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Antibiotic Assay Medium D (pH 7.9) (DM023I)

Intended Use

Antibiotic Assay Medium D (pH 7.9) (DM023I) is recommended for the microbiological assay of antibiotics in compliance with IP.

Product Summary and Explanation

Antibiotic Assay media are used in the performance of antibiotic assays. Grove and Randall have elucidated those antibiotic assays and media in their comprehensive treatise on antibiotic assays.[1] The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms.[2] Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods.[2] Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay. The cylinder plate method, first described by Abraham et al.[3] for the assay of penicillin, was later modified by Foster and Woodruff[4] and by Schmidt and Moyer.[5] Antibiotic Assay Medium D (pH 7.9) is forumlated in accordance to IP and CFR; and is employed to analyze the Neomycin, Erythromycin content as per FDA and the IP.[6,7] This medium provides a pH range of 8 while Antibiotic assay medium A provides pH range of 6.5-6.7.

Principles of the Procedure

Antibiotic Assay Medium D (pH 7.9) contains a combination of peptone, pancreatic digest of casein, yeast extract and beef extract which provides essential nutrients for the growth of test organisms. Dextrose provides the carbon source, enhances the growth of test organism. Agar provides excellent medium for antibiotic diffusion and gives well-defined zones of inhibition. Higher pH provides the optimal conditions for activity of antibiotic and also supports the growth of the test organisms.

Cylinder Plate Assay

This method is based on the diffusion of an antibiotic solution from a cylinder placed on the surface of an inoculated agar medium. After incubation, the diameter of a zone of inhibition is observed which depends, in part, on the concentration or activity of the antibiotic. The results depend on critical rates of diffusion of the antibiotic, critical growth rates of the standard organisms and critical minimal inhibitory coefficient levels of each organism. This method is used in the assay of commercial preparations of antibiotics, as well as in the quantitative determination of antibiotics in body fluids, animal feeds and other materials.

Pre-diffusion of antibiotics for 10-20 minutes in the agar by incubating at temperature below the optimal growth temperature for microorganism would facilitate better diffusion of antibiotics followed by incubation of plates for microbial growth.

Note :

Recommended for the Microbiological assay of Erythromycin, Chlortetracycline, Framycetin, Gentamicin, Kanamycin sulphate, Neomycin.

Formula / Liter

Ingredients	Gms / Liter
Peptone	6.00
Pancreatic digest of casein	4.00
Yeast extract	3.00
Beef extract	1.50
Dextrose	1.00
Agar	15.00

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Final pH: 7.9 ± 0.1 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Freshly prepared plates should be used for antibiotic assays.
4. All conditions in the microbiological assay must be controlled carefully.
   The use of standard culture medium in the test is one of the important steps for obtaining good results.

Directions

1. Suspend 30.5 grams of the medium in one liter of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow coloured homogeneous free flowing powder
Prepared Medium	Light yellow coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 3.05% solution	pH 7.9 ± 0.1 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Growth Promotion Test

As per Indian Pharmacopoeia.

Expected Cultural Response: Cultural characteristics observed after an incubation at specified temperature and specified period.

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Recovery	Antibiotics Assayed	Incubation temperature/ Period
1.	*Micrococcus luteus ATCC 9341*	50-100	good-luxuriant	>=70%	Erythromycin	32-35°C/ 24 hrs
2.	*Staphylococcus epidermidis ATCC 12228*	50-100	good-luxuriant	>=70%	Gentamycin, Neomycin	32-35°C/ 24 hrs
3.	*Bacillus pumilis NCTC 8241 5*	50-100	good-luxuriant	>=70%	Chlortetracycline, Kanamycin sulphate Framycetin,	32-35°C/ 5days

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Preparation of Stock cultures

1. Maintain stock cultures on agar slants and make transfers at 1- or 2-week intervals.
2. Using sterile purified water, saline or Antibiotic Medium No. 3, prepare the inoculum for assay by washing growth from a fresh 24-48 hour agar slant and further dilute the culture to obtain the desired organism concentration.

Cylinder Plate Assay

1. Use 20 × 100 mm glass or plastic Petri dishes with sufficient depth so that cylinders used in the assay will not be pushed into the medium by the cover.

2. Use stainless steel or porcelain assay cylinders having the following dimensions (± 0.1 mm): 8 mm outside diameter, 6 mm inside diameter and 10 mm long. Clean the cylinders carefully to remove all residues, using an occasional acid bath (i.e., with approximately 2N nitric acid or with chromic acid).
3. Four or six cylinders are generally used per plate, evenly spaced on a 2.8 cm radius.
4. For assuring accurate assays, use a level surface for working to obtain uniformly thick base and seed layers in the Petri dish.
5. Allow the base layer to solidify and then overlay the seed layer containing a proper concentration of the test organism. The amount of medium in the layers varies for different antibiotics, with most assays specifying a 21 mL base layer and a 4 mL seed layer.
6. In any case, dishes with flat bottoms are required to assure complete coverage of the bottom of the dish when small amounts of base medium are used. Tilt the plate to obtain even coverage of the base layer by the seed layer and allow it to solidify in a level position. Plates should be used the same day as prepared.

Other Tests :

Cup plate method is carried out using *B. pumilis* / kanamycin and *M. luteus* / Erythromycin

1) Dilution : 16 mg Kanamycin in 10 ml distilled water

Stock : 1:10 dilution of above solution

Concentration	Stock (ml)	Distilled water (ml)	Zone of inhibition
5	0.25	4.75	15 mm
20	1.00	4.00	20 mm
10	0	-	25 mm

2) Dilution : 9 mg Erythromycin in 10 ml distilled water

Stock : 1:10 dilution of above solution

Concentration	Stock (ml)	Distilled water (ml)	Zone of inhibition
5	0.25	4.75	22 mm
10	0.50	4.50	32 mm
10	0	5.00	41 mm

Results

1. After incubation the concentration of the antibiotic being assayed is determined by measuring the zone of inhibition obtained, with that of reference standard antibiotic.
2. Refer to appropriate references and specific test procedures.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

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Packaging
Product Name : Antibiotic Assay Medium D (pH 7.9)
Product Code : DM023I
Available Pack sizes : 100gm / 500gm

References

1. Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopedia, Inc. New York, N.Y.
2. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
3. Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings. 1941. Lancett *ii*: 177.
4. Foster and Woodruff. 1943. J. Bacteriol. *46*:187.
5. Schmidt and Moyer. 1944. J. Bacteriol. *47*:199.
6. Indian Pharmacopoeia 2010, Ministry of Health and Family welfare, Government of India, New Delhi.
7. Tests and Methods of Assay of Antibiotics and Antibiotic containing Drugs, FDA, CFR, 1983. Title 21, part 436, Subpart D, Washington, D.C. U.S Government printing office, paragraphs 436, 100-436, 106 pg 242-259 (April 1).

Further Information

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