Modified Thayer Martin Medium Base (w/o Supplement) M795

Modified Thayer Martin Medium Base is used for selective isolation and enumeration of *Neisseria* species especially *Neisseria gonorrhoeae*

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	23.000
Starch	1.000
Sodium chloride	5.000
Dextrose	2.500
Agar	20.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51.5 grams in 900 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in 90 ml amounts in flasks and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45°C and aseptically add following sterile solutions:

1.20 ml blood lysed by heating at 55-56°C for 1 hour, to 90 ml medium.

2.Antibiotic solution to a final concentration of 3 mcg vancomycin per ml medium and 7.5 mcg colistin methane sulphate per ml medium.

Principle And Interpretation

The laboratory diagnosis of gonorrhoea depends on the demonstration of intracellular diplococci in smears and on the isolation and identification of *Neisseria gonorrhoeae* by culture procedures. Many different complex media have been introduced for the isolation of *Gonococcus* but excellent results may be obtained by using the medium introduced by Thayer and Martin. The original formula, an enriched chocolate agar medium containing the antibiotics ristocetin and polymyxin B, was recommended for the isolation of *N.gonorrhoeae* and *N.meningitidis* . However, the medium was found to be inhibitory against other Neisseriae and also suppressed *Pseudomonas* and *Proteus* species. Thayer and Martin reported the successful use of vancomycin, colistin methane and nystatin. This combination showed growth of *N.gonorrhoeae* while inhibiting the growth of staphylococci and saprophytic Neisseriae (1).

Carpenter and Morton reported an improved medium to isolate gonococci in 24 hours (2). Later on the efficiency of GC medium supplemented with haemoglobin and yeast concentrate was demonstrated for isolating gonococci (3). Subsequently Thayer and Martin Medium was developed for the primary isolation of *N.gonorrhoeae* and *N.meningitidis* from specimens containing mixed flora collected from throat, vagina, rectum and urethra (4,5). Thayer and Martin (5) used vancomycin, colistin and nystatin. Martin and Lester (6) used an additional antibiotic trimethoprim to make the medium selective.

Modified Thayer Martin Medium Base is used for selective isolation and enumeration of pathogenic *Neisseria* species especially *N.gonorrhoeae* . In 1947, an improved medium for isolating *Gonococcus* in 24 hours was reported by Carpenter and Morton (2).

Peptic digest of animal tissue provide nutrients to the organisms while starch neutralizes the toxic fatty acids if present in the agar. Addition of lysed blood after heating supplies vitamins ,amino acids, coenzymes etc. which enhances the growth of pathogenic *Neisseria* . Vancomycin and colistin methane sulphate inhibit gram-positive and gram-negative bacteria respectively (8). Some strains of Capnocytophaga species may grow on this medium when inoculated with oropharyngeal specimens (7).

Modified Thayer Martin Medium Base added with chocolate agar and antibiotics minimizes the overgrowth of gonococci and meningococci by contaminants, suppresses the growth of saprophytic *Neisseria* species and stimulates the growth of pathogenic *Neisseria* . Humidity is essential for successful isolation of gonococci. All presumptive Neisseriae should be confirmed by carbohydrate fermentation tests and serological tests. Some strains of Neisseriae may fail to grow in presence of antibiotics.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium
Basal medium: Light amber coloured clear to slightly opalescent gel. After addition of sterile lysed blood and supplements : Chocolate coloured opaque gel forms in Petri plates.

Reaction
Reaction of 5.15% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M795: Cultural characteristics observed on addition of blood with subsequent heating and antibiotic solution (3mcg Vancomycin &1.5 mcg Colistin methane sulphate per ml of medium) after an incubation at 35-37°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	-
Neisseria gonorrhoeae ATCC 19424	50-100	good-luxuriant	>=50%	small, grayish-white to colourless, mucoid
Neisseria meningitidis ATCC 13090	50-100	good-luxuriant	>=50%	medium to large, blue-gray, mucoid
Proteus mirabilis ATCC 25933	>=10^3	inhibited	0%	

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

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3.Carpenter C. M., Bucca M. A., Buck T. C., Casman E. P., Christensen C. W., Crowe E., Drew R., Hill J., Lankford C. E., Morton H. E., Peizer L. R., Shaw C. I., Thayer J. D., 1949, Am. J. Syphil. Gonorrh. Vener. Dis., 33:164.
4.Martin J. E., Billings T. E., Hackney J. F. and Thayer J. D., 1967, Public Hlth. Rep., 82:361.
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