BD BBL™ Trypticase™ Soy Agar with 5% Sheep Blood// MacConkey II Agar

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QUALITY CONTROL PROCEDURES

I. INTRODUCTION

Trypticase Soy Agar with 5% Sheep Blood is used for the growth of fastidious organisms and for the visualization of hemolytic reactions. MacConkey II Agar is a selective and differential medium for the detection of coliform organisms and enteric pathogens.

II. PERFORMANCE TEST PROCEDURE

A. 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 spreadinoculate 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 3 - 5% 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. MacConkey II Agar

1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Streak the plates for isolation using cultures diluted to yield 10^3-10^5 CFU/plate.
   b. Incubate the plates at 35 ± 2°C in an aerobic atmosphere.
   c. Include **Trypticase** Soy Agar with 5% Sheep Blood plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for growth, pigmentation and selectivity.
3. Expected Results

CLSI Organisms	ATCC™	Recovery	Colony Color
**Escherichia coli*	25922	Growth	Pink
**Proteus mirabilis*	12453	Growth, inhibition of swarming (partial)	Colorless
**Salmonella enterica* subsp. *enterica* serotype Typhimurium	14028	Growth	Colorless
**Enterococcus faecalis*	29212	Inhibition (partial)	
Additional Organisms			
Pseudomonas aeruginosa	10145	Growth	Pink to green
Shigella dysenteriae	9361	Growth	Colorless to pink

*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 7.3 ± 0.2 (TSA 5 %SB) and 7.1 ± 0.2 (MacConkey II Agar).
4. Note the firmness of plates during the inoculation procedure.

5. Incubate uninoculated representative plates at 30 ± 1°C for 84 h and examine for microbial contamination.

PRODUCT INFORMATION

IV. INTENDED USE

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.

MacConkey II Agar is a selective and differential medium for the detection of coliform organisms and enteric pathogens.

V. SUMMARY AND EXPLANATION

A. Trypticase Soy Agar with 5% Sheep Blood

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 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. MacConkey II Agar

At the present time, many culture media are available to the laboratorian for the isolation, cultivation and identification of enteric bacteria. One of the earliest of these was developed by MacConkey and first described as a brief published note.[1] The landmark paper on MacConkey Agar was published in 1905 and contained detailed descriptions of the medium and the bacterial growth patterns obtained.[2] This formulation was devised in the knowledge that bile salts are precipitated by acids and certain enteric microorganisms ferment lactose whereas others do not possess this ability.

Since the publication of the early papers, the MacConkey Agar formula has been modified many times. A compilation of culture media published in 1930 lists ten modifications which were published up to that time.[3] More recent modifications include use of additives (e.g., kanamycin) and the deletion of certain ingredients (e.g., crystal violet, and neutral red).4

MacConkey Agar is recommended for use with clinical specimens likely to contain mixed microbial flora, such as urine, respiratory and wound, because it allows a preliminary grouping of enteric and other gram-negative bacteria.[5,6] It is also utilized in the microbiological examination of foods.[7]

The **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.

VI. PRINCIPLES OF THE PROCEDURE

A. Trypticase Soy Agar with 5% Sheep Blood

The combination of casein and soy peptones in the **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.[8] Hemolytic reactions of streptococci are proper and growth of *Haemophilus hemolyticus*, a nonpathogen whose hemolytic colonies are indistinguishable from those of betahemolytic streptococci, is inhibited.

Trypticase Soy Agar with 5% Sheep Blood 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 (**Taxo™** A) for presumptive identification of group A streptococci (*S. pyogenes*).

B. MacConkey II Agar

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.

VII. REAGENTS

Trypticase Soy Agar with 5% Sheep Blood

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	14.5 g
Papaic Digest of Soybean Meal	5.0 g
Sodium Chloride	5.0 g
Agar	14.0 g
Growth Factors	1.5 g
Defibrinated Sheep Blood	5%

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

MacConkey II Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	17.0 g
Pancreatic Digest of Casein	1.5 g
Peptic Digest of Animal Tissue	1.5 g
Lactose	10.0 g
Bile Salts	1.5 g
Sodium Chloride	5.0 g
Neutral Red	0.03 g
Crystal Violet	0.001 g
Agar	13.5 g

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

Warnings and Precautions:

For *in vitro* Diagnostic Use in Taiwan

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" [9-12] 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

A variety of swabs and containers have been devised for collecting specimens. Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory. Several holding media or transport systems, such as **BBL** specimen collection and transport products, have been devised to prolong the survival of microorganisms when a significant delay is expected between collection and definitive culturing.

Refer to appropriate texts for details of specimen collection and handling procedures.[13,14]

The laboratory must be furnished with sufficient clinical information to enable the microbiologist to select the most suitable media and appropriate techniques.

IX. PROCEDURE

Material Provided: Trypticase Soy Agar with 5% Sheep Blood and MacConkey II Agar (**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: 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

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 **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 MacConkey II Agar is as follows:

E. coli.....	Pink to rose-red (may be surrounded by a zone of precipitated bile)
Enterobacter/Klebsiella.....	Mucoid, pink
Proteus.....	Colorless, swarming in areas of isolated colonies is inhibited
Salmonella.....	Colorless
Shigella.....	Colorless
Pseudomonas.....	Irregular, colorless to pink
Gram-positive bacteria.....	No growth to slight growth

XI. LIMITATIONS OF THE PROCEDURE

It has been reported that some *Enterobacteriaceae* and *Pseudomonas aeruginosa* are inhibited on MacConkey Agar when incubated in a CO_2 -enriched atmosphere.[15]

Not all strains of *E. coli* ferment lactose.

Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and other identification procedures. Consult appropriate texts for detailed information and recommended procedures.[5,16-19]

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII. AVAILABILITY

Cat. No.	Description
251290	BD **BBL™ Trypticase™** Soy Agar with 5% Sheep Blood// MacConkey II Agar, Pkg. of 20 plates

XIII. REFERENCES

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XIV. FURTHER INFORMATION

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