Condalab

Reference: 6030 **Technical Data Sheet**

Product: **TTC 1% SUPPLEMENT**

Specification

Redox type indicator of microbial growth.

Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vials Vial with: 6 ± 0.1 g	22±0.25 x 55±0.5 mm glass vials, tag labelled, White plastic cap - 10 vials per box.	49 months	2-8 ºC

Composition

Compositon (g/vial)

TTC.....0.05

Note: Each vial is sufficient to supplement 2L of Chapman Agar

Reconstitute the original freeze-dried vial by adding
Sterile Distilled Water.....5 ml

Description /Technique

Description:

2,3,5-triphenyltetrazolium chloride (TTC) is a dye largely used for the presumptive detection, isolation and enumeration of microbial colonies like *E. coli* and other coliforms in solid culture media, by the membrane filtration technique in waters for human consumption. Also, the use of TTC is highly recommended for milk testing, because there is a high percentage of microorganisms unable to reduce TTC in pasteurized milk, which cannot be detected by laboratory procedures.
This dye is colorless in the oxidized form and red when reduced by live microorganisms, due to formation of formazan, an insoluble red pigment which is kept inside granules in the cells. Depending on TTC concentration, it may has a very little inhibition of microbial growth in Gram positive organisms.

Technique:

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add 2,5 ml to 1000 ml of TTC Chapman Agar (Cat. 1076), autoclaved and cooled to 45-50 ºC. Mix well and distribute into sterile containers.
When the TTC is required to be added to another media, like KF Streptococcal Agar (Cat. 1034) or KF Streptococcal Agar with Bromocresol Purple (Cat. 1101), refer to the specific instructions of the medium for the quantity of TTC 1% supplement that should be added.

Instructions for use:

For the detection and enumeration of *Escherichia coli* and coliform bacteria in water samples:
- Filter two samples of water over two different membranes and incubate on TTC Chapman Agar (Cat. 1076) at 36±2 ºC and 44±4 ºC respectively for 21±3 hours.

Typical colonies have the appearance as follow:
- *E. coli* and *Citrobacter spp* present yellow colonies with orange-colored center.
- *Enterobacter spp* forms red colored colonies and dark yellow with orange-colored center. The medium is yellow. - *Klebsiella spp* form red colored or yellow, but without center. The medium is yellow.
- Lactose non fermentative bacteria grow with purple colonies and change the medium to blue.

Klebsiella and *Enterobacter* species can also produce yellow-green colonies.
The results will always refer to counts per 100 ml of sample, considering if it has been necessary to make dilutions.
- Count as lactose-positive bacteria the colonies that present a yellow development of the medium under the membrane.
- Subculture the characteristic colonies obtained, in non-selective agar and Tryptophan Culture Broth (Cat. 1237).
- Carry out the oxidase test and incubate the tubes of Tryptophan Culture Broth at 44±0,5 ºC for 21±3 hours.
- Indole production is determined by adding a few drops of Kovac's Reagent (Cat. 5205) to the incubated Tryptophan Culture Broth tubes. A positive test is indicated by the development of red color in the reagent layer.
- The colonies that are oxidase negative will be considered as coliform bacteria and the colonies that are negative oxidase and positive indol will be considered as *E.coli.*

Quality control

Physical/Chemical control

Color : White-yellowish pH: at 25ºC

Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely
Add 1 vial to 2L of medium base. DO NOT HEAT once supplemented.
Cool the media at 50 ºC and Pouring into MF dishes.

Membrane Filtration /Practical range 100 ± 20 CFU. min. 50 CFU (productivity)./10^4-10^6 CFU (selectivity).

Aerobiosis. Incubation at 36 ± 2 ºC, reading at 21±3 h

Microorganism	Growth
Enterococcus faecalis ATCC® 19433	Inhibited
Escherichia coli ATCC® 25922, WDCM 00013	Good (≥ 70%) Colonies Yellow-orange under MF.
Ps. aeruginosa ATCC® 27853	Good- Red colonies w. blue center.
E. coli NCTC® 13167, WDCM 00179	Good (≥ 70%) Colonies Yellow-orange under MF.

Sterility Control

Incubation 48 hours at 30-35 ºC and 48 hours at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

Bibliography

Kennor, G.A., Clark, H.F. and Kabler, P.W. (1961) J. Appl. Microbiol. 9: 15-20. Slanetz, L.W. and Bartley, C.H. (1957) J. Bact. 74: 591-595.
Chapman, G.H. (1947) J. Bact. 53: 504.
Nordic Committee on Food Analysis (1968) Leaflet 68.22/01/202022/01/2020