Crystal Violet Tetrazolium Agar Base

M586

Intended Use:

Recommended for detection of Gram-negative psychrotrophic bacteria causing food spoilage.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Yeast extract	2.500
Dextrose (Glucose)	1.000
Crystal violet	0.001
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.5 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 and aseptically add 5 ml of sterile 1% solution of 2, 3, 5-Triphenyl Tetrazolium Chloride (FD057). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Microorganisms which are able to grow at refrigeration temperatures are usually referred to as psychrophilic. Species of *Achromobacter, Alcaligens, Flavobacterium* and *Pseudomonas* are included among the psychrotrophic bacteria as these organisms are able to grow relatively rapidly at commercial refrigeration temperatures (4). Many psychrotrophic microorganisms when present in large numbers can cause a variety of off-flavors as well as physical defects in foods. Their growth rate is highly dependent on temperature, and therefore, if the temperature is reduced, their growth rate is also slowed down. Thus the spoilage of refrigerated food is very much dependent on temperature (1,7). Crystal Violet Tetrazolium Agar Base is used for the detection of gram-negative psychrophilic bacteria causing food spoilage. It is based on the formulation by Olson (5) and recommended by APHA (6) for detecting gram-negative psychrotrophic bacteria.

Tryptone and yeast extract provide various nitrogenous nutrients to the organisms while dextrose serves as the carbon source. Crystal violet inhibits most of the gram-positive organisms and therefore inclusion of crystal violet in the medium does not affect the growth of psychrotrophic organisms, which are mostly gram-negative.

Standard methods for the detection of gram-negative psychrotrophic bacteria should be followed (6).

Type of specimen

Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (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 further 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 greyish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light purple coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 2.35% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed with added 1% T.T.C. solution (FD057) after an incubation at 20-30°C for 18-48 hours

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=50%	maroon
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Yersinia enterocolitica ATCC 27729	50-100	good-luxuriant	>=50%	maroon

Key : *Corresponding WDCM numbers.

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. Use before expiry date on the label.
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 (2,3).

Reference

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2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.
4. Mossel D. A. A., and Zwart H., 1960, J. Appl. Bacteriol., 23:185-188.
5. Olson H. C., 1963, J. Dairy Sci., 46:362.
6. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Tomkin R. B., 1973, Food Technol., 27:54.

Revision : 03 / 2019