



## Modified Tergitol 7 Agar Base (Tergitol -7 Agar Base, Modified) M616I

### Intended Use:

Recommended for selective isolation and enumeration of coliform organisms in water by membrane filtration method. The composition and performance criteria are in accordance with ISO 9308-1:2000.

### Composition\*\*

Ingredients	g/ L
Peptone	10.000
Yeast extract	6.000
HM extract ⊖	5.000
Lactose	20.000
Tergitol 7 (Sodium heptadecyl sulphate)	0.100
Bromo thymol blue	0.050
Agar	16.000
Final pH ( at 25°C)	7.2±0.2

\*\*Formula adjusted, standardized to suit performance parameters

⊖ Equivalent to Meat extract

### Directions

Suspend 57.15 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Add 2.5 ml of 1% Triphenyl Tetrazolium Chloride (TTC) (FD057). Mix well and pour into sterile Petri plates.

### Principle And Interpretation

Tergitol-7 Agar is a selective and differential medium for the detection and enumeration of coliforms in water. Chapman (1,2) modified his original formula of Tergitol-7 Agar by addition of Triphenyl Tetrazolium Chloride (TTC). It is now recommended by ISO Committee (3).

Tergitol-7 is a selective agent (4) which inhibits gram positive organisms and minimises swarming of *Proteus* species enabling better coliform recovery. Lactose fermentation is observed by change in colour of bromo thymol blue, the pH indicator. Triphenyl Tetrazolium Chloride (TTC) allows earlier recognition and identification of *Escherichia coli* and *Klebsiella aerogenes* in water and food (5).

Peptone, HM extract and yeast extract serve as sources of carbon, nitrogen and other essential nutrients including vitamin B complex. Bromothymol blue is the pH indicator. TTC is rapidly reduced by coliforms except *Escherichia coli* and *Klebsiella aerogenes* to insoluble formazan which gives red colour to the colonies. The lactose fermenters show greenish yellow colonies with yellow zones while lactose non-fermenters show red colonies surrounded by blue zones.

Filter the specimen to be analyzed through two membranes. Place the membrane upon two TTC Tergitol Agar plates. Incubate one plate at 37°C for 24 hours (total coliforms) and the other at 44°C for 18-24 hours (faecal coliforms). The yellow colonies with deep yellow halo after incubation at 44°C should be identified as faecal coliform bacteria.

### Type of specimen

Water samples

### Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6). After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations :

1. Further biochemical and serological tests must be carried out for complete identification.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

### Appearance

Cream to light green homogeneous free flowing powder

### Gelling

Firm, comparable with 1.6% Agar gel.

### Colour and Clarity of prepared medium

Green coloured clear to slightly opalescent gel forms in Petri plates

### Reaction

Reaction of 5.71% w/v aqueous solution at 25°C. pH : 7.2±0.2

### pH

7.00-7.40

### Cultural Response

**Productivity** : Cultural response was observed with added TTC Solution 1% (FD057) after an incubation at 36 ± 2°C for 21 ±3 hours. Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar (Tryptone Soya Agar).

**Specificity** : Cultural response was observed with added TTC Solution 1% (FD057) after an incubation at 36 ± 2°C for 21 ±3 hours.

**Selectivity** : Cultural response was observed with added TTC Solution 1% (FD057) after an incubation at 36 ± 2°C for 21 ±3 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony (on plain medium)	Colour of colony (with addition of FD057)
<b>Productivity</b>					
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	≥50%	yellow	yellow with red centre
<i>Escherichia coli</i> ATCC 8739 (00012*)	50-100	luxuriant	≥50%	yellow	yellow with red centre
<i>Escherichia coli</i> (00179*)	50-100	luxuriant	≥50%	yellow	yellow with red centre
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	luxuriant	≥50%	yellow	reddish brown
<i>Citrobacter freundii</i> ATCC 43864 (00006)*	50-100	luxuriant	≥50%	yellow	yellow with red centre
<b>Specificity</b>					
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	10 <sup>3</sup> - 10 <sup>4</sup>	growth		colourless with bluish zone	red with bluish zone
^ <i>Pseudomonas paraaeruginosa</i> ATCC 9027 (00026*)	10 <sup>3</sup> - 10 <sup>4</sup>	growth		colourless with bluish zone	red with bluish zone

### Selectivity

*Enterococcus faecalis*  $\geq 10^4$  inhibited  
ATCC 19433 (00009)\*

*Enterococcus faecalis*  $\geq 10^4$  inhibited  
ATCC 29212 (00087)\*

Key : (\*) Corresponding WDCM numbers.

(#) Formerly known as *Enterobacter aerogenes*

^ Formerly known as *Pseudomonas aeruginosa*

### Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

### Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

### Reference

- 1.Chapman G.H., 1947, J. Bact., 53:504.
- 2.Chapman G.H., 1951, Am. J. Public Health, 41:1381.
- 3.ISO 9308-1:2000. Water quality. Detection and enumeration of *Escherichia coli* and coliform bacteria.PART 1. Membrane filtration method.
- 4.Pollard A.L., 1946, Science., 103:758.
- 5.Mossel D.A.A., 1962, J. Appl. Bact., 25:20.
- 6.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
- 7.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
- 8.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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### Disclaimer :

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