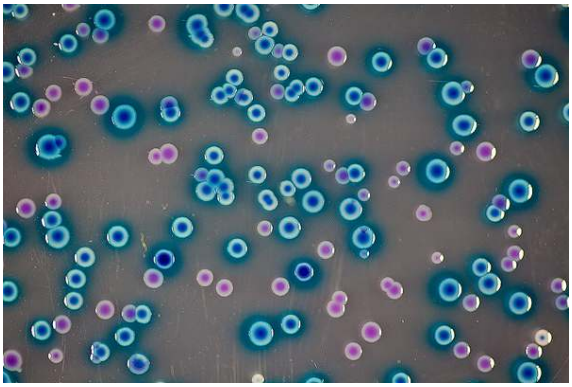
INSTRUCTIONS FOR USE

. ChromArt

CHROMOGENIC E.COLI O157 AGAR

Dehydrated culture medium



Chromogenic *E.Coli* O157 Agar*: E.coli* O157 (purple colonies), and *E.coli* non-O157 (blue colonies)

1 - INTENDED USE

In vitro diagnostic device. Selective and chromogenic medium for the isolation and differentiation of *Escherichia coli* O157:H7, from food.

2 - COMPOSITION - TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptones	17.0 g
Bile salts n°3	1.5 g
Chromogenic compounds	0.5 g
Agar	12.0 g

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

E.coli O157:H7 was first recognized as a pathogen in 1982 during an outbreak investigation of haemorrhagic colitis.[1] Although more than 300 verotoxins or Shiga toxins producing serotypes are known, the infection is mainly caused by the motile serotype *E.coli* O157: H7 and its non-motile variant O157:NM (O157:H-).[2] The severity of illness presents different degrees, from uncomplicated diarrhoea to haemorrhagic colitis, up to haemolytic-uremic syndrome and thrombotic thrombocytopenic purpura; the infectious dose for O157:H7 is estimated to be 10-100 cells; the infection is particularly serious for the most vulnerable subjects, such as children and the elderly.[3] The strain virulence is substantially due to the production of one or both of the Shiga toxins Stx1 and Stx2 and, more rarely, of their variants. Infections are mostly food or water borne and have implicated undercooked ground beef, raw milk, cold sandwiches, water, unpasteurized apple juice and sprouts and vegetables.[4] Direct contact with animals belonging to the reservoir species and person to person transmission may play a role in the spread of infection.[5]

E.coli O157:H7 strains are phenotypically distinct from *E.coli* as they exhibit slow or no fermentation of sorbitol and do not have glucuronidase activity; these characteristics led to the design of various culture media for primary isolation such as CT-SMAC (Cefixime Tellurite Sorbitol Mac Conkey Agar) and CT-SMAC MUG.[6] Following the findings about the isolation of sorbitol positive and β-glucuronidase positive *E.coli* O157 and strains that do not grow on CT-SMAC, [7,8,9] several chromogenic media, based on more specific differential mechanisms, have been proposed.

Chromogenic E.coli O157 Agar includes a mixture of chromogenic compounds to detect the enzymatic activities of *Enterobacteriaceae* (β-glucuronidase and β-glucosidase) and a specific enzymatic activity of *E.coli* O157.

The selective action of Chromogenic E.coli O157 Agar is due to the presence of bile salts n°3, which inhibit the growth of Gram-positive bacteria. To increase the selective properties and the specificity of the results, potassium tellurite and cefixime can be added to the medium: according to the data of Zadik[10] this addition completely or partially inhibits the growth of 67% of *E.coli* non-O157 and almost completely the growth of sorbitol non-fermenting Gram negative bacteria other than *E.coli* O157.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 31 g in 1000 mL of cold purified water. Heat to boiling, stirring constantly and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and distribute into sterile Petri dishes. If cefixime-tellurite addition is required, reconstitute one vial of Cefixime Tellurite O157 Supplement (REF 42ISEC) with 5 mL of sterile purified water and, under aseptic conditions, add to 500 mL of pre-cooled medium base.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	greyish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chromogenic E.coli O157 Agar	Dehydrated medium	4055812	500 g (16,1 L) CND: W0104010101
		4055811	100 g (1,6 L) CND: W0104010101

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies. Cefixime Tellurite O157 Supplement REF 42ISEC.

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8 - SPECIMENS

Chromogenic E.coli O157 Agar, with or without Cefixime Tellurite O157 Supplement, is intended for the bacteriological processing of non-clinical samples; refer to the applicable international standards.[2,11]

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

1. A test amount is enriched in nine times the weight of pre-warmed Modified Tryptic Soy Broth (REF 402155M2) plus novobiocin 20 mg/L (Novobiocin Antimicrobic Supplement -REF 4240045) at 41.5°C ± 1°C for 6 h and subsequently for a further 12 to 18 h.
2. *E. coli* O157 cells are separated and concentrated using immunomagnetic beads coated with antibodies to *E. coli* O157 after 6 h and again, if necessary, after a further 12 to 18 h incubation.
3. 50 µl of immunomagnetic concentrated broth are sub-cultured onto CT-SMAC and onto Chromogenic E.coli O157 Agar.
4. Chromogenic E.coli O157 Agar is incubated in aerobic atmosphere at 35-37°C for 18-24 hours. CT-SMAC should be incubated following the IFU recommended procedure.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Pink to violet colonies can be presumptively identified as *E.coli* O157.
E.coli strains not belonging to the serogroup O157, *Citrobacter, Enterobacter, Klebsiella, Serratia* grow with blue or green-blue colonies.
Purify the typical colonies from Chromogenic E.coli O157 Agar by streaking onto Nutrient Agar and incubate at 37°C for 18 to 24 h.
For confirmation, ISO16654[12] requires indole test (+) and agglutination with *E. coli* O157 antiserum.
FDA BAM[2] requires β-galactosidase (+), β-glucuronidase (-) and indole (+) tests and the presence of the O157 and H7 antigens.
The pink to violet colony with the biochemical profile of *E.coli* and positive for the antisera O157 and H7 is confirmed as *E.coli* O157:H7.
If the isolate is O157 positive but H7 negative it may be a non-motile variant (O157:NM) and therefore requires a confirmation test of its toxigenic potential (for example with PCR technique). The colony can also be sub-cultured to blood agar plate to induce mobility and re-tested with H7 antiserum.
O157:H7 and O157:NM isolates that produce verocytotoxin are considered pathogenic. However, an O157:NM strain that does not produce shiga toxins or other EHEC (Enterohaemorrhagic *E.coli*) virulence factors is probably non-pathogenic. There are many *E. coli* O157 serotypes that carry other than H7 antigens (e.g.: H3, H12, H16, H38, H45, etc), and these often do not carry EHEC virulence factors.[2]
For a complete explanation of the identification criteria and methods, refer to the literature cited for clinical samples[11] and for food samples[2].

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli O157 ATCC 43894	35-37°C / 18-24 H / A	growth, violet colonies
E. coli ATCC 25922	35-37°C / 18-24 H / A	growth, blue colonies
S.aureus ATCC 25923	35-37°C / 18-24 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - LIMITATIONS OF THE METHOD

- Chromogenic E.coli O157 Agar does not detect EHEC strains of *E.coli* other than O157:H7.
- Follow the recommended times and temperatures as *E. coli* O157 does not grow at 44-45°C and because delayed observation of the colonies can lead to errors of interpretation.
- *Salmonella* spp., especially in the absence of the Cefixime Tellurite Supplement, may grow with colonies with a pale violet centre, that may not be distinguishable from *E.coli* O157 colonies.
- Some enterococci can develop small colonies with prolonged incubation beyond 24 hours.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

13 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Material Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredients for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.

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- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +2°C /+8°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap were damaged or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

15 - REFERENCES

1. Centers for Disease Control. 1984. Update: sporadic hemorrhagic colitis. Morbid. Mortal. Weekly Rep. 33:28
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7. Díaz S, Vidal D, Herrera-León S, Sánchez S. Sorbitol-fermenting, β-Glucuronidase-positive, shiga toxin-negative Escherichia coli O157:H7 in free-ranging red deer in south-central Spain. Foodborne Pathog Dis 2018; 8:1313-1315
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9. Gunzer F, Bohm H, Russmann H et al. Molecular detection of sorbitol-fermenting Escherichia coli 0157 in patients with hemolytic-uremic Ssyndrome. J Clin Microbiol 1992; 30:1807.
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TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/05
Revision 2	CE mark elimination	2020/08

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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