



Antibiotic Assay Medium No.3 (Antibiotic Assay Medium C) MAP042 (MU042/MM042)

Intended Use:

Used as the broth medium in turbidimetric or serial dilution assay of a wide variety of antibiotics in accordance with USP/IP.

Composition**

Ingredients	g / L
Peptone	5.000
Yeast extract	1.500
HM peptone B #	1.500
Dextrose (Glucose)	1.000
Sodium chloride	3.500
Dipotassium hydrogen phosphate	3.680
Potassium dihydrogen phosphate	1.320
pH after sterilization	7.0±0.05

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 17.5 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Advice: Recommended for the Microbiological assay of Amikacin, Capreomycin, Chloramphenicol, Chlortetracycline, Cycloserine, Demeclocycline, Dihydrostreptomycin, Doxycycline, Gramicidin, Kanamycin, Methacycline, Oxytetracycline, Rolitetracycline, Streptomycin, Tetracycline, Tobramycin and Troleandomycin according to official methods.

Principle And Interpretation

Grove and Randall have elucidated the antibiotic assays and medias in their comprehensive treatise on antibiotic assays (1). Antibiotic assay Medium No. 3 is used as the broth medium in turbidimetric or serial dilution assay of a wide variety of antibiotics. This medium is formulated in accordance with USP and IP (2,3).

Turbidimetric antibiotic assay is based on the change or inhibition of growth of a test microorganisms in a liquid medium containing a uniform concentration of an antibiotic. After incubation of the test organism in the working dilutions of the antibiotics, the amount of growth is determined by measuring the light transmittance using spectrophotometer. The concentration of antibiotic is determined by comparing amounts of growth obtained with that given by the reference standard solutions. Use of this method is appropriate only when test samples are clear.

Peptone, HM peptone B and yeast extract provide essential nutrients and growth factors for enhanced microbial growth. Sodium chloride maintains the osmotic equilibrium and retains the cell viability and cell integrity. Phosphates in the medium provide good buffering action. Dextrose serves as the carbon and energy source for luxuriant growth.

Type of specimen

Antibiotics as per USP & IP

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (2,3).

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. Use of this method is appropriate only when test samples are clear.

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

Colour and Clarity of prepared medium

Light yellow coloured clear solution without any precipitate.

pH

6.95-7.05

Growth Promotion Test

In accordance with USP/IP.

Cultural Response

Cultural characteristics observed after incubation at specified temperature.

Organism	Inoculum (CFU)	Growth	Inoculum medium for maintenance	Antibiotics assayed	Incubation temperature / period
As per USP					
<i>Enterococcus hirae</i> ATCC 10541 (00011*)	50-100	luxuriant	Gramicidin	Gramicidin	36-37.5°C/16-18h
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	luxuriant		Capreomycin, Dihydrostreptomycin,	36-37.5°C/16-24h
<i>Escherichia coli</i> ATCC 10536	50-100	luxuriant		Chloramphenicol	32-35°C/24h
<i>Staphylococcus aureus</i> ATCC 29737	50-100	luxuriant		Chlortetracycline, Oxytetracycline, Tetracycline	32-35°C/24h
<i>Staphylococcus aureus</i> ATCC 9144 (00035*)	50-100	luxuriant	Tylosin		35-39°C/16-18 h
As per IP					
Organism	Inoculum (CFU)	Growth		Antibiotics assayed	Incubation temperature / period
<i>Escherichia coli</i> ATCC 9637	50-100	luxuriant		Colistimethate sodium	35-37°C/24h
<i>Enterococcus hirae</i> ATCC 10541 (00011*)	50-100	luxuriant		Gramicidin Tyrothricin	36-38°C/16-18h
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	luxuriant		Capreomycin Streptomycin	36-37°C/24h
<i>Staphylococcus aureus</i> ATCC 6538 (00032*)	50-100	luxuriant		Gramicidin Vancomycin	32-35°C/16-18h 37-39°C/24h
<i>Staphylococcus aureus</i> ATCC 29737	50-100	luxuriant		Amikacin, Doxycycline, Kanamycin sulphate, Oxytetracycline, Tetracycline, Tobramycin, Tylosin	32-35°C/24h

Key:-*- Corresponding WDCM numbers

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 (4,5).

Reference

1. Grove and Randall, 1955, Assay Methods of Antibiotics, Medical Encyclopedia, Inc. New York
2. The United States Pharmacopoeia-National Formulary (USP-NF), 2022.
3. Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.
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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Disclaimer :

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