

Also known as

Tergitol 7 Agar; T7 Agar

Specification

Medium for the detection of coliforms by membrane filtration in water analyses according to ISO 9308-1:2000 standard.

Formula * in g/L

Meat peptone.....	10,000
Meat extract.....	5,000
Lactose	20,000
Yeast extract.....	6,000
Bromothymol blue	0,050
Tergitol® 7.....	0,100
Agar.....	15,000

Final pH 7,2 ±0,1 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 56,2 g in 1 L of distilled water and bring to the boil. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Cool to 45-50°C. Add 2-3 mL/L of filtered sterile 1% aqueous 2, 3, 5-triphenyltetrazolium chloride (TTC) (Art. No DSHB3074) and pour plates. Do not reheat.

Description

This medium is formulated for the presumptive identification of coliforms in drinking water, by membrane filtration according to ISO 9308-1:2000. Due to the instability of the triphenyltetrazolium it is provided in a separate container, sterilized and ready to use (Art. No. DSHB3074). Poured plates can be stored refrigerated for up to 8 days without losing their effectiveness. They should not be used if any signs of dehydration or drying appear.

Technique

While using the membrane filter technique for the presumptive identification of coliforms in water, it should be kept in mind that the minimum volume to be filtered depends on the type of water being tested. If necessary dilute with sterile phosphate buffer in order to obtain the number of colonies on the membrane appropriate for counting.

For every water sample two volumes must be filtered over two different membranes and incubated on Tergitol® 7 agar à 35°C and 44°C respectively.

After 48 hours typical colonies have the appearance as follow:

- *Escherichia coli*, *Citrobacter* spp.: Yellow with a centred orange nucleus under the membrane filter (MF).
- *Klebsiella* spp.: Brick red or yellow without a nucleus. The medium under the (MF) is yellow.
- *Enterobacter* spp.: Dark yellow or brick red with an orange nucleus. The medium is also yellow.
- Non lactose-fermenters: Violet or indigo colonies. The medium turns blue.

Most coliforms can not grow on this medium when incubated à 44°C, except *E. coli* which forms a colony with a characteristic appearance.

Results are always expressed per 100 ml sample including any applied dilutions. Estimation is done by taking typical colonies which have grown à 35°C as faecal coliforms, together with those grown à 44°C as *E. coli*. Nevertheless, according to legislation and despite the selectivity of medium, results can only be considered as presumptive and all coliform colonies have to be confirmed by following the criteria below:

Typical appearance in EMB Agar or Endo Agar Base and characteristic reactions in Kligler fer Agar.

For the confirmation of faecal *E. coli*, the following characteristics are used for verification: a motile, Gram negative bacillus and lactose fermenter with acid and gas production, which gives negative results on the citrate test and indole production positive.

Quality control

Incubation temperature: 36°C ±2,0

Incubation time: 21 ±3h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) / 10⁴-10⁶ CFU (Selectivity) / ≥ 10³ CFU (specificity) according to ISO 11133:2014/Amd 1:2018 . MF methods.

Microorganism
Growth
Remarks

<i>Escherichia coli</i> ATCC® 8739	Productivity > 0.50	Yellow-orange colonies under MF
<i>Enterobacter aerogenes</i> ATCC® 13048	Productivity > 0.50	Yellow-orange colonies under MF
<i>Escherichia coli</i> ATCC® 25922	Productivity > 0.50	Yellow-orange colonies under MF
<i>Enterococcus faecalis</i> ATCC® 19433	Total inhibition	Selectivity
<i>Citrobacter freundii</i> ATCC® 43864	Productivity > 0.50	Yellow-orange colonies under MF
<i>Pseudomonas aeruginosa</i> ATCC® 9027	Good (specificity)	Dark red colonies, blue center
<i>E. coli</i> NCTC® 13167	Productivity > 0.50	Yellow-orange colonies under MF

References

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- CHAPMAN G.H. (1951) A culture medium for detecting and confirming E. coli in ten hours. Am. J. Publ. Hlth 41:1381-1386.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 3rd ed. APHA. Washington.
- GUINEA, SANCHO, PARES (1979) Análisis Microbiológico de Aguas. Ed. Omega. Barcelona.
- ISO 9308-1:2000 Standard. Water Quality - Detection and enumeration of Escherichia coli and coliform bacteria - Part 1: Membrane filtration method.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- SPECK, M (Ed.) (1982) Compendium of Methods for the Microbiological Examination of Foods. 2nd ed. APHA. Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).