Antibiotic Assay Medium L- AOAC

M991

Intended Use:

Recommended for microbiological assay of Monensin using *Bacillus subtilis* as test organism.

Composition**

Ingredients	Gms / Litre
Dipotassium hydrogen phosphate	0.690
Potassium dihydrogen phosphate	0.450
Yeast extract	2.500
Dextrose (Glucose)	10.000
Agar	15.000
Final pH (at 25°C)	6.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 28.64 grams in 1000 ml purified / distilled water. 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.

Principle And Interpretation

Antibiotic Assay Medium L is formulated in accordance with AOAC (3) for the microbiological assay of Monensin in feeds, using *Bacillus subtilis* (ATCC 6633) as the test organism.

Use single inoculated agar layer. Optimum concentration of suspension of *Bacillus subtilis*, is determined before assay by preparing trial plates. Usually 0.5 ml suspension is used per 100 ml of seed agar, to obtain appropriate inhibition zones (17.5± 2.5 mm with 0.5µg/ml). For actual assay add appropriate amount of suspension to sterile, molten medium, mix and pour 6 ml into sterile Petri plate. Cover and refrigerate for about 1 hour before use.

For the standard graph or response lines prepare dilution using 50% methanol to obtain 0.25, 0.5, 1.0 and 2.0 µg monensin/ ml. Reference concentration is 0.5 µg/ml. To obtain standard curve 10 seeded agar plates are used placed with cylinders. Different standard concentrations are filled in it. Incubate at 16-18 hours at 35-37°C and measure diameters of zones of inhibition. Weigh 20gram finished feed and 5 gram premix and add it to chromatographic column. Elute with 9:1 methanol water. 200 ml elute is again diluted with 50% methanol to 0.5µg monensin /ml. This is called assay solution. Use 5 plates for each assay solution. Fill the alternate cylinders with reference concentration and assay solution after incubation at 35-37°C for 16-18 hours, measure diameters of zones of inhibition to nearest 0.1 mm. Average 10 reading of reference concentration and 10 reading of reference concentration and 10 readings of assay solution.

If assay solution gives larger average than reference concentration add difference between them to reference point on standard curve. If assay solution gives smaller value than reference concentration, substract difference between them from reference point. Using corrected value of assay solution determine amount of antibiotic.

Type of specimen

Pharmaceutical sample

Specimen Collection and Handling

For pharmaceutical sample samples follow appropriate techniques for handling specimens as per established guidelines (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.

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 2.86% w/v aqueous solution at 25°C. pH : 6.0±0.2

pH

5.80-6.20

Cultural Response

Cultural characteristics observed after an incubation at 35 - 37°C f or 16 - 18 hours .

Organism	Inoculum (CFU)	Growth	Recovery	Anibiotic assayed
Bacillus subtilis subsp. *spizizenii* ATCC 6633 (00003*)	50-100	luxuriant	>=70%	Inhibition zones with Monensin

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 (1,2).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. 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.
3. Williams. (Ed.), 2005, Official Methods of Analysis of AOAC International, 19th ed., AOAC, International, Washington D. C.

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