BD™ MacConkey II Agar / Columbia CNA Agar Improved II with 5% Sheep Blood (Biplate)

INTENDED USE

BD MacConkey II Agar / Columbia CNA Agar Improved II with 5% Sheep Blood (Biplate) is an improved medium used for the selective isolation of Gram negative and Gram positive bacteria from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

MacConkey Agar is one of the earliest formulations (published in 1900 by MacConkey) for the isolation, cultivation and identification of *Enterobacteriaceae* and certain nonfermenters. Later on, this medium was modified several times.[1,2]

The MacConkey II Agar formulation was designed in 1987 to improve the inhibition of swarming *Proteus* species, to achieve more definitive differentiation of lactose fermenters and nonfermenters, and for superior growth of enteric bacteria. In MacConkey II Agar, peptones provide nutrients. Crystal violet is included to inhibit Gram positive bacteria, especially enterococci and staphylococci. Differentiation of enteric micro-organisms is achieved by the combination of lactose and the neutral red pH indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.[3-5]

Ellner et al. in 1966 reported the development of a blood agar formulation, which has been designated as Columbia Agar[6]. 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.[6] 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. In Columbia CNA Agar Improved II with 5% Sheep Blood, a small amount of aztreonam is included to maintain a good selectivity of this medium, and the concentration of nalidixic acid has been reduced to 5.5 mg/l to increase the recovery of gram-positive cocci, especially staphylococci. The colistin concentration remained unchanged. Aztreonam is a monobactam with activity only against most Gram negative bacteria, while Gram positive organisms are not affected.[7-9]

Sheep blood allows detection of hemolytic reactions which are especially important in the presumptive diagnosis of streptococci.[10]

The main advantage of Columbia CNA Agar Improved II with 5% Sheep Blood over Columbia CNA Agar with 5% Sheep Blood is the improved growth of staphylococci which are more often detected after 18 to 24 hours of incubation and the better inhibition of resistant gram-negative bacteria, especially *Proteus* spp.

The combination of these two media in a biplate (**BD MacConkey II Agar / Columbia CNA Agar Improved II with 5% Sheep Blood**) is used for the selective isolation of Gram negative and Gram positive bacteria from clinical specimens.

REAGENTS

BD MacConkey II Agar / Columbia CNA Agar Improved II with 5% Sheep Blood (Biplate)
Formulas* Per Liter Purified Water

MacConkey II Agar		Columbia CNA Agar Improved II with 5% Sheep Blood	
Pancreatic Digest of Gelatin	17.0 g	Peptones	20.0 g
Pancreatic Digest of Casein	1.5	Yeast Extract	3.5
Peptic Digest of Animal Tissue	1.5	Tryptic digest of beef heart	3.0
Lactose	10.0	Corn Starch	1.0
Bile Salts	1.5	Sodium Chloride	5.0
Sodium Chloride	5.0	Colistin	10.0 mg
Neutral Red	0.03	Nalidixic Acid	5.5
Crystal Violet	0.001	Aztreonam	3.0
Agar	13.5	Sheep Blood, defibrinated	5%
pH 7.1 ± 0.2		pH 7.3 ± 0.2	

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

PRECAUTIONS

IVD . For professional use only. ②
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 representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate the plates, preferably in an inverted position, at 35 to 37° C aerobically for 18-24 hours.

Strains	MacConkey II Agar	Columbia CNA Agar Improved II with 5% Sheep Blood
Escherichia coli ATCC 25922	Growth good to excellent; pink to red colonies with bile precipitates	Inhibition complete
Proteus mirabilis ATCC 12453	Growth good to excellent; beige to brownish colonies, swarming inhibited	Inhibition complete
Enterococcus faecalis ATCC 29212	Inhibition (partial to) complete	Growth good to excellent; small grey colonies
Staphylococcus aureus ATCC 25923	Inhibition complete	White to yellowish colonies with beta hemolysis
Streptococcus pyogenes ATCC 19615	Not tested	Small greyish colonies; beta hemolysis
Streptococcus pneumoniae ATCC 6305	Not tested	Small green to grey colonies; alpha hemolysis
Uninoculated	Light pink, slightly opalescent	Red, opaque

PROCEDURE

Materials Provided
BD MacConkey II Agar / Columbia CNA Agar Improved II with 5% Sheep Blood (90 mm **Stacker™** biplates). Microbiologically controlled.

Materials Not Provided
Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types
The media contained in this biplate are used for the selective isolation of many Gram negative and Gram positive bacteria from all types of clinical 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.
To inoculate this biplate with specimens from swabs, first roll the swab over a small area of Columbia CNA Agar Improved II with 5% Sheep Blood, and afterwards over a small area of MacConkey II Agar. Using a fresh loop for each of the media, streak for isolation from the inoculated areas. Incubate in ambient air for 24 to 48 hours at 35-37° C. It is not recommended to incubate this product in a carbon dioxide enriched aerobic atmosphere since results on MacConkey Agar may vary form those obtained with incubation in ambient air.[11]

Since there exist Gram positive and Gram negative organisms that are inhibited on both media of this biplate or do not grow in ambient air, it is recommended to include a nonselective blood agar plate, e.g., **BD Columbia Agar with 5% Sheep Blood** which is incubated for 24 to 48 hours at 35 - 37° C in an aerobic atmosphere enriched with carbon dioxide.

Results
Typical growth results on **BD MacConkey II Agar / Columbia CNA Agar Improved II with 5% Sheep Blood (Biplate)** are as follows:

Organisms	**MacConkey II Agar**	**Columbia CNA Agar Improved II with 5% Sheep Blood**
E. coli	Pink to rose-red (may be surrounded by a zone of precipitated bile)	Inhibition (partial to) complete
Enterobacter	Mucoid, pink	Inhibition (partial to) complete
Klebsiella	Mucoid, pink	Inhibition (partial to) complete
Proteus	Colorless, swarming inhibited	Inhibition (partial to) complete; swarming inhibited
Salmonella	Colorless	Inhibition complete
Shigella	Colorless	Inhibition complete
Pseudomonas	Irregular, colorless to pink	Inhibition (partial to) complete
Staphylococci	Inhibition partial to complete	Growth; white to yellow, small to medium-sized colonies, with or without beta-hemolysis
Streptococci	Inhibition complete	Growth; tiny to medium-sized colonies with or without beta or alpha hemolysis
Enterococci	Inhibition partial to complete	Growth; tiny to medium-sized colonies; may have greyish borders, usually non-hemolytic

Other Gram negative and Gram positive bacteria, not listed above, may also grow on these media. For details and interpretation of growth, consult the references.[4,10,12]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD MacConkey II Agar is one of the standard media used for primary plating of clinical specimens and for a variety of nonclinical materials. On this medium, all organisms of the family *Enterobacteriaceae* and a variety of other Gram negative rods, e.g., *Pseudomonas* and related genera, will grow. Nonfermenters or other Gram negative rods susceptible to the selective ingredients do not grow on this medium. Consult the respective chapters in the references before using the medium for specific organisms.[4,10,12]

BD Columbia CNA Agar Improved II with 5% Sheep Blood is an improved selective medium for the isolation and cultivation of many aerobically growing Gram positive micro-organisms, e.g., streptococci, staphylococci, *Listeria* spp and others, from clinical specimens. 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.

In internal performance evaluations, 38 strains (clinical isolates and collection strains) of Gram positive bacteria belonging to the species mentioned in Table 1 and many Gram negative bacteria have been tested for growth on **BD MacConkey II Agar / Columbia CNA Agar Improved II with 5% Sheep Blood (Biplate). BD Columbia Agar with 5% Sheep Blood** (=COL) was used as a growth reference medium. Plates were incubated in an aerobic atmosphere for 18 to 24 hours at 35-37° C.
Quinolone-resistant *Proteus* strains were completely inhibited on Columbia CNA Agar Improved II with 5% Sheep Blood but produced heavy growth on MacConkey II Agar and Columbia Agar. The colony sizes and hemolytic zones on Columbia CNA Agar Improved II with 5% Sheep Blood were comparable to those on Columbia Agar. All gram-positive strains except *Corynebacterium diphtheriae* produced growth on Columbia CNA Agar Improved II with 5% Sheep Blood within 18-20 hours of aerobic incubation and were completely inhibited on MacConkey II Agar. *C. diphtheriae* needed 42 hours of incubation. Testing included staphylococci that needed 2 days of incubation on regular Columbia CNA Agar.

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

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

* 48 hours incubation needed for detection on **CNA-II** and **CNA.**

Limitations: 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 sporeformers such as *Bacillus* spp., may be inhibited on Columbia CNA Agar Improved II with 5% Sheep Blood.
Corynebacteria may need 42 to 48 hours of incubation Columbia CNA Agar Improved II with 5% Sheep Blood.
Certain streptococci, e.g. *Streptococcus intermedius* and *Streptococcus milleri* need a CO_2 enriched or anaerobic atmosphere for growth.
Columbia Agar base has relatively high carbohydrate content. Therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha hemolysis on Columbia CNA Agar Improved II with 5% Sheep Blood.

Although a great variety of Gram negative and Gram positive bacteria will grow on one of the media contained in **BD MacConkey II Agar / Columbia CNA Agar Improved II with 5% Sheep Blood (Biplate)**, it is recommended to include a nonselective medium for the primary isolation of all pathogens that may be present in a specimen.[10] **BD Columbia Agar with 5% Sheep Blood** is a frequently used nonselective primary plating medium that may be used for this purpose. For the isolation of fastidious organisms, such as *Neisseria* or *Haemophilus*, a chocolate agar plate, e.g. **BD Chocolate Agar (GC II Agar with IsoVitaleX)** should also be inoculated with the specimen if these organisms are expected.

It has been reported that some *Enterobacteriaceae* and *Pseudomonas aeruginosa* are inhibited on MacConkey Agar when incubated in a CO_2 -enriched atmosphere.[11] Therefore, **BD**

MacConkey II Agar / Columbia CNA Agar Improved II with 5% Sheep Blood (Biplate) should not be incubated in a in a CO_2 -enriched atmosphere.

On some plates with strong growth of staphylococci on the Columbia CNA Agar Improved II with 5% Sheep Blood medium and no growth on MacConkey II Agar, fading of the color of the MacConkey II Agar medium has been observed. This has no negative effect on the recovery and typical colony coloration of gram-negative bacteria on MacConkey II Agar.

Although certain diagnostic tests may be performed directly on these media, biochemical and, if indicated, immunological testing, using pure cultures is necessary for complete identification of the isolates.

REFERENCES

1. Levine, M., and H.W. Schoenlein. 1930. A compilation of culture media for the cultivation of microorganisms. The Williams & Wilkins Company, Baltimore.
2. MacFaddin, J.F. 1985. Media for isolation-cultivation- identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
3. Baron, E.J., L.R. Peterson, and S.M. Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis.
4. Farmer, J.J., III., K.D. Boatwright, and J.M. Janda 2007. *Enterobacteriaceae*: introduction and identification. *In:* Murray, P. R., E. J. Baron, M.L. Landry, J.H. Jorgensen, and M. A. Pfaller (ed.). Manual of clinical microbiology, 9thed. American Society for Microbiology, Washington, D.C.
5. Downes, F.P., and K. Ito. 2001. Compendium of methods for the microbiological examination of foods. 4th edition. American Public Health Association (APHA). Washington, D.C. USA.
6. Ellner, P.D., C.J. Stoessel, E. Drakeford, and F. Vasi. 1966. A new culture medium for medical bacteriology. Am. J. Clin. Pathol. 45: 502-504.
7. Wood, W., G. Harvey, E.S. Olson, and T.M. Reid. 1993. Aztreonam selective agar for Gram positive bacteria. J. Clin. Pathol. 46: 769-771.
8. Wiedemann, B., and B. A. Atkinson. 1986. Susceptibility to antibiotics: species incidence and trends. *In:* Lorian, V. (ed.), Antibiotics in Laboratory medicine, p. 962-1208. Williams and Wilkins, Baltimore, USA.
9. von Graevenitz, A. 1986. Use of antimicrobial agents as tools in epidemiology, identification, and selection of microorganisms. *In:* Lorian, V. (ed.), Antibiotics in Laboratory medicine, p. 723-738. Williams and Wilkins, Baltimore, USA.
10. Spellerberg, B., Brandt, C. 2007. *Streptococcus*. *In:* Murray, P. R., E. J. Baron, M.L. Landry, J.H. Jorgensen, and M. A. Pfaller (ed.). Manual of clinical microbiology, 9thed. American Society for Microbiology, Washington, D.C.
11. Mazura-Reetz, G. T. Neblett, and J. M. Galperin. 1979. MacConkey Agar: CO2 vs. ambient incubation. Abst. Ann. Mtg. American Society for Microbiology. C179.
12. Isenberg, H. D. (ed.). 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol.1, p. 1.6.1-1.6.7. American Society for Microbiology, Washington, D.C.

PACKAGING/AVAILABILITY

BD MacConkey II Agar / Columbia CNA Agar Improved II with 5% Sheep Blood (Biplate)

Cat. No.	**Description**
REF 257574	Ready-to-use Plated Media, cpu 20
REF 257584	Ready-to-use Plated Media, cpu 120

FURTHER INFORMATION

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