Antibiotic Assay Medium M- AOAC

M992

Intended Use:

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

Composition**

Ingredients	Gms / Litre
Yeast extract	2.500
Dextrose (Glucose)	10.000
Dipotassium hydrogen phosphate	0.690
Potassium dihydrogen phosphate	0.450
Agar	20.000
Final pH (at 25°C)	6.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.64 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. Mix well before pouring in sterile Petri plates.

Principle And Interpretation

Antibiotic Assay Medium M is formulated in accordance with AOAC (1) for the microbiological assay of Lasalocid in feeds, using *Bacillus subtillis* (ATCC 6633) as the test organism.

Prepare slant culture of *Bacillus subtillis* (ATCC 6633) on Assay Medium No. 1 and incubate for 16-24 hours at 37°C. Wash the growth with sterile distilled water and transfer it to surface of Assay Medium No. 32 and incubate at 37°C for 7 days. Wash the growth with sterile distilled water. Heat to 65°C for 30 minutes in water bath. Centrifuge, decant the supernatant and resuspend the cells. Repeat this for 3 minutes in water bath. Dilute suspension with sterile distilled water (1 + 50) to read 20%T on sprectrophotometer at 530 nm before use.

Use single inoculated agar layer. Optimum concentration of suspension of *Bacillus subtillis* is determined prior to assay to be added to Medium M to obtain inhibition zone of adequate size (17.5 ± 2.5 mm with 1.0 µg/ml). For actual assay add appropriate amount of suspension to sterile, molten medium M (pH 6.0). Mix and add 6 ml to each plate. Prepare plates 2.5 3 hours before use. Weigh 1.0 g premix. Transfer to flask and add 100 ml methanol. Shake vigorously for 3 minutes and dilute with methanol. Dilute 4 ml of this to 100 ml methanol. Further dilute 3 ml with 22 ml methanol and water to 100 ml (1 ml= ca/µg lasalocid Na/ml 25% methanol). Prepare final concentration of feed to 0.0075%. For more details refer AOAC.

Using lasalocid, sodium obtain standard response line, assay solution. Place cylinders on each plate and alternatively fill with reference concentration and other standard concentration. Incubate at 35-36°C. Calculate zone diameters of L (Low concentration giving measurable zone) and H (Highest concentration) of standard response line and connect with straight line. This corrected reference point is used for sample calculations. Average the 9 readings of reference concentration and 9 readings of assay solution. If assay solution gives larger average than reference concentration, add difference between them to reference point on standard response line. If the assay solution gives lower average than reference concentration, subtract the difference from reference point. Using the corrected value of assay solution, amount of antibiotic is determined.

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.
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

Gelling
Firm, comparable with 2.0% Agar gel

Colour and Clarity
Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.36% 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 for 18-48 hours.

Organism	Growth	Inhibition zones with
Bacillus subtilis @SBB 5522 (00003*)	Luxuriant	Lasalocid

Reference

1. Williams (Ed.), 2005, Official Methods of Analysis of AOAC International, 19th ed., AOAC, International, Washington D. C.

Revision : 1 / 2011