Antibiotic HiVeg Assay Medium No. 11 (Neomycin, Erythromycin HiVeg Assay Agar)

MV004

Antibiotic HiVeg Assay Medium No.11 (Neomycin, Erythromycin HiVeg Assay Agar) is used for microbiological assay of antibiotics.

Composition**

Ingredients	Gms / Litre
HiVeg peptone	6.000
HiVeg hydrolysate	4.000
Yeast extract	3.000
HiVeg extract	1.500
Dextrose	1.000
Agar	15.000
Final pH (at 25°C)	8.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.5 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Advice:Erythromycin,Chlortetracycline, Dihydrostreptomycin sulphate,Erythromycin estolate, Framycetin, Gentamicin, Gentamicin sulphate, Kanamycin sulphate, Kanamycin monosulphate, Kanamycin acid sulphate, Netilmicin sulphate Netilmicin, Neomycin, Paromomycin, Sisomycin, Spiramycin, Streptomycin sulphate

Other Tests :

Cup plate method is carried out using B. pumilis / kanamycin and M. flavis / erythromycin

1) Dilution : 16 mg Kanamycin in 10 ml distilled water

Stock : 1:10 dilution of above solution

concentration	stock (ml)	Distilled water (ml)	zone of inhibition
5	0.25	4.75	15 mm
20	1.00	4.00	20 mm
100	5.00	-	25 mm

2) Dilution : 9 mg Erythromycin in 10 ml distilled water

Stock : 1:10 dilution of above solution

Concentration	stock (ml)	Distilled water (ml)	zone of inhibition
5	0.25	4.75	22 mm
10	0.50	4.50	32 mm
100	5.00	-	41 mm

Principle And Interpretation

Antibiotic HiVeg Medium No.11 (Neomycin, Erythromycin HiVeg Assay Agar) is prepared using vegetable peptones in place

of animal peptones, making the medium BSE, TSE risks free. It can be used for the same purpose of Antibiotic Medium No.11 (Neomycin, Erythromycin Assay Agar). Grove and Randall have elaborately elucidated the methods to perform Antibiotic Assay assays and various medias used for estimating the antibiotic concentrations of various formulations (1). Schmidt and Moyer have reported the use of antibiotic assay medium for the liquid formulation used in the performance of antibiotic assay (2). These media are also in accordance with USP (3) and FDA (4). MV004 media can be used as a seed layer or base layer for various assays. It is often used as inoculum medium for *Staphylococcus aureus* . It is used for antibiotic plate assay of Ampicillin, Carbomycin, Erythromycin, Clindamycin and Gentamycin with *Micrococcus luteus* , of Oleandomycin, Paromomycin, Neomycin, Netilmycin, Sisomycin with *Staphylococcus epidermidis* . It can also be used for plate assay of Kanamycin and Neomycin with either *Staphylococcus auerus* or *Bacillus pumilus* and for plate assay of Framycetin with *Bacillus pumilus* .

Nutrients and growth factors are supplied by the ingredients like HiVeg peptone, HiVeg hydrolysate, yeast extract and HiVeg 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 40-45°C and spread evenly over the surface of solidified base agar. All conditions in the microbiological assay must be controlled carefully.

Note: For Antibiotic Assay Methods and Selection of Antibiotic HiVeg Assay Medias, Refer Section Antibiotic HiVeg Assay Media.

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 gel forms in Petri plates

Reaction
Reaction of 3.05% w/v aqueous solution at 25°C. pH : 8.3±0.2

pH
8.10-8.50

Cultural Response
Cultural characteristics was observed after an incubation at 35-37°C for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
Cultural Response				
Micrococcus luteus ATCC 9341	50-100	luxuriant	>=70%	Erythromycin While assaying Tylosin, Tylosin tartarate,Vancomycin hydrochloride, adjust the pH to 8.0±.0.2
Staphylococcus aureus ATCC 6538p	50-100	luxuriant	>=70%	Kanamycin monosulphate,Kanamycin acid sulphate, Netilmicin sulphate
Staphylococcus epidermidis ATCC 12228	50-100	luxuriant	>=70%	Gentamicin, Neomycin,Netilmicin, Paromomycin, Sisomycin

Bacillus pumilis ATCC 14884	50-100	luxuriant	>=70%	Chlortetracycline ,Framycetin, Kanamycin sulphate
Bacillus subtilis ATCC 6633	50-100	luxuriant	>=70%	Dihydrostreptomycin sulphate, Erythromycin estolate,Kanamycin monosulphate, Kanamycin acid sulphate,Spiramycin, Streptomycin sulphate
Bacillus subtilis NCTC 8236	50-100	luxuriant	>=70%	Dihydrostreptomycin sulphate Streptomycin sulphate
Bacillus subtilis NCTC 8241	50-100	luxuriant	>=70%	Erythromycin estolate,Gentamicin sulphate

Storage and Shelf Life

Store below 30°C in tightly closed container 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. United States Pharmacopoeia 2011, USP 34/NF 29, US Pharmacopoeial Convention Inc, Rockville, MD
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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