BD™ Columbia Agar with 5% Sheep Blood

INTENDED USE

BD Columbia Agar with 5% Sheep Blood is a highly nutritious general purpose medium for the isolation and cultivation of nonfastidious and fastidious microorganisms from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Ellner et al.[1] in 1966 reported the development of a new blood agar formulation, which has been designated as Columbia Agar. **BD Columbia Agar with 5% Sheep Blood** derives its superior growth-supporting properties from the combination of two peptones, and yeast extract as a supplier of the B complex vitamins. Corn starch is included to absorb toxic by-products contained in the specimen and serves as an energy source for organisms possessing alpha-amylases. Sheep blood allows detection of hemolytic reactions and supplies the X factor (heme) necessary for the growth of many pathogenic species.

On this medium, colonies tend to be larger and growth is more luxuriant than on media containing other blood agar bases. Columbia Blood Agar is recommended as a primary isolation medium in the MiQ standards and in other diagnostic manuals.[2,3] In many European countries, this medium has become the most frequently used primary isolation medium for clinical specimens.

REAGENTS

BD Columbia 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
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 representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate the inoculated plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide. Examine plates after 18 to 24 h for amount of growth, colony size and hemolytic reactions.

Strains	Growth Results
Streptococcus pyogenes ATCC™ 19615	Growth good to excellent, beta hemolysis weak to good
Streptococcus pneumoniae ATCC 6305	Growth good to excellent, alpha hemolysis
Staphylococcus aureus ATCC 25923	Growth good to excellent; may or may not be beta hemolytic
Escherichia coli ATCC 25922	Growth good to excellent; may or may not be beta hemolytic
Uninoculated	Red (blood color)

PROCEDURE

Materials Provided

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

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as described.

Specimen Types

This is a universal isolation medium and can be used for all types of aerobically incubated clinical specimens (see also **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 for isolation from this inoculated area. Appropriate selective media for detection of specific pathogens, e.g., **BD MacConkey II Agar** for the isolation of *Enterobacteriaceae*, should be included.
Since many pathogens require carbon dioxide on primary isolation, **BD Columbia Agar with 5 % Sheep Blood** plates should be incubated in an aerobic atmosphere containing approximately 3 to 10 % CO_2. Incubate plates at 35 ± 2°C for 18 to 72 h. Read for the first time after 18 to 24 hours and re-incubate if necessary.

Results

After incubation, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of micro-organisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

The number of species growing on this medium is very large. Therefore, no comprehensive information on the appearance of the organisms on this medium can be given here. Consult the appropriate references for information on appearance and further differential tests of the organisms isolated.[2-7]
Typical colonial morphology of frequently isolated organisms on **BD Columbia 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
Corynebacteria	Small to large, white to gray or yellow, with or without hemolysis
Listeria monocytogenes	Small to medium-sized, grayish, with weak beta hemolysis
Enterobacteriaceae	Medium-sized to large, grey colonies, with or without hemolysis
Candida spp.	Small, white

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Columbia Agar with 5% Sheep Blood is a primary isolation medium on which most microorganisms, such as *Enterobacteriaceae*, *Pseudomonas* and other non-fermenting Gram negative rods, streptococci, enterococci, staphylococci, coryneforms, *Candida* species, and many others will grow.[2,5,6]

The medium lacks V factor (nicotinamide adenine dinucleotide, NAD) since sheep blood contains NADase which destroys the NAD. For this reason, *Haemophilus influenzae* which requires both the X and V factors, will not grow on this medium.

Neisseria gonorrhoeae does not grow well on this medium. Instead, **BD Chocolate Agar (GC II Agar with IsoVitaleX)** should be used for its recovery.

Also, the medium is not suitable for the isolation and growth of *Mycobacterium*, *Legionella*, *Bordetella* and other organisms with highly specific nutritive requirements.

The number and types of bacterial species occurring as infectious agents is very large. Therefore, before the medium is routinely used for rarely isolated or newly described micro-organisms, its suitability must first be tested by the user by cultivating pure cultures of the organism in question.

Due to the rather high carbohydrate (starch) content of Columbia Agar Base, beta-hemolytic streptococci may exhibit alpha rather than beta hemolytic reactions or may exhibit weak hemolytic reactions on media based on this formulation.

Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures is recommended for complete identification. Consult appropriate references for further information. [3,5,6]

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.
2. MiQ - Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik, edited by Mauch, H., R. Lüttiken, and S. Gatermann for the Deutsche Gesellschaft für Hygiene und Mikrobiologie (DGHM). Volumes 3, 6, and 7. Urban & Fischer, Munich, Germany.
3. 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.
4. Chapin, K.C., and T.-L. Lauderdale. 2003. Reagents, stains, and media. *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.
5. 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.
6. Baron, E. J, L. R. Peterson, and S. M. Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed., p. 415. Mosby-Year Book, Inc. St. Louis, MO.
7. MacFaddin, J. F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 86-92. Williams & Wilkins, Baltimore, MD.

PACKAGING/AVAILABILITY

BD Columbia Agar with 5 % Sheep Blood

Cat. No. 254005 Ready-to-use Plated Media, cpu 20
Cat. No. 254071 Ready-to-use Plated Media, cpu 120

FURTHER INFORMATION

For further information please contact your local BD representative.

Becton Dickinson GmbH
Tullastrasse 8 – 12
D-69126 Heidelberg/Germany
Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16
Reception_Germany@europe.bd.com

http://www.bd.com
http://www.bd.com/europe/regulatory/