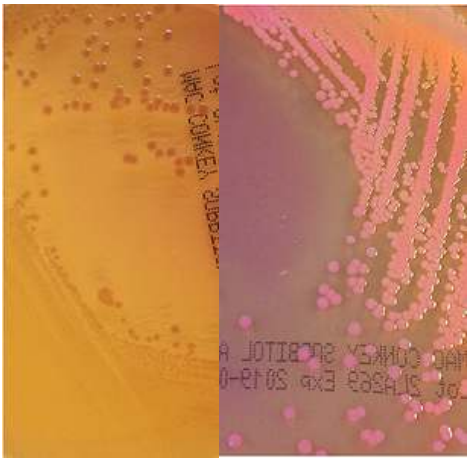
INSTRUCTIONS FOR USE

MAC CONKEY SORBITOL AGAR

Ready-to-use plates



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

1 - INTENDED USE

In vitro diagnostic device. Selective and differential medium for the detection of *Escherichia coli* O157:H7 in clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA *

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
Purified water	1000 mL

*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, 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]; Mac Conkey Sorbitol Agar complies with the formulation of the medium base reported by ISO 16654[8] and FDA-BAM [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[9], 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.

Mac Conkey Sorbitol Agar is intended for the isolation and differentiation of *E.coli* O157:H7 from faecal specimens.[10,11]

4- PHYSICAL CHARACTERISTICS

Prepared plates appearance	red-violet, limpid or slightly opalescent
Final pH at 20-25°C	7.1 ± 0.2

5 - MATERIALS PROVIDED

Product	Type	REF	Pack
Mac Conkey Sorbitol Agar	Ready-to-use plates	541669S	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Mac Conkey Sorbitol Agar (SMAC agar), is intended for the bacteriological processing of faecal specimens. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.[11-13]

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9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
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.
An enrichment step is recommended for all diarrhoeal specimens and any with obvious blood semi-formed or liquid faeces, for specimens from children under 5 years, and in case of outbreaks.[12,13]
Carry out the 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 SMAC agar 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.

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 SMAC agar by streaking onto Nutrient Agar and incubate at 35-37°C for 18 to 24 h.
For confirmation, oxidase (-), β-galactosidase (+), β-glucuronidase (-) indole (+) tests and the presence of O157 and H7 antigens by latex agglutination may be performed. [2,8,11]
For a complete explanation of the identification criteria and methods, refer to the literature cited for clinical samples.[11,13]

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, the end user can perform its own Quality Control 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
Escherichia coli ATCC 25922	35-37°C / 18-24 H / A	growth, red 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 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready to use plates of Mac Conkey Sorbitol Agar and of the raw material used for the production of prepared plates (dehydrated Mac Conkey Sorbitol Agar REF 401669S), are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by semi-quantitative ecometric technique with the target strains *E.coli* O157:H7 ATCC 43888, *E.coli* O157:H7 NCTC 12900, *E.coli* O157:H7 ATCC 43894. After incubation at 35-37°C for 18-24 hours, 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 suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *E.coli* ATCC 25922, and *S.aureus* ATCC 25923. *E.coli* grows with red colonies while *S.aureus* is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- There are several well-known EHEC (Entero Haemorragic *E.coli*) 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 SMAC agar. Refer to the cited literature for the determination of these strains in clinical samples.[11]
- Laboratories that use CT-SMAC agar (Cefixime Tellurite Sorbitol MacConkey) should also inoculate a SMAC agar plate to isolate tellurite sensitive STEC strains.[11]
- *E.coli* O157 sorbitol positive and β-glucuronidase positive strains have been reported.[14,15] For the management of these strains refer to the cited literature.[11-13]
- Follow the recommended time and temperature 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 is 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.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 ready-to use plates 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.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled biocontamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

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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	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 0	First publication, in compliance with IVDR 2017/746	2021/02
Revision 1	Removal of obsolete classification	2023/04

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

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