Citrate Azide Agar

M1908

Intended Use:

Recommended for selective cultivation of Enterococci in dairy products.

Composition**

Ingredients	Gms / Litre
Yeast extract	10.000
Tryptone	10.000
Sodium citrate	20.000
Sodium azide	0.400
Tetrazolium blue	0.010
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 55.41 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE**. Overheating will destroy the selective properties. Cool to 45 - 50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Enterococci may be considered an essential part of the autochthonous microflora of humans and animals. *Enterococcus faecalis* and *Enterococcus faecium* are relatively heat-resistant and may characteristically survive in traditional milk pasteurization procedures. Most of the Enterococci are relatively resistant to freezing, and, unlike *Escherichia coli,* they readily survive this treatment (1). Citrate Azide Agar is a selective media for the identification of Enterococci in dairy, water and other foodstuffs (2).

Tryptone and yeast extract in the medium provide nitrogen, carbon, long chain amino acids, vitamins and other essential growth nutrients. The high concentrations of sodium citrate inhibit the growth of the accompanying microbial flora. Tetrazolium blue is reduced by Enterococci to form blue coloured colonies. Sodium azide helps in the selective isolation of Enterococci. The test sample can be directly streaked on the surface of the agar.

Type of specimen

Food and dairy samples; Water samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4,5).
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 test 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 yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured (may have slight blue tinge), clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.5% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for18-48 hours.

Organism	Growth	Inoculum (CFU)	Recovery	Colour of colony
Enterococcus faecalis ATCC 33186 (00210*)	good-luxuriant	50-100	>=50%	blue
Escherichia coli ATCC 25922 (00013*)	inhibited	>=10^4		
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	inhibited	>=10^4		
Enterococcus faecalis ATCC 29212 (00087*)	good-luxuriant	50-100	>=50%	blue

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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.Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.

2.Frank & Yousef,2004, Standard Methods for the Examination of Dairy Products,17th Ed.

3.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

4.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

5.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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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