

QUALITY CONTROL PROCEDURES

I INTRODUCTION

Blood Agar is an enriched medium for the isolation and growth of fastidious microorganisms and for the determination of hemolytic reactions.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Using a 0.01 mL calibrated loop, streak-inoculate the slant surfaces using 10⁻¹ dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures.
 - b. Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.
2. Examine tubes after 24 h for growth and hemolysis.
3. Expected Results

CLSI Organisms	ATCC™	Recovery
* <i>Streptococcus pyogenes</i>	19615	Growth, beta hemolysis
* <i>Streptococcus pneumoniae</i>	6305	Growth, alpha hemolysis
* <i>Staphylococcus aureus</i>	25923	Growth
* <i>Escherichia coli</i>	25922	Growth

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes at 20 – 25°C and 30 – 35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Blood Agar (**Trypticase** Soy Agar with 10% Sheep Blood) in slant form is used for cultivating and maintaining fastidious microorganisms.

V SUMMARY AND EXPLANATION

The nutritional composition of **Trypticase** Soy Agar has made it a popular medium, both unsupplemented and as a base for media containing blood. **Trypticase** Soy Agar with 10% Sheep Blood is used for the recovery and cultivation of fastidious microbial species and for the determination of hemolytic reactions which are important differentiating characteristics for bacteria, especially *Streptococcus* species.

VI PRINCIPLES OF THE PROCEDURE

The combination of casein and soy peptones in the **Trypticase** Soy Agar base renders the Blood Agar medium highly nutritious by supplying organic nitrogen, particularly amino acids and larger-chained peptides. The sodium chloride maintains osmotic equilibrium. Defibrinated sheep blood is the most widely used blood for enriching agar base media.¹ Hemolytic reactions of streptococci are proper and growth of *Haemophilus hemolyticus*, a nonpathogen whose hemolytic colonies are indistinguishable from those of beta-hemolytic streptococci, is inhibited.

VII REAGENTS

Blood Agar Slants

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	14.5 g	Agar	14.0 g
Papaic Digest of Soybean Meal	5.0 g	Growth Factors.....	1.5 g
Sodium Chloride	5.0 g	Sheep Blood (Defibrinated)	10%

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2 – 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{2,3} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Blood Agar Slants

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Streak the agar slant surface with a pure culture. Incubate the tubes at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere with or without supplementation with carbon dioxide.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Microbial growth obtained after sufficient incubation can be used as an inoculum for growth or biochemical studies. The slant cultures also can be used as stock cultures for organism maintenance purposes.

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.²⁻⁴

XII PERFORMANCE CHARACTERISTICS


Prior to release, all lots of Blood Agar slants are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are inoculated with **Trypticase** Soy Broth cultures diluted to 10^{-1} of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumoniae* (ATCC 6305) and *Streptococcus pyogenes* (ATCC 19615). Inoculated tubes with loosened caps are incubated at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere supplemented with carbon dioxide. Tubes are read for growth and hemolysis after 18 – 24 h incubation. Growth of all organisms is moderate to heavy. *S. pneumoniae* exhibits alpha hemolysis while *S. aureus* and *S. pyogenes* produce beta hemolysis.


XIII AVAILABILITY

Cat. No.	Description
220830	BBL™ Blood Agar Slants, Pkg. of 10 size K tubes
220831	BBL™ Blood Agar Slants, Ctn. of 100 size K tubes

XIV REFERENCES

1. Vera, H.D., and D.A. Power. 1980. Culture media, p. 969. In E.H. Lennette, A. Balows, W.J. Hausler, Jr., and J.P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
2. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
3. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
4. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

 Becton, Dickinson and Company
7 Loveton Circle
Sparks, MD 21152 USA
800-638-8663
www.bd.com/ds

 Benex Limited
Rineanna House
Shannon Free Zone
Shannon, County Clare, Ireland

ATCC is a trademark of the American Type Culture Collection.
BD, BD Logo, BBL and Trypticase are trademarks of Becton, Dickinson and Company. ©2011 BD.