HealthLink
800-638-2625

Product No. 1220
Revision No. 01

PRODUCT INFORMATION AND QUALITY CONTROL SHEET
CNA 5% SHEEP BLOOD / MacCONKEY AGAR BIPLATE

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 .

MacConkey Agar is a selective and differential medium for the isolation and differentiation of gram negative enteric bacilli.

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.

Since this bile salt, neutral red, and lactose agar was described by MacConkey in 1905, its formulation has been modified many times. The current formulation was designed to exhibit better differentiation of lactose fermenting versus non lactose fermenting organisms, enhance the growth of enteric pathogens, and improve the inhibition of swarming Proteus 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.

The selective property of the MacConkey medium is due to the addition of bile salts and crystal violet which inhibit the growth of gram positive organisms. Differentiation of lactose fermenting versus non lactose fermenting is achieved with the indicator neutral red. Colonies will appear either colorless or pink to red based on the organisms ability to ferment this carbohydrate.

IV. TYPICAL FORMULA AND APPEARANCE
(Approximate formula* per liter of processed water)

CNA 5% AGAR

Appearance = opaque, cherry red	
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%

MacConkey AGAR

Appearance =pinkish-purple, slightly opalescent	
Pancreatic Digest of Gelatin	17.0g
Pancreatic Digest of Casein	1.5
Peptic Digest of Animal Tissue	1.5
Lactose	10.0
Bile Salts	1.5
Sodium Chloride	5.0
Neutral Red	0.03
Crystal Violet	0.001
Agar	13.5

XI. EXPECTED RESULTS
*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.

VI. STORAGE/SHELF LIFE
Plated media should be stored at 2-8^{0}C (36-46^{0}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
CNA 5% SB / MacConkey Agar Plates.

IX. MATERIALS REQUIRED BUT NOT PROVIDED
Incubator maintaining 33-37^{0}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 to cover the entire agar surface. 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.)

A non-selective medium, such as Tryptic Soy Agar with 5% sheep blood, should also be considered for inoculation to increase the chance of recovering gram negative bacteria present in low numbers and to reflect other bacterial flora present in the specimen.

NCCLS Control Organisms (ATCC Strains)

CNA 5% AGAR	
Streptococcus pyogenes (ATCC 19615)	Growth, beta hemolysis
Streptococcus pneumoniae (ATCC 6305)	Growth, alpha hemolysis
Staphylococcus aureus (ATCC 25923)	Growth
Proteus mirabilis (ATCC 12453)	Inhibition (partial)
MacConkey AGAR	
Escherichia coli (ATCC 25922)	Growth, pink colonies
Proteus mirabilis (ATCC 12453)	Growth, colorless colonies, inhibition of swarming
Salmonella typhimurium (ATCC 14028)	Growth, colorless colonies
Enterococcus faecalis (ATCC 29212)	Inhibition (partial)

XII. LABORATORY RESULTS
These medium are 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.

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 antiinfective therapy prior to specimen collection, improper culture incubation temperatures and atmospheres, improper length of culture incubation, and improper storage and handling of culture media.

Definitive identification of organisms and antimicrobial sensitivity determination requires additional testing which may include: Gram stain, oxidase, catalase, and other biochemical tests. Additional biochemical information may be obtained from reference microbiology texts.[1,2,3]

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^{0}C.
2. Daily, document that laboratory incubator maintains temperature within the recommended range: 33-37^{0}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 QA/QC log sheets or have technical questions answered, please call between the hours of 9:00 am to 5:00 pm EST.

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