BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) and BBL™ MacConkey II Agar with MUG–I Plate™

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R_x Only

QUALITY CONTROL PROCEDURES

I INTRODUCTION

BD BBL™ Trypticase™ Soy Agar with 5% Sheep Blood is used for the growth of fastidious organisms and for the visualization of hemolytic reactions. **BD BBL™** MacConkey II Agar with MUG is used for the presumptive identification of *Escherichia coli*.

II PERFORMANCE TEST PROCEDURE

A. BD BBL Trypticase Soy Agar with 5% Sheep Blood

1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Using a volumetric pipettor or equivalent method, deliver 0.1 mL of a dilution yielding 30–300 CFU to each plate and spread-inoculate using a sterile glass spreader.
   b. Incubate the *Staphylococcus* and *Escherichia* strains at 35 ± 2 °C in an aerobic atmosphere and the *Streptococcus* strains at 35 ± 2 °C in an aerobic atmosphere supplemented with carbon dioxide.
2. Examine plates after 18–24 h for growth, colony size and hemolytic reactions.
3. Expected Results

CLSI Organisms	ATCC®	Recovery
**Streptococcus pyogenes*	19615	Growth, beta hemolysis
**Streptococcus pneumoniae*	6305	Growth, alpha hemolysis
**Staphylococcus aureus*	25923	Growth
**Escherichia coli*	25922	Growth

*Recommended organism strain for User Quality Control.

B. BD BBL MacConkey II Agar with MUG

1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Streak the plates for isolation using 18- to 24-h broth cultures diluted 10^{-1}. For *Proteus mirabilis*, make two additional ten-fold dilutions prior to streaking.
   b. Incubate the plates at 35 ± 2 °C in an aerobic atmosphere.
   c. Include **BD BBL Trypticase** Soy Agar with 5% Sheep Blood plates as nonselective controls for all organisms.
2. Examine plates after 18–48 h for growth, fluorescence, pigmentation and selectivity.
3. Expected Results

Organisms	ATCC	Recovery	Colony Color	Fluorescence
**Escherichia coli*	25922	Growth	Rose-red	+
Proteus mirabilis	12453	Growth	Colorless, inhibition of swarming	–
**Salmonella enterica* subsp. *enterica* serotype Typhimurium	14028	Growth	Colorless	–
**Enterococcus faecalis*	29212	Inhibition (partial to complete)	N/A	–

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under “Product Deterioration.”
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.4 ± 0.2 (TSA II) and 7.1 ± 0.2 (**BD BBL** MacConkey II Agar with MUG).
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BD BBL Trypticase Soy Agar with 5% Sheep Blood is used for cultivating fastidious microorganisms and for the visualization of hemolytic reactions produced by many bacterial species.

BD BBL MacConkey II Agar with MUG is used for the presumptive identification of *Escherichia coli*.

V SUMMARY AND EXPLANATION

A. BD BBL Trypticase Soy Agar with 5% Sheep Blood

The nutritional composition of **BD Trypticase** Soy Agar has made it a popular medium, both unsupplemented and as a base for media containing blood. **BD BBL Trypticase** Soy Agar with 5% Sheep Blood is extensively 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.

B. BD BBL MacConkey II Agar with MUG

The **BD BBL** MacConkey II Agar formulation was made available in 1983. It was specially designed to improve the inhibition of swarming *Proteus* species, to achieve more definitive differentiation of lactose fermenters and nonfermenters, and for the promotion of superior growth of enteric pathogens.

Trepeta and Edberg[1] modified MacConkey Agar by the incorporation of MUG (4-methylumbelliferyl β-D-glucuronide). The resulting medium allowed the authors to presumptively identify *E. coli* from the primary plating medium within 5 min.

VI PRINCIPLES OF THE PROCEDURE

A. BD BBL Trypticase Soy Agar with 5% Sheep Blood

The combination of casein and soy peptones in the **BD Trypticase** Soy Agar base render the 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.[2] 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.

BD BBL Trypticase Soy Agar with 5% Sheep Blood (TSA II) provides excellent growth and beta hemolysis by *Streptococcus pyogenes* (Lancefield group A) and also provides excellent growth and appropriate hemolytic reactions with other fastidious organisms. It is suitable for use with low concentration (0.04 unit) bacitracin discs (**BD Taxo™** A) for presumptive identification of group A streptococci (*S. pyogenes*).

B. BD BBL MacConkey II Agar with MUG

BD BBL MacConkey II Agar is a selective and differential medium. It is only slightly selective since the concentration of bile salts, which inhibits gram-positive microorganisms, is low in comparison with other enteric plating media. Crystal violet also is included in the medium to inhibit the growth of gram-positive bacteria, especially enterococci and staphylococci.

Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.

Most strains (96 to 97%) of *E. coli* produce an enzyme, β-D-glucuronidase.[3] The enzyme hydrolyzes MUG to yield 4-methylumbelliferone, a compound which fluoresces under long-wave (366 nm) UV light. The addition of MUG to the formulation allows β-D-glucuronidase-positive strains of *E. coli* to fluoresce blue-green when examined under UV light.

VII REAGENTS

BD BBL Trypticase Soy Agar with 5% Sheep Blood (TSA II)

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	Defibrinated Sheep Blood	5%

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

BD BBL MacConkey II Agar with MUG

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	17.0 g	Sodium Chloride	5.0 g
Pancreatic Digest of Casein	1.5 g	Neutral Red	0.03 g
Peptic Digest of Animal Tissue	1.5 g	Crystal Violet	0.001 g
Lactose	10.0 g	MUG (4-methylumbelliferyl-β-D-glucuronide)	0.1 g
Bile Salts	1.5 g	Agar	13.5 g

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

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

If excessive moisture is observed, invert bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[4–7] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation time. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates 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.[8,9] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: BD BBL Trypticase Soy Agar with 5% Sheep Blood (TSA II) and **BD BBL** MacConkey II Agar with MUG (**BD I Plate**)

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

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate plates, protected from light, at 35 ± 2 °C for 18–24 h. With respiratory specimens, incubate in an aerobic atmosphere supplemented with carbon dioxide. With other specimens, incubate aerobically *without* added CO_2.

User Quality Control:

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 guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

Typical results on **BD BBL Trypticase** Soy Agar with 5% Sheep Blood are as follows:

1. Hemolytic streptococci may appear as translucent or opaque, grayish, small (1 mm), or large, matt and mucoid (2–4 mm) colonies, encircled by a zone of hemolysis. Gram stains should be made and examined to check the macroscopic findings. (Other organisms which may cause hemolysis include *Listeria*, various corynebacteria, hemolytic staphylococci, *Escherichia coli* and *Pseudomonas*.)

   In reporting, approximate quantitation of the number of colonies of hemolytic streptococci may be helpful to the clinician.
2. Pneumococci usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of "green" (alpha) hemolysis.
3. Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.
4. *Listeria*. Small zones of beta hemolysis are produced. They may be distinguished by their rod shape in stains, and by motility at room temperature.
5. Other organisms representing minimal flora and clinically significant isolates can also be expected to grow on this nonselective formulation.

Typical colonial morphology on **BD BBL** MacConkey II Agar with MUG is as follows:

Colonies of lactose-fermenting bacteria appear pink to rose-red in color and may be surrounded by a zone of bile precipitation while lactose-nonfermenting colonies are colorless. Examine the medium under long-wave UV light (366 nm). β-D-glucuronidase positive colonies have a blue-green fluorescence; β-D-glucuronidase negative colonies do not fluoresce.

XI LIMITATIONS OF THE PROCEDURE

It has been reported that some *Enterobacteriaceae* and *Pseudomonas aeruginosa* are inhibited on **BD BBL** MacConkey Agar when incubated in a CO_2-enriched atmosphere.[10]

Not all strains of *E. coli* ferment lactose or produce β-D-glucuronidase. Some strains of *Salmonella* and *Shigella* produce β-D-glucuronidase and will fluoresce.[11] A small percentage of *Yersinia* and streptococci have been reported to fluoresce.[12]

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.[8,9,13]

XII PERFORMANCE CHARACTERISTICS

BD BBL Trypticase Soy Agar with 5% Sheep Blood

BD BBL Trypticase Soy Agar (TSA) with 5% Sheep Blood was used as a control in a study using broth enhanced culture (Todd Hewitt) and Optical Immunoassay method for the diagnosis of β-hemolytic streptococcal infection. Five hundred two (502) specimens were tested. TSA with 5% Sheep Blood had a sensitivity and specificity of 92.5% and 99.4%, respectively.[14] Nguyen et al. used **BD BBL Trypticase** Soy Agar with 5% Sheep Blood as the 'gold standard' for the detection of group B *Streptococcus* from the lower genital tract of pregnant women.[15] In another study, Rossmann et al. successfully reisolated *Lautropia mirabilis* on **BD BBL Trypticase** Soy Agar with 5% Sheep Blood from the oral cavities of human immunodeficiency virus infected children.[16] Of the 85 children evaluated in this study, 35 (41.4%) were positive for *L. mirabilis*. Isenberg et al. used **BD BBL Trypticase** Soy Agar with 5% Sheep Blood as a control to evaluate the recovery of *Enterococcus* from a selective medium under study.[17] Two hundred fifty (250) group D streptococcal strains isolated from clinical material and 8 strains obtained from the National Communicable Disease Center (Atlanta, Ga.) were used.

BD BBL MacConkey II Agar with MUG

In a clinical study performed at a hospital and university school of medicine, MUG was incorporated into **BD BBL** MacConkey II Agar to detect the presence of β-glucuronidase. It was found that the time to identify *E. coli* strains was reduced from one hour to five minutes and the ability to identify this organism in mixed specimens was enhanced.[1]

XIII AVAILABILITY

Cat. No.	Description
221949	**BD BBL™ Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) and **BD BBL™** MacConkey II Agar with MUG-**BD I Plate™**

XIV REFERENCES

1. Trepeta, R.W., and S.C. Edberg. 1984. Methylum belliferyl-β-D-glucuronide-based medium for rapid isolation and identification of *Escherichia coli*. J. Clin. Microbiol. *19*:172-174.
2. 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.
3. Killian, M., and P. Bulow. 1976. Rapid diagnosis of *Enterobacteriaceae*. I Detection of bacterial glycosidases. Acta Pathol. Microbiol. Scand. Sec. B, *84*:245-251.
4. National Committee for Clinical Laboratory Standards. 2001. Approved Guideline M29-A2. Protection of laboratory workers from occupationally acquired infections, 2nd ed. NCCLS, Wayne, Pa.
5. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. *17*:53-80.
6. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
7. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
8. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
9. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
10. Mazura-Reetz, G., T.R. Neblett, and J.M. Galperin. 1979. MacConkey agar: CO_2 vs. ambient incubation, abstr. C 179, p. 339. Abstr. 79th Annu. Meet. Am. Soc. Microbiol. 1979.
11. Feng, P.C.S., and P.A. Hartmen. 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. *43*:1320-1329.
12. Robison, B.J. 1984. Evaluation of a fluorogenic assay for detection of *Escherichia coli* in foods. Appl. Environ. Microbiol. *48*:285-288
13. 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.
14. Fries, S.M. 1995. Diagnosis of group A streptococcal pharyngitis in a private clinic: comparative evaluation of an optical immunoassay method and culture. J. Pediatr. *126*:933-936.
15. Nguyen, T.M., et al. 1998. Detection of group B streptococcus: comparison of an optical immunoassay with direct plating and broth-enhanced culture methods. J. Matern. Fetal. Med. *7*:172-176.
16. Rossmann, S.N. et.al. 1998. Isolation of *Lautropia mirabilis* from oral cavities of human immunodeficiency virus-infected children. J. Clin. Microbiol. *36*:1756-1760.
17. Isenberg, H.D., D. Goldberg, and J. Sampson, 1970. Laboratory studies with a selective medium. Appl. Microbiol. *20*: 433-436.

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