PRODUCT INFORMATION AND QUALITY CONTROL SHEET
CHOCOLATE AGAR

INTENDED USE
Chocolate Agar is an enriched medium for the isolation and cultivation of fastidious microorganisms, especially *Neisseria* and *Haemophilus* species from a variety of clinical specimens.

SUMMARY AND EXPLANATION
Bacto GC Medium Base was designed in 1947. Christensen and Schoenlein demonstrated that this base, enriched with hemoglobin and supplements yielded accelerated early growth of *Neisseria gonorrhoeae.*[1] This medium was improved upon by substituting a chemical supplement for the yeast concentrate.[2] In addition to aiding the growth of gonococci, other fastidious organisms, e.g. *Haemophilus* species was also improved.

PRINCIPLES OF THE PROCEDURE
Chocolate agar contains a GC agar base, bovine hemoglobin, and a chemically defined enrichment. Casein and meat peptones contained in the GC agar base provide the nitrogenous elements, phosphate buffers maintain the pH and corn starch neutralizes any toxic fatty acids that may be present in the agar. Hemin (X factor) is provided by the bovine hemoglobin, necessary for growth of *Haemophilus* species. The chemically defined enrichment solution provides nicotinamide adenine dinucleotide/NAD (V factor), vitamins, amino acids, co-enzymes, dextrose, ferric ions and other factors for improved growth of *Neisseria* species.

TYPICAL FORMULA AND APPEARANCE
Appearance = opaque, chocolate brown
(Approximate formula* per liter of processed water)

Pancreatic Digest of Casein	7.5g
Selected Meat Peptone	7.5
Corn Starch	1.0
Dipotassium phosphate	4.0
Monopotassium phosphate	1.0
Sodium Chloride	5.0
Agar	12.0
Hemoglobin	10.0
Chocolate enrichment solution	10 ml

*adjusted and/or supplemented to meet performance criteria.

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.

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.

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.[3,4,5]

MATERIALS PROVIDED
Chocolate Agar Plates – 10 each

MATERIALS REQUIRED BUT NOT PROVIDED
Incubator maintaining 35 - 37^{0}C.
Ancillary culture media, reagents and laboratory equipment as required.

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 fastidious microorganisms require carbon dioxide on primary isolation, plates should be incubated in an atmosphere of approximately 3-10% CO_2 (atmospheric incubator or zip-lock bag with generator). Incubate plates media side up at 35-37^{0}C for 18-24 hours.

EXPECTED RESULTS

NCCLS CONTROL ORGANISMS (ATCC STRAINS)

Neisseria gonorrhoeae Growth
(ATCC 43069)

Haemophilus influenzae Growth
(ATCC 10211)

Streptococcus pneumoniae Growth
(ATCC 6305)

Neisseria meningitidis Growth
(ATCC 13090)

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 and Gram stain. Definitive identification of certain organisms and antimicrobial sensitivity determination requires further testing with supplementary materials. Additional biochemical information may be obtained from reference microbiology texts.[3,4,5]

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

Chocolate agar is an enriched medium. Pathogenic organisms may be overgrown by nonpathogenic or normal specimen flora.

REFERENCES

1. Difco Manual, *Dehydrated Culture Media and Reagents for Microbiology,* 10th ed. 1984. Difco Laboratories, Detroit, MI.

2. Power, D.A. (ed.) and P.J. McCuen. 1988. Manual of **BBL** products and laboratory procedures, 6th ed. Becton Dickinson Microbiology Systems, Cockeysville, MD.

3. Finegold, S.M. and W.S. Martin. 1982. Bailey and Scott's Diagnostic Microbiology, 6th ed. C.V. Mosby Company, St, Louis.

4. 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.

5. 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: 35-37^{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 in "STORAGE/SHELF LIFE" section.
2. Peel off the lower portion of a product bag label 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 have technical questions answered; please call between the hours of 9:00 am to 5:00 pm EST.

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