BD™ Columbia CNA Agar with 5% Sheep Blood

INTENDED USE

BD Columbia CNA Agar with 5% Sheep Blood is a selective medium used for the isolation of gram-positive bacteria (especially staphylococci and streptococci) from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.
Ellner et al. in 1966 reported the development of a blood agar formulation, which has been designated as Columbia Agar.[1] This medium which achieves larger colonies and more luxuriant growth than on comparable blood agar bases, is utilized for media containing blood and for selective formulations. Ellner et al. found that a medium containing 10 mg of colistin and 15 mg of nalidixic acid per liter in a Columbia agar base, enriched with 5% sheep blood, supports the growth of staphylococci, hemolytic streptococci and enterococci while inhibiting the growth of *Proteus, Klebsiella* and *Pseudomonas* species.[1-3]
Columbia Agar provides a highly nutritious base medium. The addition of the antimicrobial agents, colistin and nalidixic acid renders the medium selective for gram-positive micro-organisms, especially streptococci and staphylococci. In **BD Columbia CNA Agar with 5% Sheep Blood**, the concentration of nalidixic acid has been reduced to 10 mg/l to increase the recovery of gram-positive cocci from clinical specimens. Sheep blood allows detection of hemolytic reactions which are especially important in the presumptive diagnosis of streptococci.[2-6]

REAGENTS

BD Columbia CNA Agar with 5% Sheep Blood
Formula* Per Liter Purified Water

Pancreatic Digest of Casein	12.0 g
Peptic Digest of Animal Tissue	5.0
Yeast Extract	3.0
Beef Extract	3.0
Corn Starch	1.0
Sodium Chloride	5.0
Agar	13.5
Colistin	10.0 mg
Nalidixic Acid	10.0
Sheep Blood, defibrinated	5%

pH 7.3 ± 0.2
*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.
Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.
Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate the medium with the strains listed below. (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate at 35 ± 2° C for 18 to 24 hours, preferably in an aerobic atmosphere enriched with carbon dioxide.

Strains	Growth Results
Staphylococcus aureus ATCC™ 25923	Good to excellent growth, may be beta-hemolytic
Streptococcus pneumoniae ATCC 6305	Good to excellent growth, alpha hemolysis
Streptococcus pyogenes ATCC 19615	Good to excellent growth, beta hemolysis
Enterococcus faecalis ATCC 29212	Good to excellent growth
Proteus mirabilis ATCC 12453	Inhibition partial to complete; no swarming
Uninoculated	Red (blood color)

PROCEDURE

Materials Provided

BD Columbia CNA Agar with 5% Sheep Blood (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This is a selective medium for isolation of many Gram positive bacteria in aerobic bacteriology that can be used for all types of clinical specimens. For details see **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE.**

Test Procedure

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. In order to provide detection of all pathogens contained in the specimen, it must also be streaked onto appropriate nonselective media, e.g., **BD Columbia Agar with 5% Sheep Blood** and onto other selective media, e.g., **BD MacConkey II Agar**.[4,7]

Incubate at 35 ± 2° C for 42 to 48 hours, preferably in an aerobic atmosphere enriched with carbon dioxide, and read the plates after 18 to 24 and after 42 to 48 hours.

Results

Typical colonial morphology on **BD Columbia CNA Agar with 5% Sheep Blood** is as follows:

Streptococci (non-group D)	Small, white to grayish. Beta or alpha hemolysis.
Enterococci (Group D)	Small, but larger than group A streptococci, grayish. Alpha (rarely beta) hemolysis.
Staphylococci	Large, white to gray or cream to yellow, with or without hemolysis
Micrococci	Large, white to gray or yellow to orange, with or without hemolysis
Corynebacteria	Small to large, white to gray or yellow, with or without hemolysis
Candida spp.	Small, white
Listeria monocytogenes	Small to medium-sized, grayish, with weak beta hemolysis
Gram-negative bacteria	No growth to trace growth

Other Gram positive bacteria, not listed above, may also grow on the medium. For details and interpretation of growth, consult the references.[2,4-6]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Columbia CNA Agar with 5% Sheep Blood is a standard medium for the isolation and cultivation of many aerobically growing Gram positive micro-organisms, e.g., streptococci, staphylococci, coryneforms, *Listeria* spp and others.[2,4,5] When incubated anaerobically, it may also be used for the isolation of Gram positive strict anaerobes.[3,7]

The number and types of bacterial species occuring as infectious agents is very large. Therefore, before the medium is routinely used for rarely isolated and newly described micro-organisms, its suitability must first be tested by the user by cultivating pure cultures of the organism in question.
Gram negative bacteria exhibiting resistance to the selective ingredients may grow on this medium.
Candida species and other fungi are not inhibited on this medium.
Although they are Gram positive bacteria, aerobic spore-formers such as *Bacillus* spp., may be inhibited on **BD Columbia CNA Agar with 5% Sheep Blood**.
Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures is necessary for complete identification.[4,6]

Columbia Agar base has a relatively high carbohydrate content and, therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha hemolysis.

REFERENCES

1. Ellner, P.D., C.J. Stoessel, E. Drakeford, and F. Vasi. 1966. A new culture medium for medical bacteriology. Am. J. Clin. Pathol. 45:502-504.
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3. Chapin, K.C., and T.-L. Lauderdale. 2003. Reagents, stains, and media: bacteriology. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
4. Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.).2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
5. Ruoff, K.L., R.A. Whiley, and D. Beighton. 2003. *Streptococcus*. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8thed. American Society for Microbiology, Washington, D.C.
6. Isenberg, H. D. (ed.). 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol.1, p. 1.6.1-1.6.7. American Society for Microbiology, Washington, D.C.
7. Thomson, R.B., and J.M. Miller. 2003. Specimen collection, transport, and processing: bacteriology. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.

PACKAGING/AVAILABILITY

BD Columbia CNA Agar with 5% Sheep Blood

Cat. No. 254007	Ready-to-use plated media, 20 plates
Cat. No. 254072	Ready-to-use plated media, 120 plates

FURTHER INFORMATION

For further information please contact your local BD representative.

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