



Antibiotic Assay Medium A

MM003

Antibiotic Assay Medium A is used for microbiological assay of β -lactam and other antibiotics as per the specification of Indian Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Peptone	6.000
Tryptone # #	4.000
Yeast extract	3.000
Meat extract B #	1.500
Dextrose	1.000
Agar	15.000
pH after sterilization	6.55 \pm 0.05

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of casein

Equivalent to Beef extract

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

Advice : Recommended for the microbiological assay of Bacitracin, Novobiocin, Streptomycin, Vancomycin and Netilmicin sulphate and as a maintenance medium for the assay of Amikacin, Bacitracin, Carbenicillin, Capreomycin, Chlortetracycline, Colistimethate sodium, Colistin sulphate, Doxycycline, Erythromycin, Framycetin, Gentamicin, Gramicidin, Kanamycin B sulphate, Neomycin, Netilmicin sulphate, Novobiocin, Oxytetracycline, Polymyxin-B, Sisomicin, Spiramycin, Streptomycin, Teicoplanin, Tetracycline, Tobramycin, Tylosin, Tyrothricin, Vancomycin.

Principle And Interpretation

This medium is widely employed as seed agar in the preparation of plates for microbiological agar diffusion assays by FDA cylinder plate method for wide variety of antibiotics. This medium is also used as inoculum and maintenance medium for several test organisms. Composition of this medium is in accordance to Indian Pharmacopoeia (1) and CFR (2)

Essential nutrients, vitamins, mineral, trace elements and growth factors are supplied by peptone, tryptone yeast extract and meat extract B. 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.

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar 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. The use of standard culture media in the test is one of the important step for the good results.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

After sterilization, reaction of 3.05% w/v aqueous solution. pH : 6.55 \pm 0.05

pH

6.50-6.60

Growth Promotion Test

As per Indian Pharmacopoeia

Cultural Response

Cultural characteristics observed after an incubation at specified temperature for specified period.

Cultural Response

Organism	Inoculum (CFU)	Growth	Inoculum medium	Antibiotics assayed	Incubation temperature / period
Cultural Response					
<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	good-luxuriant	Polymyxin B		32-35°C/ 24 hours
<i>Bacillus cereus var mycoides</i> ATCC 11778	50-100	good-luxuriant	Oxytetracycline , Tetracycline		32-35°C/ 5 days
<i>Bacillus pumilis</i> ATCC 14884	50-100	good-luxuriant	Chlortetracycline, Framycetin, Kanamycin sulphate		32-35°C/ 5 days
<i>Bacillus subtilis</i> ATCC 6633	50-100	good-luxuriant	Streptomycin	Streptomycin	32-35°C/ 5 days
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	good-luxuriant	Streptomycin, Capreomycin		36-37°C/ 24 hours
<i>Micrococcus luteus</i> ATCC 9341	50-100	good-luxuriant	Erythromycin		32-35°C/ 24 hours
<i>Micrococcus luteus</i> ATCC 10240	50-100	good-luxuriant	Bacitracin	Bacitracin	32-35°C/ 24 hours
<i>Pseudomonas aeruginosa</i> ATCC 25619	50-100	good-luxuriant	Carbenicillin		36-37.5°C/ 24 hours
<i>Staphylococcus aureus</i> ATCC 29737	50-100	good-luxuriant	Amikacin, Doxycycline, Kanamycin sulphate, Oxytetracycline, Tetracycline, Tobramycin, Tylosin, Gramicidin, Netilmicin sulphate		32-35°C/ 24 hours
<i>Staphylococcus aureus</i> ATCC 6538	50-100	good-luxuriant			32-35°C/ 24 hours
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	good-luxuriant	Novobiocin, Gentamicin, Neomycin, Sisomicin	Novobiocin	32-35°C/ 24 hours

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, Govt 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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