TECHNICAL DATA SHEET

MACCONKEY SORBITOL (CT-SMAC) AGAR

DETECTION OF *ESCHERICHIA COLI* O157

1 INTENDED USE

MacConkey Sorbitol (CT-SMAC) agar is a selective media used for the isolation and differentiation of *Escherichia coli* O157 in water, milk, beef products and other food preparations.

2 HISTORY

The serotype O157 of *Escherichia coli* is a pathogenic serotype, identified for the first time in 1982 as the agent responsible for hemorrhagic colitis. Animal origin feeds is the principle source of human contamination. Beef and pork tissue, poultry and certain non-pasteurized dairy products have been incriminated in toxi-infections linked to *Escherichia coli* O157. This microorganism has also been isolated from potatoes, apple cider and tap water.

In 1985, Farmer et Davis confirmed the work of Wells *et al.* (1983), by demonstrating that the serotype O157 as characterized by its incapacity to ferment sorbitol, while over 80% of *E. coli* strains are sorbitol-positive. In 1986, March & Ratnam reinforced the interest in the use of MacConkey-Sorbitol agar for the detection of *Escherichia coli* O157. In 1991, Chapman introduced cefixime to inhibit *Proteus*. In 1993, Zadik combined the action of potassium tellurite with that of cefixime. The addition of these components to the agar increases the frequency of detection of O157 colonies by eliminating a large spectrum of the secondary microflora that may be present in the sample.

3 PRINCIPLES

Tryptone and Peptic digest of meat favor the growth of *Escherichia coli* O157.

Sorbitol-negative microorganisms (notably O157) produce transparent colonies.

Sorbitol-positive microorganisms are detected by the colonies turning red (under the action of neutral red).

Secondary microflora are inhibited by the association between the bile salts, crystal violet, cefixime and potassium tellurite.

A few strains of *Escherichia coli* O157 are sorbitol-positive and will not be detected directly.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Tryptone ..... 17,0 g
- Peptic digest of Meat ..... 3,0 g
- D-Sorbitol ..... 10,0 g
- Bile salts n°3 ..... 1,5 g
- Sodium chloride ..... 5,0 g
- Neutral red..... 30,0 mg
- Crystal violet..... 1,0 mg
- Cefixime ..... 0,05 mg
- Potassium tellurite..... 2,5 mg
- Bacteriological agar..... 13,5 g

pH of the ready-to-use media at 25 °C : 7,1 ± 0,2.

For 50 g of dehydrated base media BK147

- Tryptone ..... 17,0 g
- Peptic digest of meat ..... 3,0 g
- D-Sorbitol ..... 10,0 g
- Bile salts n°3 ..... 1,5 g
- Sodium chloride ..... 5,0 g
- Neutral red ..... 30,0 mg
- Crystal violet ..... 1,0 mg
- Bacteriological agar ..... 13,5 g

For one vial of supplement BS037
(Qs 500 mL)

- Cefixime ..... 0,025 mg
- Potassium tellurite ..... 1,25 mg

5 PREPARATION

- Dissolve 50,0 g of dehydrated based media (BK147) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring until complete dissolution.
- Divide into 100 mL aliquots (per vial).
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.
- Reconstitute the selective supplement (BS037) with 5 mL of sterile water.
- Shake the flask or vortex in order to obtain complete dissolution, all the while avoiding the formation of foam.
- Aseptically add 1 mL of the reconstituted selective supplement (BS037) per 100 mL vial of base media.
- Mix well.
- Pour into sterile Petri plates and let solidify on a cold surface.

✓ **Reconstitution :**
50,0 g/L

✓ **Sterilization :**
15 min at 121 °C

✓ **Supplement preparation**
5 mL eau stérile

✓ **Add to base :**
1 mL / 100 mL

6 INSTRUCTIONS FOR USE

- Dry the plates in an incubator, covers partially removed.
- After enrichment in modified Tryptone soja broth with novobiocin (BK150) and after immune-magnetic separation, inoculate the plates and spread the microbilles.
- Incubate at 37 °C for 18 to 24 hours.

✓ **Inoculation :**
On surface

✓ **Incubation :**
18 h to 24 h at 37 °C

7 RESULTS

Escherichia coli O157 forms smooth, transparent colonies that may present an orange halo of alkalinzation.
As the specificity of the media isn't total, the suspect colonies must be submitted to serological agglutination tests on slides with the help of specific O157 antiserums.

See ANNEX 1 : PHOTO SUPPORT .

8 QUALITY CONTROL

Dehydrated base media : beige-pink powder, free-flowing and homogeneous.
Selective supplement : white pellet, giving after reconstitution an transparent, limpid solution.
Prepared media : red-violet agar

Typical culture response after 21 hours of incubation at 37 °C (NF EN ISO 11133) :

Microorganisms		Growth	Characteristics
Escherichia coli O157:H7	WDCM 00014	Good, score 2	transparent colonies
Escherichia coli sorbitol-positive	WDCM 00013	Weak, score 0-1	pink-red colonies
Staphylococcus aureus	WDCM 00034	Inhibited, score 0	-

9 STORAGE / SHELF LIFE

Dehydrated base media : 2-30 °C.
Cefixime-Tellurite Selective supplement : 2-8 °C.
The expiration dates are indicated on the labels.

Reconstituted supplement (*) : 30 days at 2-8 °C.
Prepared base media in vials (*) : 180 days at 2-8 °C.
Prepared complete media in plates (*) : 30 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated base media (without cefixime or potassium tellurite) :
500 g bottle BK147HA

Cefixime-Tellurite Selective supplement :
10 vials qsp 500 mL BS03708

11 BIBLIOGRAPHY

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March, S. B., and S. Ratnam. 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157 : H7 associated with hemorrhagic colitis. J. Clin. Microbiol. 23 : 869-872.

Chapman, P. A., C. A. Siddons, P. M. Zadik, and L. Jewes. 1991. An improved selective medium for the isolation of *Escherichia coli* O157. J. Med. Microbiol. 35 : 107-110.

Zadik, P. M., P. A. Chapman, and C. A. Siddons. 1993. Use tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. J. Med. Microbiol. 39 : 155-158.

NF EN ISO 16654. Juillet 2001. Microbiologie des aliments. Méthode horizontale pour la recherche des *Escherichia coli* O157.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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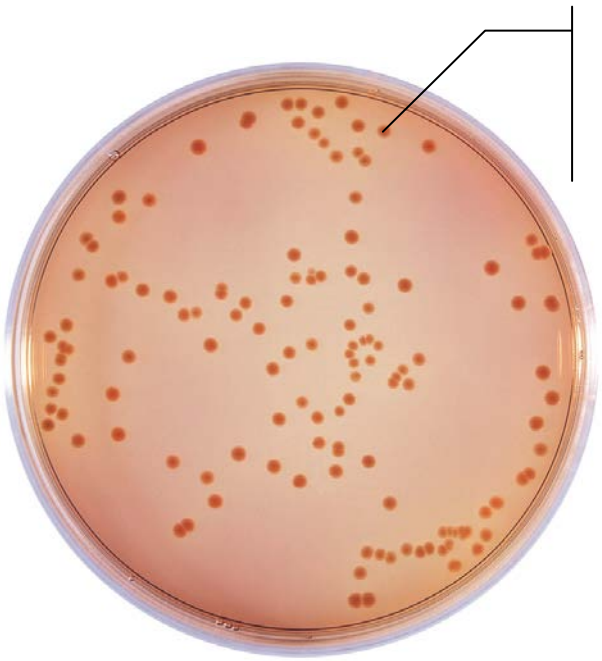
ANNEX 1 : PHOTO SUPPORT

MacCONKEY Sorbitol (CT-SMAC) Agar

Detection of *Escherichia coli* O157.

Results :

Growth obtained after 24 hours of incubation at 37 °C.



Note : Colonies are slightly colored artificially by the absorption of the colorants present in the media formulation. Colonies of *E. coli* **non-**O157:H7 are red and of much greater size.