TM 1973 – ANTIBIOTIC ASSAY MEDIUM M - AOAC

INTENDED USE

For microbiological assay of Lasalocid using *Bacillus subtilis.*

PRODUCT SUMMARY AND EXPLANATION

Antibiotic Assay Medium M is formulated in accordance with AOAC 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.

COMPOSITION

Ingredients	Gms / Ltr
Yeast extract	2.500
Dextrose	10.000
Dipotassium hydrogen phosphate	0.690
Potassium dihydrogen phosphate	0.450
Agar	20.000

PRINCIPLE

Essential amino acids, mineral and growth factors are supplied with yeast extract in this medium. Dextrose provides carbon and energy source for enhancing the growth of test organism. Good buffering action is maintained by phosphates in the medium.

INSTRUCTION FOR USE

- Dissolve 33.64 grams in 1000 ml purified / distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 45-50°C.
- Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium : Yellow coloured clear to slightly opalescent gel forms in Petri plates.
pH (at 25°C) : 6.0±0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Inhibition zone with	Incubation Temperature	Incubation Period
Bacillus subtilis subsp. spizizenii	6633	50-100	Luxuriant	>=70	Lasalocid	35-37°C	18-48 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

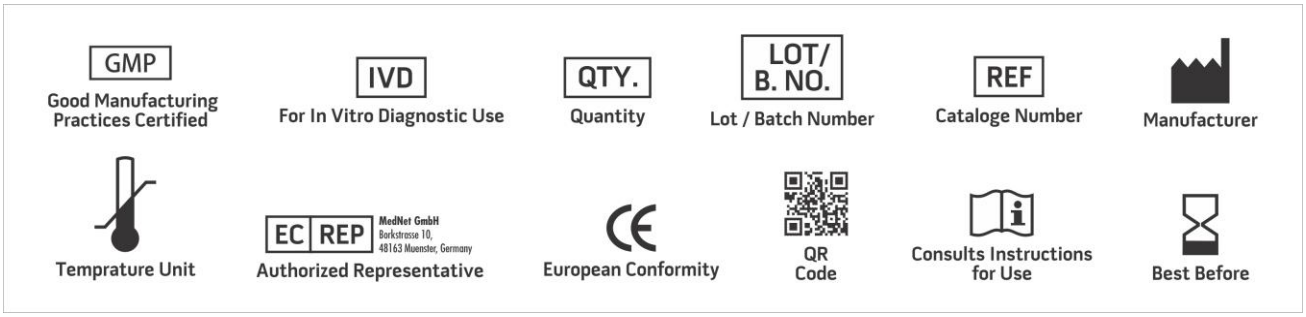
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
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