



Antibiotic Assay Medium A

ME003

Intended Use:

Recommended for microbiological diffusion assay of several antibiotics in accordance with European Pharmacopoeia.

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)	7.0±0.1

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 30.40 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml distilled/ purified/ R 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 or dispense as desired.

Note: Recommended for the microbiological assay of Bacitracin at pH 7.0. For other antibiotics: Josamycin, Josamycin propionate & Rifamycin sodium at pH 6.6. Gentamicin sulphate, Kanamycin monosulphate, Kanamycin acid sulphate, Neomycin sulphate, Netilmicin sulphate, Spiramycin, Streptomycin sulphate, Erythromycin & Dihydrostreptomycin sulphate at pH 7.9. Tylosin, Tylosin tartarate & Vancomycin hydrochloride at pH 8.0.

Principle And Interpretation

This medium is used as inoculum and maintenance medium for different test organisms for antibiotic assays. Composition of this medium is in accordance with European Pharmacopoeia (1).

Essential nutrients, vitamins, mineral, trace elements and growth factors are supplied by peptone, tryptone, yeast extract and HM peptone B. Glucose monohydrate in the medium serves as the carbon source for stimulating the growth of the test microorganism. Agar provides excellent medium for antibiotic diffusion and gives well defined zones of inhibition. Freshly prepared plates should be preferably used for assaying antibiotics. Test organisms is 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. One of the critical and important step for obtaining good results is use of appropriate standard culture media.

Type of specimen

Antibiotics as per European Pharmacopoeia

Specimen Collection and Handling

Follow appropriate techniques for handling specimens as per established guidelines (1,2).

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.

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

Yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

After sterilization, reaction of 3.05% w/v aqueous solution. pH : 7.0±0.1

pH

6.90-7.10

Growth Promotion Test

As per European Pharmacopoeia

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed	Incubation Temperature
** <i>Bacillus spizizenii</i> ATCC 6633 (00003*)	50-100	good-luxuriant	≥70%	Josamycin, Josamycin propionate	35-37°C
\$ <i>Kocuria rhizophila</i> ATCC 9341	50-100	good-luxuriant	≥70%	Rifamycin sulphate	35-39°C
<i>Micrococcus luteus</i> ATCC 10240	50-100	good-luxuriant	≥70%	Bacitracin zinc (Adjust the pH to 7.0 ± 0.1)	35-39°C

Key : *Corresponding WDCM numbers.

\$- Formerly known as *Micrococcus luteus*

**Formerly known as *Bacillus subtilis* subsp. *spizizenii*

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 (3,4).

Reference

1. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
2. National Committee for Clinical Laboratory Standards, 2000, Approved Standard: M7-A5. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow aerobically, 5th Ed., NCCLS, Wayne, Pa.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. 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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