



Dehydrated Culture Media
Bases / Media Supplements

Technical Information

Antibiotic Assay Medium M- AOAC

Product Code: DM 1992

Application: - 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

Principle & Interpretation

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

Prepare slant culture of *Bacillus subtilis* (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 spectrophotometer at 530 nm before use.

Use single inoculated agar layer. For this optimum concentration of suspension of *Bacillus subtilis* 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 two to three hours before use. Weigh 1.0 g premix. Transfer to flask and add 100 ml methanol. Shake vigorously for 3 minutes and 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 obtains 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.

Methodology

Suspend 33.64 grams of powder media in 1000 ml distilled water. Shake well & heat 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.



Quality Control

Physical 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 3.3 6% w/v aqueous solution at 25°C. pH : 6.0±0.2

pH range 5.80-6.20

Cultural Response/ characteristics

DM 1992: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Growth zones with Cultural Response	Inhibition
<i>Bacillus subtilis</i> ATCC 6633	luxuriant	Lasalocid

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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