



Ready Prepared Media

Technical Information

Blood Agar Plate w/ Gentamicin (5mcg/plate)

Product Code: PM 6304

Application: Recommended for the selective isolation of *Streptococcus pneumoniae*.

Composition**

Ingredients	Gms / Litre
Proteose peptone	15.000
HL extract #	2.500
Yeast extract	5.000
Sodium chloride	5.000
Agar	15.000
Gentamicin	5mcg/plate
Sheep blood	50.000 ml
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters.

Equivalent to Liver extract

Principle & Interpretation

Blood Agar Base No. 2 is a highly nutritive medium. Microorganisms producing haemolysin give visible haemolytic zones on this medium. Blood Agar with Gentamicin is recommended for the selective isolation of *Streptococcus pneumoniae* (1). The medium gave increased isolation rates of beta-hemolytic streptococci, other streptococci, *Bacteroides*, *clostridia*, and yeasts. It also proved valuable in detecting gentamicin-resistant gram-negative bacilli when these were present in heavy mixed culture (2).

HL extract and yeast extract helps enhance the growth and haemolytic reactions of fastidious organisms like Streptococci and Pneumococci. Proteose peptone serves as the nitrogen source while HL extract and yeast extract provide essential carbon, vitamin, nitrogen and amino acid sources. Sodium chloride maintains the osmotic equilibrium. Blood provides additional growth factors and also serves as basis for determining haemolytic reactions. Gentamicin inhibits other gram positive bacteria. Isolation of *Streptococcus pneumoniae* from respiratory secretions can be increased by using a 5% sheep blood agar plate supplemented with gentamicin.

Type of specimen

Clinical material : Isolated microorganism

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4). After use, contaminated materials must be sterilized by autoclaving before discarding.



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Warning and Precautions

In Vitro diagnostic use. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium .
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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..

Methodology

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Quality Control

Appearance

Sterile Sheep blood agar with 5% defibrinated sheep blood in 90 mm disposable plate with smooth surface and absence of black particles/cracks/bubbles

Colour

Cherry red coloured opaque medium

Quantity of Medium

25ml of medium in 90 mm plate.

pH

7.10 - 7.50

Sterility Check

Passes release criteria

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours (# Incubated anaerobically).

Organism	Inoculum(CFU)	Growth	Recovery
Streptococcus pneumoniae ATCC 6303	50-100	Luxuriant	>=50%
#Clostridium sporogenes ATCC 11437	50-100	Luxuriant	>=50%
#Clostridium perfringens ATCC 13124	50-100	Luxuriant	>=50%
Streptococcus pyogenes ATCC 19615	50-100	Luxuriant	>=70%
Staphylococcus aureus	>=10 ³	Inhibited	0%



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subsp. aureus ATCC
25923 (00034*)
Staphylococcus aureus $\geq 10^3$ Inhibited 0%
subsp. aureus ATCC6538 (00032*)
Key : (*) - Corresponding WDCM numbers

Storage and Shelf Life

- On receipt store between 2-8°C Use before expiry date on the label.
- 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).

Further Reading

1. R.E., Schmid, Washington JA 2nd, Anhalt JP; Gentamicin-blood agar for isolation of *Streptococcus pneumoniae* from respiratory secretions. J Clin Microbiol. 1978 May;7(5):426-7.
2. W.A., Black, Van Buskirk F; Gentamicin blood agar used as a general-purpose selective medium. J. Appl Microbiol. 1973 Jun;25 (6):905-7.
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.

Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at CDH is true and accurate
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