

PRODUCT INFORMATION AND QUALITY CONTROL SHEET

TSA 5% SHEEP BLOOD / CHOCOLATE AGAR BIPLATE

I. INTENDED USE

Trypticase Soy Agar (TSA) supplemented with sheep blood is an enriched general purpose medium for the cultivation of microorganisms and the visualization of hemolytic reactions.

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

The nutritional composition of Trypticase Soy Agar has made it a popular medium, both un-supplemented and as a base for media containing blood. Trypticase Soy Agar with 5% or 10% sheep blood is used extensively for the cultivation of fastidious microbial species and for the determination of hemolytic reactions which are important differentiation characteristics for bacteria, especially *Streptococcus* species.

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

The presence of casein and soy peptones in the Trypticase Soy Agar base makes this medium highly nutritious by supplying organic nitrogen, particularly amino acids and larger chained peptides. The sodium chloride maintains osmotic equilibrium.

Defibrinated sheep blood, added to enrich the base medium, provides excellent growth and beta hemolysis of *Streptococcus pyogenes* in addition to appropriate hemolytic reactions of other organisms. It is a suitable medium for use with low concentration bacitracin discs (0.04 unit) for presumptive identification of group A Streptococci, and for performing the CAMP test for presumptive identification of group B Streptococci (*S. agalactiae*).

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

(Approximate formula* per liter of processed water)

TSA 5% AGAR

Appearance = opaque, cherry red	
Pancreatic Digest of Casein	14.5g
Papaic Digest of Soybean Meal	5.0
Sodium Chloride	5.0
Agar	14.0
Defibrinated Sheep Blood	5%

CHOCOLATE AGAR

Appearance = opaque, chocolate brown	
Pancreatic Digest of Casein	7.5g
Selected Meat Peptone	5.0
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.

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

TSA 5% / Chocolate Agar Plates – 10 each per sleeve

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.)

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 18-24 hours.

XI. EXPECTED RESULTS

NCCLS Control Organisms (ATCC Strains)

TSA 5% AGAR

<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>Escherichia coli</i> (ATCC 25922)	Growth

CHOCOLATE AGAR

<i>Neisseria gonorrhoeae</i> (ATCC 43069)	Growth
<i>Haemophilus influenzae</i> (ATCC 10211)	Growth
<i>Streptococcus pneumoniae</i> (ATCC 6305)	Growth
<i>Neisseria meningitidis</i> (ATCC 13090)	Growth

XII. LABORATORY RESULTS

These media 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 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.

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.^{3,4,5}

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. Lippincott 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°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:

Inspect plates according to instructions contained on 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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January, 1999

Product No. 1267 Rev. No. 01