Biomed Global

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Columbia 5% Horse Blood Agar/ MacConkey III | Ready-to-use Media

a product by Biomed MDX

IVD

Intended Use:

Columbia Agar with 5% Horse Blood and MacConkey III (Biplate) medium is a dual-purpose medium used to isolate and cultivate bacteria from clinical specimens. Columbia Agar with 5% Horse Blood allows for the differentiation of bacteria based on their hemolytic reactions, while MacConkey III is a selective and differential medium for the detection and isolation of gram-negative organisms.

Principle of the Procedure:

Columbia 5% Horse Blood Agar :

Columbia agar with 5% Horse blood is a differential and enriched medium widely employed in clinical microbiology. Its composition of Columbia agar base supplemented with 5% defibrinated Horse blood provides essential nutrients and growth factors, supporting the cultivation of a broad spectrum of microorganisms, including fastidious species. The Horse blood component also facilitates the differentiation of bacteria based on their hemolytic properties. Columbia Agar Base is a foundational medium for cultivating a wide range of bacteria, including both fastidious and non-fastidious organisms. Introduced in 1966, it provides a rich environment for microbial growth. Modifications can be introduced to enhance its utility. For example, specific additives can be incorporated to selectively inhibit the growth of certain bacterial groups, allowing for the isolation of specific target organisms from complex samples.

MacConkey III:

Peptone supplies nutrients and agar is the solidifying agent. Bile salts are inhibitory to non-intestinal bacteria and help to prevent the swarming of *Proteus spp*. Bile salts and crystal violet inhibit the growth of Gram-positive cocci. Lactose is added as a carbon source. Differentiation of bacteria is achieved by the combination of lactose and the indicator dye neutral red, which is red at acid pH and yellow at alkaline. Bacteria which ferment lactose appear as red - pink-coloured colonies which may be surrounded by zones of precipitated bile salts. Precipitation is caused by the action of the acid produced by lactose fermentation on the bile salts. Bacteria which do not ferment lactose, such as Salmonella, usually appear as colourless to straw colonies. Sodium chloride maintains osmotic balance in the medium. Therefore, MacConkey III Agar can be used with clinical specimens likely to contain mixed flora such as urine and wounds as it allows the preliminary grouping of Gram-negative bacteria into lactose fermenters and non-fermenters.

Product Summary:

Columbia 5% Horse Blood Agar:

Columbia Agar Base is a foundational medium for cultivating a wide range of bacteria, including both fastidious and non-fastidious organisms. Introduced in 1966, it provides a rich environment for microbial growth[1]. Modifications can be introduced to enhance its utility. For example, specific additives can be incorporated to selectively inhibit the growth of certain bacterial groups, allowing for the isolation of specific target organisms from complex samples.

MacConkey III:

Lactose fermenters are microorganisms that ferment lactose and those that are unable to ferment lactose are called non-lactose fermenters[2]. *Escherichia coli* (*E. coli*) are non-spore forming bacteria that are able to grow in aerobic and anaerobic conditions[2]. Salmonella is a bacterial pathogen that can be isolated from faeces, blood, bone marrow, bile, urine, food, animal feed and environmental materials. Ingestion of contaminated food and water can cause foodborne infections, including gastroenteritis, typhoid fever, paratyphoid fever or even death in humans. All Salmonella serotypes can cause disease in humans[3]. *Acinetobacter baumannii* is a Gram-negative nosocomial pathogen that can persists on dry surfaces longer than any other Gram-negative bacteria. It can persist on moist and dry surfaces for more than 20 days contributes to its widespread in a hospital setting[4]. *Acinetobacter spp*. are commonly isolated from locations such as hand, groin, toe webs etc[5]. Due to the high antibiotic resistance shown. by this bacterium an early identification is often recommended. *Acinetobacter spp*. have been isolated in connection with community acquired and nosocomial pneumonias, urogenital tract, eye and soft tissue infections and are difficult to treat particularly due to their broad antibiotic resistance.

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Formulation* (PER LITER):

Columbia 5% Horse Blood Agar		MacConkey III	
Special peptone	23.0g	Peptones	20.0g
Starch	1.0g	Lactose	10.0g
Sodium Chloride	5.0g	Bile salts No. 3	1.5g
Agar	10.0g	Sodium chloride	5.0g
Horse Blood	50.0g	Neutral red	0.03g
		Crystal violet	0.001g
		Agar	15.0g

pH 7.3 +/- 0.2 pH 7.1 +/- 0.2

*Adjust and/or supplemental as required to meet performance criteria

Procedure

Materials Provided

90mm Columbia 5% Horse Blood Agar/MacConkey III

Materials Required but Not Provided

Ancillary culture media, reagents, and laboratory equipment as required.

Test Procedure

1. Collect a sample of the undiluted, well-mixed using a calibrated loop (0.01 or 0.001 ml) for each of the two media of this biplate.
2. First, streak a sample on Columbia 5% Horse Blood Agar, then the second sample on MacConkey III Agar.
3. Incubate plates at 35°C ± 2°C for 18 to 24 hours.
4. Observe the result according to user requirements.
5. Dispose of all used reagents and contaminated materials as infectious waste. Laboratories must handle and dispose of all waste safely according to regulations.

Results

Examine for colonies exhibiting colonial morphology. Appropriate biochemical or immunological tests may be required for final identification.

Quality Control

Inoculate representative samples with the following strains. Incubate the inoculated plates at 35 ± 2°C for 18 to 24 hrs. to allow colonies to develop on the medium.

Columbia 5% Horse Blood Agar:

Strains	ATCC®	Growth
Escherichia coli	25922	Growth at 24 hours, beta hemolysis
Streptococcus pyogenes	19615	Growth at 24 hours, beta hemolysis
Streptococcus pneumoniae	6305	Growth at 24 hours, alpha hemolysis
Candida albicans	60193	Growth at 24 hours, no hemolysis
Enterococcus faecalis	29212	Growth at 24 hours, gamma hemolysis
Uninoculated plate	-	No Growth

MacConkey III:

Strains	ATCC®	Growth
Escherichia coli	25922	Pink to red growth
Proteus mirabilis	12453	Growth Colorless Inhibition of swarming
Salmonella choleraesuis subsp. choleraesuis serotype Typhimurium	14028	Growth Colourless
Staphylococcus aureus	25923	No growth
Enterococcus faecalis	29212	No growth
Uninoculated plate	-	No growth

Transportation:

Temperature fluctuations may occur during transportation. However, these fluctuations do not affect the performance, quality, or safety of the media.

Storage and Shelf Life:

Upon receipt, store plates at 2 to 8°C, in their original sleeve wrapping until just before 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.

Warning and Precautions:

For in vitro diagnostic use. For Professional Use Only. Do Not Reuse.

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

Limitation of the Procedure

This medium is for laboratory use only and is not intended for the diagnosis of disease or other conditions. Identifications are presumptive and colonies should be identified using appropriate methods [5-8]

Reference

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3. Public Health England. 2021. “Identification of Salmonella species.'' UK Standards for Microbiology Investigations UK SMI ID 24, Issue 4. Accessed 19 Jan 2022. https://www.gov.uk/government/publications/smi-id-24-identification-of-salmonella-species
4. Gerner-Smidt, P. (1995). Taxonomy and epidemiology of Acinetobacter infections. Rev Med Microbiol., 6, 186-195.
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6. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven, Philadelphia.
7. Zimbro, M. J., Power, D. A., Miller, S. M., Wilson, G. E., & Johnson, J. A. (Eds.). (2009). Difco™ and BBL™ manual: Manual of microbiological culture media (2nd ed.). Becton, Dickinson and Company.

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Packaging Symbol

Symbol	Definition
REF	Catalogue number
IVD	In Vitro Diagnostic Medical Device
LOT	Batch code
	Date of manufacture
	Temperature limit
	Use-by date
	Keep away from sunlight
	Do not re-use
	Fragile, handle with care
	Consult instructions for use or consult electronic instructions for use
	Do not use if packaging damaged and consult instructions for use
	Manufacturer

Further Information:

For further information please contact your Biomed MDX representative.

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