PRODUCT SPECIFICATION SHEET

Antibiotic Assay Medium No.11 (Neomycin, Erythromycin Assay Agar) (Erythromycin Seed Agar) (DM023U)

Intended Use

Antibiotic Assay Medium No.11 (Neomycin,Erythromycin Assay Agar) (Erythromycin Seed Agar) (DM023) is recommended for microbiological assay of antibiotics.

Product Summary and Explanation

Antibiotic assay media are prepared according to the specifications of the *USP*[1], European Pharmacopeia[2] and *AOAC* International.[3] The antibiotic media are identified numerically with names assigned by Grove and Randall in *Assay Methods of Antibiotics*.[4] The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms.[1] Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods.[1] Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay. The cylinder plate method, first described by Abraham et al.[5] for the assay of penicillin, was later modified by Foster and Woodruff[6] and by Schmidt and Moyer.[7]

This medium is recommended for the Microbiological assay of Erythromycin, Chlortetracycline, Dihydrostreptomycin sulphate, Erythromycin estolate, Framycetin, Gentamicin, Gentamicin sulphate, Kanamycin sulphate, Kanamycin monosulphate, Kanamycin acid sulphate, Netilmicin sulphate, Netilmicin, Neomycin, Paromomycin, Sisomycin, Spiramycin, Streptomycin sulphate.

Principles of the Procedure

Antibiotic Assay Medium No. 10 contains a combination of peptic digest of animal tissue and casein enzymic hydrolysate, 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. Dibasic potassium phosphate in the medium enhances buffering action and sodium chloride maintains osmotic equilibrium. 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.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue (Peptone)	6.00
Casein enzymic hydrolysate	4.00
Yeast extract	3.00
Beef extract	1.50
Dextrose	1.00
Agar	15.00
Final pH: 8.3 ± 0.2	
Formula may be adjusted and/or supplemented as required to meet performance	

PRODUCT SPECIFICATION SHEET

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.
5. The use of standard culture medium in the test is one of the important steps for obtaining good results.

Directions

1. Suspend 30.50 grams of the medium in one liter of distilled water containing.
2. Heat to boiling, to dissolve the medium completely, with frequent agitation to avoid charring.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specification

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light yellow coloured, clear to slightly opalescent gel forms in Petri plates
Reaction of 3.05% solution	pH 8.3 ± 0.2
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics was observed after an incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Antibiotics Assayed
1.	*Micrococcus luteus ATCC 9341*	50-100	good-luxuriant	>=70%	Erythromycin While assaying Tylosin, Tylosin tartarate, Vancomycin hydrochloride, adjust the pH to 8.0±.0.2
2.	*Staphylococcus aureus ATCC 6538p*	50-100	good-luxuriant	>=70%	Kanamycin monosulphate, Kanamycin acid sulphate, Netilmicin sulphate
3.	*Staphylococcus epidermidis ATCC 12228*	50-100	good-luxuriant	>=70%	Gentamicin, Neomycin, Netilmicin, Paromomycin, Sisomycin
4.	*Bacillus pumilis ATCC 14884*	50-100	good-luxuriant	>=70%	Chlortetracycline, Framycetin, Kanamycin sulphate
5.	*Bacillus subtilis ATCC 6633*	50-100	good-luxuriant	>=70%	Dihydrostreptomycin sulphate, Erythromycin estolate, Kanamycin monosulphate, Kanamycin acid sulphate, Spiramycin, Streptomycin sulphate
6.	*Bacillus subtilis NCTC 8236*	50-100	good-luxuriant	>=70%	Dihydrostreptomycin sulphate, Streptomycin sulphate
7.	*Bacillus subtilis NCTC 8241*	50-100	good-luxuriant	>=70%	Erythromycin estolate, Gentamicin sulphate

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 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.
3. In some turbidimetric assays, an 18- 24hour culture of the test organism grown in Antibiotic Medium 3, diluted to obtain the optimal number of organisms, is used.
4. For using as a test organism *Bacillus subtilis*, inoculate the organism on Antibiotic Medium 1 and incubate at 37°C for 1 week. Wash spores from the agar surface, and heat the spores at 56°C for 30 minutes. Using sterile purified water wash the spores three times, heat again at 65°C for 30 minutes, and then dilute to the optimal concentration. Inoculum prepared following this method should produce a sharp zone in the assay.
5. For preparing spore suspension of *B. subtilis*, Antibiotic Medium 1 modified by the addition of 300 mg manganese sulfate ($MnSO_4 \cdot H_2O$) per liter is used which aids in the sporulation of *B. subtilis*.
6. When *B. cereus* var. *mycoides* is required, inoculate the organism on Antibiotic Medium 1 and incubate at 30°C for 1 week. Wash and prepare the spores as for *B. subtilis*, above.

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. flaves / 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
100	5.00	-	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
100	5.00	-	41 mm

Results
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
For a complete discussion of antibiotic assay methods, refer to appropriate procedures outlined in the references.[1,2,3]

Packaging
Product Name : Antibiotic Assay Medium No.11 (Neomycin, Erythromycin Assay Agar) (Erythromycin Seed Agar)
Product Code : DM023
Available Pack sizes : 100 gm/ 500gm

References

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3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
4. Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopedia, Inc. New York, N.Y.
5. Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings. 1941. Lancett *ii*: 177.
6. Foster and Woodruff. 1943. J. Bacteriol. 46:187.
7. Schmidt and Moyer. 1944. J. Bacteriol. 47:199.

Further Information
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