



Dehydrated Culture Media
Bases / Media Supplements

Technical Information

Antibiotic Medium No. 2

Product Code: DM 1005U

Application: - Antibiotic Medium No.2 is recommended 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

Principle & Interpretation

This medium is commonly recommended 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 is required for the growth of test organisms. This medium supplies solidified substratum for growth of organisms and supports the over layering 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.

Methodology

Suspend 25.5 grams of dehydrated media in 1000 ml distilled water. Mix thoroughly & heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes

Quality Control

Appearance

Cream to yellow coloured homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity

Amber coloured slightly opalescent gel forms in Petri plates.

pH Range

6.50-6.70

Cultural Response

DM 1005U: 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> ATCC10240	50-100	luxuriant	>=70%	Bacitracin
<i>Staphylococcus aureus</i> ATCC 9144	50-100	luxuriant	>=70%	Tylosin
<i>Staphylococcus aureus</i> ATCC 29737	50-100	luxuriant	>=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	>=70%	Novobiocin
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	luxuriant	>=70%	Capreomycin, Streptomycin, Troleandomycin
<i>Enterococcus hirae</i> ATCC10541	50-100	luxuriant	>=70%	Gramicidin, Thiostrepton, Tobramycin
<i>Escherichia coli</i> ATCC10536	50-100	luxuriant	>=70%	Chloramphenicol, Spectinomycin

Storage and Shelf Life

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

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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

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