BD™ DNase Test Agar

INTENDED USE

BD DNase Test Agar and is used for differentiating micro-organisms based on deoxyribonuclease (DNase) activity. In clinical microbiology, this medium is not used as an isolation medium on which specimens are streaked directly but requires the use of pure cultures such as those previously isolated from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

In 1956, Weckman and Catlin showed a correlation between increased DNase activity of *Staphylococcus aureus* and positive coagulase activity.[1] They suggested that DNase activity could be used to identify potentially pathogenic staphylococci. DiSalvo confirmed their results by obtaining excellent correlation between the coagulase and DNase activity of staphylococci isolated from clinical specimens.[2] Jeffries, Holtman and Guse incorporated DNA in an agar medium to study DNase production by bacteria and fungi.[3]

In **BD DNase Test Agar**, tryptone provides nutrients for growth. Sodium chloride maintains the osmotic balance. High molecular deoxyribonucleic acid enables the detection of deoxyribonuclease (DNase) that depolymerizes DNA. After incubation of the medium with the test strain, the plate is flooded with hydrochloric acid which precipitates the polymerized DNA, making the medium opaque. Organisms that degrade DNA produce a clear zone around the growth area.

This medium is mainly used in the identification of staphylococci but may also be used for the detection of DNase activity in other microorganisms.

REAGENTS

BD DNase Test Agar

Formula* Per Liter Purified Water

Bacto™ Tryptone	20.0
Sodium Chloride	5.0
Deoxyribonucleic Acid	2.0
Agar	15.0

pH 7.3 +/- 0.2

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains. Inoculate the strains with a loopful of growth from a blood agar plate such as **BD Columbia Agar with 5% Sheep Blood** in a band. Up to four organisms may be inoculated on one plate. Incubate 18 to 24 hours in an aerobic atmosphere. After incubation, flood the plate with 1 N hydrochloric acid. Allow the acid to penetrate into the medium for 2 minutes. DNase positive colonies will be surrounded by clear zones in the medium.

Strain	Test Results
Staphylococcus aureus ATCC™ 25923	DNase positive
Staphylococcus epidermidis ATCC 12228	DNase negative
Serratia marcescens ATCC 13880	DNase positive
Klebsiella pneumoniae ATCC 33495	DNase negative
Uninoculated	Light to medium amber, may be slightly opalescent

PROCEDURE

Materials Provided

BD DNase Test Agar (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.
1 N hydrochloric acid (HCl).

Specimen Types

This medium is intended to be used for the differentiation of microorganisms and is not an isolation medium on which clinical specimens are streaked directly. Pure cultures (i.e previously isolated from clinical specimens) are needed for this test (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Test Procedure

Inoculate the medium with a loopful of growth from a blood agar plate such as **BD Columbia Agar with 5% Sheep Blood** in a band or spot-inoculate with a loop. Up to four organisms may be inoculated on one plate. It is recommended to include a negative control, e.g., *Staphylococcus epidermidis*, and a positive control, e.g., *S. aureus*. Incubate the plates for 18 to 24 hours aerobically at 35 to 37° C. If strains of other bacterial species or fungi are tested, incubate according to their requirements.
After incubation, flood the plates with sufficient 1 N hydrochloric acid (HCl). Allow the acid to penetrate the whole medium surface for 2 min.

Results

After application and penetration of hydrochloric acid into the medium, DNase positive organisms such as *Staphylococcus aureus* or *Serratia marcescens* will be surrounded by clear zones of depolymerized DNA while the medium farer away from the inoculation band will be opaque and whitish due to polymerized DNA. Colonies of DNase negative organisms will not show any clearing around the colonies.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD DNase Test Agar is a standard medium for the determination of deoxyribonuclease.[5,6] It is used mainly in the identification of *Staphylococcus aureus* and its differentiation from *S. epidermidis* or other DNase negative staphylococci, and for the differentiation of *Serratia* from *Klebsiella*/*Enterobacter*.[3-5]
Organisms other than *Staphylococcus aureus* and *Serratia marcescens* may be DNase positive. Further tests are needed to identify these or other organisms.

REFERENCES

1. Weckman, B. G., and B. W. Catlin. 1957. Deoxyribonuclease activity of micrococci from clinical sources. J. Bacteriol. 73: 747-753.
2. DiSalvo, J. W. 1958. Deoxyribonuclease and coagulase activity of micrococci. Med. Tech. Bull. U. S. Armed Forces Med. J. 9: 191.
3. Jeffries, C. D., Holtman, D. F., and D. G. Guse. 1957. Rapid method for determining the activity of microorganisms on nucleic acid. J. Bacteriol. 73: 590- 591.
4. Schreier, J.B. 1969. Modification od deoxyribonuclease test medium for rapid identification of *Serratia marcescens*. Am. J. Clin. Pathol. 51: 711.
5. MacFaddin, J. D. 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol. 1, p. 275-284. Williams & Wilkins, Baltimore, MD.
6. Murano, E.A., and J. A. Hudnall. 2001. Media, reagents, and stains. *In:* Downes, F.P., and K. Ito (ed.). Compendium of methods for the microbiological examination of foods, 4th edition. American Public Health Association, Washington. D.C.

PACKAGING/AVAILABILITY

BD DNase Test Agar

Cat. No. 255506 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.

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