# BD BBL™ Columbia CNA Agar with 5% Sheep Blood

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## QUALITY CONTROL PROCEDURES

### I. INTRODUCTION

Columbia CNA Agar with 5% Sheep Blood is a selective and differential medium for the isolation and differentiation of grampositive microorganisms from clinical and nonclinical specimens.

### II. PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with 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 plates at 35 ± 2°C in an aerobic atmosphere supplemented with 3-5% carbon dioxide.
   c. Include ***Trypticase.*** Soy Agar with 5% Sheep Blood (TSA5%SB) plates as nonselective controls for all organisms.
2. Examine plates after 18-24 for growth, colony size, hemolytic reaction, pigmentation, and selectivity.
3. Expected Results

| **CLSI Organisms** | **ATCC™** | **Recovery** |
|---|---|---|
| **Streptococcus pyogenes* | 19615 | Growth, beta hemolysis |
| **Streptococcus pneumonia* | 6305 | Growth, alpha hemolysis |
| **Staphylococcus aureus* | 25923 | Growth |
| **Proteus mirabilis* | 12453 | Inhibition (partial) |

*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.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 30 ± 1°C for 84 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.

### V. SUMMARY AND EXPLANATION

Ellner et al., in 1966, reported the development of a blood agar formulation, which has been designated as Columbia Agar.[1]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.

### VI. PRINCIPLES OF THE PROCEDURE

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.[2]

## VII. REAGENTS

**Columbia CNA Agar with 5% Sheep Blood**

Approximate Formula* Per Liter Purified Water

| | |
|---|---|
| Pancreatic Digest of Casein ..... | 12.0 g |
| Peptic Digest of Animal Tissue ..... | 5.0 g |
| Yeast Extract ..... | 3.0 g |
| Beef Extract ..... | 3.0 g |
| Corn Starch ..... | 1.0 g |
| Sodium Chloride..... | 5.0 g |
| Agar ..... | 13.5 g |
| Colistin ..... | 10.0 mg |
| Nalidixic Acid ..... | 10.0 mg |
| Sheep Blood, defibrinated ..... | 5% |

*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 the 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"[3-6] 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 times. 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, cracking 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.[7,8]

Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

## IX. PROCEDURE

**Material Provided:** Columbia CNA Agar with 5% Sheep Blood

**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 for 24-48 h at 35 ± 2°C in an aerobic atmosphere supplemented with 3.5% 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

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 |

## 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.[7-12]

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 |
|---|---|
| 251352 | BD **BBL™** Columbia CNA Agar with 5% Sheep Blood, Pkg. of 20 plates |

## XIII. REFERENCES


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## XIV. FURTHER INFORMATION

For further information please contact your local BD representative.

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