



Antibiotic Assay Medium C

M555B

Intended Use:

Recommended for turbidimetric assay of a wide variety of antibiotics in accordance with BP.

Composition**

Ingredients	Gms / Litre
Peptone	6.000
HM peptone B #	1.500
Yeast extract	3.000
Sodium chloride	3.500
Glucose monohydrate	1.000
Dipotassium hydrogen phosphate	3.680
Potassium dihydrogen phosphate	1.320
pH after sterilization	* 7.0±0.1

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

* While assaying Josamycin & Josamycin propionate, adjust the pH to 8.0 ±0.1

Directions

Suspend 19.9 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml R-water/ purified /distilled water. Heat with frequent agitation to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Adjust the pH of the medium, using freshly prepared buffer solution as recommended by the British pharmacopoeia for the antibiotic assayed.

Advice : Recommended for the microbiological assay of Colistimethate sodium, Dihydrostreptomycin sulphate, Erythromycin estolate, Erythromycin ethylsuccinate, Framycetin sulphate, Gentamicin sulphate, Gramicidin, Kanamycin acid sulphate, Kanamycin monosulphate, Neomycin sulphate, Rifamycin sodium, Spiramycin, Streptomycin sulphate, Tylosin, Tylosin tartarate, Tyrothricin and Vancomycin hydrochloride according to British Pharmacopoeia.

Principle And Interpretation

This medium is used in turbidimetric assay of several antibiotics. The composition of the medium is in accordance to the specifications detailed in the British Pharmacopoeia (1). Turbidimetric methods for determining the potency of antibiotics are inherently more accurate and more precise than comparable agar diffusion procedures (2)

Peptone, HM peptone B and yeast extract provide essential nutrients and growth factors for enhanced microbial growth. Sodium chloride maintains the osmotic equilibrium while phosphates are incorporated in the medium to provide good buffering action. Glucose monohydrate serves as the carbon and energy source for faster growth.

Turbidimetric antibiotic assay is based on the change or inhibition of growth of a test microorganisms in a liquid medium containing a uniform concentration of an antibiotic (3). Use of this method is appropriate only when test samples are clear.

Quality Control

Appearance

Cream to yellow coloured homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured clear solution without any precipitate

pH

6.90-7.10

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours . (Key: 1.* - While assaying Josamycin and Josamycin propionate adjust the pH of the medium to 8.0 ± 0.1 2.# - While assaying Vancomycin hydrochloride, the incubation temperature is maintained at 37-39°C)

Organism	Inoculum (CFU)	Growth	Serial dilution with
<i>Escherichia coli</i> ATCC 9637	50-100	luxuriant	Colistimethate sodium
<i>Escherichia coli</i> ATCC 10536	50-100	luxuriant	Rifamycin sodium
<i>Enterococcus hirae</i> ATCC 10541	50-100	luxuriant	Gramicidin, Tyrothricin
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	luxuriant	Dihydrostreptomycin sulphate, Streptomycin sulphate
<i>Staphylococcus aureus</i> ATCC 6538p	50-100	luxuriant	Erythromycin estolate, Erythromycin ethylsuccinate, Erythromycin stearate, Framycetin sulphate, Gentamicin sulphate, Gramicidin, Kanamycin monosulphate, Kanamycin acid sulphate, Neomycin sulphate, Spiramycin, Tobramycin, *Josamycin, Josamycin propionate, #Vancomycin hydrochloride
<i>Staphylococcus aureus</i> ATCC 9144	50-100	luxuriant	Tylosin, Tylosin tartarate

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. British Pharmacopoeia, 2011, The Statutory Office, British Pharmacopoeia
2. Rippere RA. Some principles of microbiological turbidimetric assays of antibiotics. J Assoc Off Anal Chem.1979 62(4):951-6.
3. Chapin-Robertson and Edberg, 1991, Measurement of Antibiotics in Human Body fluids:Techniques and significance. Antibiotics in Laboratory medicine, New York pp 305

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