



Citrate Azide Agar

M1908

It is used 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 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.

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 for 18-24 hours.

Cultural Response

Organism	Growth	Inoculum (CFU)	Recovery	Colour of colony
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Cultural Response

<i>Enterococcus faecalis</i> ATCC 33186	good-luxuriant	50-100	$\geq 50\%$	blue
<i>Escherichia coli</i> ATCC 25922	inhibited	$\geq 10^3$		
<i>Staphylococcus aureus</i> ATCC 25923	Inhibited	$\geq 10^3$		
<i>Enterococcus faecalis</i> ATCC 29212	good-luxuriant	50-100	$\geq 50\%$	blue

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label

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.

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