



Chocolate No. 2 Agar Base

M1548

Chocolate No. 2 Agar Base, with supplements is recommended for the cultural isolation of *Neisseria* and *Haemophilus* species from a variety of clinical specimens.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	7.500
Meat extract	7.500
Sodium chloride	5.000
Dipotassium phosphate	4.000
Corn starch	1.000
Monopotassium phosphate	1.000
Agar	12.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 19 grams in 245 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add equal amount of sterile 2% Haemoglobin solution (FD022) (250 ml). Aseptically add rehydrated contents of one vial of Vitamino Growth Supplement, Modified (FD215). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Gonococci are fastidious organisms with exacting nutritional and environmental requirements (5). The cultivation medium for gonococci should ideally be a rich nutrient base with blood, either partially lysed or completely lysed. The diagnosis and control of gonorrhoea have been greatly facilitated by improved laboratory methods for detecting, isolating and studying *N. gonorrhoeae*.

Chocolate No.2 Agar is used for isolation and cultivation of fastidious microorganisms especially *Neisseria* and *Haemophilus* species from a variety of clinical specimens. (1,2) Casein enzymic hydrolysate and meat extract provides nitrogenous sources for growth of fastidious organisms. Cornstarch neutralizes toxic fatty acids that may be released during growth. The Vitamin Supplement has necessary growth factors, vitamins, aminoacids and coenzymes. (2) Dipotassium phosphate and monopotassium phosphate helps to maintain pH of medium whereas sodium chloride maintains osmotic equilibrium thereby maintaining integrity of cells.

The added supplements provides necessary X factor (from Haemoglobin) and V factor (from Growth Supplement) required by the fastidious organisms (3,4).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel

Colour and Clarity of prepared medium

Basal medium: Light amber coloured clear to slightly opalescent gel. After addition of haemoglobin : Chocolate brown coloured opaque gel forms in Petri plates.

Reaction

Reaction of 3.8% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

Please refer disclaimer Overleaf.

7.10-7.50

Cultural Response

M1548: Cultural characteristics observed with added 2% haemoglobin (FD022) and Vitamino Growth Supplement, Modified (FD215), after an incubation at 35-37°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
<i>Neisseria gonorrhoeae</i> ATCC 19424	50-100	luxuriant	>=70%
<i>Neisseria meningitidis</i> ATCC 5013090	50-100	luxuriant	>=70%
<i>Haemophilus influenzae</i> ATCC 19418	50-100	luxuriant	>=70%

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1. Carpenter and Morton, 1947, Proc. N.Y. State Assoc. Public Health Lab., 27:58
2. Carpenter et al. 1949, Am. J. Syphil. Gonorrh. Veneral Dis., 33:164
3. Martin, Billings, Hacney and Thayer. 1967. Public Health Rep, 82:361.
4. Vastine, Dawson, Hoshiwara, Yonega, Daghfous and Messadi. 1974. Appl. Microbiol. 28:688.
5. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), 1996, Mackie and McCartney, Practical Medical Microbiology, 14th Ed., Churchill Livingstone

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