DNASE TEST AGAR WITH TOLUIDINE BLUE

INTENDED USE

DNase Test Agar with Toluidine Blue is differential media used for the detection of deoxyribonuclease activity to aid in the identification of bacteria isolated from clinical specimens.

SUMMARY AND EXPLANATION

The DNase test is used to detect the degradation of deoxyribonucleic acid (DNA).[1,2] The test is useful for differentiating *Serratia* from *Enterobacter*, *Staphylococcus aureus* from coagulase-negative staphylococci, and *Moraxella catarrhalis* from *Neisseria* species.[1]

In 1957, Jeffries et al. described a rapid agar plate method for demonstrating DNase activity of microorganisms.[3] This procedure utilized a semi-synthetic medium with nucleic acid solution incorporated in the medium. Enzymatic activity is detected by flooding the plate with 1 N hydrochloric acid (HCl). A clear zone surrounding growth indicates a positive reaction.

DNase Test Agar is based on a medium developed by DiSalvo to adapt the rapid plate method for staphylococci.[4] Rather than using semi-synthetic medium, DiSalvo incorporated DNA into **Trypticase**™ Soy Agar and subsequently reported a correlation between coagulase production and DNase activity.

DNase Test Agar with Toluidine Blue contains a metachromatic dye to eliminate the necessity of reagent addition to the agar following incubation.[5] Toluidine blue may be toxic to some gram-positive cocci and, therefore, should be used primarily with *Enterobacteriaceae*.

PRINCIPLE

Peptones provide amino acids and other complex nitrogenous substances to support bacterial growth. Sodium chloride maintains osmotic equilibrium. DNA is the substrate for DNase activity. DNase is an extracellular enzyme that breaks the DNA down into subunits composed of nucleotides.

Toluidine blue forms a complex with intact (polymerized) DNA. In the intact DNA complex, the toluidine blue has the normal blue color. DNase activity depolymerizes the DNA, breaking down the dye-DNA complex. In the presence of nucleotides produced from the DNase depolymerization, the dye takes on its metachromatic color, forming pink to red zones around bacterial growth. A negative test is indicated when the medium remains blue.

REAGENTS (FORMULA)

Tryptose	20.0	g
DNA	2.0	g
Sodium Chloride	5.0	g
Toluidine Blue	0.1	g
Agar	15.0	g
Deionized Water	1000.0	ml

PROCEDURE

Inoculate by making a single streak line using inoculum from an agar slant or plate. One plate may be inoculated with up to eight isolates by spot inoculation (1/8 to 1/4 inch) or streak inoculation (a single 1- to 2-inch line). Incubate at 35 ± 2°C for 24-48 hours. Plates should be incubated in an inverted position.

EXPECTED RESULTS

On DNase Test Agar with Toluidine Blue, DNase activity is indicated by pink to red zones surrounding growth. The color of the medium remains unchanged if the test is negative.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Serratia marcescens ATCC 13880	35 ± 2°C for 18-24 hours	Growth with Positive Reaction
Klebsiella pneumoniae ATCC 33495	35 ± 2°C for 18-24 hours	Growth with Negative Reaction

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BIBLIOGRAPHY

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5. Smith, Hancock and Rhoden. 1969. Appl. Microbiol. *18*:991.

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