

## Columbia 5% Sheep Blood Agar Plate

MP144

### Intended Use:

Recommended for isolation and cultivation of fastidious organisms.

### Composition\*\*

Ingredients	g'TN
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Agar	15.000
Defibrinated Sheep Blood	50ml
Final pH ( at 25°C)	7.3±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

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

### Principle And Interpretation

Columbia Blood Agar Base was devised by Ellner et al (1). This medium contains special peptone which supports rapid and luxuriant growth of fastidious and non-fastidious organisms. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Fildes found that Nutrient Agar supplemented with a digest of sheep blood supplied both of these factors and the medium would support the growth of *H. influenzae* (2,3). The inclusion of bacitracin makes the enriched Columbia Agar Medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from upper respiratory tract (4). Columbia Agar Base is used as the base for the media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.

Corn starch serves as an energy source and also neutralizes toxic metabolites. Sheep blood permits the detection of haemolysis and also provides heme (X factor) which is required for the growth of many bacteria. However it is devoid of V factor (Nicotinamide adenine dinucleotide) and hence *Haemophilus influenzae* which needs both the X and V factors, will not grow on this medium.

### Type of specimen

Clinical samples - throat swabs, pus, etc.

### Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions

In Vitro diagnostic use only. 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.As this medium have a relatively high carbohydrate content, beta-haemolytic Streptococci may exhibit a greenish haemolytic reaction which may be mistaken for the alpha haemolysis.

2.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

3. 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.

4. Biochemical characterization is required on colonies of pure culture for complete identification.

## 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

Sterile Columbia Agar W/5% sheep blood in 90mm disposable plates with smooth surface and absence of black particles/ cracks/bubbles

### Colour of medium

Red coloured medium

### Quantity of medium

25 ml of medium in 90mm disposable plates.

### pH

7.10-7.50

### Sterility Check

Passes release criteria

### Growth Promotion Test

In accordance with the harmonized method of USP/EP/BP/JP.

### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
<i>Neisseria meningitidis</i> ATCC 13090	50-100	luxuriant	≥70%	none
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	luxuriant	≥70%	beta / gamma
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	50-100	luxuriant	≥70%	gamma
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	50-100	luxuriant	≥70%	beta / gamma
<i>Streptococcus pneumoniae</i> ATCC 6303	50-100	luxuriant	≥70%	alpha
<i>Streptococcus pyogenes</i> ATCC 19615	50-100	luxuriant	≥70%	beta
<i>Clostridium sporogenes</i> ATCC 19404 (00008*)	50-100	luxuriant	≥50 %	
<i>Clostridium sporogenes</i> ATCC 11437	50-100	good-luxuriant	≥50 %	
<i>Clostridium perfringens</i> ATCC 13124 (00007*)	50-100	luxuriant	≥50 %	
<i>Clostridium perfringens</i> ATCC 12934	50-100	luxuriant	≥50 %	

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

## Reference

1. Bailey R. K., Voss J. L. and Smith R. F., 1979, J. Clin. Microbiol., 9 ; 65-71
2. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Chapin K. C. and Doern G. V., 1983, J. Clin. Microbiol., 17:1163.
4. Ellner P. P., Stoessel C. J., Drakeford E. and Vasi F., 1966, Am. J. Clin. Pathol., 45:502.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2<sup>nd</sup> 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.

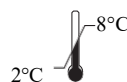
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