DNase Test Agar Base

M482

Intended use

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

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Soya peptone	5.000
Deoxyribonucleic acid (DNA)	2.000
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 42 grams in 1000 ml purified / distilled water. Heat with frequent agitation 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°C and pour into sterile petriplates. Add 0.1 gm Toluidine Blue (FD051) before sterilizing the medium or flood the plates with 0.1% Toluidine Blue (FD051) solution after incubation as desired.

Principle And Interpretation

DNase Test Agar 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. The correlation between DNase activity and coagulase activity was first studied by Weckman and Catlin (9). Jeffries et al demonstrated DNase activity by the agar plate method employing a semi-synthetic medium (4). Positive DNase activity was visualized as clear zones (around colonies) when the plates were flooded with 1 N hydrochloric acid. DiSalvo (2) confirmed the correlation between coagulase activity and DNase activity by incorporating DNA into the medium along with calcium chloride to activate the enzyme. Di Salvo incorporated DNA and calcium chloride to activate DNase enzyme. Schreier modified DNase medium by adding toluidine blue by (7). This modified medium achieved faster identification of *Serratia marcescens* and could differentiate *Serratia* from other members of the *Enterobacteriaceae* .

Type of specimen

Food and dairy samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6,10).
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. DNase test must be accompanied by other biochemical tests for confirmation of *Staphylococcus.*
2. Small zones of clearing can be due to presence of other enzymes.
3. Organisms other than *Staphylococcus aureus* and *Serratia marcescens* can be DNase positive.

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

Basal medium :Light amber ; After addition of Toluidine blue(FD051) : Blue 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 with added Toluidine Blue (FD051) 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, change in colour from blue to pink purple around the growth when toluidine blue is used / clear zone surrounding colonies when plates are flooded w/1N HCL
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	positive, change in colour from blue to pink purple around the growth when toluidine blue is used/ clear zone surrounding colonies when plates are flooded w/1N HCL
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	positive, change in colour from blue to pink purple around the growth when toluidine blue is used / clear zone surrounding colonies when plates are flooded w/1N HCL

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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. 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. Use before expiry date on the label.
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 (3,5).

Reference

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6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Schreir, 1969, Am. J. Clin. Pathol., 51:711.
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9. Weckman and Catlin, 1957, J. Bact., 73:747.
10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.

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