



Technical Data

Antibiotic Assay Medium D

M556

Intended Use:

Recommended for microbiological assay of Erythromycin using *Klebsiella pneumoniae*.

Composition**

Ingredients	Gms / Litre
HMH extract #	1.500
Yeast extract	1.500
Casitose##	5.000
Glucose monohydrate	1.000
Sodium chloride	3.500
Dipotassium hydrogen phosphate	3.680
Potassium dihydrogen phosphate	1.320
Potassium nitrate	2.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Heart extract

Equivalent to Peptone-Casein

Directions

Suspend 19.40 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified /distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Antibiotic Assay Medium D is used for the microbiological assay of Erythromycin estolate using *Klebsiella pneumoniae*. Grove and Randall have elucidated the antibiotic assays and media in their comprehensive treatise on antibiotic assays.(1). Turbidimetric methods for determining the potency of antibiotics are inherently more accurate and more precise than comparable agar diffusion procedures. Combination of peptone, HMH extract and yeast extract supplies nutrients and essential mineral and growth factors for enhanced microbial growth. Potassium nitrate serves as inorganic source of nitrogen for the growth of test organism. Sodium chloride maintains the osmotic equilibrium while phosphates are incorporated in the medium to provide good buffering action. Glucose monohydrate serves as the carbon and energy source for faster growth. Turbidimetric antibiotic assay is based on the change or inhibition of growth of a test microorganism in a liquid medium containing a uniform concentration of an antibiotic.

Type of specimen

Pharmaceutical sample

Specimen Collection and Handling

For pharmaceutical sample samples follow appropriate techniques for handling specimens as per established 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. Freshly prepared medium plates must be used or it may result in erroneous results.
2. 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 homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured clear solution without any precipitate

Reaction

Reaction of 1.94% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

Organism	Inoculum (CFU)	Growth	Serial dilution with
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	luxuriant	Erythromycin stearate

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 (2,3).

Reference

1. Grove and Randall, 1955; Assay methods of Antibiotics, Medical Encyclopedia, Inc. New York.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.

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