BD™ Columbia CNA Agar with 5% Sheep Blood, Improved II

INTENDED USE

BD Columbia CNA Agar with 5% Sheep Blood, Improved II is a selective medium used for the isolation of gram-positive micro-organisms, especially staphylococci and streptococci, from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Ellner et al. in 1966 reported the development of a blood agar formulation, which has been designated as Columbia Agar.[1] This medium which achieves larger colonies and more luxuriant growth than on comparable blood agar bases, is utilized for media containing blood and for selective formulations. Ellner et al. found that a medium containing 10 mg of colistin and 15 mg of nalidixic acid per liter in a Columbia agar base, enriched with 5% sheep blood, supports the growth of staphylococci, hemolytic streptococci and enterococci while inhibiting the growth of *Proteus, Klebsiella* and *Pseudomonas* species.[1-3] Over the years, the resistance of bacteria to antimicrobial agents has increased. This is especially true for Gram negative rods that should be inhibited, but often produce growth, on Columbia CNA Agar with 5% Sheep Blood. To maintain good selectivity of this medium, a small amount of aztreonam is included in **BD Columbia CNA Agar with 5% Sheep Blood, Improved**. Aztreonam is a monobactam with activity only against most Gram negative bacteria, while Gram positive organisms are not affected.[4-6] In **BD Columbia CNA Agar with 5% Sheep Blood, Improved II**, the concentration of nalidixic acid has been reduced to 5.5 mg/l to increase the recovery of gram-positive cocci, especially staphylococci, from clinical specimens.

Columbia Agar provides a highly nutritious base medium. The addition of the antimicrobial agents, colistin, nalidixic acid and aztreonam renders the medium selective for gram-positive micro-organisms, especially streptococci and staphylococci. Sheep blood allows detection of hemolytic reactions which are especially important in the presumptive diagnosis of streptococci.[2,3, 7-9]

REAGENTS

BD Columbia CNA Agar with 5% Sheep Blood, Improved II

Formula* Per Liter Purified Water

Pancreatic Digest of Casein	12.0 g	Agar	13.5 g
Peptic Digest of Animal Tissue	5.0	Colistin	10.0 mg
Yeast Extract	3.0	Nalidixic Acid	5.5
Beef Extract	3.0	Aztreonam	3.0
Corn Starch	1.0	Sheep Blood, defibrinated	5%
Sodium Chloride	5.0	pH 7.3 ± 0.2	

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

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate the medium with the strains listed below. (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate at 35 ± 2° C for 18 to 24 hours, in an aerobic atmosphere enriched with carbon dioxide.

Strains	Growth Results
Staphylococcus aureus ATCC™ 25923	Good to excellent growth, may be beta-hemolytic
Streptococcus pneumoniae ATCC 6305	Good to excellent growth, alpha hemolysis
Streptococcus pyogenes ATCC 19615	Good to excellent growth, beta hemolysis
Enterococcus faecalis ATCC 29212	Good to excellent growth
Proteus mirabilis ATCC 12453	Inhibition complete
Uninoculated	Red (blood color)

PROCEDURE

Materials Provided

BD Columbia CNA Agar with 5% Sheep Blood, Improved II (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This is a universal selective medium for isolation of many Gram positive bacteria, especially staphylococci and streptococci in aerobic bacteriology that can be used for all types of bacteriological specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Test Procedure

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. In order to provide detection of all pathogens contained in the specimen, it must also be streaked onto appropriate nonselective media, e.g., **BD Columbia Agar with 5% Sheep Blood** and onto other selective media, e.g., **BD MacConkey II Agar**.[7-10]

Incubate **BD Columbia CNA Agar with 5% Sheep Blood, Improved II** at 35 - 37° C for 18 - 24 hours, in an aerobic atmosphere enriched with carbon dioxide. A second reading after 40 – 48 hours may be necessary for slow-growing gram positive organisms.

Results

Typical colonial morphology on **BD Columbia CNA Agar with 5% Sheep Blood, Improved II** 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, grayish. Alpha (rarely beta) hemolysis.
Staphylococci	Large, white to gray or cream to yellow, with or without hemolysis
Corynebacteria*	Small to large, white to gray or yellow, with or without hemolysis
Candida spp.	Small, white
Listeria monocytogenes	Small to medium-sized, grayish, with weak beta hemolysis
Gram-negative bacteria	No growth to trace growth

* See **Limitations of the Procedure**

Other Gram positive bacteria, not listed above, may also grow on the medium (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Columbia CNA Agar with 5% Sheep Blood, Improved II is an improved medium for the isolation and cultivation of many aerobically growing Gram positive micro-organisms, e.g., streptococci, staphylococci, *Listeria* spp and others. The medium allows a faster detection of staphylococci, enterococci and streptococci and a better inhibition of Gram negative bacteria than Columbia CNA Agar with 5% Sheep Blood.

Performance Characteristics [11]

In internal performance evaluations, more than 45 strains (clinical isolates and collection strains) of Gram positive bacteria belonging to the species mentioned in Table 1 have been tested for growth on **BD Columbia CNA Agar with 5% Sheep Blood, Improved II** (=CNA-II) and compared to **BD Columbia CNA Agar with 5% Sheep Blood** (=CNA). **BD Columbia Agar with 5% Sheep Blood** (=COL) was used as a growth reference medium. Plates were incubated in a CO_2 enriched aerobic atmosphere for 18 to 24 hours.
Quinolone-resistant *Proteus* strains were completely inhibited on CNA-II but produced heavy growth on CNA and COL. Also, a variety of other Gram negative rods (*Klebsiella pneumoniae*, which were producers of extended spectrum beta-lactamases, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*) were tested. All of these strains did not grow on CNA-II**,** but some produced light to medium growth on CNA, and all produced heavy growth on COL.
More Gram negative bacteria were inhibited on CNA-II than on CNA, while the isolation of Gram positive bacteria was identical on both selective media or better on CNA-II.
Additionally, several strains of *Staphylococcus aureus* that showed weak growth on CNA were tested on CNA-II. These strains all produced acceptable to excellent growth on CNA-II. The colony sizes and hemolytic zones on CNA-II were comparable to those on COL.

Table 1: Gram positive species tested and recovered on **BD Columbia CNA Agar with 5% Sheep Blood, Improved II** (incubation: aerobic with 5 to 8% carbon dioxide)

*Corynebacterium diphtheriae**	*Staphylococcus hyicus*	*Streptococcus. milleri*
Enterococcus faecalis	*Staphylococcus saccharolyticus***	*Streptococcus. mitis*
Enterococcus faecium	*Staphylococcus saprophyticus*	*Streptococcus pneumoniae*
Enterococcus durans	*Staphylococcus schleiferi*	*Streptococcus pyogenes*
Enterococcus hirae	*Staphylococcus xylosus*	*Streptococcus sanguis*
Listeria monocytogenes	*Staphylococcus warneri*	*Streptococcus* group C
Staphylococcus aureus	*Streptococcus agalactiae*	*Streptococcus* group F
Staphylococcus capitis	*Streptococcus bovis*	*Streptococcus* group G
Staphylococcus cohnii	*Streptococcus constellatus***	
Staphylococcus epidermidis	*Streptococcus intermedius*	

* 48 hours incubation needed for detection on **CNA-II** and **CNA.**
** These are species that grow better when grown anaerobically; anaerobic incubation for 42 to 48 hours needed for detection on **BD Columbia CNA Agar with 5% Sheep Blood, Improved II** and on **BD Columbia CNA Agar with 5% Sheep Blood**

A variety of anaerobic Gram positive cocci (*Peptostreptococcus* and related genera) were also tested on CNA-II and compared to CNA (incubation 42 to 72 hours, in an anaerobic atmosphere). While most of the test strains grew on both media, several test strains produced weaker growth on CNA-II than on CNA.

Limitations of the Procedure

Gram negative bacteria exhibiting resistance to the selective ingredients may grow on this medium.
Candida species and other fungi are not inhibited on this medium.
Although they are Gram positive bacteria, aerobic spore-formers such as *Bacillus* spp., may be inhibited on **BD Columbia CNA Agar with 5% Sheep Blood** and on **BD Columbia CNA Agar with 5% Sheep Blood, Improved II**.
Certain corynebacteria and micrococci will grow only weakly or not at all on **BD Columbia CNA Agar with 5% Sheep Blood, Improved II**. This medium must not be used for the isolation of strictly anaerobic bacteria. Instead, **BD Schaedler CNA Agar with 5% Sheep Blood** or **BD Columbia CNA Agar with 5% Sheep Blood** should be used for this purpose.

The number and types of bacterial species occurring as infectious agents is very large. Therefore, before the medium is routinely used for rarely isolated and newly described micro-organisms, its suitability must first be tested by the user by cultivating pure cultures of the organism in question.
Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures is necessary for complete identification.[7-9]

Columbia Agar base has a relatively high carbohydrate content and, therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha hemolysis.

REFERENCES

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11. Data on file. 2004. Becton Dickinson GmbH, Heidelberg/Germany.

PACKAGING/AVAILABILITY

BD Columbia CNA Agar with 5% Sheep Blood, Improved II

REF	257303	Ready-to-use plated media, 20 plates
REF	257306	Ready-to-use plated media, 120 plates

FURTHER INFORMATION

For further information please contact your local BD representative.

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