SHEEP BLOOD COLUMBIA AGAR

INTENDED USE

Columbia Agar Base, without or with the addition of 5% (or 10%) sheep blood, is a highly nutritious, general-purpose medium for the isolation and cultivation of nonfastidious and fastidious microorganisms from a variety of clinical and nonclinical materials.

Columbia Agar Base is utilized as the base for media containing blood and for selective media formulations in which various combinations of antimicrobial agents are used as additives.

SUMMARY AND EXPLANATION

Ellner et al.,[1] in 1966, reported the development of a blood agar formulation, which has been designated as Columbia Agar. The base achieves the more rapid and luxuriant growth obtained from casein hydrolysate media with the sharply defined hemolytic reactions, more typical colonial morphology and improved pigment production achieved with media containing infusion peptone.

Sheep blood allows detection of hemolytic reactions and supplies the X factor (heme) necessary for the growth of many bacterial species but lacks V factor (nicotinamide adenine dinucleotide), since it 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. Fildes found that supplementing nutrient agar with a digest of sheep blood supplied both of these factors and the medium would support the growth of *H. influenzae*.[2,3] The inclusion of bacitracin makes the enriched Columbia Agar medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from the upper respiratory tract.[4]

Columbia Agar with 5% sheep blood is a general all-purpose enriched primary isolation medium that allows growth of all clinically significant anaerobes and facultative anaerobes.[5,6] Columbia Agar supplemented with 5% sheep blood is recommended when processing clinical specimens for unusual organisms, such as *Bartonella bacilliformis*, the causative agent of Oroya fever and Peruvian wart. Columbia Agar supplemented with 5% sheep blood and 20 µg of ampicillin per mL is used in isolating *Aeromonas* sp. from stool samples of patients showing clinical symptoms of gastroenteritis.[7]

PRINCIPLE

Columbia Agar Base supplemented with sheep, rabbit or horse blood derives its superior growth-supporting properties from the combination of peptones prepared from pancreatic digest of casein, meat peptic digest and heart pancreatic digest. Yeast extract and corn starch are also included in the formulation and serve as energy sources with yeast extract being a supplier of the B-complex vitamins. Sodium chloride maintains osmotic balance in the medium.

It should be noted that Columbia Sheep 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.

REAGENTS (FORMULA)

Pancreatic Digest of Casein	10.0	g
Proteose Peptone	5.0	g
Yeast Extract ….....	5.0	g
Beef Heart, Infusion	3.0	g
Corn Starch	1.0	g
Sodium Chloride	5.0	g
Agar	15.0	g
Deionized Water	1000.0	ml

PROCEDURE

Refer to appropriate standard references for details on test methods to obtain isolated colonies from specimens or samples using Columbia Agar.[5] Incubate the plates at 35 ± 2°C for 18-72 hours under appropriate atmospheric conditions, or as instructed in the standard reference.[5]

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO_2.

EXPECTED RESULTS

After the recommended incubation period, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a “dilution” technique, diminishing numbers of microorganisms 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.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Escherichia coli ATCC 25922	35 ± 2°C for 18-24 hours	Growth, Beta hemolysis
Neisseria meningitidis ATCC 13090	35 ± 2°C for 18-24 hours	Growth, Gamma hemolysis
Streptococcus pneumoniae ATCC 6305	35 ± 2°C for 18-24 hours	Growth, Alpha hemolysis

BIBLIOGRAPHY

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