DNASE TEST AGAR w/ and w/o ADDITIVES

INTENDED USE

Remel DNase Test Agar w/ and w/o Additives are solid media recommended for use in qualitative procedures to detect deoxyribonuclease (DNase) activity in microorganisms.

SUMMARY AND EXPLANATION

In 1957, Jeffries et al. developed a medium for demonstrating deoxyribonuclease (DNase) activity of microorganisms possessing this extracellular enzyme.[1] Such microorganisms depolymerize DNA in the medium resulting in a clear zone around the colonies when the plate is flooded with 1N hydrochloric acid (HCl). DNase Test Agar has been recommended for identification of a variety of bacteria including staphylococci, enteric gram-negative bacilli, and pseudomonads.[2-5] Modifications of DNase Test Agar, described in 1969, incorporated an indicator system consisting of a dye which eliminated the need to flood the plate with HCl. On DNase Test Agar w/ Methyl Green, DNase-producing organisms form colonies surrounded by a clear zone in an otherwise green medium.[6] Schreier added toluidine blue to DNase Test Agar for separation of *Serratia* from *Klebsiella* and *Enterobacter* spp.[7] On DNase Test Agar w/ Toluidine Blue, DNase-producing colonies (e.g., *Serratia*) are surrounded by a pink zone on a blue agar plate.[8] Because DNase Test Agar with an indicator system does not require addition of HCl, the test isolate is not subjected to the bactericidal effects of the acid and is still viable. The plate can be reincubated if negative at one day and colonies may be selected directly from the agar surface for additional testing.

PRINCIPLE

Casein and soy peptones supply nitrogen, amino acids, and peptides necessary for bacterial growth. Sodium chloride provides essential electrolytes which maintain osmotic equilibrium. DNase-producing organisms depolymerize DNA into nucleotide fractions such as, mononucleotides and oligonucleotides. Manifestation of DNase activity varies with the DNase Test Agar in use. In the case of DNase Test Agar, 1N HCl added to the plate after incubation reacts with DNA (polymerized) in the medium, yielding free nucleic acid and a cloudy precipitate. In areas where DNA has been depolymerized, around and below DNase-producing colonies, the agar is clear in contrast to the rest of the plate. Methyl green in DNase Test Agar w/ Methyl Green forms a stable colored complex in the presence of DNA. If the DNA is depolymerized, methyl green fades to a colorless compound and DNase-producing colonies are surrounded by clear zones in an otherwise green agar plate. Toluidine blue in DNase Test Agar w/ Toluidine Blue reacts with DNA and forms a blue complex. When DNA is depolymerized, the structure of toluidine dye is changed resulting in a pink color around DNase-producing colonies.

REAGENTS (CLASSICAL FORMULAE)*

Casein Peptone	15.0 g	Deoxyribonucleic Acid (DNA)	2.0 g
Sodium Chloride	5.0 g	Agar	15.0 g
Soy Peptone	5.0 g	Demineralized Water	1000.0 ml

pH 7.3 ± 0.2 @ 25°C

The following optional ingredients are available per liter of medium:

Methyl Green	0.05 g	Toluidine Blue	0.1 g

*Adjusted as required to meet performance standards.

PROCEDURE

1. Inoculate DNase Test Agar w/ or w/o Additives from a pure, 18-24 hour culture of the isolate to be tested. Using a heavy inoculum, apply a pencil line streak to the medium from rim to center of plate. Four or more test isolates can be streaked on one plate.
2. Incubate plates in ambient air for 18-24 hours at room temperature or at 33-37°C.
3. Following incubation, flood DNase Test Agar with 1N HCl and examine for clearing around growth line. DNase Test Agar w/ Methyl Green and DNase Test Agar w/ Toluidine Blue do not require the addition of 1N HCl. Refer to Interpretation of the Test to determine the results of growth on these media.

Pour Tube: Melt the pour tube in a boiling water bath and cool to 45-50°C. Mix and dispense into a sterile petri dish and proceed with the instructions above.

INTERPRETATION OF THE TEST

DNase Test Agar:

Positive Test - Following addition of 1N HCl a zone of clearing forms around the growth line, the rest of the plate remains opaque
Negative Test - Following addition of 1N HCl there is no clearing around the growth line, the entire plate is opaque

DNase Test Agar w/ Methyl Green:

Positive Test - Growth line surrounded by a colorless zone in an otherwise green agar plate
Negative Test - No zone surrounding the growth line, the entire plate is green

DNase Test Agar w/ Toluidine Blue:

Positive Test - Growth line surrounded by a pink zone in contrast to the rest of the plate which is blue
Negative Test - No pink zone surrounding the growth line, the entire plate is blue

(Continued on back)

QUALITY CONTROL

All lot numbers of DNase Test Agar w/ and w/o Additives have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
DNase Test Agar:		
Serratia marcescens ATCC® 8100	Ambient, 18-24 h @ 33-37°C	Positive
Staphylococcus aureus ATCC® 25923	Ambient, 18-24 h @ 33-37°C	Positive
Escherichia coli ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Negative
Staphylococcus epidermidis ATCC® 12228	Ambient, 18-24 h @ 33-37°C	Negative
DNase Test Agar w/ Methyl Green:		
Serratia marcescens ATCC® 8100	Ambient, 18-24 h @ 33-37°C	Positive
Staphylococcus aureus ATCC® 25923	Ambient, 18-24 h @ 33-37°C	Positive
Escherichia coli ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Negative
Staphylococcus epidermidis ATCC® 12228	Ambient, 18-24 h @ 33-37°C	Negative
DNase Test Agar w/ Toluidine Blue:		
Serratia marcescens ATCC® 8100	Ambient, 18-24 h @ 33-37°C	Positive
Staphylococcus aureus ATCC® 25923	Ambient, 18-24 h @ 33-37°C	Positive
Escherichia coli ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Negative
Staphylococcus epidermidis ATCC® 12228	Ambient, 18-24 h @ 33-37°C	Negative

LIMITATIONS

1. Some strains of staphylococci and other gram-positive bacteria may be inhibited on DNase Test Agar w/ Toluidine Blue.[9]
2. Some stains of *S. aureus* do not grow well on DNase Test Agar, however, growth is not required for detection of DNase activity.[9]
3. DNase Test Agar w/ and w/o Additives is intended as a supplemental test for identification of various organisms. Additional biochemical testing may be required for definitive identification of the test isolate.[9]

BIBLIOGRAPHY

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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