



Antibiotic Assay Medium D

MM004

Antibiotic Assay Medium D is used for microbiological assay of antibiotics in accordance with Indian Pharmacopoeia .

Composition**

Ingredients	Gms / Litre
Peptone	6.000
Pancreatic digest of casein	4.000
Yeast extract	3.000
Beef extract	1.500
Dextrose	1.000
Agar	15.000
pH after sterilization	7.9±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.5 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

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

Advice : 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)	D/W(ml)	Zone of Inhibition
5	0.25	4.75	15 mm
20	1.00	4.00	20 mm
10	0	5.00	25 mm

2) Dilution : 9 mg Erythromycin in 10 ml distilled water Stock : 1:10 dilution of above solution.

Concentration	Stock (ml)	D/W(ml)	Zone of Inhibition
5	0.25	4.75	22 mm
10	0.50	4.50	32 mm
10	0	5.00	41 mm

Principle And Interpretation

This medium is formulated in accordance to IP and CFR; and is employed to analyze the Neomycin, Erythromycin content as per FDA and the IP (1,2). This medium provides a pH range of 8 while Antibiotic assay medium A provides pH range of 6.5-6.7.

Peptone, pancreatic digest of casein, yeast and beef extract supplies essential nutrients, vitamins, mineral, trace elements and growth factors. Dextrose in the medium serves as the carbon source for stimulating the growth of the test microorganism. 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 test organisms.

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar pre-cooled to 40-45°C and spread evenly over the surface of solidified base agar. All conditions in the microbiological assay must be controlled carefully.

Quality Control

Appearance

Please refer disclaimer Overleaf.

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 3.05% w/v aqueous solution. pH : 7.9±0.1

pH

7.80-8.00

Growth Promotion Test

As per Indian Pharmacopoeia.

Cultural Response

Cultural characteristics observed after an incubation at

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed	Incubation temperature/ Period
Cultural Response					
<i>Micrococcus luteus</i> ATCC 9341	50-100	luxuriant	≥70%	Erythromycin	32-35°C/ 24 hrs
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	luxuriant	≥70%	Gentamycin, Neomycin	32-35°C/ 24 hrs
<i>Bacillus pumilis</i> ATCC 14884	50-100	luxuriant	≥70%	Chlortetracycline, Kanamycin sulphate, Framycetin,	32-35°C/ 5 days

Storage and Shelf Life

Store below 30°C in tightly closed container and use freshly prepared medium . Use before expiry date on the label.

Reference

1. Indian Pharmacopoeia 2010, Ministry of Health and Family welfare, Government of India, New Delhi.
2. 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, p. 242-259, (April 1).

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