

PRODUCT INFORMATION AND QUALITY CONTROL SHEET

COLUMBIA CNA AGAR WITH 5% SHEEP BLOOD

I. INTENDED USE

Columbia CNA Agar supplemented with sheep blood is a selective and differential medium for the isolation and differentiation of gram positive organisms.

II. SUMMARY AND EXPLANATION

Ellner et al., described a modification of Columbia Agar, an enriched general support growth medium, which consisted of the addition of colistin and nalidixic acid and supplementation with 5% sheep blood. This medium was found to support the growth of staphylococci, hemolytic streptococci, and enterococci while inhibiting the growth of *Proteus*, *Pseudomonas*, and *Klebsiella* species.

III. PRINCIPLES OF THE PROCEDURE

Growth support characteristics of Columbia Agar are derived from the presence of peptones prepared from pancreatic digest of casein, peptic digest of animal tissue and beef extract. Yeast extract and corn starch are included as energy sources. Sheep blood provides both X factor (hemin) and a visualization of hemolytic reactions. Colistin and nalidixic acid, antimicrobial agents, create a selective environment for gram positive organisms by either disrupting the cell membrane or blocking DNA replication of susceptible gram negative organisms.

IV. TYPICAL FORMULA AND APPEARANCE

Appearance = opaque, cherry red
(Approximate formula* per liter of processed water)

Pancreatic Digest of Casein	12.0g
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	10.0mg
Nalidixic Acid	10.0
Defibrinated Sheep Blood	5%

*adjusted and/or supplemented to meet performance criteria.

V. PRECAUTIONS

This product is for IN VITRO diagnostic use only. Culture specimens may contain microorganisms which can be potentially infectious to the user. Strict adherence to aseptic techniques and established precautions against microbiological hazards should be followed throughout the procedure. Carefully dispose of all items which contact patient specimens or isolated bacteria. (See Material Safety Data Sheet for further information.)

VI. STORAGE/SHELF LIFE

Plated media should be stored at 2-8°C (36-46°F), media side up, in the unopened or resealed package protected from light. DO NOT FREEZE OR EXPOSE TO HIGH TEMPERATURES. Allow unopened plates to warm to room temperature prior to inoculation. Prior to and during inoculation procedures, plates should be handled in a manner that minimizes product exposure to the environment. Product which has exceeded the assigned expiration date noted on the label should not be used.

Do not use plates that exhibit evidence of drying, cracking, discoloration, microbial contamination or any other signs of deterioration. The presence of excessive condensate may indicate plates which have been damaged by exposure to temperature extremes.

VII. SPECIMEN COLLECTION

The quality of culture results depends primarily on the adequacy and condition of the specimen submitted for examination.

Proper specimen collection techniques must be followed to ensure the most accurate culture results. Sterile swabs and collection containers should be used. Plates should be inoculated promptly after specimen collection. If a delay in inoculation is unavoidable, transport medium should be employed. Specimens should be collected prior to the initiation of antimicrobial therapy.

Detailed information on proper specimen collection may be obtained from microbiology reference materials.

VIII. MATERIALS PROVIDED

Columbia CNA Agar Plates with 5% Sheep Blood and lot specific Quality Control Certificate.

IX. MATERIALS REQUIRED BUT NOT PROVIDED

Incubator maintaining 33-37°C.
Ancillary culture media, reagents and laboratory equipment as required.

X. PROCEDURE

Inoculate the specimen as soon as possible after it is received in the laboratory. The streak plate method is used primarily to isolate pure cultures from specimens containing mixed flora. If material is being cultured directly from a swab, roll the swab over a small area of the plate surface at the edge (approximately 1/4 to 1/3 of the plate); then streak in a zig-zag fashion with a sterile loop from this inoculated area in two sections to cover the entire agar surface; flaming or flipping loop between sections. Avoid applying excessive pressure to the agar surface during inoculation to prevent gouging and splitting of the agar media. (Note: Agar surfaces should be smooth and moist but free of excessive moisture which could cause confluent growth patterns.)

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere of approximately 3-10% CO₂ (atmospheric incubator or zip-lock bag with generator). Incubate plates media side up at 33-37°C for 24-48 hours.

XI. EXPECTED RESULTS

NCCLS CONTROL ORGANISMS (ATCC STRAINS)

* <i>Streptococcus pyogenes</i> (ATCC 19615)	Growth, beta hemolysis
* <i>Streptococcus pneumoniae</i> (ATCC 6305)	Growth, alpha hemolysis
* <i>Staphylococcus aureus</i> (ATCC 25923)	Growth
* <i>Proteus mirabilis</i> (ATCC 12453)	Inhibition (partial)

XII. LABORATORY RESULTS

This medium is intended to be used as a primary isolation medium. Presumptive identification of organisms may be made on the basis of typical organism morphology, hemolytic reactions and Gram stain. Definitive identification of organisms and antimicrobial sensitivity determination requires further testing. Additional biochemical information may be obtained from reference microbiology texts.^{1,2,3}

XIII. LIMITATIONS

The ability to detect microorganisms by culture techniques can be affected by the following factors: improper specimen collection, storage and inoculation, initiation of anti-infective therapy prior to specimen collection, improper culture incubation

temperatures and atmospheres, improper length of culture incubation, and improper storage and handling of culture media.

XIV. REFERENCES

1. Finegold, S.M. and W.S. Martin. 1982. Bailey and Scott's Diagnostic Microbiology, 6th ed. C.V. Mosby Company, St. Louis.
2. Koneman, E.S., S.D.Allen, V.R. Dowell, Jr. and H. M. Sommers. 1983. Color Atlas and Textbook of Microbiology, 2nd ed. J.B. Lippincot Company, Philadelphia.
3. Lennette, E.H., ed. 1985. Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, D.C.

USER QUALITY ASSURANCE/ QUALITY CONTROL PROCEDURES AND INFORMATION

HealthLink recommends that the following quality assurance and quality control procedures be performed on each batch of product.

I. QUALITY ASSURANCE

The following quality assurance procedures must be performed to assure the product will perform according to its intended use within the assigned expiry date:

1. Daily, document that product storage refrigerator maintains temperature within the recommended range: 2-8°C.
2. Daily, document that laboratory incubator maintains temperature within the recommended range: 22-35°C.

II. QUALITY CONTROL

The following incoming inspection procedures must be performed for each batch (batch = same lot, same shipment) of culture media received in the laboratory :

1. Inspect plates according to instructions contained on the "Quality Control Log Sheet." (See also Section VI "STORAGE/SHELF LIFE")
2. Peel off the lower portion of a product bag label (Quality Control Certificate) for the lot being accepted into the laboratory and affix it to the Quality Control Log Sheet.
3. Initial and date the Quality Control Log Sheet.

Note: Notify Technical Service immediately if media does not meet the inspection criteria.

TECHNICAL SERVICE

HealthLink provides a toll free technical service line (1-800-638-2625) to assist with product usage. To receive additional product information, procedural instructions, QA/QC log sheets, Material Safety Data Sheets, or to have technical questions answered; please call between the hours of 9:00 am to 5:00 pm EST.

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