1,2,3,6} 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.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD CHROMagar O157 is a chromogenic medium for the selective isolation and presumptive identification of *E. coli* O157:H7 from clinical, food, veterinary and environmental sources.

Performance Results¹²

Clinical Testing: A total of 110 frozen fecal isolates and 16 stool cultures (10 fresh and 6 archived) were evaluated at a metropolitan hospital using BBL CHROMagar O157, Sorbitol MacConkey (SMAC) and Sorbitol MacConkey with Cefixime and Tellurite (SMAC-CT). The frozen fecal isolates consisted of 50 *E. coli* O157:H7, 15 *E. coli* non-O157, 8 Shiga-toxin positive *E. coli* non-O157 and 37 other *Enterobacteriaceae* and nonfermentive gram-negative rods. Seven of the 16 stools tested were found to be positive for *E. coli* O157:H7. The following sensitivities and specificities were observed:

	Sensitivity (No.)	Specificity (No.)
BBL CHROMagar O157	98 % (56/57)	100 % (69/69)
SMAC	96 % (55/57)	80% (55/69)
SMAC-CT	100 % (57/57)	93% (64/69)

Agrifood Testing

BBL CHROMagar O157 was validated by the AOAC-Research Institute under the Performance Tested Methods Program.¹² **BBL CHROMagar O157** was evaluated for the detection of *E. coli* O157:H7 in raw ground beef and unpasteurized apple cider using seeded samples. The recovery of *E. coli* O157:H7 on **BBL CHROMagar O157** was compared to the FDA BAM, USDA FSIS and ISO reference plated media. The reference recommended enrichment and screening procedures were followed for the reference media and **BBL CHROMagar O157**.

Immunomagnetic separation (IMS) was performed according to the USDA and ISO methods. Of the 180 food samples tested, 45 were tested using FDA BAM and USDA FSIS methods, and 90 were tested using ISO methods. **BBL CHROMagar O157** produced a sensitivity of 100% and a specificity of 100% as compared to the reference methods for both food matrices. No false negatives were found in testing the food matrices. No statistical difference was found in recovery using the **BBL CHROMagar O157** method compared to the reference plated media based on Chi-square analysis. Known isolates, including 54 strains of *E. coli* O157:H7 (3 of which were non-motile strains) and 32 non-*E. coli* O157:H7 strains, were evaluated on **BBL CHROMagar O157** with a sensitivity and specificity of 100%. The results of these studies demonstrate that **BBL CHROMagar O157** is an effective medium for the recovery and detection of *E. coli* O157:H7 in raw ground beef and unpasteurized apple cider using FDA BAM, USDA FSIS and ISO methods. See Table 1 for summary of validation method comparison study results.

Table 1: Summary of Validation Method Comparison Results

Food Matrix	Method	Inoculum Level	Total Samples	Total Positive	Reference Positive	CHROMagar O157 Positive	Method Agreement ¹	Chi-Square ³
Raw Ground Beef	USDA Beef	High	20	15	12	15	85% ²	1.33
		Low	20	13	10	13	85% ²	1.33
		Control	5	0	0	0	-	-
Raw Ground Beef	ISO Beef	High	20	17	16	17	95% ²	0.00
		Low	20	10	9	10	95% ²	0.00
		Control	5	0	0	0	-	-
Unpasteurized Apple Cider	ISO Cider	High	20	19	19	19	100%	0.00
		Low	20	14	14	14	100%	0.00
		Control	5	0	0	0	-	-
Unpasteurized Apple Cider	FDA Cider	High	20	13	13	13	100%	0.00
		Low	20	10	10	10	100%	0.00
		Control	5	0	0	0	-	-

¹ Represents the percentage of confirmed positive and negative samples, combined, which were equivalent between the reference and **BBL CHROMagar O157** methods.

² Additional positive samples detected by the **BBL CHROMagar O157** method: 3 additional positives when testing raw ground beef by the USDA/FSIS method and 1 additional positive when testing raw ground beef by the ISO method.

³ Chi-square values of < 3.84 indicate no significant difference at p<0.05.

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**. 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 hours before reporting as negative. 12

Confirmatory tests are necessary for definitive identification. 1-3,6

This medium must not be used for the isolation of enteric pathogens other than *E. coli* O157:H7.

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12. Data on file, BD Diagnostic Systems.

PACKAGING/AVAILABILITY

BD CHROMagar O157 Medium

Cat. No. 254105 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.



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