



DNase Test Agar w/ Toluidine blue

M1041

Intended use

DNase Test Agar w/ toluidine blue is recommended for the detection of deoxyribonuclease activity of bacteria and fungi, and especially for identification of Staphylococci.

Composition**

Ingredients	Gms / Litre
Tryptose	20.000
Deoxyribonucleic acid (DNA)	2.000
Sodium chloride	5.000
Toluidine blue	0.100
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42.1 grams in 1000 ml purified / distilled water. Heat with frequent agitation to dissolve the medium 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.

Principle And Interpretation

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 (8) in Micrococci and found the correlation with coagulase activity as coagulase positive species were DNase positive. Di Salvo (2) 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 (6). Modified medium achieved faster identification of *Serratia marcescens* and could differentiate *Serratia* from other members of the *Enterobacteriaceae*

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 (7).

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.

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 (1,5,9). 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. 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

Light yellow to light blue homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

M1041: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth	D-Nase Activity
<i>Serratia marcescens</i> ATCC 8100	50-100	luxuriant	positive reaction, pink to red zone around the growth
<i>Staphylococcus aureus</i> subsp.aureus ATCC 25923 (00034*)	50-100	luxuriant	positive reaction, pink to red zone around the growth
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	50-100	luxuriant	negative reaction
<i>Streptococcus pyogenes</i> ATCC 19615	50-100	luxuriant	positive reaction, pink to red zone 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 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Di Salvo, 1958, Med. Tech. Bull., U.S. Armed Forces Med. J., 9:191.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. 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.
6. Schreir, 1969, Am. J. Clin. Pathol., 51:711.
7. Streitfeld, Hoffman and Janklow, 1962, J. Bact., 84:77.
8. Weckman and Catlin, 1957, J. Bact., 73:747.
9. 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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Disclaimer :

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