DNase Test Agar Base w/o DNA

M741

Intended Use:

Recommended with the addition of DNA it is used for detection of deoxyribonuclease activity of bacteria and fungi.

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Soya peptone	5.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.0 grams in 1000 ml purified / distilled water. Add 2 grams of DNA, 0.025 grams Bromothymol blue and 10 grams of mannitol. Heat, to boiling, to dissolve the medium completely. Sterilize by autoclaving at 12 to 15 lbs pressure (118°C to 121°C) for 15 minutes. Cool to 45-50°C and pour into sterile Petri plates.

Principle And Interpretation

DNase Test Agar Base 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 nonpigmented Serratia species isolated from clinical sources that might be improperly identified as *Enterobacter* and *Klebsiella* species. DNase activity was observed by Weckman and Catlin (7) in Micrococci and found the correlation with coagulase activity as coagulase positive species were DNase positive. Di Salvo (1) confirmed the results of Weckman and Catlin and observed accurate correlation of DNase and coagulase activity. In his experiment Di Salvo incorporated DNA and calcium chloride to activate DNase enzyme. Schreier modified DNase medium by adding toluidine blue (5). This modified medium achieved faster identification of *Serratia marcescens* and could differentiate *Serratia* from other members of the *Enterobacteriaceae*. DNase Test Agar Base without DNA can be used to detect DNase activity as well as mannitol fermentation by the addition of mannitol and a pH indicator dye i.e. bromothymol blue (4).

Tryptone and soya peptone provides essential nutrients. The depolymerization of the DNA (DNase activity) may be detected by flooding the surface of the medium with 1 N HCl (6) and observing for clear zones around the colonies on the medium (with added DNA and mannitol and no bromothymol blue). In the absence of DNase activity, cloudy precipitate is formed due to reaction of HCl with nucleic acids.When bromothymol blue is used, yellow zones are formed.

Further confirmatory tests for the identification should be carried out.

Type of specimen

Isolated Microorganism from food samples

Specimen Collection and Handling:

For isolated Microorganism samples, follow appropriate techniques for sample collection and processing as per guidelines (1,7). 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. The test organisms must be in pure culture and 18-24 hours old.
2. Further confirmatory tests for the identification should be carried out

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

After addition of Bromothymol blue : Blue coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 5.2% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed with added 2 grams of DNA, 0.025 grams Bromothymol blue and 10 grams of mannitol after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	D-Nase Activity
Serratia marcescens ATCC 8100	50-100	luxuriant	positive reaction ,change in colour from green to yellow around the growth
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant	positive reaction, change in colour from green to yellow around the growth
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	positive reaction,change in colour from green to yellow around the growth

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 (2,3).

Reference

1. Di Salvo, 1958, Med. Tech. Bull., U.S. Armed Forces Med. J., 9:191.
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3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual Clinical Microbiology, 11th Edition. Vol. 1.
4. MacFaddin J. F., 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1. Williams & Wilkins, Baltimore, Md.
5. Schreir, 1969, Am. J. Clin. Pathol., 51:711.
6. Streitfeld, Hoffman and Janklow, 1962, J. Bact., 84:77.
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Revision : 02 / 2019