BD™ Columbia Agar With 5% Horse Blood

INTENDED USE

Columbia Agar with 5% Horse Blood is a highly nutritious general purpose medium for the isolation and cultivation of nonfastidious and fastidious micro-organisms 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. Columbia Agar with 5% Horse 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. Horse blood allows detection of hemolytic reactions and supplies both the X factor (heme) and the V factor (nicotinamide adenine dinucleotide, NAD), necessary for the growth of many bacterial species, including *Haemophilus influenzae*, which requires both the X and V factors. Columbia Blood Agar has a relatively high carbohydrate content and, therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha hemolysis. However, this greenish hemolytic reaction of streptococci is less frequently observed on Columbia Agar supplemented with horse blood than on the same medium supplemented with sheep blood.[2] It should be noted that beta-hemolytic reactions depend on the type of blood added; as an example, enterococci which only very rarely hemolyse sheep blood, will produce a well visible beta hemolysis on horse blood. *Staphylococcus aureus* which is usually beta hemolytic on sheep blood, will often be non-hemolytic on horse blood.

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.[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% Horse Blood

Formula* Per Liter Purified Water

Pancreatic Digest of Casein	12.0 g
Peptic Digest of Animal Tissue	5.0
Yeast Extract	3.5
Beef Extract	3.0
Corn Starch	1.0
Sodium Chloride	5.0
Agar	13.5
Horse 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, beta hemolysis
Streptococcus pneumoniae ATCC 6305	Growth, alpha hemolysis
Enterococcus faecalis ATCC 29212	Growth, beta hemolysis
Staphylococcus aureus ATCC 25923	Growth; may be beta-hemolytic
Escherichia coli ATCC 25922	Growth
Uninoculated	Red (blood color)

PROCEDURE

Materials Provided

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

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This is a universal isolation medium and can be used for all types of aerobically incubated bacteriological 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* and other Gram negative rods, should be included.
Since many pathogens require carbon dioxide on primary isolation, **BD Columbia Agar with 5 % Horse 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. Consult the appropriate references for the appearance and further differential tests of the organisms isolated.[2,3]
Typical colonial morphology of frequently isolated organisms on **BD Columbia Agar with 5% Horse 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. 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

The medium is suitable for the isolation and cultivation of many aerobically growing micro-organisms, such as *Enterobacteriaceae*, *Pseudomonas* and other non-fermenting Gram negative rods, streptococci, staphylococci, coryneforms, *Candida* species, and many others.[2-4]

Columbia Agar supplemented with horse blood provides clearer beta hemolysis of streptococci than Columbia Agar with sheep blood.[2]
Hemolytic properties described in diagnostic textbooks usually refer to sheep blood. On horse blood containing media, like on **BD Columbia Agar with 5 % Horse Blood**, these features might be different (see also **PRINCIPLES AND EXPLANATION OF THE PROCEDURE)**.
Colonies of *Haemophilus haemolyticus* which is part of the normal throat flora, are beta-hemolytic on horse and rabbit blood agar and must be distinguished from colonies of beta-hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of H. *haemolyticus.*[5]
Neisseria gonorrhoeae does not grow well on this medium. Instead, Chocolate Agar should be used for the recovery of these species.
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.
Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are necessary for complete identification. Consult appropriate references for further information.[4,5]

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. 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.
3. 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.
4. 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.
5. 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.

PACKAGING/AVAILABILITY

BD Columbia Agar with 5% Horse Blood
Cat. No. 256006 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

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