Technical Data

DNase Test Agar w/ Methyl Green

M1419

Intended Use:

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

Composition**

Ingredients	Gms / Litre
Tryptose	20.000
Deoxyribonucleic acid (DNA)	2.000
Sodium chloride	5.000
Methyl green	0.050
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42.05 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. Mix well and pour into sterile Petri plates.

Principle And Interpretation

DNase test Agar is used for detecting deoxyribonuclease activity of bacteria and fungi and particularly for identification of pathogenic Staphylococci (1). DNase producing organisms exhibit clear zone around growth against green background. Reagent addition is not required (2). This medium is based on modification of the procedure for detecting DNase-producing bacteria as per Smith, Hanoch, and Rhoden (4) and Jefferies, Holtman and Guse (3). The medium supports growth of both gram positive and gram-negative bacteria.

Tryptose serves as nitrogenous source for the organisms. DNase produced by microorganisms depolymerizes the DNA substrate in the medium. Methyl green fades into a colourless compound producing distinct clear zones surrounding colonies (or band/ spot inocula) in an otherwise green coloured medium. Methyl green requires a highly polymerized DNA substrate (5) and it combines with polymerized DNA forming a stable, green complex at pH 7.5 (6,7,8). As hydrolysis progresses, methyl green is released and when not combined at this pH it fades and becomes a colourless compound. Therefore clear zones are observed (7,9).

Type of specimen

Food samples, Pure isolates of bacteria and fungi

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (1,2,8).
For pure isolates of bacteria and fungi samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(3)
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. Some organism may show poor growth due to nutritional variation.

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

Light yellow to greeenish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Green coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.2% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

Organism	Inoculum (CFU)	Growth	DNase Activity
Serratia marcescens ATCC 8100	50-100	luxuriant	positive, clear halo around the growth.
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant	positive, clear halo around the growth.
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	positive, clear halo around the growth.

Reference

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4. Jeffries C.D.; Holtman, D.F.; and Guse, D.G (1957) J. Bacteriol., 73, 590.
5. Lachica, R.V.F. and Deibel, R. H (1969). Appl. Environ, Microbiol., 32 (4), 633.
6. Kurnick, N.B (1947). Cold Spring Harbor Symp. Quant. Biol., 12, 141.
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Revision : 02 / 2016

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