

## ANTIBIOTIC MEDIUM N° 1 (SEED AGAR) EUROPEAN PHARMACOPOEIA, USP

**CAT N°: 1520** Standard medium used for the preparation of the seed layer in antibiotic assays

### FORMULA IN g/l

Peptone	6.00	Beef Extract	1.50
Pancreatic Digest of Casein	4.00	Dextrose	1.00
Yeast Extract	3.00	Bacteriological Agar	15.00

**Final pH 6.6 ± 0.1 at 25°C**

\*This medium has the same formula as Antibiotic Medium N° 11 (Cat. 1 528), with the difference that the pH of the medium has been adjusted to 6.6.

### PREPARATION

Suspend 30.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 8-15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

### USES

ANTIBIOTIC MEDIUM N° 1 is used for testing the potency of antibiotics through their inhibitory effects on microorganisms. The cylinder assay plate is based on the diffusions of the antibiotics under test, measuring the size of the inhibition zones of microorganisms.

Peptones, Yeast extract and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological Agar is the solidifying agent.

### ASSAY PLATES

Seed Agar is used as an inoculum substrate. It is cooled to 48°C and inoculated accordingly with the specific antibiotics under test. Use 2 ml of the liquid culture to inoculate 100 ml of the Seed Agar. Agitate the mixture gently to produce a homogeneous distribution and pour 4 ml on each plate of solidified Base Agar (21 ml).

It is very important that the seed layer is evenly distributed over the entire surface of the Base Agar. Once the seed layer is solid, the cylinders are placed evenly spaced on a 2.5 - 3 mm radius. The standard sample is added as described above. This method is used for testing the potency of bacitracin and penicillin preparations.

Seed Agar is used for the basic layer as well as the seed layer for the assay of chloramphenicol in plates. A medium with a higher pH and the same formula is used for the assay of erythromycin, carbomycin and neomycin: Neomycin Test Agar (Antibiotic Medium N° 11, Cat. 1528).

### ENUMERATION OF MICROORGANISMS

Seed Agar can be used to determine the number of microorganisms in many antibiotic preparations.

### DETERMINATION OF ANTIBIOTICS IN MILK

Disk diffusion methods are utilized to detect the presence of residual antibiotics. The milk used to manufacture fermented products is tested for inhibitory substances, such as residual antibiotics for the treatment of mastitis of the animal, which can interfere with the normal activity of the initial culture.

## MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of  $35 \pm 2^{\circ}\text{C}$  and observed after 18 - 24 hours.

Microorganisms	Growth	Inhibition zones
<i>Staphylococcus aureus</i> ATCC 6538	Good	Cephalothin, Chloramphenicol, Penicillin
<i>Micrococcus luteus</i> ATCC 9341	Good	Cephalothin, Chloramphenicol, Penicillin

## 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.

European Pharmacopoeia 7.0



## STORAGE

Once opened keep powdered medium closed to avoid hydration.



2°C

25°C