Antibiotic Medium Nº 2 (Agar Base)

Cat. 1002

Used as basal medium for microbiological assay of antibiotics.

Practical information

Aplications	Categories
Antibiotic Assay	Base layer

Industry: Pharmaceutical/Veterinary

Principles and uses

Antibiotic Medium Nº 2 is the standard agar base used for the microbiological assay of antibiotics in pharmaceutical preparations, body fluids, animal feed preparations and other materials.

The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms. Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods. The antibiotic media are identified numerically with names assigned by Grove and Randall in “Assay methods of antibiotics”. The use of standardized culture media and strict control of all test conditions are essential requirements in the microbiological assay of antibiotics in order to obtain satisfactory test results.

This medium has the same formula as Antibiotic Medium Nº 5 (Cat. 1524) and Antibiotic Medium Nº 8 (Cat. 1004), with the difference that the pH of the medium has been adjusted to 6,6. To carry out the antibiotic test, the Antibiotic Medium should be prepared on the same day as the test is done. The sample can be tested by the two methods of dilution and assay plate diffusion.

Gelatin peptone and beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Bacteriological Agar is the solidifying agent.

Formula in g/L

Bacteriological agar	15	Beef extract	1,5
Gelatin peptone	6	Yeast extract	3

Preparation

Suspend 25,5 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize at 121 ºC for 15 minutes.

Instructions for use

- Liquefy a the medium and inoculate it at a suitable temperature, for the example 48 ºC to 50 ºC, with a known quantity of a suspension of microorganism sensitive to the antibiotic to be examined.
- Agitate the mixture gently to produce a homogeneous distribution and Immediately pour into Petry dishes a quantity of the inoculated medium to form a layer 2-5 mm thick. Alternatively, the medium may consist of 2 layers, only the upper layer being inoculated.
- Prepare a solution of the reference substance and of the antibiotic to be examined having known concentrations and presumed to be equal activity.
- Apply the solutions to the surface of the medium, for example, in sterile cylinders of porcelain, stainless steel, or in cavities prepared in the agar.
- The same volume of the solution must be added to each cylinder or cavity.
- Alternatively, use a sterile absorbent paper disc, impregnate the discs with the solutions of the reference substance or the solutions of the antibiotics to be examined and place on the surface of the agar.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rest	Fine powder	Cream	Amber, sligtly opalescent	6,6±0,1

Microbiological test

Incubation conditions: (35±2 ºC / 18-24 h).

Microrganisms	Specification	Characteristic reaction
Micrococcus luteus ATCC 10240	Good growth	Inhibition zones with bacitracin
Staphylococcus epidermidis ATCC 12228	Good growth	Inhibition zones with noboviacin
Staphylococcus aureus ATCC 6538	Good Growth	Inhibition zones with methicillin and dicloxacillin

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Grove and Randall. Assay Methods of Antibiotics, Medical Encyclopedia Inc. New York 1955. United States Pharmacopoeia Convention. 1955. The United States Pharmacopoeia, 23rd Ed. Biological Tests and Assays, p. 1690-1696. The United States Pharmacopoeia Convention, Rockville, Md.