Citrate Azide Tween Carbonate Base

M1618

Citrate Azide Tween Carbonate Base is recommended for the identification of Enterococci in meat, meat products, dairy products and other foodstuffs.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	15.000
Yeast extract	5.000
Potassium dihydrogen phosphate	5.000
Sodium citrate	15.000
Tween 80	1.000
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 28 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C. Aseptically add the rehydrated contents of 1 vial of CATC Supplement (FD235). 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. Because of its wide distribution, Enterococci can also occur in different food commodities, especially those of animal origin (1).! Enterococcus faecalis and *Enterococcus faecium* are relatively heat-resistant and may characteristically survive in traditional milk pasteurization procedures.

E. faecium is markedly heat-tolerant and is a spoilage agent in marginally processed canned hams. Most of the Enterococci are relatively resistant to freezing, and, unlike Escherichia coli @, they readily survive this treatment (2). A wide variety of selective media for *Enterococcus* has been recommended and used. Indicator substances added to the media are useful for the recognition of Enterococci and for the rapid identification of single species on the basis of colony appearance. Citrate Azide Tween Carbonate Base is a selective media formulated by Burkwall and Hartmann (3). It was later modified by Reuter (4) for the identification of Enterococci in meat, meat products, dairy products and other foodstuffs.

Casein enzymic hydrolysate and yeast extract in the medium provide nitrogen, vitamins and amino acids. Tween 80 acts as a neutralizer, which inactivates residual disinfectants if present in the collected sample. The high concentrations of citrate inhibit the growth of the accompanying microbial flora. Triphenyl Tetrazolium Chloride (TTC) is reduced by Enterococci to form a red formazan, which imparts red colour to the 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, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 5.6% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response

M1618: Cultural characteristics observed with added CATC Supplement (FD235),after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
Streptococcus pyogenes ATCC 12344	50-100	none -poor	<=10%	
Streptococcus agalactiae ATCC 13813	50-100	none-poor	<=10%	
Enterococcus faecalis ATCC 29212	50-100	good-luxuriant	>=50%	red
Enterococcus faecalis ATCC 33186	50-100	good-luxuriant	>=50%	red
Enterococcus faecium ATCC 6057	50-100	good	40-50%	red colonies may or may not be observed
Streptococcus bovis DSM 20065	50-100	none-poor	<=10%	
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=10^3	Inhibited	0%	

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. Belzer R.: Vergleichende Untersuchungen von Enterokokkenselektivnährböden-Inaug. Dissert., Univ. München, 1983.
2 . Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.
3. Burkwall M. K., and Hartman P. A., 1964, Appl. Microbiol., 12; 18-23.
4. Reuter G., Arch F., Lebensmittelhyg., 1968, 19; 53-57 and 84-89.

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