



Antibiotic Medium No. 2

MU005

Antibiotic Medium No.2 is used as basal medium for microbiological assay of antibiotics in accordance with United States Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Peptone	6.000
Yeast extract	3.000
Beef extract	1.500
Agar	15.000
pH after sterilization (at 25°C)	6.6±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25.5 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.

Principle And Interpretation

This medium is commonly used as base agar for microbiological agar diffusion assays for wide variety of antibiotics. Agar diffusion assays can be performed by cylinders, punched-hole or paper disc tests. This medium is identical numerically with the name assigned by Grove and Randall (1). This medium is prepared according to the specifications detailed in the USP and CFR (2,3).

Peptone, yeast and beef extract nitrogenous, vitamins and mineral requirement for the growth of test organisms. This medium provides solidified substratum for growth of organisms and supports the overlaying of soft agar.

To perform an antibiotic assay the Antibiotic assay medium No.2 is used as Base Agar. This medium should be prepared on the same day as the test. For the cylinder method, a base layer of 21 ml is required. Once the base medium has solidified, Antibiotic assay medium No.1 as seed agar, inoculated with the standardized culture can be overlaid. Even distribution of the layer is important.

Quality Control

Appearance

Cream to yellow coloured homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Amber coloured slightly opalescent gel forms in Petri plates.

pH

6.50-6.70

Cultural Response

MU005: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Basal layer
<i>Micrococcus luteus</i> ATCC 10240	50-100	luxuriant	≥70%	Bacitracin
<i>Staphylococcus aureus</i> ATCC 9144	50-100	luxuriant	≥70%	Tylosin

Please refer disclaimer Overleaf.

<i>Staphylococcus aureus</i> ATCC 29737	50-100	luxuriant	$\geq 70\%$	Amikacin, Cephalothin, Cephapirin, Cloxacillin, Cycloserine, Chlortetracycline, Demeclocycline, Doxycycline, Kanamycin, Methacycline, Nafcillin, Oxytetracycline, Rolitetracycline, Tetracycline
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	good-luxuriant	$\geq 70\%$	Novobiocin
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	luxuriant	$\geq 70\%$	Capreomycin, Streptomycin, Troleandomycin
<i>Enterococcus hirae</i> ATCC 10541	50-100	luxuriant	$\geq 70\%$	Gramicidin, Thiostrepton, Tobramycin
<i>Escherichia coli</i> ATCC 10536	50-100	luxuriant	$\geq 70\%$	Chloramphenicol, Spectinomycin

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. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.1.
2. United States Pharmacopoeia / National Formulary 2011, US Pharmacopoeial Convention, Inc., Rockville, MD.
3. 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).

Revision : 2 / 2015

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.