

## Columbia 5% Horse Blood/Chocolate Agar

### | Ready-to-use Media

a product by **Biomed MDX**



#### Intended Use:

Columbia Agar with 5% Horse Blood and Chocolate Agar (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 Chocolate Agar is a non-selective enriched growth medium used for the isolation and cultivation of fastidious microorganisms.

#### 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

##### Chocolate Agar:

This growth medium is specifically formulated to help isolate certain microorganisms, especially when taken from respiratory samples. Built upon a base that supplies fundamental nutrients like peptones, carbohydrates, and salts crucial for bacterial development, the medium is enhanced with broken-down horse blood. This process releases vital growth components: hemin (X factor) and nicotinamide adenine dinucleotide (NAD or V factor). The inclusion of NAD is critical for the cultivation of demanding microorganisms. However, further microbiological tests are required to identify and diagnose specific microorganisms definitively.

#### 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<sup>1</sup>. Columbia Agar with Horse Blood is primarily used to show hemolysis of fastidious organism esp *Streptococcus spp* that can produce Beta, alpha and Gamma lysis pattern clearly on the plate. 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.

##### Chocolate Agar:

Rich growth medium, especially useful for isolating fastidious respiratory bacteria. Contains essential nutrients and growth factors from lysed horse blood, promoting the development of demanding microorganisms. Further testing is needed for specific identification.

**Formulation\* (PER LITER):**

Columbia 5% Horse Blood Agar		Chocolate Agar	
Special peptone	23.0g	Special peptone	23.0g
Starch	1.0g	Starch	1.0g
Sodium Chloride	5.0g	Sodium Chloride	5.0g
Agar	10.0g	Agar	10.0g
Horse Blood	50mL	Horse Blood	70mL
		Nicotinamide Adenine Dinucleotide (NAD)	0.05g

pH 7.3 +/- 0.2

pH 7.3 +/- 0.2

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

## Procedure

### Materials Provided

90mm Columbia 5% Horse Blood Agar/Chocolate Agar

### 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 Chocolate 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 wastesafely 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
<i>Escherichia coli</i>	25922	Growth at 24 hours, beta hemolysis
<i>Streptococcus pyogenes</i>	19615	Growth at 24 hours, beta hemolysis
<i>Streptococcus pneumoniae</i>	6305	Growth at 24 hours, alpha hemolysis
<i>Candida albicans</i>	60193	Growth at 24 hours, no hemolysis
<i>Enterococcus faecalis</i>	29212	Growth at 24 hours, gamma hemolysis
Uninoculated plate	-	No Growth

**Chocolate Agar:**

Strains	ATCC®	Growth
<i>Haemophilus influenza</i>	10211	Growth
<i>Neisseria gonorrhoeae</i>	43069	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<sup>5-8</sup>

## Reference

1. 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.
2. Ellner, P. D., Stoessel, C. J., Drakeford, E., & Vasi, F. A. (1966). New Culture Medium for Medical Bacteriology.
3. 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.
4. Ladhani, S., Slack, M. P., Heath, P. T., Von Gottberg, A., Chandra, M., Ramsay, M. E., & European Union Invasive Bacterial Infection Surveillance participants. (2010). Invasive *Haemophilus influenzae* disease, Europe, 1996–2006. *Emerging infectious diseases*, 16(3), 455.

**Packaging Symbol**

Symbol	Definition
	Catalogue number
	In Vitro Diagnostic Medical Device
	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:**

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