

PRODUCT INFORMATION AND QUALITY CONTROL SHEET

CHOCOLATE AGAR SLANT

I. 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.

II. 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.

III. 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.

IV. TYPICAL FORMULA AND APPEARANCE

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

Proteose peptone	15.0g
Corn Starch	1.0
Dipotassium phosphate	4.0
Monopotassium phosphate	1.0
Sodium Chloride	5.0
Agar	13.0
Hemoglobin	10.0
Chocolate enrichment solution	10 ml
Final pH	7.2 ± 0.2 @ 25°C

*adjusted and/or supplemented to meet performance criteria.

V. PRECAUTIONS

This product is for *IN VITRO* diagnostic use only. Culture specimens may contain microorganisms that 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 that contact patient specimens or isolated bacteria.

VI. STORAGE/SHELF LIFE

Media should be stored at 2-8°C (36-46°F). DO NOT FREEZE OR EXPOSE TO HIGH TEMPERATURES. Allow unopened tubes to warm to room temperature prior to inoculation. Prior to and during inoculation procedures, tubes 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 tubes that exhibit evidence of drying, cracking, discoloration, microbial contamination or any other signs of deterioration.

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. Tubes 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}

VIII. MATERIALS PROVIDED

Chocolate Agar slants (10/box)

IX. MATERIALS REQUIRED BUT NOT PROVIDED

Incubator maintaining 35 - 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. Chocolate Agar slants may be used for the cultivation and maintenance of pure cultures. Inoculate the slants with a swab or a loopful of culture.

Since many fastidious microorganisms require carbon dioxide on primary isolation, tubes should be incubated in an atmosphere of approximately 3-10% CO₂ (atmospheric incubator or zip-lock bag with generator).

XI. EXPECTED RESULTS

NCCLS CONTROL ORGANISMS (ATCC STRAINS)

<i>Neisseria gonorrhoeae</i>	Growth (small, grayish-white to
(ATCC 43069)	colorless mucoid colonies)
<i>Haemophilus influenzae</i>	Growth (moist, smooth gray
(ATCC 10211)	colonies)

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 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}

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.

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

XIV. 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 that 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: 2-8⁰C.
2. Daily, document that laboratory incubator maintains temperature within the recommended range: 22-35⁰C.

II. USER QUALITY CONTROL

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

1. Inoculate two tubes with the following NCCLS Quality Control Organisms and compare growth to below:
Neisseria gonorrhoeae Growth (small, grayish-white to
(ATCC 43069) colorless mucoid colonies
Haemophilus influenzae Growth (moist, smooth gray
(ATCC 10211) colonies)
2. Inspect tubes for evidence of drying, cracking, discoloration, microbial contamination or any other signs of deterioration.

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.

HealthLink
3611 St. Johns Bluff Rd. So. Ste. 1
Jacksonville, FL 32224

1-800-638-2625

January, 2004

Product No. 1715 Rev. No. New