3. Cool the medium to approximately 45°C and pour into plates using about 20 mL per plate. The plates may be used at once or refrigerated for a few days.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate and incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Expected Results

Typical colonial appearance on DCLS Agar is as follows:

Escherichia coli	Large, flat, pink to rose red with a zone of precipitated bile salts
Enterobacter/Klebsiella	Large, mucoid, pink
Proteus	Colorless to red
Salmonella	Colorless to pale pink
Shigella	Colorless to pale pink
Pseudomonas	Colorless to brown or green
Gram-positive bacteria	No growth

References

1. Leifson. 1935. J. Pathol. Bacteriol. *40*:581.
2. Hajna and Damon. 1956. Appl. Microbiol. *4*:341.

Availability

BBL™ DCLS Agar

Cat. No. 211144 Dehydrated – 500 g

Europe
Cat. No. 254012 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

D

D/E Neutralizing Agar • D/E Neutralizing Broth

Intended Use

D/E (Dey/Engley) Neutralizing Agar has the ability to neutralize antimicrobial chemicals and is used for environmental sampling for the detection and enumeration of microorganisms present on surfaces of sanitary importance. Prepared plates are provided for environmental monitoring. Sterile Pack and Isolator Pack **RODAC**™ prepared plates are particularly useful for monitoring surfaces in clean rooms and other environmentally-controlled areas and are also recommended for use in air sampling equipment such as the Surface Air System. **Finger Dab**™ Sterile Pack and Isolator Pack plates are intended for sampling gloved hands. **Hycheck**™ hygiene contact slides are used for assessing the microbiological contamination of surfaces and fluids.

D/E Neutralizing Broth is for the neutralization and testing of antiseptics and disinfectants according to the procedure of Engley and Dey.[1]

User Quality Control

Identity Specifications

Difco™ D/E Neutralizing Agar

Dehydrated Appearance:	Bluish-gray, homogeneous, appears moist and lumpy.
Solution:	5.4% solution, soluble in purified water upon boiling. Solution is lavender, opaque with a fine precipitate.
Prepared Appearance:	Lavender, opaque with a fine precipitate.
Reaction of 5.4% Solution at 25°C:	pH 7.6 ± 0.2

Difco™ D/E Neutralizing Broth

Dehydrated Appearance:	Bluish-gray, homogeneous, appears moist and lumpy.
Solution:	3.9% solution, soluble in purified water upon warming. Solution is purple, opaque with an even suspension of particles.
Prepared Appearance:	Purple, opaque with an even suspension of particles.
Reaction of 3.9% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response

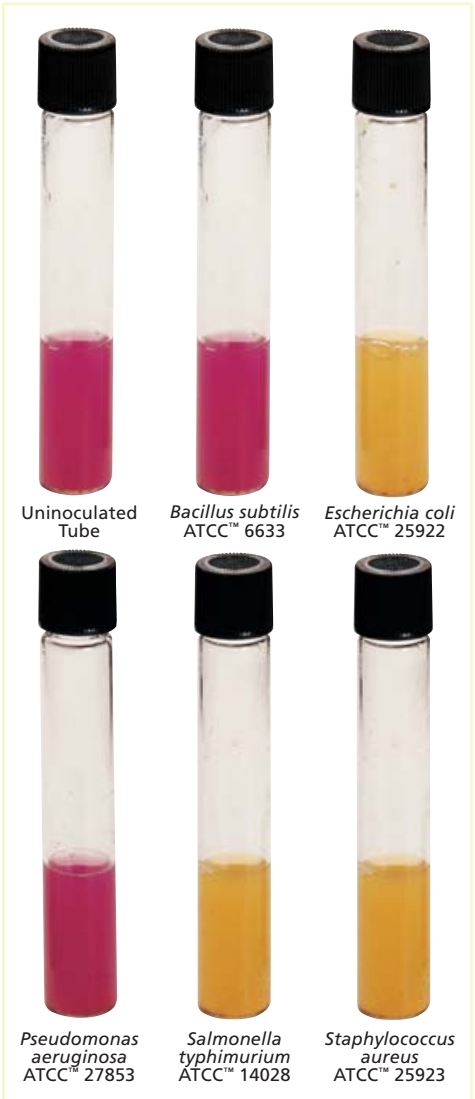
Difco™ D/E Neutralizing Agar or D/E Neutralizing Broth

Prepare the medium per label directions. Inoculate plates and incubate at 35 ± 2°C for up to 40-48 hours. Prepare tubes with and without the addition of disinfectants; e.g., mercurials and quaternary ammonium compounds. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus subtilis	6633	10^2-10^3	Good
Escherichia coli	25922	10^2-10^3	Good
Pseudomonas aeruginosa	27853	10^2-10^3	Good
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good

Neutralization Test

Prepare D/E Neutralizing Agar per label directions. Inoculate 50 mL of D/E Neutralizing Agar with 0.1 mL of a heavy suspension of test organism and dispense into 150 ×15 mm Petri dishes of D/E Neutralizing Agar and Plate Count Agar. Place 1/2 inch sterile blank disks on each plate. Dispense 0.1 mL of each disinfectant solution onto two disks per medium. Incubate at 35 ± 2°C for 40-48 hours. D/E Neutralizing Agar should exhibit no zones of inhibition or zones significantly smaller than those found on Plate Count Agar.



Summary and Explanation

Environmental contact sampling plates (**RODAC** plates) are specially constructed so that the D/E Neutralizing Agar medium can be over-filled, producing a meniscus or dome-shaped surface that can be pressed onto a surface for sampling its microbial burden. These plates are used in a variety of programs to establish and monitor cleaning techniques and schedules.[2-5] After touching the surface to be sampled with the medium, the dish is covered and incubated at an appropriate temperature. The presence and number of microorganisms is determined by the appearance of colonies on the surface of the agar medium. Collection of samples from the same area before and after cleaning and treatment with a disinfectant permits the evaluation of the efficacy of sanitary procedures because of the neutralizing ability of the medium.

The **Hycheck** hygiene contact slide is a double-sided paddle containing two agar surfaces for immersing into fluids or sampling surfaces. There are two slides containing D/E Neutralizing Agar: one slide contains D/E Neutralizing Agar on both sides; and another slide contains D/E Neutralizing Agar along with Tryptic Soy Agar.

D/E Neutralizing Broth is used for environmental sampling where neutralization of the chemical is important to determine its bactericidal or bacteriostatic activity. This medium will neutralize a broad spectrum of antiseptic and disinfectant chemicals, including quaternary ammonium compounds, phenolics, iodine and chlorine preparations, mercurials, formaldehyde and glutaraldehyde.[1]

Principles of the Procedure

Peptone, yeast extract and dextrose are sources of nutrients required for the replication of microorganisms. The peptone provides nitrogenous compounds, including essential amino acids. Yeast extract is a rich source of B-complex vitamins. Dextrose is an energy source. Five neutralizers in this medium will inactivate a variety of disinfectant and antiseptic chemicals: sodium bisulfite neutralizes aldehydes; sodium thioglycollate neutralizes mercurials; sodium thiosulfate neutralizes iodine and chlorine;[1] lecithin neutralizes quaternary ammonium compounds; and polysorbate 80, a non-ionic surface active agent, neutralizes substituted phenolics.[6-9] Bromcresol purple is incorporated as an indicator for dextrose utilization.

In the medium supplemented with penicillinase, the addition of penicillinase inactivates penicillinase-sensitive beta-lactam antibiotics.

In the prepared plated medium, the entire double-bagged (Sterile Pack) or triple-bagged (Isolator Pack) product is subjected to a sterilizing dose of gamma radiation so that the contents inside the outer bag are sterile.[10] This allows the inner bag(s) to be aseptically removed and brought into an environmentally-controlled area without introducing contaminants. Since the agar medium has been sterilized after packaging, the presence of microbial growth after sampling and incubation can be relied upon to represent the presence of environmental contaminants

and not pre-existing microorganisms in the medium that may have been introduced during manufacture. The plate has a marked grid to facilitate counting organisms.

Due to the high concentration of lecithin in the broth medium (which renders the medium opaque), turbidity cannot be used to detect growth. Therefore, bromcresol purple and dextrose are added to the medium. Those organisms that ferment dextrose will turn the medium from purple to yellow. Growth of *Pseudomonas* species, which do not ferment dextrose, can be detected by the formation of a pellicle on the surface of the broth.[1]

Formulae

Difco™ D/E Neutralizing Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	5.0	g
Yeast Extract	2.5	g
Dextrose	10.0	g
Sodium Thioglycollate	1.0	g
Sodium Thiosulfate	6.0	g
Sodium Bisulfite	2.5	g
Polysorbate 80	5.0	g
Lecithin	7.0	g
Bromcresol Purple	0.02	g
Agar	15.0	g

Difco™ D/E Neutralizing Broth

Consists of the same ingredients without the agar.

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

Directions for Preparation from Dehydrated Product

Difco™ D/E Neutralizing Agar

1. Suspend 54 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 the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ D/E Neutralizing Broth

1. Dissolve 39 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Agar

Selected surfaces are sampled by firmly pressing the agar medium against the test area. Hold the plates with thumb and second finger and use index finger to press plate bottom firmly against surface. Pressure should be the same for every sample. Do not move plate laterally; this spreads contaminants over the agar surface making resolution of colonies difficult. Slightly curved surfaces may be sampled with a rolling motion. Areas (walls, floors, etc.) to be assayed may be divided into sections or grids and samples taken from specific points within the grid.

Grid method:

1. Subdivide surface (floor or wall) into 36 equal squares per 100 square feet of area by striking five equidistant dividing lines from each of two adjacent sides.
2. These dividing lines intersect at twenty-five points.
3. Number these intersections consecutively in a serpentine configuration.
4. Use red numerals for odd numbers, black numerals for even numbers.
5. Omit number 13 which falls in the center of the total area.
6. Sample odd points at one sampling period, even points at the next sampling period.
7. For areas greater than 100 square feet, extend grid to include entire area.
8. For areas smaller than 25 square feet, divide the areas into twenty-five equal squares (sixteen intersections). Sample eight even-numbered or odd-numbered intersections at each sampling period.
9. For areas smaller than 25 and 100 square feet, divide into 36 equal squares as in #1.
10. Mark plates with intersection numbers.

Incubate exposed plates at 35-37°C for 48 hours, and 25°C for 7 days as required.

Broth

Add 1 mL of disinfectant solution to one tube of D/E Neutralizing Broth. Add culture as desired. Incubate tubes at 35°C. Examine for growth, indicated by a color change from purple to yellow or by pellicle formation.

To determine whether viable organisms are present in a "bacteriostatic" or "bactericidal" solution, inoculate samples from the broth onto D/E Neutralizing Agar or Standard Methods Agar plates. Incubate plates at 35-37°C for 48 hours.

Expected Results

Agar

After incubation, count visible colonies on plated medium. Counting of plates containing a profusion of growth can lead to considerable error. A basic decision to be made is whether distinct colony margins can be observed. Spreading colonies should be counted as one but care taken to observe other distinct colonies intermingled in the growth around the plate periphery or along a hair line. These should also be counted as one colony, as should bi-colored colonies and halo-type spreaders.

It is generally agreed that 200 colonies is the approximate maximum that can be counted on these plates. Colony counts may be recorded by:

1. Simply keeping individual counts.
2. Number of viable particles per square foot (agar area of **RODAC**™ plates is 3.97 square inches).
3. Means and standard deviations.

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

1. Engley and Dey. 1970. Chem. Spec. Manuf. Assoc. Proc., Mid-Year Meet., p. 100.
2. Vesley and Michaelson. 1964. Health Lab. Sci. *1*:107.
3. Pryor and McDuff. 1969. Exec. Housekeeper, March.
4. Dell. 1979. Pharm. Technol. *3*:47.
5. 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.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Quisno, Gibby and Foter. 1946. Am. J. Phar. *118*:320.
8. Erlandson and Lawrence. 1953. Science *118*:274.
9. Brummer. 1976. Appl. Environ. Microbiol. *32*:80.
10. 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™ Hycheck™ 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.