



Antibiotic Assay Medium No. 4

MU140

Intended Use:

Antibiotic Assay Medium No. 4 is used for detection of Penicillin in milk samples and in microbiological assay of different antibiotics in accordance with United States Pharmacopoeia.

Composition**

Ingredients	g / L
Peptone	6.000
Yeast extract	3.000
Beef extract	1.500
Dextrose	1.000
Agar	15.000
pH after sterilization (at 25°C)	6.6±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.5 grams in 1000 ml of purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

This dehydrated culture medium is suitable for plate counts in pharmaceutical and related products and for the microbial assay and detection of antibiotics like penicillin in milk. This medium is formulated in accordance to the specifications and procedures listed by the Food and Drug Administration and USP (1, 2). This medium is identical numerically with name assigned by Grove and Randall (3).

Peptone, yeast and beef extract provide nutritional requirement for growth of the indicator organisms like *Bacillus stearothermophilus*, *Micrococcus luteus*. This medium is similar to Antibiotic assay medium no. 2 except for the additional ingredient dextrose. Dextrose in the medium serves as an easily available source of carbon stimulating luxuriant growth of the test organisms.

Generally presence of penicillin in milk is detected by the cylinder plate method, using *Micrococcus luteus* as the test organism, and by paper disk method, using *Bacillus stearothermophilus*. The cylinder plate method is recommended as the standard for quantification of β -lactam residues. A description of the cylinder plate method for detecting penicillin in dry powdered milk is given by Kramer et al. (4). The same basic procedure is also recommended to the assay of penicillin in fluid milk.

Freshly prepared plates should be used for antibiotic assays. The use of this medium assures well defined zones of the test organism. All conditions in the microbiological assay must be controlled carefully. The use of standard culture medium in the test is one of the important steps for obtaining good results.

Type of specimen

Antibiotics as per USP

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 clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 2.65% w/v aqueous solution (after sterilization). pH : 6.6±0.1

pH

6.50-6.70

Cultural Response

Growth Promotion is carried out in accordance with USP. Cultural characteristics observed after an incubation at 32-35°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar and fungal growth on Sabouraud Dextrose Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Incubation temperature	Incubation period
Cultural Response					
<i>Micrococcus luteus</i> ATCC 10240	50-100	good-luxuriant	≥50%	32-35°C	18-24 hours
<i>Bacillus stearothermophilus</i> ATCC 7953	50-100	good-luxuriant	≥50%	55°C	18-24 hours

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. 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. 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).
2. The United States Pharmacopoeia-National Formulary (USP-NF), 2022.
3. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopaedia, Inc. New York.
4. Kramer, J., G.G. Carter, B. Arret, J. Wilner, W.W. Wright, and A. Kirshbaum. 1968. Antibiotic residues in milk, dairy products and animal tissues: methods, reports and protocols. Food and Drug Administration, Washington, DC.
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.

Revision : 03/2025

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