Antibiotic Assay Medium A with pH 7.9

ME004

Intended Use:

Recommended for microbiological assay of antibiotics in accordance with EP.

Composition**

Ingredients	g / L
Peptone	6.000
Tryptone #	4.000
Yeast extract	3.000
HM peptone B #	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
Equivalent to Pancreatic digest of casein
Equivalent to Beef extract

Directions

Suspend 30.4 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml water R/purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Note: Recommended for the microbiological assay of Gentamicin sulphate, Kanamycin monosulphate, Kanamycin acid sulphate, Netilmicin sulphate, Spiramycin, Streptomicin 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, tryptone, yeast extract and HM peptone B. 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.

Type of specimen

Antibiotics as per European Pharmacopoeia

Specimen Collection and Handling

Follow appropriate techniques for handling specimens as per established guidelines (3,4). 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. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.
2. Fastidious organisms may not grow on this medium and may require supplementation of blood.
3. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
4. Freshly prepared plates should be used for antibiotic assays.

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

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

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				
##*Kocuria rhizophila* 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 (00036*)	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 spizizenii* ATCC 6633 (00003*)	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

Key : *Corresponding WDCM numbers.

**Formerly known as *Bacillus subtilis* subsp. *spizizenii* ## Formerly known as *Micrococcus luteus*

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 (5,6).

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, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
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).
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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