BD™ Mac Conkey II Agar / Columbia CNA Agar with 5% Sheep Blood (Biplate)

INTENDED USE

BD Mac Conkey II Agar / Columbia CNA Agar with 5% Sheep Blood (Biplate) is 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.[1,2] Later on, this medium was modified several times.[3,4]

MacConkey Agar is only slightly selective since the concentration of bile salts, which inhibits Gram positive micro-organisms, is low in comparison with other enteric plating media. This medium is recommended for use with clinical specimens likely to contain mixed microbial flora, such as urine, respiratory, wound, and others, because it allows a preliminary grouping of enteric and other gram-negative bacteria in lactose fermenters and lactose nonfermenters.[4-6] MacConkey Agar is also utilized in the microbiological examination of foods.[7]

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.[4]

Ellner et al. in 1966 reported the development of a blood agar formulation, which has been designated as Columbia Agar.[8] 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.[8,9]

Columbia Agar provides a highly nutritious base medium. The addition of the antimicrobial agents, colistin and nalidixic acid renders the medium selective for gram-positive micro-organisms, especially streptococci and staphylococci. Sheep blood allows detection of hemolytic reactions.[4,5,9]

The combination of these two media in a biplate is used for the selective isolation of Gram negative and Gram positive bacteria from clinical specimens.

REAGENTS

BD Mac Conkey II Agar / Columbia CNA Agar with 5% Sheep Blood (Biplate)

Formulas* Per Liter Purified Water

MacConkey II Agar		Columbia CNA Agar 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	0.01

Neutral Red	0.03	Nalidixic Acid	0.015
Crystal Violet	0.001	Agar	15.0
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.
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 aerobically for 24 hours at 35 to 37° C.

Strains	**MacConkey II Agar**	**Columbia CNA Agar 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 partial to complete, swarming inhibited
Salmonella Typhimurium ATCC 14028	Growth good to excellent; beige colonies	Not tested
Shigella flexneri ATCC 12022	Growth good to excellent; beige colonies	Not tested
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 (blood color)

PROCEDURE

Materials Provided

BD Mac Conkey II Agar / Columbia CNA Agar 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**).[10]

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 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 ± 2° C. It is not recommended to incubate MacConkey Agar in a carbon dioxide enriched aerobic atmosphere.[11]
Since there exist Gram positive and Gram negative organisms that are inhibited on both media, it is necessary 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 ± 2° C in an aerobic atmosphere enriched with carbon dioxide.

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

Organisms	Mac Conkey II Agar	Columbia CNA Agar 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 partial to complete
Shigella	Colorless	Inhibition partial to 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.[5,9]
Further biochemical and, if indicated, immunological testing using pure cultures is necessary for complete identification of the isolates.[5,6,9]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE
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.[5-7,9] 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.[5,9,10]
It has been reported that some *Enterobacteriaceae* and *Pseudomonas aeruginosa* are inhibited on MacConkey Agar when incubated in a CO_2 -enriched atmosphere.[11]

Columbia CNA Agar with 5% Sheep Blood is a standard medium for the isolation and cultivation of many aerobically growing Gram positive micro-organisms, e.g., streptococci, staphylococci, coryneforms, *Listeria* spp. and others.[5,9]
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 Columbia CNA Agar with 5% Sheep Blood.
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.

Although a great variety of Gram negative and Gram positive bacteria will grow on one of the media contained in **BD Mac Conkey II Agar / Columbia CNA Agar with 5%Sheep Blood (Biplate)**, it is necessary 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.
.
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. MacConkey, A.T. 1900. Note on a new medium for the growth and differentiation of the *Bacillus coli communis* and the *Bacillus typhi abdominalis*. The Lancet, Part II:20.
2. MacConkey, A. 1905. Lactose-fermenting bacteria in faeces. J. Hyg. 5:333-379.
3. Levine, M., and H.W. Schoenlein. 1930. A compilation of culture media for the cultivation of microorganisms. The Williams & Wilkins Company, Baltimore.
4. MacFaddin, J.F. 1985. Media for isolation-cultivation- identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
5. Baron, E.J., L.R. Peterson, and S.M. Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis.
6. Farmer, J.J., III. 2003. *Enterobacteriaceae*: introduction and identification. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8thed. American Society for Microbiology, Washington, D.C.
7. 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.
8. 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.
9. Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
10. Thomson, R.B., and J.M. Miller. 2003. Specimen collection, transport, and processing: bacteriology. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
11. Mazura-Reetz, G. T. Neblett, and J. M. Galperin. 1979. MacConkey Agar: CO_2 vs. ambient incubation. Abst. Ann. Mtg. American Society for Microbiology. C179.

PACKAGING/AVAILABILITY

BD Mac Conkey II Agar / BD Columbia CNA Agar with 5%Sheep Blood (Biplate)
Cat. No. 254447 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.

BD Diagnostic Systems
Tullastrasse 8 – 12
D-69126 Heidelberg/Germany
Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16
Reception_Germany@europe.bd.com

BD Diagnostic Systems Europe
Becton Dickinson France SA
11 rue Aristide Bergès
38800 Le Pont de Claix/France
Tel: +33-476 68 3636 Fax: +33-476 68 3292 http://www.bd.com