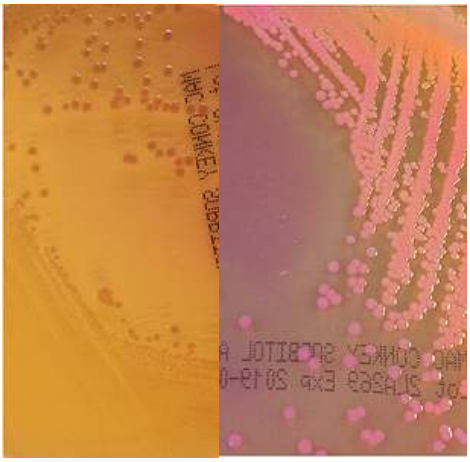
INSTRUCTIONS FOR USE

MAC CONKEY SORBITOL AGAR
CEFIXIME TELLURITE O157 SUPPLEMENT

Dehydrated culture medium and selective supplement



Mac Conkey Sorbitol Agar: at left sorbitol non fermenting *E.coli* O157, at right sorbitol fermenting *E.coli*

1 - INTENDED USE

In vitro diagnostic devices. Selective and differential medium and selective supplement for the isolation of *Escherichia coli* O157:H7, from clinical specimens and from food.

2 - COMPOSITION

MAC CONKEY SORBITOL 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
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	1,25 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, 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 Agar is prepared according to a modification of the formula described by Rappaport and Henig[7]; the selective supplement Cefixime Tellurite O157 Supplement is prepared on the basis of the observations published by Zadik[8].

Mac Conkey Sorbitol Agar, supplemented with cefixime and potassium tellurite (CT-SMAC), complies with the formulation reported in ISO 16654[9] for the isolation of *E. coli* O157, as well as with the FDA-BAM requirements[2].

Mac Conkey Sorbitol Agar is identical to Mac Conkey Agar except that lactose has been replaced with sorbitol. *E.coli* 0157:H7 does not ferment sorbitol or ferments it beyond 24 hours of incubation and grows with colourless colonies, lactose fermenter non-O157 strains grow with red-purple colonies, often surrounded by an opaque pink-red halo.

The determination of *E. coli* 0157: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%.

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[10] 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.

Mac Conkey Sorbitol Agar, supplemented with cefixime and potassium tellurite, is intended for the isolation and differentiation of E.*coli* O157:H7 from clinical specimens such as faeces[11,12] and from food[2,9].

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. Store prepared plates in plastic bags at 3 ± 2°C up to 2 weeks.[9]

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

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6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Mac Conkey Sorbitol Agar CND: W0104010101; EDMA:14.01.01.01: RDM: 1873876/R	Dehydrated medium	401669S2	500 g (9,8 L)
Cefixime Tellurite O157 Supplement CND: W0104010104; EDMA: 14.01.01.04	Freeze-dried supplement	42ISEC	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 Agar, with or without Cefixime Tellurite O157 Supplement, is intended for the bacteriological processing of faecal specimens. Good laboratory practices for collection, transport and storage of the specimens should be applied.[11,12] For non-clinical samples, refer to the applicable international standards.[2,9]

9 - TEST PROCEDURE

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

Faeces (routine investigation)[11,12]

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. Incubate in aerobic atmosphere at 35-37°C for 16-24 hours.

Faeces (all diarrhoeal specimens and any with obvious blood semi-formed or liquid faeces, children under 5 years, outbreaks) [11,12]

Carry out an enrichment in Modified Tryptic Soy Broth (REF 402155M2) supplemented with novobiocin 20 mg/L (Novobiocin Antimicrobic Supplement-REF 4240045), with incubation at 35-37°C for 16-24 hours. Subculture a loop of enrichment broth onto a CT-SMAC plate, streak the inoculum over the four quadrants of the plate to obtain isolated colonies. Incubate in aerobic atmosphere at 35-37°C for 16-24 hours

Food[9]

1. 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.
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 a second selective isolation agar of laboratory choice (e.g. Mac Conkey Sorbitol MUG Agar REF 401669 or Chromogenic E.coli O157 Agar REF 405581). CT-SMAC is incubated at 37±1°C for 18 to 24 h. The second agar of choice should be incubated following the IFU's recommended procedures.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Colourless colonies (sorbitol negative) can be presumptively identified as *E.coli* O157.
Purify the sorbitol negative colonies from CT-SMAC by streaking onto Nutrient Agar and incubate at 35-37°C for 18 to 24 h.
For confirmation, ISO16654[9] 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 sorbitol 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[12] 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 / 16-24 H / A	growth, colourless colonies
Escherichia coli ATCC 25922	35-37°C / 16-24 H / A	growth partially inhibited, red colonies
S.aureus ATCC 25923	35-37°C / 16-24 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 Agar, supplemented with Cefixime Tellurite Supplement, is tested for productivity and selectivity by comparing the results with previously approved Reference Batches.

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 and *E.coli* O157:H7 NCTC 12900, *E.coli* O157:H7 ATCC 43894. After incubation the colour of the colonies and the amount of growth are evaluated and recorded. Target-strains grow with colourless colonies 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 25823. *E.coli* and *K.pneumoniae* are partially inhibited and grow with red colonies, *E.hermannii* is partially inhibited and grow with colourless colonies, *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. 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 have been reported.[13,14] For the management of these strains refer to the cited literature.[11,12]
- 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.).
- 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.
- The culture medium and the supplement are intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are qualitative *in vitro* diagnostics, 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.
- 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 10-30°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, presence of large lumps).
- **Selective supplement**: upon receipt store at 2-8°C until the expiry date. Do not use beyond this date. Once opened and reconstituted, the vial contents must be used immediately.

16 - REFERENCES

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TABLE OF APPLICABLE SYMBOLS

REF o 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

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

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