HealthLink
800-638-2625

Product No. 1221
Revision No. New

# PRODUCT INFORMATION AND QUALITY CONTROL SHEET

# CNA 5% SHEEP BLOOD / EMB 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 .

Levine EMB 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.

Holt-Harris and Teague devised eosine methylene blue agar which, through the use of eosin and methylene blue as indicators, provided good differentiation of lactose fermenting versus non-lactose fermenting microorganisms. Levine modified this original formula by eliminating sucrose and increasing the concentration of agar which provided better differentiation between *Escherichia* and *Enterobacter* species, and reduced the effect 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 dyes eosin Y and methylene blue act as selective agents by being slightly inhibitory to gram positive bacteria. Further, they enable differentiation between lactose fermenting and non lactose fermenting microorganisms based on the presence or absence of dye uptake in the bacterial colonies. Lactose fermenting coliforms are seen as blue-black colonies and non-lactose fermenting colonies are colorless, transparent, or amber in color. Some gram positive organisms (fecal streptococci, staphylococci and yeasts) will produce pinpoint colonies on this agar.

## 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% |

LEVINE EMB AGAR

Appearance = wine red with greenish cast, slightly opaque-cent with fine precipitate

| | |
|---|---|
| Pancreatic Digest of Gelatin | 10.0g |
| Lactose | 10.0 |
| Dipotassium Phosphate | 2.0 |
| Eosine Y | 0.4 |
| Methylene Blue | 0.065 |
| Agar | 15.0 |

*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°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

CNA 5% SB / EMB Agar Plates.

## 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 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.

**XI. EXPECTED RESULTS**

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) |
| LEVINE EMB AGAR | |
| *Escherichia coli* (ATCC 25922) | Growth, blue-black colonies with green metallic sheen |
| *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°C.

2. Daily, document that laboratory incubator maintains temperature within the recommended range: 33-37°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.

**HealthLink**
**3611 St. Johns Bluff Road So. Ste. 1**
**Jacksonville, FL 32246**

**1-800-638-2625**

**September, 1998**

Product No. 1221 Rev. No. New