Antibiotic Medium No. 2

MU005

Intended Use:

Recommended for microbiological assay of antibiotics in accordance with USP.

Composition**

Ingredients	g / L
Peptone	6.000
Yeast extract	3.000
HM peptone B #	1.500
Agar	15.000
pH after sterilization (at 25°C)	6.6±0.1

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

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. Cool to 45-50°C. Mix well and pour into sterile Petri plates or dispense as desired.

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 HM peptone B nitrogenous, vitamins and mineral requirement for the growth of test organisms. This medium provides solidified substratum for growth of organisms and supports the over layering of soft agar.

Type of specimen

Antibiotics as per USP

Specimen Collection and Handling

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. After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Freshly prepared plates must be used or it may result in erroneous results.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

Organism	Inoculum (CFU)	Growth	Recovery	Basal layer
Micrococcus luteus ATCC 10240	50-100	luxuriant	>=70%	Bacitracin
Staphylococcus aureus ATCC 9144 (00035*)	50-100	luxuriant	>=70%	Tylosin
Staphylococcus aureus ATCC 29737	50-100	luxuriant	>=70%	Amikacin, Cephalothin, Cephapirin, Cloxacillin, Cycloserine, Chlortetracycline, Demeclocycline, Doxycycline, Kanamycin, Methacycline, Nafcillin, Oxytetracycline, Rolitetracycline, Tetracycline
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	good-luxuriant	>=70%	Novobiocin
Klebsiella pneumoniae ATCC 10031	50-100	luxuriant	>=70%	Capreomycin, Streptomycin, Troleandomycin
Enterococcus hirae ATCC 10541 (00011*)	50-100	luxuriant	>=70%	Gramicidin, Thiostrepton, Tobramycin
Escherichia coli ATCC 10536	50-100	luxuriant	>=70%	Chloramphenicol, Spectinomycin

Key : *- Corresponding WDCM numbers

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and use freshly prepared medium. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference
1. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.1.
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).
3. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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