

CHOCOLATE AGAR

INTENDED USE:

Chocolate Agar are non-selective media for the isolation and cultivation of fastidious microorganisms, especially Neisseria and Haemophilus species, from a variety of clinical specimens.

PRINCIPLE AND INTERPRETATION:

Chocolate Agar (Blood Agar No. 2 Base) contains Proteose peptone, liver digest and yeast extract as sources of nitrogen and vitamins. Sodium chloride maintains the osmotic stability.

Heated (heat-denatured) horse blood supplies both the X factor (heme) and, since it does not contain NADase, the V factor (nicotinamide adenine dinucleotide, NAD) necessary for the growth of Haemophilus influenzae and supplies additional nutrients.

COMPOSITION:

Ingredients	Gr/Liter	
Nutrient substrate	20 gr	
Sodium chloride	5 gr	
Agar	15 gr	
Defibrinated Horse Blood	90 ml	

***Formula adjusted, standardized to suit performance parameters **pH**: $7,2 \pm 0,2$

PRECAUTIONS:

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

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.

Incubate the plates for 24 to 48 hours in a moist environment.

QUALITY CONTROL:

1.Sterility Control:

Incubation 48 hours at 30-35°C and 72 hours at 20-25°C: NO GROWTH

2.Phsical/Chemical Control

pH: 7,2 ± 0,2 Apperance: Dark brown

3.Microbiological Control: Cultural response on Chocolate Agar at 35± 2 °C after 24-48 hours incubation.

Microorganism	Inoculum (CFU)	Results	
		Growth	Reaction
Haemophilus influenzae ATCC 10211	10-100	Good	White small colonies
Neissseria gonorrhoeae ATCC 49226	10-100	Good	White small colonies
Streptococcus pyogenes ATCC 19615	10-100	Good	Cream colonies
Streptococcus pneumoniae ATCC 6305	10-100	Good	Cream colonies



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LIMITATIONS OF THE PROCEDURE:

These media are used for the isolation of Haemophilus influenzae from clinical specimens from body sites that contain normal flora.

These media must not be used for the primary isolation of Haemophilus species from cerebrospinal fluid or from specimens from other primary sterile body sites. Since Haemophilus species are a regular part of the respiratory tract flora, their presence in specimens from

the upper respiratory tract such as throat swabs and sputa does not necessarily indicate that these organisms are the cause of an infection. Serological tests are required to demonstrate that the isolate belongs to serogroups often involved in infections.

STORAGE CONDITIONS AND SHELF LIFE:

Store the prepared medium at 2 - 12°C. Use before expiry date on the label. Do not use beyond stated expiry date.

DISPOSAL:

Incubated prepared medium may contain active bacteria and micro-organisms. Do not open infected medium. Infected plate should be autoclaved, incinerated or opened and soaked in a chlorine-based disinfectant (liquid bleach) for 20 minutes prior to disposal.

PACKAGING:

Katalog Number: 02007 Packaging: Single wrap Content: 10 plates/each package

REFERENCES:

1. Kilian, M. 2003. Haemophilus. 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.

 Mannheim, W. et al.: Pasteurellaceae. In: Mikrobiologische Diagnostik (ed. F. Burkhardt). Thieme Verlag, Stuttgart 1992.
Vastine, D.W., C.R. Dawson, I. Hoshiwara, C. Yonega, T. Daghfous, and M. Messadi. 1974. Comparison of media for the isolation of Haemophilus species from cases of seasonal conjunctivitis associated with severe endemic trachoma. Appl. Microbiol. 28:688- 690.
Chapin, K., and G.V. Doern. 1983. Selective media for recovery of Haemophilus influenzae from species contaminated with upper respiratory tract microbial flora. J. Clin. Microbiol. 17:1163-1165.

