Antibiotic Assay Medium No. 32

M1141

Intended Use:

Recommended for preparing inoculum of *Bacillus subtilis* during assay of Dihydrostreptomycin and Vancomycin.

Composition**

Ingredients	Gms / Litre
Peptone	6.000
Tryptone	4.000
Yeast extract	3.000
HM peptone B #	1.500
Dextrose (Glucose)	1.000
Manganese sulphate	0.300
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 30.8 gms in 1000 ml purified / distilled water. Heat to boiling to disslove 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 Dihydrostreptomycin and Vancomycin

Principle And Interpretation

This medium is formulated in accordance to FDA (5) and is a modification of Antibiotic assay Medium No.1. This medium is used to develop incoulum of *Bacillus subtilis* for antibiotic assay.
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. Manganese sulphate in this medium facilitates the sporulation and growth of *Bacillus subtilis* (1,2,6), which is generally used as test organisms for plate assay of Dihydrostreptomycin and Vancomycin.

Type of specimen

Pharmaceutical sample

Specimen Collection and Handling

For pharmaceutical sample samples follow appropriate techniques for handling specimens as per established guidelines (5). 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 medium 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
Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.08% 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 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
Bacillus subtilis subsp. *spizizenii* ATCC 6633 (00003*)	50-100	good-luxuriant	>=70 %	Dihydrostreptomycin,Vancomycin

Key : (*) 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. 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. Charney, J., Fisher, W.P. and Hegarty, C.P. 1951. J. Bacteriol. 62:145.

2. Curran, H.R. and Evans, F.R. 1954. J. Bacteriol. 67: 489.

3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual Clinical Microbiology, 11th Edition. Vol. 1.

5. 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, (April1).

6. Vasantha & Freese, 1979, J.Gen.Microbiol. 112:329-336

Revision : 02/2020