



P.O. Box 788
 Waterville, Maine 04903-0788
 227 China Road
 Winslow, Maine 04901

Administrative Offices
 Phone: 207-873-7711
 Fax: 207-873-7022

Customer Service
 Phone: 1-800-244-8378
 Fax: 207-873-7022

TECHNICAL PRODUCT INFORMATION

Catalog No.: P1150Chocolate Agar w/Enrichments
 Catalog No.: P3025 Blood Agar/Chocolate Bi-plate
 Catalog No.: T1250Chocolate Agar Slant

INTENDED USE:

Chocolate Agar is recommended for the cultivation and isolation of *Neisseria* and *Haemophilus* species. CO² favors primary isolation. The medium is best for organisms which require X and V Factor.

HISTORY/SUMMARY:

Interest in the cultural procedure for the diagnosis of gonococcal infections was stimulated by Ruys and Hens McLeod et al², Leahy and Carpenter, Leahy and Wilson⁵ and Carpenter⁶ who clearly demonstrated the superiority of this method over the microscopic technique. Further studies in cooperation with Carpenter, and McLeod⁷ and Herrold resulted in the development of Chocolate Agar prepared with Proteose No. 3 Agar and Hemoglobin, which proved to be satisfactory for isolating the organism from all types of gonococcal infections.

Chapin and Doern found that in only 6 of 17 cases was *Haemophilus influenzae* recovered from sputum specimens cultured by using conventional techniques including enriched chocolate agar (CHOC) media, despite the fact that gram stained smears of sputum specimens often revealed a predominance of pleomorphic gram-negative bacilli. Observations such as these have lead to the development of selective media which inhibit upper respiratory tract microbial flora while permitting growth of *Haemophilus influenzae*. Approaches utilized most frequently incorporate bacitracin into various enriched basal media (^{11, 12, 13, 14}).

PRINCIPLES:

Chocolate Agar, Enriched has been proven to be as effective in the number of isolations of *Neisseria gonorrhoeae* as was any other non-selective medium recommended for this purpose. Further studies describing the advantage and superiority of this medium lead to the acceptance of Chocolate Agar, Enriched as a standard medium for the cultivation of *Neisseria gonorrhoeae*.

Chocolate Agar, Enriched, though an excellent culture medium for gonococci, is not a selective medium and therefore when mixed cultures are encountered, growth of contaminants is not restricted which may result in over-growth of gonococci by bacterial and fungal flora often found in specimens from urethral and cervical sites.

FORMULA:

Ingredients per liter of purified water	
Casein peptone/Meat peptone	15.0 g
Corn Starch	1.0 g
Dispotassium phosphate	4.0 g
Monopotassium phosphate	1.0 g
Sodium Chloride	5.0 g
Agar	10.0 g
Hemoglobin	10.0 g

X & V Enrichment	10.0 ml
------------------	---------

Final pH: 7.2 ± 0.2 @ 25°C.

PRECAUTIONS:

Since living organisms used with this material can be infectious to the user, proper handling and disposal methods should be established by the laboratory director. This product is for In Vitro Diagnostic Use.

STORAGE:

This media should be stored at 2-8°C. Use media prior to expiration date. Do not use media that shows signs of deterioration.

PROCEDURE:

The specimen to be cultivated should be streaked onto a chocolate agar plate or onto the agar slant. Identification of the isolate may be accomplished as directed in standard references, such as the Manual of Clinical Microbiology. Plates and tubes should be incubated at least three days before discarding as negative. The media should be incubated at 35°C for 24 hours in CO₂.

PERFORMANCE CHARACTERISTICS:

Organisms	Incubation	Results
*ATCC# 43069 <i>Neisseria gonorrhoeae</i>	CO ₂ 18 to 24 hrs @ 35 °C	Growth
*ATCC# 10211 <i>Haemophilus influenzae</i>	CO ₂ 18 to 24 hrs @ 35 °C	Growth

*NCCLS Recommended organism¹⁵

QUALITY CONTROL:

It is recommended that the laboratory confirm the performance characteristics of this media. All lot numbers of Chocolate Agar have been tested using the above quality control organisms and have been found to be acceptable. This quality assurance testing for Chocolate Agar conforms with or exceeds NCCLS standards.

REFERENCES:

1. Muench, Wochschr., 80:846, 1933.
2. J. Path. Bact., 38:221, 1934.
3. J. Infect. Dis., 61:129, 1937
4. Am. J. Syphilis, 22:347, 1938.
5. Am. J. Syphilis, 22:55, 1938.
6. 7th Ann. Yearbook (1936-37), pg 133, Suppl. Am. J. Publ. Hlth., 27:3, 1937
7. Am. J. Syphilis, 22:347, 1938.
8. Am. J. Syphilis, 22:55, 1938.
9. Blair, J.E. et al. 1970 Manual of Clinical Microbiology, ASM Bethesda.
10. Bailey, R.W. and E.G. Scott, 1970 Diagnostic Microbiology 3rd Ed. C.V. Mosby Co., St. Louis
11. Hovig, B., and E.H. Aandahl. 1969. A Selective Method for the Isolation of *Haemophilus* in Material from the Respiratory Tract. Acta Pathol. Microbiol. Scand. 77:677-684.
12. Killian, M., J. Heine-Jensen, and P. Bulow, 1972. *Haemophilus* in Upper Respiratory Tract of Children.
13. Michaels, R.H., F.E. Stonebraker, and J.B. Robtham. 1975. Use of Antiserum Agar for the Detection of *Haemophilus Influenzae* Type B in the Pharynx. Pediatric Res. 9:513-516.
14. Chapin, K.J. and Doern, G., 1983. Selective Media for Recovery of *Haemophilus influenzae* from Specimens Contaminated with Upper Respiratory Tract Microbiol. Flora. J. of Clin. Micro. June 83, 17:P1163-1165.
15. National Committee for Clinical Laboratory Standards. Quality Assurance Standard for Commercially Prepared Microbiological Culture Media; Approved Standard. NCCLS publication M22-A3, Vol. 24 No.19 Table 2, 2004.

