Antibiotic Assay Medium M- AOAC

M992

Antibiotic Assay Medium M is recommended for microbiological assay of Lasalocid using *Bacillus subtilis* ATCC 6633 as test organism.

Composition**

Ingredients	Gms / Litre
Yeast extract	2.500
Dextrose	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 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.

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
M992: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Growth	Inhibition zones with
Cultural Response		
Bacillus subtilis ATCC 6633	Luxuriant	Lasalocid

Storage and Shelf Life
Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry date on the label.

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

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