Antibiotic Assay Medium D

MAP556

(ME556/M556B)

Intended Use:

Recommended for the microbiological assay of Erythromycin using *Klebsiella pneumoniae* as a test organism in accordance with EP/BP.

Composition**

Ingredients	g / L
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
pH after sterilization	7.0±0.1

**Formula adjusted, standardized to suit performance parameters
Equivalent to Heart extract
$ Equivalent to Peptone casein

Directions

Suspend 19.4 grams of dehydrated medium powder in 1000 ml purified/R-water/distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Adjust the pH of the medium using freshly prepared buffer solution as recommended by the European pharmacopoeia for the antibiotic assayed.

Principle And Interpretation

This medium is formulated in accordance to European Pharmacopoeia and British Pharmacopoeia (1,2). This medium is widely used for turbidimetric assay of erthromycin estolate using *Klebsiella pneumoniae* as test organism. Turbidimetric methods for determining the potency of antibiotics are inherently more accurate and more precise than comparable agar diffusion procedures. Combination of Casitose, HMH extract and yeast extract supply 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. Use of this method is appropriate only when test samples are clear.

Type of specimen

Antibiotics as per EP & BP

Specimen Collection and Handling

To perform the antibiotic assay the Base Agar should be prepared on the same day as the test. For the cylinder method, a base layer of 21 ml is required. Once the base medium has solidified, seed layer inoculated with the standardized culture can be overlaid. Even distribution of the layer is important. 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 should be used for antibiotic assays.
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 to slightly opalescent solution

Reaction

After sterilization,reaction of 1.95% w/v aqueous solution. pH : 7.0±0.1

pH

6.90-7.10

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Serial dilution with
Klebsiella pneumoniae ATCC 10031	50-100	luxuriant	Erythromycin estolate

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 (4,5).

Reference

1. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
2. The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
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 of Clinical Microbiology, 11th Edition. Vol. 1.

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