Technical Information

Columbia 5% Sheep Blood Agar Plate

Product Code: PM 1144

Application: Recommended for isolation and cultivation of fastidious organisms.

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Columbia Blood Agar Base was devised by Ellner et al (4). 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* (5,6). Columbia Agar Base is used as the base for the media containing blood .

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 - blood, respiratory exudates.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic 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. Carry out confirmatory tests of all the colonies.

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 Columbia Agar W/5% sheep blood in 90mm disposable plates.
pH
7.10-7.50
Quantity of medium
25 ml of medium in 90 mm disposable plates.
Colour of medium
Red coloured coloured medium
Sterility Test
Passes release criteria
Growth Promotion Test
In accordance with the harmonazied method of USP/EP/BP/JP.
Cultural Response
Cultural characteristics observed after incubation at 35-37°C for 24-48 hours.

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

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

- On receipt store between 20-30°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).

Further Reading

1. Bailey R. K., Voss J. L. and Smith R. F., 1979, J. Clin. Microbiol., 9 ; 65-71
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
4. Ellner P. P., Stoessel C. J., Drakeford E. and Vasi F., 1966, Am. J. Clin. Pathol., 45:502.
5. Fildes P., 1920, Br. J. Exp. Pathol., 1:129.
6. Fildes P., 1921, Br. J. Exp. Pathol., 2:16.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. 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.
9. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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