

Revisions

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Part Number: L009884		Category and Description Package Insert (Lab Use), CHROMagar O157	Sheet: 1 of 3 <hr/> Scale: N/A	A

INTENDED USE

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

SUMMARY AND EXPLANATION

E. coli O157:H7 is the most frequently isolated pathogen from bloody stools.^{1,2} However, absence of bloody diarrhea does not rule out the presence of *E. coli* O157:H7.³ This serotype causes a broad range of illness from mild non-bloody diarrhea to severe bloody diarrhea (hemolytic colitis), hemolytic uremic syndrome and death.^{1,2} 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.^{1,2} In addition, transmission may occur person to person, as well as from recreational water sources.^{1,2}

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.

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.

REAGENTS

BBL CHROMagar O157

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

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

Warnings and Precautions:

For Laboratory 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 *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, sample containers and other contaminated materials must be sterilized by autoclaving before discarding.

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.

PROCEDURE

Material Provided: **BBL CHROMagar O157**

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

Sample Collection and Handling

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

Test Procedure

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

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 ± 2°C for 18 – 24 h in an inverted position (agar-side up).

User Quality Control

Examine plates for signs of deterioration as described under “Product Deterioration.” Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions. The following test strains are recommended:

Test Strains	Expected Results
<i>Escherichia coli</i> O157:H7 ATCC™ 700728	Growth: Light mauve to mauve colonies
<i>Escherichia coli</i> ATCC 25922	Complete inhibition to partial growth of non-mauve colonies
<i>Enterobacter cloacae</i> ATCC 13047	Growth: Blue-green to blue colonies

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.

RESULTS

After proper incubation, read plates against a white background. Interpretation of plate results must be completed within 18 – 24 hours 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:H7.⁴⁻⁶ 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.

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**.

Confirmatory tests are necessary for definitive identification.⁴⁻⁶

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.⁷

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

PERFORMANCE CHARACTERISTICS⁷

AgriFood Testing

BBL CHROMagar O157 was validated by the AOAC-Research Institute under the Performance Tested Methods Program.⁷ **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.

*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.

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	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	High	20	17	16	17	95% ²	0.00
		Low	20	10	9	10	95% ²	0.00
		Control	5	0	0	0	–	–
Unpast. Apple Cider	ISO	High	20	19	19	19	100%	0.00
		Low	20	14	14	14	100%	0.00
		Control	5	0	0	0	–	–
Unpast. Apple Cider	FDA	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.


AVAILABILITY

Cat. No. Description

214984 BBL™ CHROMagar™ O157 Prepared Plates – Pkg. of 20

REFERENCES

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7. Data on file, BD Diagnostics.

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