DNase Test Agar with Toluidine Blue

Intended Use

Recommended for the detection of deoxyribonuclease activity of bacteria and fungi, and especially for identification of Staphylococci.

Typical Composition (g/litre)

Tryptose 20.0 ; Deoxyribonucleic acid 2.0 ; Sodium Chloride 5.0 ; Toluidine blue 0.100 ; Agar 15.0

Mode of Action

DNase Test Agar w/ toluidine blue is used for detecting deoxyribonuclease activity of bacteria and fungi and particularly for identification of pathogenic Staphylococci.

Tryptose provide essential nutrients. DNase depolymerizes the DNA resulting in the formation of a clear zone around the microbial growth which is visualized by flooding the plate with hydrochloric acid. When toluidine blue is added to the medium itself, DNase activity results in the production of a bright pink reaction due to the metachromatic property of toluidine blue. Some strains of Staphylococci may be inhibited on DNase Test Agar due to toluidine blue.

Preparation

Suspend 42.1 grams in 1 liter purified / distilled water. Heat with frequent agitationto dissolve the media completely. Sterilize by autoclaving at 118°C to 121°C for 15 minutes. Cool to 45-50°C mix well and pour into sterile Petri plates.

Final pH (at 25°C) 7.3±0.2

Storage

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.

Specimen

Food and dairy samples

Experimental Procedure and Evaluation

DNase Test Agar w/ toluidine blue is used for detecting deoxyribonuclease activity of bacteria and fungi and particularly for identification of pathogenic Staphylococci. With added toluidine blue, it is used in differentiation and identification of non-pigmented Serratia species isolated from clinical sources that might be improperly identified as Enterobacter and Klebsiella species. DNase activity was observed by Weckman and Catlin in Micrococci and found the correlation with coagulase activity as coagulase positive species were

DNase positive. In an experiment Di Salvo incorporated DNA and calcium chloride to activate DNase enzyme. Schreier modified DNase medium by adding toluidine blue. Modified medium achieved faster identification of Serratia marcescens and could differentiate Serratia from other members of the Enterobacteriaceae.

Quality Control

Organism	Inoculum	Growth	DNase Activity
Serratia marcescens ATCC 8100	50 - 100	Luxuriant	Pink to red zone around the growth (+) reaction.
Staphlococcus epidermidis ATCC 12228	50 - 100	Luxuriant	Pink to red zone around the growth (+) reaction.
Streptococcus pyogenes ATCC 19615	50 - 100	Luxuriant	Negative reaction
Staphylococcus aureus ATCC 25923	50 - 100	Luxuriant	Pink to red zone around the growth (+) reaction.

Reference

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