Antibiotic Assay Medium A with pH 7.9

ME004

Antibiotic Assay Medium A with pH 7.9 is used for microbiological assay of antibiotics in pharmaceutical and food related preparations in accordance with European Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Peptone	6.000
Pancreatic digest of casein	4.000
Yeast extract	3.000
Beef extract	1.500
Glucose monohydrate	1.000
Agar	15.000
Final pH (at 25°C)	7.9±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.40 grams in 1000 ml R/purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Advice: Recommended for the microbiological assay of Gentamicin sulphate, Kanamycin monosulphate, Kanamycin acid sulphate, Neomycin sulphate, Netilmicin sulphate, Spiramycin, Streptomycin sulphate, Tylosin,Tylosin tartarate, Vancomycin hydrochloride .

Principle And Interpretation

Antibiotic Assay media are used in the performance of antibiotic assays. Grove and Randall have elucidated those antibiotic assays and media in their comprehensive treatise on antibiotic assays (1). Schmidt and Moyer have reported the use of antibiotic assay medium for the liquid formulation used in the performance of antibiotic assay (2). This medium is recommended by EP (3) and FDA (4).

Nutrients and growth factors are supplied by the ingredients like peptone, pancreatic digest of casein, yeast extract and beef extract. Dextrose provides the carbon and energy source. Agar provides excellent medium for antibiotic diffusion and gives well-defined zones of inhibition. Higher pH provides the optimal conditions for activity of antibiotic and also supports the growth of the test organisms.

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar pre-cooled to 40-45oC and spread evenly over the surface of solidified base agar. All conditions in the microbiological assay must be controlled carefully.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light yellow coloured clear to slightly opalescent

Reaction
After sterilization, reaction of 3.04% w/v aqueous solution. pH : 7.9±0.1

pH
7.80-8.00

Cultural Response
ME004: Cultural characteristics observed after an incubation at specified temperature for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed & incubation temp.
When incubated anaerobically				
Micrococcus luteus ATCC 9341	50-100	good-luxuriant	>=70%	Tylosin, Tylosin tartarate (adjust the pH to 8.0±.0.1) - 32-35°C
Staphylococcus aureus ATCC 6538p	50-100	good-luxuriant	>=70%	Kanamycin monosulphate - 30-37°C, ,, Kanamycin acid sulphate-35-39°C, Netilmicin sulphate - ,,32-35°C,,
Staphylococcus epidermidis ATCC 12228	50-100	good-luxuriant	>=70%	Gentamicin sulphate - 35-39°C
Bacillus pumilis NCTC 8241	50-100	good-luxuriant	>=70%	Gentamicin sulphate - 35-39°C
Bacillus subtilis ATCC 6633	50-100	good-luxuriant	>=70%	Kanamycin monosulphate - 30-37°C, Kanamycin acid sulphate - 35-39°C, Spiramycin - 30-32°C, Streptomycin sulphate,,- 30-37°C Vancomycin hydrochloride (adjust the pH to 8.0±.0.1) -,,37-39°C
Bacillus subtilis NCTC 8236	50-100	good-luxuriant	>=70%	Streptomycin sulphate,,30-37°C

Storage and Shelf Life

Store below 30°C and use freshly prepared medium . Use before expiry date on the label.

Reference

1. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc, New York.
2. Schmidt and Moyer, 1944; J. Bact, 47:199.
3. European Pharmacopoeia, 2009, European Dept. for the Quality of Medicines.
4. Tests and Methods of Assay of Antibiotics and Antibiotic containing Drugs, FDA, CFR, 1983. Title 21, part 436, Subpart D, Washington, D.C. U.S Government printing office, paragraphs 436, 100-436, 106 pg 242-259 (April 1).

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