Antibiotic Assay Medium C

M555

Intended Use:

As the broth medium in tubidimetric assay of a wide variety of antibiotics.

Composition**

Ingredients	Gms / Litre
Peptone	6.000
HM peptone B #	1.500
Yeast extract	3.000
Sodium chloride	3.500
Dextrose (Glucose)	1.000
Dipotassium hydrogen phosphate	3.680
Potassium dihydrogen phosphate	1.320
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

Directions

Suspend 20 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
Note : Adjust the medium pH to 8.0±0.2 for assaying Josamycin and Josamycin sulphate.

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.

Principle And Interpretation

This medium is used in turbidometic assay of several antibiotics. The composition of the medium is in accordance with the specifications detailed in the European and British Pharmacopeia (1,3). Turbidimetric methods for determining the potency of antibiotics are inherently more accurate and more precise than comparable agar diffusion procedures (6) Peptone, HM peptone B and yeast extract provides 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. Dextrose 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 microorganims in a liquid medium containing a uniform concentration of an antibiotic (2).

Type of specimen

Pharmaceutical sample

Specimen Collection and Handling

For pharmaceutical sample samples follow appropriate techniques for handling specimens as per established guidelines (1,3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Freshly prepared medium plates must be used or it may result in erroneous results.
2. Use of this method is appropriate only when test samples are clear.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured clear solution without any precipitate

Reaction
Reaction of 2.0% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

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

Organism	Inoculum (CFU)	Growth	Serial dilution with
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	Colistimethate sodium, Rifamycin sodium
Escherichia coli ATCC 9637	50-100	luxuriant	Colistimethate sodium
Escherichia coli ATCC 10536	50-100	luxuriant	Rifamycin sodium
Enterococcus hirae ATCC 10541 (00011*)	50-100	luxuriant	Gramicidin, Tyrothricin
Klebsiella pneumoniae ATCC 10031	50-100	luxuriant	Dihydrostreptomycin sulphate, Streptomycin sulphate
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50-100	luxuriant	Erythromycin estolate,Framycetin sulphate,Genatamicin sulphate, Gramicidin,Kanamycin monosulphate, Kanamycin acid sulphate, Neomycin sulphate,Spiramycin,Tylosin,Tylosin hydrochloride, Vancomycin hydrochloride While assaying Josamycin and Josamycin sulphate adjust the pH of the medium to 8.0±0.2

Staphylococcus aureus ATCC 6538P	50-100	luxuriant	Erythromycin estolate,Framycetin sulphate,Genatamicin sulphate, Gramicidin,Kanamycin monosulphate, Kanamycin acid sulphate, Neomycin sulphate,Spiramycin, Vancomycin hydrochloride. While assaying Josamycin and Josamycin sulphate adjust the pH of the medium to 8.0 ± 0.1
Staphylococcus aureus ATCC 9144 (00035*)	50-100	luxuriant	Tylosin, Tylosin tartarate

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and use freshly prepared medium. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. British Pharmacopoeia, 2016, British Pharmacopoeia Commission

2. Chapin-Robertson and Edberg, 1991, Measurement of Antibiotics in Human Body fluids:Techniques and significance. Antibiotics in Laboratory medicine, New York pp 305

3. European Pharmacopoeia, 2017, European Department, for the quality of Medicines.

4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual Clinical Microbiology, 11th Edition. Vol. 1.

6. Rippere RA. Some principles of microbiological turbidimetric assays of antibiotics. J Assoc Off Anal Chem.1979 62(4):951-6.

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