



Chocolate No. 2 Agar Base

M1548

Intended Use:

Recommended for the cultural isolation of *Neisseria* and *Haemophilus* species from a variety of clinical specimens.

Composition**

Ingredients	g / L
Tryptone	7.500
HM extract#	7.500
Sodium chloride	5.000
Dipotassium hydrogen phosphate	4.000
Corn starch	1.000
Potassium dihydrogen phosphate	1.000
Agar	12.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat extract

Directions

Suspend 19.0 grams in 245 ml purified / 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% FO Growth Supplement (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 (1). 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 (2,3). Tryptone and HM extract provides nitrogenous sources for growth of fastidious organisms. Corn starch neutralizes toxic fatty acids that may be released during growth. The Vitamino Supplement has necessary growth factors, vitamins, aminoacids and coenzymes (3). Phosphates 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 (4,5).

Type of specimen

Clinical samples - Sputum ,Vaginal secretions, Penile secretions

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. It is an enriched medium on which mixed pathogenic bacteria may be grown with other nonpathogenic bacteria. For selective recovery of *N. gonorrhoeae* may be achieved using a selective supplement, FD024 is recommended.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

7.10-7.50

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery
<i>Neisseria gonorrhoeae</i> ATCC 19424	50-100	luxuriant	≥70%
<i>Neisseria meningitidis</i> ATCC 13090	50-100	luxuriant	≥70%
<i>Haemophilus influenzae</i> ATCC 19418	50-100	luxuriant	≥70%

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

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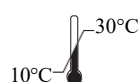
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