



Antibiotic Assay Medium No.1 (Antibiotic Assay Medium A) MAP003 (MU003/MM003)

Intended Use:

Recommended for microbiological assay of β -lactam and other antibiotics in pharmaceutical and food related preparations in accordance with USP/IP.

Composition**

Ingredients	g / L
Peptone	6.000
Tryptone#	4.000
Yeast extract	3.000
HM peptone B ##	1.500
Dextrose (Glucose)	1.000
Agar	15.000
pH after sterilization	6.6 \pm 0.1

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

Note: Recommended for the microbiological assay of Bacitracin, Carbenicillin, Cloxacillin, Colistimethate, Colistin, Erythromycin, Gentamicin, Neomycin, Nafcillin, Novobiocin, Paromomycin, Penicillin-G, Polymycin B, Sisomicin, Capreomycin, Chloramphenicol and Chlortetracycline.

Principle And Interpretation

This medium is also used as inoculum and maintenance medium for different test organisms for antibiotic assays. Composition of this medium is in accordance with USP and IP (1,2) and is recommended by FDA (3) and identified numerically with the name assigned by Grove and Randall (4).

Essential nutrients, vitamins, mineral, trace elements and growth factors are supplied by Peptone, Tryptone, yeast extract and HM peptone 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.

Type of specimen

Antibiotics as per USP and IP (1,2)

Specimen Collection and Handling

Freshly prepared plates should be preferably used for assaying antibiotics. Test organisms is inoculated in sterile seed agar pre-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. One of the critical and important step for obtaining good results is use of appropriate standard culture media. 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

Yellow coloured slightly opalescent gel forms in Petri plates.

pH

6.50-6.70

Cultural Response

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

Organism As per IP	Inoculum (CFU)	Growth	Inoculum medium for maintenance	Antibiotics assayed	Incubation temperature / period
<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 spizizenii</i> ATCC 6633 (00003*)	50-100	good-luxuriant	Framycetin, Spiramycin, Kanamycin B, Streptomycin, Teicoplanin, Vancomycin	Streptomycin, Vancomycin	32-35°C/ 5 days
<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	good-luxuriant	Colistimethate sodium, Colistin sulphate, Polymyxin B		32-35°C/ 24 hours
<i>Escherichia coli</i> ATCC 10536	50-100	good-luxuriant	Colistimethate sodium, Colistin sulphate		35-39°C/ 24 hours
<i>Escherichia coli</i> ATCC 9637	50-100	good-luxuriant	Colistimethate sodium		35-37°C/ 24 hours
<i>Enterococcus hirae</i> ATCC 10541 (00011*)	50-100	good-luxuriant	Gramicidin, Tyrothricin		36-38°C/ 16-18 hours
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	good-luxuriant	Capreomycin, Streptomycin		36-37°C/ 24 hours
<i>SKokuria rhizophila</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> <i>subsp. aureus</i> ATCC 6538 (00032*)	50-100	good-luxuriant	Netilmicin sulphate, Vancomycin Gramicidin	Netilmicin sulphate	32-35°C/24 hours 37-39°C/24 hours 32-35°C/16-18 hours
<i>Staphylococcus aureus</i> ATCC 29737	50-100	good-luxuriant	Amikacin, Doxycycline, Oxytetracycline, Tetracycline, Tobramycin, Tylosin, Kanamycin sulphate		32-35°C/ 24 hours
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	50-100	good-luxuriant	Gentamicin, Neomycin, Novobiocin, Sisomicin	Novobiocin	32-35°C/ 24 hours

Organism	Inoculum (CFU)	Growth	Inoculum medium	Antibiotics assayed	Incubation temperature / period
As per USP					
<i>S. Kokuria rhizophila</i> ATCC 9341	50-100	good-luxuriant	Amoxicillin, Erythromycin		32-35°C/ 24 hours
<i>Micrococcus luteus</i> ATCC 10240	50-100	good-luxuriant	Bacitracin	Bacitracin	32-35°C/ 24 hours
<i>Staphylococcus aureus</i> ATCC 29737	50-100		Cloxacillin, Tetracycline, Oxytetracycline, Nafcillin, Penicillin G, Chlorotetracycline	Cloxacillin, Nafcillin, Penicillin G	32-35°C/ 24 hours
<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	good-luxuriant	Colistimethate sodium, Colistin, Polymyxin B		32-35°C/ 24 hours
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	50-100	good-luxuriant	Gentamicin, Neomycin, Novobiocin, Paromomycin	Novobiocin	32-35°C/ 24 hours
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	good-luxuriant	Capreomycin, Neomycin, Dihydrostreptomycin		36-37.5°C/ 16-24 hours
<i>Escherichia coli</i> ATCC 10536	50-100	good-luxuriant	Chloramphenicol		32-35°C/ 24 hours

Key- (*)- Corresponding WDCM numbers,

(\$)- Formerly known as *Micrococcus luteus* **Formerly known as *Bacillus subtilis* subsp. *spizizenii*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. 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 (5,6).

Reference

1. The United States Pharmacopoeia-National Formulary (USP-NF), 2022.
2. Indian Pharmacopoeia, 2018, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.
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).
4. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopaedia, Inc. New York.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. 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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