



## Antibiotic Assay Medium No. 2 (Base Agar)

M005

### Intended use

Antibiotic Medium No. 2 (Base Agar) is used as a basal medium for microbiological assay of antibiotics.

### Composition\*\*

Ingredients	Gms / Litre
Peptone	6.000
HM Peptone B #	1.500
Yeast extract	3.000
Agar	15.000
Final pH ( at 25°C)	6.6±0.2

\*\*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.

Advice : Recommended for the microbiological assay of Spiramycin

### 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 equivalent to Antibiotic Assay Medium No.B as per Indian Pharmacopoeia (3). Peptone, yeast and HM peptone B provide the nitrogenous and carbonaceous compounds, long chain amino acids, 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.

To perform the 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.

### Type of specimen

Pharmaceutical preparations.

### Specimen Collection and Handling

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (3).

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 homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

#### Colour and Clarity of prepared medium

Amber coloured, clear to slightly opalescent gel forms in Petri plates

#### Reaction

Reaction of 2.55% w/v aqueous solution at 25°C. pH : 6.6±0.2

#### pH

6.40-6.80

#### Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed	Basal layer
<i>Bacillus subtilis subsp. spizizenii</i> ATCC 6633 (00003*)	50-100	luxuriant	≥70%	Spiramycin	
<i>Micrococcus luteus</i> ATCC 10240	50-100	luxuriant	≥70%		Bacitracin
<i>Staphylococcus aureus</i> ATCC 9144 (00035*)	50-100	luxuriant	≥70%		Tylosin
<i>Staphylococcus aureus</i> ATCC 29737	50-100	luxuriant	≥70%		Amikacin, Cephalothin, Cephapirin, Chlortetracycline, Nafcillin, Oxytetracycline, Rolitetracycline
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	50-100	good-luxuriant	≥70%		Tetracycline
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	luxuriant	≥70%		Capreomycin, Streptomycin, Troleandomycin, Gramicidin, Thiostrepton, Tobramycin
<i>Enterococcus hirae</i> ATCC 10541 (00011*)	50-100	luxuriant	≥70%		
<i>Escherichia coli</i> ATCC 10536	50-100	luxuriant	≥70%		Chloramphenicol, Spectinomycin

\*- 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. Use before expiry date on the label.

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 (3,4).

## Reference

1. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.1.
2. Indian Pharmacopoeia 2018, Ministry of Health and Family Welfare, Govt. of India, Delhi.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2<sup>nd</sup> Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carrol , 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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### Disclaimer :

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