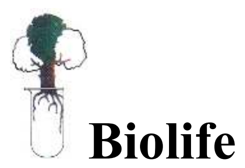
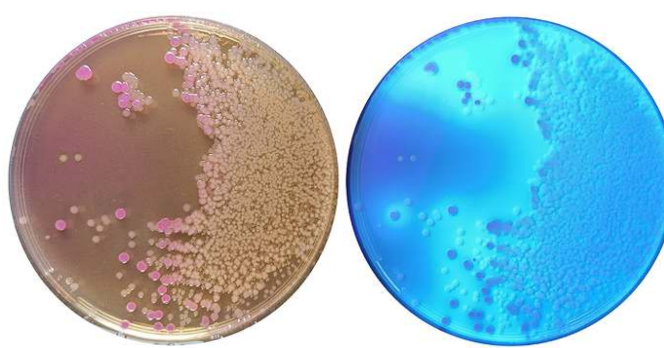


MAC CONKEY SORBITOL MUG AGAR
CEFIXIME TELLURITE O157 SUPPLEMENT

Dehydrated culture medium and selective supplement



Mac Conkey Sorbitol MUG Agar:*E.coli* and *E.coli* O157 colonies under normal light and Wood's lamp

1 - INTENDED USE

Selective and differential medium and selective supplement for the isolation of *Escherichia coli* O157:H7.

2 - COMPOSITION

MAC CONKEY SORBITOL MUG AGAR

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

Tryptone	17.000 g
Peptocomplex	3.000 g
D-Sorbitol	10.000 g
Bile Salts No. 3	1.500 g
Sodium chloride	5.000 g
Neutral red	0.030 g
Crystal violet	0.001 g
4-Methylumbelliferyl- β-D-glucuronide (MUG)	0.100 g
Agar	14.500 g

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

CEFIXIME TELLURITE O157 SUPPLEMENT (FOR 500 ML OF MEDIUM)

VIAL CONTENTS

Cefixime	0.025 mg
Potassium tellurite	1.250 mg

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.[6]

Mac Conkey Sorbitol MUG Agar is prepared according to a modification of the MacConkey formula described by Rappaport and Henig[7] for substituting lactose with sorbitol and by Szabo[8], for the inclusion of 4-methylumbelliferyl- β-D-glucuronide (MUG). The selective supplement Cefixime Tellurite O157 Supplement is prepared on the basis of the observations published by Zadik[9].

E. coli O157:H7 does not ferment sorbitol or ferments it beyond 24 hours of incubation, does not hydrolyse MUG and grows with colourless colonies, not fluorescent under long-wave ultraviolet light (Wood's lamp), lactose fermenter non-O157 strains grow with red-purple colonies, often surrounded by an opaque pink-red halo and with a slightly blue fluorescence under Wood's lamp.

The determination of *E. coli* O157:H7 on faecal samples with MacConkey Agar with sorbitol, according to the data of March[10], has a sensitivity of 100%, a specificity of 85% and an accuracy of 86%.

According to Okrend[11], the addition of a substrate to determine the β-glucuronidase enzyme decreases falsely suspect colonies by 36% compared to Mac Conkey Sorbitol Agar.

The selective action of Mac Conkey Sorbitol Agar is due to the presence of bile salts n°3, which inhibit the growth of Gram-positive bacteria; this inhibitory activity is enhanced by the addition of crystal violet. 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[9] this addition completely or partially inhibits the growth of 67% of *E.coli* non-O157 and almost completely the growth of others sorbitol non-fermenting Gram negative bacteria.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 25.5 g in 500 mL of cold purified water. Heat to boiling, stirring constantly and sterilize by autoclaving at 121°C for 15 minutes. Cool to 44-47°C and distribute into sterile Petri dishes. If cefixime-tellurite addition is required, reconstitute one vial of the lyophilised supplement with 5 mL of sterile purified water and, under aseptic conditions, add to 500 mL of pre-cooled medium base. Mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	greyish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid or slightly opalescent
Final pH at 20-25 °C	7.1 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Mac Conkey Sorbitol MUG Agar	Dehydrated medium	4016692	500 g (9,8 L)
Cefixime Tellurite O157 Supplement	Freeze-dried supplement	4240030	10 vials, each for 500 mL of medium

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.

8 - SPECIMENS
Mac Conkey Sorbitol MUG Agar, with or without Cefixime Tellurite O157 Supplement, is intended for the bacteriological processing of non-clinical specimens; good laboratory practices for collection, transport and storage of the samples should be applied.[13] Refer to the applicable international standards.[2,12]

9 - TEST PROCEDURE
- A test amount is enriched in nine times the weight of pre-warmed Modified Tryptic Soy Broth (REF 402155M2) plus novobiocin 20mg/L (Novobiocin Antimicrobic Supplement -REF 4240045) at 41.5°C ± 1°C for 6 h and subsequently for a further 12 to 18 h.
- *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.
- 50 µl of immunomagnetic concentrated broth are sub-cultured onto CT-SMAC MUG and onto a second selective isolation agar of laboratory choice (e.g. Chromogenic E. coli O157 Agar REF 4055812). CT-SMAC MUG is incubated at 37±1°C for 18 to 24 h. The second agar of choice should be incubated following the IFU recommended procedures.

10 - READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies at the normal light; check the plates by using a hand-held 366-nm light or by placing the plate on a long-wave UV lightbox UV (Wood's Lamp).
Colourless and not fluorescent colonies (sorbitol negative, β-glucuronidase negative) can be presumptively identified as *E.coli* O157.
Purify the typical colonies from CT-SMAC MUG by streaking onto Nutrient Agar and incubate at 35-37°C for 18 to 24 h.
For confirmation, ISO16654[12] requires indole test (+) and agglutination with *E. coli* O157 antiserum.
In addition to β-glucuronidase negative test, FDA BAM[2] requires β-galactosidase (+), indole (+) tests and the presence of the O157 and H7 antigens.
The sorbitol negative and β-glucuronidase negative 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[14] 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
Escherichia coli O157 ATCC 43894	35-37°C / 18-24 H / A	growth, colourless colonies not fluorescent under Wood's lamp
Escherichia coli ATCC 25922	35-37°C / 18-24 H / A	growth partially inhibited, red colonies fluorescent under Wood's lamp
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 - PERFORMANCES CHARACTERISTICS
Prior to release for sale a representative sample of all lots of dehydrated Mac Conkey Sorbitol MUG Agar, supplemented with Cefixime Tellurite Supplement, is tested for productivity and selectivity by comparing the results with previously approved Reference Batch.
Productivity is tested by semi-quantitative ecometric technique, by incubating at 35-37°C for 18-24 hours, with the target strains *E.coli* O157:H7 ATCC 43888, *E.coli* O157:H7 NCTC 12900, *E.coli* O157:H7 ATCC 43894. After incubation the colour and fluorescence emission of the colonies and the amount of growth are evaluated and recorded. Target-strains grow with colourless colonies without fluorescence emission and the growths are comparable in both batches.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10^{-1} to 10^{-6} of a 0.5 McFarland suspension of the non-target strains *E.coli* ATCC 25922, *K.pneumoniae* ATCC 27736, *E.hermannii* ATCC 33650, *E.faecalis* ATCC 19433 and *S.aureus* ATCC 25923. *E.coli* is partially inhibited and grow with red colonies, fluorescent under Wood's lamp, *K.pneumoniae* is partially inhibited and grow with red colonies not fluorescent under Wood's lamp, *E.hermannii* is partially inhibited and grow with colourless colonies not fluorescent under Wood's lamp, *E.faecalis* and *S.aureus* are totally inhibited at the dilution 10^{-1}.

13 - LIMITATIONS OF THE METHOD
- There are several well known EHEC strains that have caused illness worldwide, e.g.: O26, O111, O121, O103, O145, O45, etc. However, these strains ferment sorbitol and are not distinguishable on CT-SMAC MUG. For the determination of these strains in food, refer to the cited literature.[2]
- *E.coli* O157 sorbitol positive and β-glucuronidase positive strains and strains that do not grow on CT-SMAC MUG have been reported.[15,16] For the management of these strains refer to the cited literature.[14]
- 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.
- Some enterococci can develop small colonies with prolonged incubation beyond 24 hours.

- The presence of colourless colonies on the medium is not in itself indicative of the presence of *E.coli* O157 as other sorbitol negative bacteria can grow with colourless colonies (*Escherichia hermannii, Proteus, Pseudomonas, Acinetobacter* etc.).
- Although the intended use and the test procedure of the medium refers to the detection of E. coli O157:H7 in food and therefore the product should not be regarded as an *in vitro* diagnostic, the literature reports the use of the medium for human clinical specimens.[13,14] Clinical applications should be validated by the user.
- 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. If relevant, perform antimicrobial susceptibility testing.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are for microbiological control, for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- 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 the 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 medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

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 are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

Selective supplement

Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes) and the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. Centers for Disease Control. 1984. Update: sporadic hemorrhagic colitis. Morbid. Mortal. Weekly Rep. 33:28
2. U.S. Food and Drug Administration. Bacteriological Analytical Manual. Chapter 4a Diarrheagenic Escherichia coli. Rev October 2018
3. Griffin, P. M., S. M. Ostroff, R. V. Tauxe, K. D. Greene, J. G. Wells, J. H. Lewis, and P. A. Blake. 1988. Illnesses associated with Escherichia coli O157:H7 infections. A broad clinical spectrum. Ann. Intern. Med. 109:705–712.
4. Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of Escherichia coli O157:H7 outbreaks, United States, 1982–2002. Emerg Infect Dis. 2005;11:603–609.
5. Bach SJ, McAllister TA, Veira DM, Gannon VPJ, Holley RA. Transmission and control of Escherichia coli O157:H7—a review. Can J Anim Sci 2002; 82:475–490.
6. Thompson JS, Hodge DS, Borczyk AA. Rapid biochemical test to identify verocytotoxin-positive strains of Escherichia coli serotype O157. J Clin Microbiol. 1990;28:2165–2168.
7. Rappaport F, Henig E. Media for the isolation and differentiation of pathogenic Escherichia coli (serotypes 0111 and 055). J Clin Path 1952; 5:361-362.
8. Szabo RA, Todd EC, Jean A., 1986. Method to isolate E. coli O157:H7 from food. J. Food Protect 1986; 10:768–772.
9. Zadik PM, Chapman PA, and Siddons CA. Use of tellurite for the selection of verocytotoxigenic Escherichia coli O157. J. Med. Microbiol 1993; 39:155-158
10. March SB, Ratnam S. Sorbitol-MacConkey Medium for detection of Escherichia coli 0157:H7 associated with hemorrhagic cholitis. J Clin Microbiol 1986; 23: 869-872
11. Okrend AJG, Rose BE, Lattuada CP. Use of 5-Bromo-4-Chloro-3-Indoxyl-β-D-Glucuronide in MacConkey Sorbitol Agar to aid in the isolation of Escherichia Coli 0157:H7 From Ground Beef. J Food Prot 1990; 53:941-943.
12. ISO 16654:2001. Microbiology of food and animal feeding stuffs- Horizontal method for detection of E.coli O157
13. Public Health England. Investigation of Faecal Specimens for Enteric Pathogens. B38. Issue 8.1. 2014
14. Public Health England. Identification of Vero cytotoxin-producing Escherichia coli including Escherichia coli O157. ID22. Issue 4. 2015.
15. 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
16. Health Protection Agency (HPA). CDR Weekly. Sorbitol-fermenting Vero cytotoxin-producing E. coli (VTEC 0157). CDR 16(21) 2006b.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/04
Revision 2	Update of “intended use”, “test procedure”, “precautions and warnings” and “storage conditions and shelf life”	2022/05
Revision 3	Insert of Cefixime Tellurite O157 Supplement by Biolife	2024/12

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, E-mail: export@biolifeitaliana.it;
Web: www.biolifeitaliana.it