Antibiotic Assay Medium G

M553

Intended Use:

Recommended for microbiological assay of Bleomycin sulphate using *Mycobacterium smegmatis*, as a test organism.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
HM extract #	10.000
Sodium chloride	3.000
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Meat extract

Directions

Suspend 38 grams in 1000 ml purified/distilled water containing 10ml glycerol. Heat to boiling to dissolve 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 or as desired.

Advice : Recommended for the microbiological assay of Bleomycin sulphate

Principle And Interpretation

This medium is formulated in accordance to European and British Pharmacopoeia (1,2). This medium is employed widely as base agar for agar diffusion assay of Bleomycin using *Mycobacterium smegmatis*. It is also used for preparing the inoculum of *Mycobacterim smegmatis* for assay.

The nutrients essential for growth of test organism is provided by Peptone and HM extract in this medium. Agar provides excellent solid substratum for support and over layering of seed agar, for the assay of Bleomycin. Addition of glycerol is important for provision of carbon to the test organism.

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.

Type of specimen

Pharmaceutical sample

Specimen Collection and Handling

For pharmaceutical sample follow appropriate techniques for handling specimens as per established guidelines (1,2). 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
Light yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 3.8% 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 18-48 hours

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
Mycobacterium smegmatis ATCC 607	50-100	Luxuriant	>=50%	Bleomycin sulphate

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 (3,4).

Reference

1. British Pharmacopoeia, 2016, The Staionery Office, British Pharmacopoeia

2. European Pharmacopoeia, 2017, European Department, for the Quality of Medicines.

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.

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