TECHNICAL DATA SHEET

TERGITOL 7 AGAR

ENUMERATION OF *ESCHERICHIA COLI* AND COLIFORMS IN WATER

1 INTENDED USE

Tergitol 7 Agar is used for the enumeration of *Escherichia coli* and coliform bacteria in water, notably potable water, through the membrane filtration method.
This media is also used for testing swimming pool water.
The typical composition corresponds to that defined in 2 mandatory directives : NF EN ISO 9308-1 for water destined for human consumption and NF T90-431 for swimming pool water testing.

2 HISTORY

In 1946, Pollard showed the bactericidal action of Tergitol 7 against Gram-positive bacteria. The medium was then developed by Chapman, whose enumeration of coliform bacteria were about 30% higher than those done on other selective media. The formula was then modified by the same author by adding TTC (triphenyltetrazolium chloride), which was found to be useful for the rapid recognition of *Escherichia coli* and *Enterobacter aerogenes*.

3 PRINCIPLES

Coliform bacteria form colonies that are yellow or orange inside a yellow halo. The halo results from the acidification of lactose under the membrane filter in the presence of a colored indicator, bromothymol blue.

Tergitol 7 inhibits Gram-positive bacteria, limits invasion by *Proteus* and favors recovery of coliform bacteria.

TTC (2,3,5 triphenyltetrazolium chloride) is present in the form of a freeze-dried supplement. Bacteria that reduce the TTC (including *Proteus* &t *Pseudomonas*) display red colonies due to the formation of insoluble formazan.

Bacteria that do not ferment lactose form colonies surrounded by a blue halo.

4 TYPICAL COMPOSITION

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

For 1 liter of complete media :

- Pancreatic digest of meat..... 10,0 g
- Meat extract ..... 5,0 g
- Yeast extract ..... 6,0 g
- Lactose..... 20,0 g
- Tergitol 7 ..... 0,1 g
- Bromothymol blue ..... 50,0 mg
- 2, 3, 5 triphenyltetrazolium chloride ..... 25,0 mg
- Bacteriological agar..... 10,0 g

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

For 51,1 g of dehydrated based media BK123

- Pancreatic digest of Meat ..... 10,0 g
- Meat extract ..... 5,0 g
- Yeast extract ..... 6,0 g
- Lactose..... 20,0 g
- Tergitol 7 ..... 0,1 g
- Bromothymol blue ..... 50,0 mg
- Bacteriological agar ..... 10,0 g

Pour un flacon de supplément BS026

Triphenyltetrazolium ..... 12,5 mg
(chloride)..... 12,5 mg

5 PREPARATION

- Dissolve 51,1 g of dehydrated media (BK123) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring slowly until complete dissolution.
- Dispense 100 mL into flasks or vials.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.
- Rehydrate each via of supplement necessary (BS026) wtih 5 mL of sterile distilled water.
- Mix or use a vortex to obtain complete dissolution, while avoidung overdue foaming.
- Aseptically add 1 mL of reconsituted TTC supplement (BS026) per 100 mL vial of base media.
- Mix well.
- Pour into sterile Petri plates Ø 55 mm (the thickness of the agar must be equal or greater than 5 mm).
- Let solidify on a cold, flat surface.
- Do not dry the plates.

✓ **Reconstitution :**
51,1 g/L

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

✓ **Rehydratation BS026 :**
5 mL water / vial

✓ **Add :**
1 mL / 100 mL base

6 INSTRUCTIONS FOR USE

- Aseptically filter 100 mL of water to test through a membrane (or 250 mL for bottled water).
- To the surface of plates prepared as above or by using pre-poured plates (BM147 or BM093) brought to room temperature, deposit the membrane on the surface of the agar, filtered side up and making sure that the membrane and agar are in close contact.
- Incubate at 36 ± 2 °C for 21 ± 3 hours.

✓ **Inoculation :**
Membrane filtration

✓ **Incubation :**
21 ± 3 h at 36 ± 2 °C

NOTES

For plates that do not display typical colonies after 21 ± 3 hours, it is possible to prolong the incubation up to 44 ± 4 hours.
To limit the secondary flora, it is possible to incubate another plate at 44 °C.

7 RESULTS

Examine the membranes and consider as typical all bacteria, if a corresponding yellow halo in the medium under the membrane is present.
Confirm the presence of coliforms via an oxidase test.
Confirm the presence of *Escherichia coli* through the oxidase test plus an indole test.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated base media : beige to greenish powder, free-flowing and homogeneous.
TTC 12,5 mg supplement : white pellet, giving after reconstitution a colorless solution.
Complete media : blue-green agar.

Typical culture response after 21 hours of incubation at 36 °C (NF EN ISO 11133) :

Microorganisms		Growth (Productivity Ratio : P_R)
Escherichia coli	WDCM 00179	$P_R \geq 70$ %, yellow colonies under membrane
Enterobacter aerogenes	WDCM 00175	$P_R \geq 70$ %, yellow colonies under membrane
Citrobacter freundii	WDCM 00006	$P_R \geq 70$ %, yellow colonies under membrane
Pseudomonas aeruginosa	WDCM 00025	Red colonies, blue color in the media
Enterococcus faealis	WDCM 00087	Totally inhibited, score 0

9 STORAGE / SHELF LIFE

Dehydrated base media : 2-30 °C.
TTC 12,5 mg Supplement: 2-8 °C, shielded from light.
Pre-poured media in Petri plates : 2-8 °C.
The expiration dates are indicated on the labels.

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

10 PRESENTATION

Dehydrated base media (without TTC) :
500 g bottle BK123HA

TTC 12,5 mg Supplement:
10 vials BS02608

Pre-poured media in Petri plates (Ø 55 mm) :
20 plates BM14708
120 plates BM09308

11 BIBLIOGRAPHY

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NF T90-421. Aout 2006. Qualité de l'eau. Examens bactériologiques des eaux de piscine.

NF EN ISO 11133. Juillet 2014. Microbiologie des aliments, des aliments pour animaux et de l'eau - Préparation, production, stockage et essais de performance des milieux de culture. Tirage 2 (2016-01-01).

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

Tergitol 7 Agar

Detection and enumeration of *Escherichia coli* and other coliform bacteria.

Results :

Growth obtained after 21 hours of incubation at 36 °C.

Escherichia coli

Characteristic colony : Yellow to orange color. A yellow halo under the membrane.