

RTA.KK.157 Revision Date/Revision Number:-/0 Issue Date: 17.05.2016

CHOCOLATE AGAR W/ HORSE BLOOD + BACITRACIN

INTENDED USE:

Chocolate Agar with Horse Blood and Bacitracin is a selective medium for the isolation of Haemophilus species.

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.

Bacitracin disrupt both gram positive and gram negative bacteria by interfering with cell wall and peptidoglycan synthesis.

COMPOSITION:

Ingredients	Gr/Liter	
Nutrient substrate	20 gr	
Sodium chloride	5 gr	
Agar	15 gr	
Defibrinated Horse Blood	90 ml	
Bacitracin	20.000 IU	

^{***}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

 $pH: 7,2 \pm 0,2$

Apperance: Dark brown

3.Microbiological Control: Cultural response on Chocolate Agar w/Horse Blood and Bacitracin 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
Staphylococcus aureus ATCC 25923	100-1000	Inhibition	-
Staphylococcus epidermidis ATCC 12228	100-1000	Inhibition	-



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LIMITATIONS OF THE PROCEDURE:

For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen.

It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum.

Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

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.

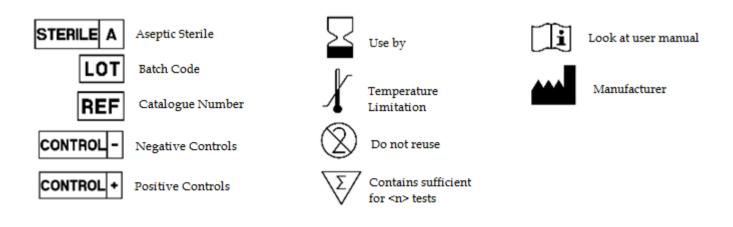
PACKAGING:

Katalog Number: 02008 Packaging: Single wrap

Content: 10 plates/each package

REFERENCES:

- 1. Carpenter, C.M., and H.E. Morton. 1947. An improved medium for isolation of the gonococcus in 24 hours. Proc. N.Y. State Assoc. Public Health Labs. 27:58-60.
- 2. Carpenter, C.M., M.A. Bucca, T.C. Buck, E.P. Casman, C.W. Christensen, E. Crowe, R. Drew, J. Hill, C.E. Lankford, H.E. Morton, L.R. Peizer, C.I. Shaw, and J.D. Thayer. 1949. Evaluation of twelve media for the isolation of the gonococcus. Am. J. Syphil. Gonorrh. Venereal Diseases 33:164-176.
- 3. Power, D.A. (ed.), and P.J. McCuen. 1988. Manual of BBL products and laboratory procedures, 6th ed. Becton Dickinson Microbiology Systems, Cockeysville, Md.
- 4. Martin, J.E., T.E. Billings, J.F. Hackney, and J.D. Thayer. 1967. Primary isolation of N. gonorrhoeae with a new commercial medium. Public Health Rep. 82:361-363.
- 5. 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.
- 6. 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.





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