COLUMBIA BLOOD AGAR

- For in vitro use only - Catalogue No. PB70

Our Columbia Agar supplemented with 5% defibrinated sheep blood is a general-purpose medium for the isolation and cultivation of non-fastidious and fastidious bacteria.

In 1966, Ellner et al. reported the development of a superior blood agar base, which has been termed Columbia Agar. Columbia Agar, unlike most other blood agar bases, contains both casein hydrolysate and meat digests. Both types of enzymatic digests have their advantages: casein hydrolysate encourages the development of large colonies, while meat digests provide well defined zones of hemolysis and good colonial differentiation. The variety of peptones and extracts makes Columbia Agar especially nutritious, and the addition of defibrinated sheep blood allows for the determination of hemolytic reactions. Although Columbia Agar can be used to cultivate a variety of fastidious organisms, bacteria that require NAD (V factor), such as *Haemophilus*, do not grow since NADase is present in sheep blood.

Formula per Litre of Medium

Pancreatic Digest of Casein	12.0 g
Peptic Digest of Animal Tissue	5.0 g
Sodium Chloride	5.0 g
Beef Extract	3.0 g
Yeast Extract	3.0 g
Corn Starch	1.0 g
Agar	13.5 g
Defibrinated Sheep Blood	50.0 mL

pH 7.3 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature.
2. Using an inoculum from a fresh, pure culture, streak the plate as to obtain isolated colonies.
3. Incubate at 35°C in an aerobic environment with or without 5 to 10% CO_2 depending on the organism being cultivated.
4. Examine after 18-24 hours.

Interpretation of Results

Our Columbia Blood Agar can be used as a primary-plating medium. Primary isolation is performed to separate and isolate organisms present in a sample. This separation allows for characterization of colony types and may indicate the presence of clinically significant bacteria. When examining plates a hand lens or stereoscopic microscope should be available for examining very small colonies. The different types of colonial morphology appearing on the agar plate should be noted as well as the number of each morphotype present. Hemolysis is also a very useful differential characteristic that is best viewed when a bright light is transmitted from behind the plate. Three main types of hemolysis can be described on this medium:

1. Alpha-hemolysis (α) – Partial hemolysis that results in a greenish discoloration around the colony
2. Beta-hemolysis (β) – Complete lysis of red blood cells resulting in a clear zone around the colony
3. Gamma-hemolysis (γ) – No hemolysis resulting in no change in the medium

Additional tests should be performed on isolated colonies from pure culture in order to complete identification.

- *Due to the high carbohydrate content of this medium some beta-hemolytic streptococci may appear to be alpha-hemolytic or adopt a greenish hue*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
Streptococcus pneumoniae ATCC 6305	Growth, α-hemolysis
Streptococcus pyogenes ATCC 19615	Growth, β-hemolysis
Staphylococcus aureus ATCC 25923	Growth
Escherichia coli ATCC 25922	Growth

Storage and Shelf Life

Our Columbia Blood Agar should be stored away from direct light at 4 to 8°C. The medium side should uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 12 weeks from the date of manufacture.

References

1. Ellner PD, Stoessel CJ, Drakeford E, Vasi F. A new culture medium for medical bacteriology. Am. J. Clin. Pathology 1966; 45:502-4.
2. Morello JA, Ellner PD. A new medium for blood cultures. Appl Microbiol 1969; 17:68.
3. Buck GE, Kelly MT. Effects of moisture content of the medium in colony morphology of *Campylobacter fetus* subsp. *jejuni*. J Clin Microbiol 1981; 14:585.
4. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 10th ed. St Louis: Mosby, 1998.
5. Murray PR, Baron E, Pfaller M, Tenover F, Yolken R. Manual of Clinical Microbiology. 7th ed. Washington: ASM, 1999.

Original: August 2000
Revised / Reviewed: October 2014