

Subculture colonies of interest so that positive identification can be made by means of biochemical testing and/or microscopic examinations of organism smears.

Broth

If the disinfectant solution is bacteriostatic, it should be neutralized in the broth medium and the test organisms introduced into the broth will grow. Growth is indicated by a color change of the medium from purple to yellow, or pellicle formation.

Growth on the plates from negative broth tubes indicates a bacteriostatic substance. No growth on the plates from negative broth tubes indicates a bactericidal substance. All positive broth tubes should be positive on the plates.

References

- Engley and Dey. 1970. Chem. Spec. Manuf. Assoc. Proc., Mid-Year Meet., p. 100.
- Vesley and Michaelson. 1964. Health Lab. Sci. 1:107.
- Pryor and McDuff. 1969. Exec. Housekeeper, March.
- Dell. 1979. Pharm. Technol. 3:47.
- Hickey, Beckelheimer and Parrow. 1993. In Marshall (ed.), Standard methods for the examinations of dairy products, 16th ed. American Public Health Association, Washington, D.C.
- Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
- Quisno, Gibby and Foret. 1946. Am. J. Phar. 118:320.
- Erlanson and Lawrence. 1953. Science 118:274.
- Brummer. 1976. Appl. Environ. Microbiol. 32:80.
- Association for the Advancement of Medical Instrumentation. 1984. Process control guidelines for gamma radiation sterilization of medical devices. AAMI, Arlington, Va.

Availability

Difco™ D/E Neutralizing Agar

COMPF SMD

Cat. No. 268620 Dehydrated – 500 g*
268610 Dehydrated – 10 kg*

BBL™ D/E Neutralizing Agar

COMPF SMD

United States and Canada

Cat. No. 299969 Prepared Plates – Ctn. of 100*
221232 Sterile Pack **RODAC™** Plates – Pkg. of 10*
222209 Sterile Pack **RODAC™** Plates – Ctn. of 100*
292227 Sterile Pack **RODAC™** Plates
with Penicillinase – Pkg. of 10*
292645 Isolator Pack **RODAC™** Plates – Pkg. of 10*
292646 Isolator Pack **RODAC™** Plates – Ctn. of 100*
292647 Isolator Pack **Finger Dab™** Plates – Pkg. of 10*

Europe

Cat. No. 257013 Prepared Plates – Pkg. of 20*

Difco™ D/E Neutralizing Broth

AOAC

Cat. No. 281910 Dehydrated – 500 g*

BBL™ D/E Neutralizing Broth

AOAC

Cat. No. 298318 Prepared Tubes, 9 mL (A Tubes) – Ctn. of 100*

Difco™ Hyccheck™ Hygiene Contact Slides

Cat. No. 290411 D/E Neutralizing Agar//D/E Neutralizing Agar (20 slides)*
290391 D/E Neutralizing Agar//Tryptic Soy Agar (20 slides)*

*Store at 2-8°C.

DNase Test Agars

DNase Test Agar • DNase Test Agar with Methyl Green DNase Test Agar with Toluidine Blue

Intended Use

DNase Test Agar, DNase Test Agar with Methyl Green and DNase Test Agar with Toluidine Blue are 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 Methyl Green contains a dye to eliminate the necessity of adding reagent to the agar plate following incubation.⁵

DNase Test Agar with Toluidine Blue contains a metachromatic dye to eliminate the necessity of reagent addition to the agar following incubation.⁶ Toluidine blue may be toxic to some gram-positive cocci and, therefore, should be used primarily with *Enterobacteriaceae*.

Principles of the Procedure

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.

The depolymerization of the DNA may be detected by flooding the surface of the medium with 1 N HCl and observing for clear zones in the medium surrounding growth. In the absence of DNase activity, the reagent reacts with the intact nucleic acid, resulting in the formation of a cloudy precipitate.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ DNase Test Agar

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 4.2% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent, may have a slight precipitate.

Prepared Appearance: Light to medium amber, slightly opalescent, may have a slight precipitate.

Reaction of 4.2% Solution at 25°C: pH 7.3 ± 0.2

Difco™ DNase Test Agar with Methyl Green

Dehydrated Appearance: Light beige with slight green tint, free-flowing, homogeneous.

Solution: 4.2% solution, soluble in purified water upon boiling. Solution is green, very slightly to slightly opalescent with slight precipitate.

Prepared Appearance: Green, very slightly to slightly opalescent with slight precipitate.

Reaction of 4.2% Solution at 25°C: pH 7.3 ± 0.2

Cultural Response

Difco™ DNase Test Agar or DNase Test Agar with Methyl Green

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for up to 48 hours. (Note: Prepare extra plates by streaking with a line of undiluted culture across the medium.) For DNase Test Agar, flood the streak plates with 1N HCl and examine for clear zones around the streaks (positive reactions). For DNase Test Agar with Methyl Green, examine the streak plates for decolorized zones around the streaks (positive reactions).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
<i>Serratia marcescens</i>	8100	30-300	Good	+
<i>Staphylococcus aureus</i>	25923	30-300	Good	+
<i>Staphylococcus epidermidis</i>	12228	30-300	Good	-
<i>Streptococcus pyogenes</i>	19615	30-300	Good	+

The HCl reagent is not needed to detect DNase activity on DNase Agar with Methyl Green. Methyl green forms a complex with intact (polymerized) DNA to form the green color of the medium. DNase activity depolymerizes the DNA, breaking down the methyl green-DNA complex, which results in the formation of colorless zones around colonies of the test organism. A negative test is indicated by the absence of a colorless zone around the colonies.

The HCl reagent is not needed to detect DNase activity on DNase Agar with Toluidine Blue. 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

Identity Specifications

BBL™ DNase Test Agar

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 4.2% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.

Prepared Appearance: Light to medium, yellow to tan, clear to slightly hazy.

Reaction of 4.2% Solution at 25°C: pH 7.3 ± 0.2

BBL™ DNase Test Agar with Toluidine Blue

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 4.2% solution, soluble in purified water upon boiling. Solution is medium to dark, blue, trace hazy to hazy.

Prepared Appearance: Medium to dark, blue, trace hazy to hazy.

Reaction of 4.2% Solution at 25°C: pH 7.3 ± 0.2

Cultural Response

BBL™ DNase Test Agar or DNase Test Agar with Toluidine Blue

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 18-24 hours. For DNase Test Agar, flood the plates with 1N HCl and examine for deoxyribonuclease activity. For DNase Test Agar with Toluidine Blue, examine for deoxyribonuclease activity.

ORGANISM	ATCC™	RECOVERY/ REACTION DNASE TEST AGAR	RECOVERY/REACTION DNASE TEST AGAR W/TOLUIDINE BLUE
<i>Enterobacter aerogenes</i>	13048	N/A	Good/-
<i>Klebsiella pneumoniae</i>	33495	Good/-	Good/-
<i>Serratia marcescens</i>	13880	Good/+	Good/+
<i>Staphylococcus aureus</i>	25923	Good/+	N/A
<i>Staphylococcus epidermidis</i>	12228	Good/-	N/A

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.

Formulae

Difco™ DNase Test Agar

Approximate Formula* Per Liter

Tryptose	20.0	g
Deoxyribonucleic Acid	2.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

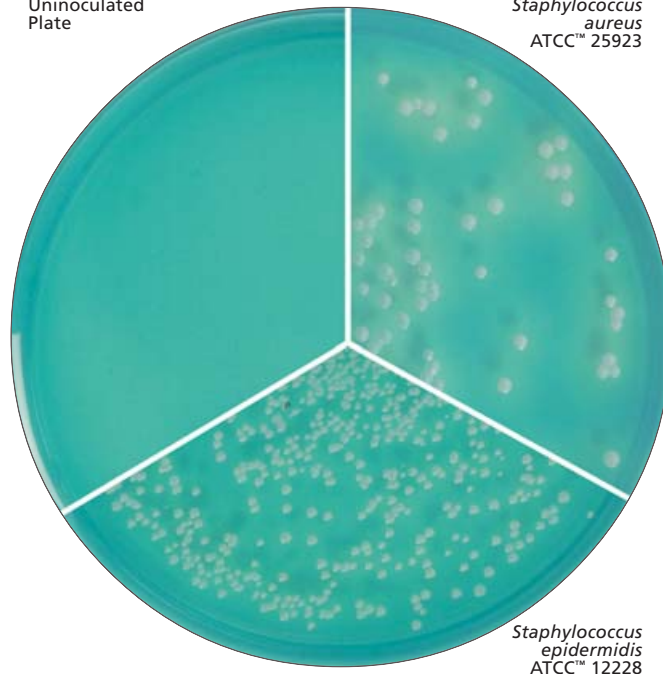


D DNase Test Agars, cont.

DNase Test Agar
Staphylococcus aureus
ATCC™ 25923



DNase Test Agar with Methyl Green
Uninoculated Plate
Staphylococcus aureus
ATCC™ 25923



DNase Test Agar with Toluidine Blue
Serratia marcescens
ATCC™ 13880

**BBL™ DNase Test Agar**

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	15.0	g
Papaic Digest of Soybean Meal	5.0	g
Deoxyribonucleic Acid	2.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

Difco™ DNase Test Agar with Methyl Green

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Proteose Peptone No. 3	10.0	g
Deoxyribonucleic Acid	2.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
Methyl Green	0.05	g

BBL™ DNase Test Agar with Toluidine Blue

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Papaic Digest of Soybean Meal	10.0	g
Deoxyribonucleic Acid	2.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
Toluidine Blue	0.1	g

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

Directions for Preparation from Dehydrated Product

DNase Test Agar or DNase Test Agar with Methyl Green or DNase Test Agar with Toluidine Blue

1. Suspend 42 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

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 $35 \pm 2^\circ\text{C}$ for 24-48 hours. Plates should be incubated in an inverted position. Incubate tubes with loosened caps.

Following incubation, flood DNase Test Agar plates with 1N HCl reagent and observe for reaction. Reagent addition is not required with DNase Test Agar with Methyl Green or with DNase Test Agar with Toluidine Blue.

Expected Results

A clear area surrounding growth (band/spot inocula) on DNase Test Agar after the addition of 1N HCl indicates a positive reaction, DNase activity. A negative reaction is indicated by no clearing and a cloudy precipitate around colonies and throughout medium due to precipitated salts in the medium.

A positive reaction on DNase Test Agar with Methyl Green is a distinct clear zone surrounding growth in an otherwise green-colored medium. The color of the medium remains unchanged if the test is negative.

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.

DRBC Agar

Intended Use

DRBC Agar is used for the enumeration of yeasts and molds.

Summary and Explanation

DRBC (Dichloran Rose Bengal Chloramphenicol) Agar is based on the Dichloran Rose Bengal Chlortetracycline Agar formula described by King, Hocking and Pitt.¹ DRBC Agar conforms with APHA guidelines for the mycological examination of foods, containing chloramphenicol rather than chlortetracycline as originally proposed.² DRBC Agar is a selective medium that supports good growth of yeasts and molds.

Principles of the Procedure

Peptone provides nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Phosphate is a buffering agent. Magnesium sulfate is a source of divalent cations and sulfate. The antifungal agent, dichloran, is added to the medium to reduce colony diameters of spreading fungi. The pH of the medium is reduced from 7.2 to 5.6 for improved inhibition of the spreading fungi.¹ The presence of rose bengal in the medium suppresses the growth of bacteria and restricts the size and height of colonies of the more rapidly growing molds.

References

1. Washington. 1985. Laboratory procedures in clinical microbiology, 2nd ed. Springer-Verlag, New York, N.Y.
2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
3. Jeffries, Holtman and Guse. 1957. J. Bacteriol. 73:590.
4. DiSalvo. 1958. Med. Tech. Bull. U.S. Armed Forces Med. J. 9:191.
5. Schreier. 1969. Am. J. Clin. Pathol. 51:711.
6. Smith, Hancock and Rhoden. 1969. Appl. Microbiol. 18:991.

Availability

Difco™ DNase Test Agar

COMPF

Cat. No. 263220 Dehydrated – 500 g

BBL™ DNase Test Agar

COMPF

Cat. No. 211179 Dehydrated – 500 g

Europe

Cat. No. 255506 Prepared Plates – Pkg. of 20*

Mexico

Cat. No. 227450 Prepared Plates – Pkg. of 10*

Difco™ DNase Test Agar with Methyl Green

Cat. No. 222020 Dehydrated – 500 g

BBL™ DNase Test Agar with Methyl Green

United States and Canada

Cat. No. 297202 Prepared Plates – Pkg. of 20*

Mexico

Cat. No. 252573 Prepared Plates – Pkg. of 10*

BBL™ DNase Test Agar with Toluidine Blue

COMPF

Cat. No. 299081 Dehydrated – 500 g

United States and Canada

Cat. No. 221856 Prepared Plates – Pkg. of 10*

Mexico

Cat. No. 211789 Prepared Plates – Pkg. of 10*

*Store at 2-8°C.

The concentration of rose bengal is reduced from 50 µg/mL to 25 µg/mL as found in Rose Bengal Chloramphenicol Agar for optimal performance with dichloran. Chloramphenicol is included in this medium to inhibit the growth of bacteria present in environmental and food samples. Inhibition of growth of bacteria and restriction of spreading of more-rapidly growing molds aids in the isolation of slow-growing fungi by preventing their overgrowth by more-rapidly growing species. In addition, rose bengal is taken up by yeast and mold colonies, which allows these colonies to be easily recognized and enumerated. Reduced recovery of yeasts may be encountered due to increased activity of rose bengal at pH 5.6.¹ Agar is the solidifying agent.

Formula

Difco™ DRBC Agar

Approximate Formula* Per Liter

Protease Peptone No. 3	5.0	g
Dextrose	10.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Dichloran	2.0	mg
Rose Bengal	25.0	mg
Chloramphenicol	0.1	g
Agar	15.0	g

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