IFU

Instructions for Use

# TERGITOL 7 WITH TTC

| | | |
|---|---|---|
| Cat. no. G58 | Tergitol 7 with TTC, 15x100mm Plate, 20ml | 10 plates/bag |
| Cat. no. J120 | PEA with 5% Sheep Blood/Tergitol 7, 15x100mm Biplate, 10/10ml | 10 plates/bag |

## INTENDED USE

Hardy Diagnostics' Tergitol 7 with TTC agar is recommended as a selective and differential medium for enumeration of coliforms in food and water samples.[1-4,7] The medium conforms to the recommendations of the APHA.[5,12]

This product is not intended to be used for the diagnosis of human disease.

## SUMMARY

Previous research by Pollard shows the bactericidal action of Tergitol 7 against gram-positive bacteria.[11] Chapman later developed the medium for the selective isolation of *Escherichia coli* and other members of the coliform group. According to Chapman's published formula, a medium containing Tergitol 7, proteose peptone no. 3, yeast extract, lactose, and bromothymol blue permitted the unrestricted development of coliform bacteria, while inhibiting the growth of gram-negative spore-formers, gram-positive microorganisms and the swarming of *Proteus* spp.[1,2] He found the difference between *E. coli* and *Enterobacter aerogenes* and other coliforms to be distinct on this medium: *E. coli* produces yellow colonies with yellow zones and occasionally rust colored centers; *E. aerogenes* produces greenish-yellow colonies, while non-lactose fermenting microorganisms produce dark red colonies with bluish zones. In addition, Chapman proposed that counts of coliform organisms on Tergitol 7 Agar were found to be as much as 30% higher than on other types of selective media.[6]

Chapman later modified his original formula by adding 40mg of triphenyltetrazolium chloride (TTC) per liter, and the modified formulation was found to be helpful in rapidly differentiating *E. coli* and *E. aerogenes*. On medium containing TTC, confirmation of the presence of *E. coli* and *E. aerogenes* was possible in as few as six to ten hours.[2] Chapman also reported that Tergitol 7 Agar with added TTC was suitable for the selective isolation of *Candida* spp. and other contaminating fungi.[2]

Hardy Diagnostics' Tergitol 7 with TTC agar is a modification of Chapman's original formulation and contains 25mg of 2,3,5 Triphenyltetrazolium Chloride (TTC) for the rapid detection of *E. coli* and *E. aerogenes* from food and water samples. The medium is recommended by the APHA for the recovery of injured or stressed total coliform bacteria from chemically treated or contaminated waters when parellel methods indicate suboptimal recovery.[5,12,13]

## FORMULA

Ingredients per liter of deionized water:*

| | |
|---|---|
| Lactose | 10.0gm |
| Proteose Peptone No. 3 | 5.0gm |

| | |
|---|---|
| Yeast Extract | 3.0gm |
| Tergitol 7 | 0.1gm |
| Bromothymol Blue | 0.025gm |
| 2,3,5 Triphenyltetrazolium Chloride (TTC) | 0.025gm |
| Agar | 15.0gm |

Final pH 6.9 +/- 0.2 at 25ºC.

In addition, PEA with 5% Sheep Blood/Tergitol 7 (J120) is the same formulation without TTC added.

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

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

## PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[1,2,8-10,12]

Method of Use: Allow the medium to warm to room temperature prior to inoculation. Inoculate agar and streak for isolation using the four quadrant method. Incubate inoculated media in an aerobic atmosphere at 35ºC. for 18-48 hours. Observe plates for growth.

**Spread Plate Method**:

1. Prepare decimal dilutions in sterile diluent to obtain 30-300 CFU per plate.

2. Aseptically inoculate agar surface with 0.1ml of well mixed diluted sample.

3. Using a sterile spreader device, distribute the inoculum evenly over the agar surface.

4. Invert and incubate plates aerobically for 18 to 48 +/- 2.0 hours at 35ºC. and observe for typical colonies.

**Note:** Pour plates do not give satisfactory results.[6]

**Membrane Filter Method:**

1. Shake the sample bottle vigorously at least 25 times to uniformly distribute the bacteria.

2. Aseptically filter a predetermined volume of the sample through a 47mm, 0.45 +/- 0.02um pore size membrane filter and rinse the sides of the funnel at least twice with 20 to 30ml of sterile buffered rinse water.

3. Use sterile forceps to aseptically remove the membrane filter from the filter base and roll it onto the surface of the agar, filtered side up, to avoid the formation of air bubbles between the membrane and agar surfaces. Reseat the membrane if bubbles occur.

4. Incubate inverted plates aerobically at 35 +/- 0.5ºC. for 18 to 48 hours and observe for typical colonies.

## INTERPRETATION OF RESULTS

Colonies of *E. coli* should appear yellow with yellow zones and occasionally rust colored centers. Colonies of *E. aerogenes* should appear greenish-yellow. In general, lactose fermenting microorganisms should appear as yellow colored colonies.

Non-lactose fermenting microorganisms, such as *Salmonella enterica*, should appear as red colored colonies with a blue periphery. The growth of *Proteus* spp. should be partially to completely inhibited and exhibit red colonies with a blue periphery and no swarming.

Gram-positive microorganisms and gram-negative spore-forming microorganisms should be inhibited on this medium.

## LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Incubation at 44ºC. has been recommended for improved recovery of coliform bacteria.[3,7]

Subculture typical colonies onto a non-selective medium, such as TSA (Cat. no. G60), and/or into Tryptone Broth (Cat. no. R40) or Peptone Broth (Cat. no. K151). From the solid medium, an oxidase test (Cat. no. Z119 or Z93) or spot indole test (Cat. no. Z65) can be performed. From the broth medium, an indole Kovac's test (Cat. no. Z67) can be performed.

Consider all characteristic oxidase negative colonies as coliforms. Colonies that are oxidase negative, but indole positive, should be considered positive for *Escherichia coli*.

Refer to the document "Limitations of Procedures and Warranty" for more information.

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media.* The following microorganisms are routinely used for testing at Hardy Diagnostics:

| Test Organisms | Inoculation Method* | Incubation | | | Results |
|---|---|---|---|---|---|
| | | Time | Temperature | Atmosphere | |
| *Escherichia coli* ATCC® 25922** | A | 18-48hr | 35°C | Aerobic | Growth; yellow colonies |
| *Salmonella enterica* ATCC® 14028 | A | 18-48hr | 35°C | Aerobic | Growth; red colonies with blue periphery |
| *Proteus mirabilis* ATCC® 43071 | B | 18-48hr | 35°C | Aerobic | Inhibition of swarming; red colonies with blue periphery |
| *Enterococcus faecalis* ATCC® 29212** | B | 18-48hr | 35°C | Aerobic | Inhibited |

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

### USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

## REFERENCES


1 Chapman, G.H. 1947. A Superior Culture Medium for the Enumeration and Differentiation of Coliforms. *J. Bacteriol.*; 53:504.

2. Chapman, G.H. 1951. A Culture Medium for Detecting and Confirming *Escherichia coli* in Ten Hours. *Am. J. Public Health*; 41:1381.

3. Mossel, D.A.A. 1962. An Ecological Investigation on the Usefulness of Two Specific Modifications of Eijkman's Test as an Element of the Methods for the Detecting of Faecal Contamination of Foods. *J. Appl. Bacteriol.*; 25:20.

4. E.U. 1998. 98/83/EC of Council of 3rd November 1998 on The Quality of Water Intended for Human Consumption. *Off. J. Eur. Commun.* L3330:32-54.

5. Speck, Marvin L. 1992. *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. American Public Health Association, Washington, D.C.

6. MacFaddin, J.F. 1985. *Media for the Isolation, Cultivation, Identification, Maintenance of Medical Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

7. Kulp, W., et al. 1953. Use of Tergitol 7 Triphenyl Tetrazolium Chloride Agar as the Coliform Confirmatory Medium

in Routine Sanitary Water Analysis. *Am. J. Public Health*; 43:1111.

8. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

9. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

10. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I & II. American Society for Microbiology, Washington, D.C.

11. Pollard, A.L. 1946. A useful selective bactericidal property of Tergitol 7. *Science*.; 103:758-759.

12. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, APHA, Washington, D.C.

13. ISO 9308-1:1990. International Organisation for Standardisation, Geneva. ISO (1994a). *Water Quality - Detection and Enumeration of Coliform Organisms, Thermotolerant Coliform Organisms and Presumptive Escherichia coli*- Part 1: Membrane filtration method.



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HDQA 2207D [D]