



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Columbia CNA Agar with 5% Sheep Blood is a selective and differential medium for the isolation and differentiation of gram-positive microorganisms from clinical and nonclinical specimens. MacConkey II Agar is a selective and differential medium for the detection of coliform organisms and enteric pathogens.

II PERFORMANCE TEST PROCEDURE

A. Columbia CNA Agar with 5% Sheep Blood

1. Inoculate representative samples with the cultures listed below.
  - a. To each plate, add 0.1 mL of a dilution of an 18- to 24-h broth culture calculated to contain 10<sup>3</sup>–10<sup>4</sup> CFU/0.1 mL for the *Streptococcus* and *Staphylococcus* strains and 10<sup>4</sup>–10<sup>5</sup> CFU/0.1 mL for the *Proteus* strain. Spread-inoculate using a sterile glass spreader.
  - b. Incubate the plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.
  - c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 and 48 h for growth, colony size, hemolytic reaction, pigmentation, and selectivity.
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>Proteus mirabilis</i>	12453	Inhibition (partial)

\*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 an 18- to 24-h broth culture of *Enterococcus faecalis* diluted to yield 10<sup>4</sup>–10<sup>5</sup> CFU/plate. For the remaining organisms, use an 18- to 24-h broth culture diluted to yield 10<sup>3</sup>–10<sup>4</sup> CFU/plate.
  - b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
  - c. Include TSA II plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for growth, colony size, pigmentation and selectivity.
3. Expected Results

CLSI Organisms	ATCC	Recovery	Colony Color
* <i>Escherichia coli</i>	25922	Growth	Pink
* <i>Proteus mirabilis</i>	12453	Growth, inhibition of swarming (partial)	Colorless
* <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	Growth	Colorless
* <i>Enterococcus faecalis</i>	29212	Inhibition (partial)	
<b>Additional Organisms</b>			
<i>Pseudomonas aeruginosa</i>	10145	Growth	Pink to green
<i>Shigella dysenteriae</i>	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 (Columbia CNA With 5% Sheep Blood) and 7.1 ± 0.2 (MacConkey II Agar).
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

Columbia CNA Agar with 5% Sheep Blood is a selective and differential medium used for the isolation and differentiation of gram-positive microorganisms from clinical and nonclinical materials.

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

## V SUMMARY AND EXPLANATION

### A. Columbia CNA Agar with 5% Sheep Blood

Ellner et al., in 1966, reported the development of a blood agar formulation, which has been designated as Columbia Agar.<sup>1</sup> The Columbia Agar base, which achieves rapid and luxuriant growth and sharply defined hemolytic reactions, is utilized as the base for media containing blood and for selective formulations in which various combinations of antimicrobial agents are used as additives.

Ellner and his colleagues found that a medium consisting of 10 mg of colistin and 15 mg of nalidixic acid per liter in a Columbia agar base enriched with 5% sheep blood would support the growth of staphylococci, hemolytic streptococci and enterococci while inhibiting the growth of *Proteus*, *Klebsiella* and *Pseudomonas* species. In BBL™ Columbia CNA Agar with 5% Sheep Blood, the concentration of nalidixic acid has been reduced to 10 mg/L to increase the recovery of gram-positive cocci from clinical specimens.

### 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.<sup>2</sup> The landmark paper on MacConkey Agar was published in 1905 and contained detailed descriptions of the medium and the bacterial growth patterns obtained.<sup>3</sup> 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.<sup>4</sup> More recent modifications include use of additives (e.g., kanamycin<sup>5</sup>) and the deletion of certain ingredients (e.g., crystal violet<sup>6</sup>, and neutral red<sup>7</sup>).

A modified MacConkey Agar (MCIC Agar) is one of the culture media recommended by the American Public Health Association for use in the examination of seawater and shellfish.<sup>8</sup> MacConkey Agar is also utilized in the microbiological examination of foods.<sup>9</sup> It 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.<sup>10</sup>

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. Columbia CNA Agar with 5% Sheep Blood

Columbia CNA Agar with 5% Sheep Blood derives its superior growth-supporting properties from the combination of peptones prepared from pancreatic digest of casein, peptic digest of animal tissue and beef extract. Yeast extract and corn starch are also included in the formulation and serve as energy sources, with yeast extract being a supplier of the B complex vitamins.

Sheep blood allows detection of hemolytic reactions and supplies the X factor (heme) necessary for the growth of many bacterial species but lacks V factor (nicotinamide adenine dinucleotide, NAD), since it contains NADase which destroys the NAD. It should be noted that this medium has a relatively high carbohydrate content and, therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha hemolysis.

The addition of the antimicrobial agents, colistin and nalidixic acid, renders the medium selective for gram-positive microorganisms. The colistin disrupts the cell membranes of gram-negative organisms, whereas the nalidixic acid blocks DNA replication in susceptible gram-negative bacteria.<sup>11</sup>

### 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 inhibit 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

### Columbia CNA Agar with 5% Sheep Blood

Approximate Formula\* Per Liter Purified Water

Pancreatic Digest of Casein .....	12.0 g	Sodium Chloride .....	5.0 g
Peptic Digest of Animal Tissue .....	5.0 g	Agar .....	13.5 g
Yeast Extract .....	3.0 g	Colistin .....	10.0 mg
Beef Extract .....	3.0 g	Nalidixic Acid .....	10.0 mg
Corn Starch .....	1.0 g	Sheep Blood, defibrinated .....	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	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	Agar .....	13.5 g
Bile Salts .....	1.5 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"<sup>12-15</sup> 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.<sup>16,17</sup>

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:** Columbia CNA 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; and 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<sub>2</sub>.

**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. Better isolation is obtained due to the inhibitory nature of the media.

Typical colonial morphology on Columbia CNA Agar with 5% Sheep Blood is as follows:

Streptococci (non-group D) .....Small, white to grayish; beta or alpha hemolysis

Enterococci (group D) .....Small, but larger than group A streptococci, blue-gray; beta or alpha hemolysis

Staphylococci .....Large, white to gray or cream to yellow, with or without hemolysis

Micrococci .....Large, white to gray or yellow to orange, with or without hemolysis

Corynebacteria .....Small to large, white to gray or yellow, with or without hemolysis

*Candida* .....Small, white

*Listeria monocytogenes* .....Small to large, blue-gray, with beta hemolysis

Gram-negative bacteria .....No growth to trace growth

Typical colonial morphology on MacConkey II 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<sub>2</sub> atmosphere.<sup>18</sup>

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.<sup>19-23</sup>

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 PERFORMANCE CHARACTERISTICS

### Columbia CNA Agar with 5% Sheep Blood

Prior to release, all lots of Columbia CNA Agar with 5% Sheep Blood are tested for performance characteristics. Representative samples of the lot are spread-inoculated with 0.1 mL of the following cultures: *Proteus mirabilis* (ATCC 12453), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumoniae* (ATCC 6305) and *S. pyogenes* (ATCC 19615). The inocula for *S. aureus*, *S. pneumoniae* and *S. pyogenes* are diluted to yield  $10^3$ – $10^4$  colony-forming units (CFU) per 0.1 mL; the inoculum for *P. mirabilis* is diluted to yield  $10^4$ – $10^5$  CFU/0.1 mL. After inoculation, the plates are incubated at  $35 \pm 2^\circ\text{C}$  in an aerobic atmosphere supplemented with carbon dioxide. After 18–24 h incubation, *S. aureus*, *S. pneumoniae* and *S. pyogenes* show fair to heavy growth with typical colonial morphology, pigmentation, and hemolytic reactions, while *P. mirabilis* shows no growth to fair growth without swarming of the colonies.

### MacConkey II Agar

Prior to release, all lots of MacConkey II Agar are tested for performance characteristics. Representative samples of the lot are streak-inoculated with the following cultures: *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 12453), *Pseudomonas aeruginosa* (ATCC 10145), *Salmonella typhimurium* (ATCC 14028), *Shigella dysenteriae* (ATCC 9361) and *Enterococcus faecalis* (ATCC 29212). The inoculum for *E. faecalis* is diluted to yield  $10^4$ – $10^5$  colony-forming units (CFU) per plate; the inocula for all other organisms is diluted to yield  $10^3$ – $10^4$  CFU/plate. After inoculation, the plates are incubated at  $35 \pm 2^\circ\text{C}$  in an aerobic atmosphere. After 18–24 h incubation, colonies of *E. coli* are rose-red and may be surrounded by precipitated bile; *P. mirabilis* exhibits fair to heavy growth of colorless colonies and swarming of the colonies is inhibited; *P. aeruginosa* shows areas of confluent growth which may exhibit green to yellow-green pigmentation while individual colonies show pink to green pigmentation; *S. typhimurium* gives fair to heavy growth of colorless colonies; *S. dysenteriae* shows growth of colorless to pink colonies; *E. faecalis* is completely to partially inhibited (fair growth) and the colonies may be pink in color.

## XIII AVAILABILITY

Cat. No.	Description
221600	<b>BBL™</b> Columbia CNA Agar with 5% Sheep Blood and MacConkey II Agar I Plate™, Pkg. of 20 plates
221601	<b>BBL™</b> Columbia CNA Agar with 5% Sheep Blood and MacConkey II Agar I Plate™, Ctn. of 100 plates

## XIV REFERENCES

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