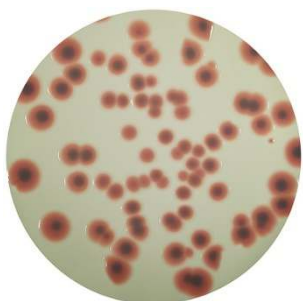


TTC TERGITOL 7 AGAR BASE TTC TERGITOL 7 AGAR TTC SOLUTIONS

Dehydrated culture medium, supplements and ready to use plates



TTC Tergitol 7 Agar- from top to bottom: colonies of *E. coli* and *Salmonella* sp. on membrane filters

1 - INTENDED USE

For the enumeration of coliforms and *Escherichia coli* in water samples by the membrane filtration technique.

2 - COMPOSITION - TYPICAL FORMULA *

DEHYDRATED TTC TERGITOL 7 AGAR BASE (AFTER RECONSTITUTION WITH 1 L OF WATER)

Yeast extract	6.00 g
Peptone	10.00 g
Beef extract	5.00 g
Lactose	20.00 g
Bromothymol blue	0.05 g
Tergitol 7	0.10 g
Agar	13.00 g

TTC 0.05% SOLUTION REF 421510 (BOTTLE CONTENT)

Triphenyl tetrazolium chloride	0.0125 g
Purified water	25 mL

TTC 1% SOLUTION REF 42111801 (BOTTLE CONTENT)

Triphenyl tetrazolium chloride	0.3 g
Purified water	30 mL

TTC TERGITOL 7 AGAR (READY-TO-USE PLATES)

TTC Tergitol 7 Agar Base	54 g
TTC 1% Solution	2.5 mL
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Tergitol 7 agar was first introduced by Chapman¹ in 1947. The medium produced a consistent and characteristic colonial morphology with *E. coli*, *Enterobacter aerogenes*, and other Gram-negative bacteria. This medium was later modified by Chapman² to include 2,3,5-triphenyltetrazolium chloride and was recommended as the medium of choice for the quantitative detection of *E. coli* in drinking water.³

TTC Tergitol Agar was recommended by the ISO 9308-1:2000 Standard,⁴ which was withdrawn and replaced by the 2014 version, for the detection and enumeration of *E. coli* and coliforms in drinking water using the membrane filtration method.

Peptone and beef extract provide nitrogen and minerals for microbial growth, yeast extract is a source of B-vitamins complex for growth stimulation, lactose is a fermentable carbohydrate and a source of carbon and energy. Tergitol-7 acts as surface-active agent which inhibits the growth of most Gram-positive bacteria as well as swarming of *Proteus*. Bromothymol blue is a pH indicator: the high concentration of acids produced during fermentation of lactose turns the bromothymol blue indicator from green to yellow. TTC is a sensitive indicator of dehydrogenase⁵ and its reduction to insoluble formazan by lactose-negative bacteria produces dark red colonies. For typical *E. coli*, under the conditions of use, TTC is not a satisfactory hydrogen acceptor and it is reduced weakly, hence the colonies are yellow-orange.⁵

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 27 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C and add, under aseptic conditions, 1.25 mL of TTC 1% Solution (REF 42111801) or the content of one bottle (25 mL) of TTC 0.05% Solution (REF 421510). Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale blue, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	green, clear
TTC solutions appearance	colourless, clear
Final pH at 20-25 °C	7.2 ± 0.1

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
TTC Tergitol Agar Base	Dehydrated medium	402160T2	500 g (9.3 L)
TTC 0.05% Solution	Liquid supplement	421510	10 x 25 mL
TTC 1% Solution	Liquid supplement	42111801	30 mL
TTC Tergitol Agar	Ready-to-use plates	492160T	3 x 10 piastre ø 55 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, membrane filters, ancillary culture media and reagents.





8 – SPECIMENS

Water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

1. Filter an appropriate volume of water depending on the expected microbial load onto two membrane filters.
2. Roll the membrane filters onto the surface of two TTC Tergitol 7 Agar plates, avoiding the formation of air bubbles between the filter and the agar surface.
3. Incubate one dish at $36 \pm 2^\circ\text{C}$ for 18-24 hours (total coliforms) and the other one at $44.0 \pm 0.5^\circ\text{C}$ for 18-24 hours (*E. coli*).
4. Subculture the characteristic colonies in a non-selective agar such as Tryptic Soy Agar (REF 402150) and in Tryptone Tryptophan Medium (REF 402165).
5. Incubate the non-selective agar at $36 \pm 2^\circ\text{C}$ for 18-24 h and perform the oxidase test.
6. Incubate the Tryptone Tryptophan Medium tube at $44.0 \pm 0.5^\circ\text{C}$ for 18-24 h and examine indole production by adding few drops of Kovacs' reagent (REF 19171000).

10 - READING AND INTERPRETATION

After incubation, examine the membranes and count as lactose-positive bacteria, the colonies developing a yellow colouration in the medium below the membrane.

Count all colonies that produce a negative oxidase reaction as coliforms.

Count all colonies producing a negative oxidase reaction and a positive indole reaction as *E. coli*.

Typical colonies on TTC Tergitol 7 Agar:

E. coli grows with yellow colonies with yellow zone sometimes with rust coloured centre.

Enterobacter/Klebsiella spp. exhibit greenish/yellow colonies.

Lactose negative organisms such as *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas* grow with red colonies with bluish zones.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T° / T - ATM	EXPECTED RESULTS
<i>E. coli</i> ATCC 25922	37°/ 18-24 H-A	growth with yellow colonies and yellow zones
<i>E. faecalis</i> ATCC 19433	37°/ 18-24 H-A	partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated and ready-to-use plates of TTC Tergitol 7 Agar (Test Batch:TB) are tested for productivity, specificity and selectivity by comparing the results with Tryptic Soy Agar.

The productivity is tested by a quantitative method with the target strains *E. coli* ATCC 25922, *E. coli* CIP 106878, *C. freundii* ATCC 43864, *E. aerogenes* ATCC 13048: the filters rolled on MF plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 37°C for 18-24 hours. The colonies are enumerated on both batches and the productivity ratio (Pr:CFU_{TB}/CFU_{TSA}) is calculated. If Pr is ≥ 0.5 and if the colonies morphology and colour are typical (yellow to orange colonies with yellow zones) the results are considered acceptable and conform to the specifications.

The specificity is assessed by semi-quantitative ecometric technique with the non-target strain *P. aeruginosa* ATCC 27853. After incubation at 37°C for 18-24 hours the non-target strain exhibits red-violet colonies with bluish zones.

The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the membranes with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target strains: *S. aureus* ATCC 25923 and *E. faecalis* ATCC 19433. The growth of *S. aureus* is totally inhibited while the growth of *E. faecalis* is partially inhibited .

13 – LIMITATIONS OF THE METHOD

- In special cases, identification of coliform bacteria may be necessary, e.g., to distinguish between faecal and aquatic/telluric strains.⁵

14 - PRECAUTIONS AND WARNINGS

- The medium base, the supplements and the ready-to-use plates are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Be careful when opening the metal ring to avoid injury.
- The supplement is sterilized by membrane filtration.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.





- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Ready to use plates

Upon receipt, store plates in their original pack at +2°C /+8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

Liquid supplements

Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Before use, examine the solution and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/flasks) and the applied storage conditions (temperature and packaging). According to ISO 9308-1:2000 the self-prepared plates can be stored at +2°C /+8°C in the dark and protected against evaporation for up to 10 days.⁴

16 – REFERENCES

- Chapman GH. A superior culture medium for the enumeration and differentiation of coliforms. J Bacteriol. 1947;53(4):504.
- Chapman GH. A culture Mmedium for detecting and confirming Escherichia coli in ten hours. Am J Public Health Nations Health. 1951; 41(11 Pt 1): 1381.
- Kulp W L, Mascoli C, Tavshanjian O. Use of Tergitol-7 Triphenyl Tetrazolium chloride agar as the coliform confirmatory medium in routine sanitary water analysis. Am J Public Health. 1953; 43:1111-1113.
- ISO 9308-1 (2000) Withdraw. Water Quality: Detection and enumeration of Escherichia coli and coliform bacteria. Part 1: Membrane filtration method.
- Kulp WL, Mascoli C, Tavshanjian O. Use of Tergitol-7 Triphenyl Tetrazolium Chloride Agar as the Coliform Confirmatory Medium in Routine Sanitary Water Analysis. Am J Public Health Nations Health. 1953 Sep; 43(9): 1111–1113.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	For single use only	This side up	Store in a dry place
Temperature limitation	Content sufficient for <n> tests	Consult instructions for use	Use by	Fragile	Keep away from direct light

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/09

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

