CHOCOLATE AGAR
(MODIFIED THAYER-MARTIN)

- For in vitro use only - Catalogue No. PT36

Our Chocolate Agar (Modified Thayer-Martin) is used for the selective isolation and cultivation of *Neisseria* species .

The first formulation of chocolate agar was devised in 1927 by McLeod, et al, which contained a combination of yeast extract and various peptones. Numerous modifications have since been made to chocolate agar to improve the recovery of *Neisseria* species and to increase its selectivity. Several key researchers which include Thayer, Martin, Lester, and Lewis devised formulations of chocolate agar which contained selective agents to help suppress the normal flora found in clinical specimens taken from the throat, vagina, rectum, and urethra.

Our formulation contains an improved casein and animal tissue digest that provide the organism with nitrogen, amino acids, and other elements essential for growth. *Neisseria* species are highly sensitive to toxic substances such as fatty acids; therefore the addition of cornstarch helps neutralize possible toxic metabolites, while potassium phosphate helps maintain an uniform pH during growth. Hemoglobin provides X-factor (hemin) required for growth of *Haemophilus* species on normal Chocolate Agar, and isovitox enrichment (Dalynn Catalogue No. VI85) provides V-factor (nicotinamide dinucleotide), cocarboxylase, and other complex compounds which enhance the growth of *Neisseria* species. Our Thayer-Martin supplement contains four antibiotics: vancomycin which inhibits gram-positive organisms, colistin which inhibits gram-negative bacteria, nystatin which inhibits yeasts and molds, and trimethoprim which is primarily added to inhibit swarming *Proteus* species.

Formula per Litre of Medium

Casein/Meat Peptone	15.0 g
Corn Starch	1.0 g
Potassium Phosphate, Dibasic	4.0 g
Potassium Phosphate, Monobasic	1.0 g
Sodium Chloride	5.0 g
Agar	10.0 g
Hemoglobin Solution (2%)	500 0 mL
Isovitox Enrichment	10.0 mL
Thayer Martin Supplement	10.0 mL

pH 7.2 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature.
2. Using an inoculum from the specimen, perform a four-quadrant streak to obtain well-isolated colonies. Alternatively, if the specimen is contained on a swab, roll the swab across part of the medium in a Z-pattern so that an adequate amount of the sample is transferred. Then taking a sterile loop streak the plate to disperse the sample throughout the medium.
3. Incubate at 35°C in a 5 to 10% CO_2 atmosphere.
4. Examine after 18-24 hours and again at 48 and 72 hours.

Interpretation of Results

Neisseria species grow while the majority of other organisms are inhibited. *N. gonorrhoeae* produces small, grey to white, mucoid colonies. *N. meningitidis* produces larger bluish-grey, mucoid colonies. Modified

Thayer-Martin Chocolate Agar is a selective, primary plating medium, therefore a subculture of potential *Neisseria* colonies onto a non-selective medium is necessary so that additional biochemical and/or serological tests can be performed from pure culture.

- *Chocolate Agar contains less agar than other solid media therefore streaking should be done carefully to avoid gouging into the agar*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Results
Neisseria gonorrhoeae ATCC 43069	Growth
Neisseria meningitidis ATCC 13090	Growth
Staphylococcus epidermidis ATCC 12228	Inhibition
Escherichia coli ATCC 25922	Inhibition
Candida albicans ATCC 10231	Inhibition

Storage and Shelf Life

Our Chocolate Agar (Modified Thayer-Martin) should be stored away from direct light at 4°C to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 8 weeks from the date of manufacture.

References

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