

Diagnostic Thioglycollate Medium (Thioglycollate Medium w/o M191 Indicator)

Intended use:

Recommended for enrichment of blood cultures.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
Soya peptone	3.000
Dextrose (Glucose)	6.000
Sodium chloride	2.500
Sodium thioglycollate	0.500
L-Cystine	0.250
Sodium sulphite	0.100
Agar	0.700
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.05 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the medium in an upright position. For maintenance of viability of cultures, add small amount of calcium carbonate into the containers before filling.

Principle And Interpretation

Thioglycollate Medium without Indicator is a semisolid medium originally formulated by Brewer (1) for the growth of aerobic and anaerobic microorganisms (2,3). Previously methylene blue was incorporated in the medium as an Eh indicator but has been omitted now to enable recognition of early growth and avoids any toxic effects of indicator. This medium supports a minimal inoculum with early visibility of growth. Obligate aerobes grow at the top of the medium, while anaerobes grow at the bottom of the medium.

This medium is nutritious and favours the growth of *Clostridium butyricum*, *Campylobacter* species, *Bacteroides* species and Pneumococci etc. from minimal inocula. *Brucella* species which fail to grow in the presence of indicator, can grow in this medium. The broth with addition of 10% v/v serum may be used for cultivation of *Trichomonas vaginalis*.

It can also be used as transportation medium for which calcium carbonate is incorporated in the medium. Calcium carbonate neutralizes the acid produced during growth and avoid rapid growth and death of gram-negative cocci, *Clostridium perfringens* and other acid-sensitive bacteria.

Tryptone, Soya peptone, dextrose, L-cystine provides nitrogenous and carbonaceous compounds, fermentable carbohydrate and trace elements. Sodium thioglycollate serves as a reducing agent. The small amount of agar helps in anaerobiosis. The reducing action provided by sodium thioglycollate and sodium sulphite binds molecular oxygen, thereby maintaining a low Eh (4). A small amount of agar is added to retard the absorption of oxygen by reducing convection currents in the medium.

Type of specimen

Isolated microorganism from clinical samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). 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. Due to varying nutritional requirements, certain strain may show slow growth.

2. Further biochemical identification is necessary for confirmation.

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

Colour and Clarity of prepared medium

Light amber coloured very slightly opalescent, viscous solution.

Reaction

Reaction of 3.0% w/v aqueous solution at 25°C. pH : 7.0±0.2

pН

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth
<i>Bacteroides vulgatus</i> ATCC 8482	50-100	poor-fair
Clostridium sporogenes ATCC 11437	50-100	good-luxuriant
<i>Candida albicans</i> ATCC 10231 (00054*)	50-100	good-luxuriant
Bacillus subtilis subsp. spizizenii ATCC 6633(00003*)	50-100	good-luxuriant
<i>Micrococcus luteus</i> ATCC 10240	50-100	good-luxuriant
Neisseria meningitidis ATCC 13090	50-100	good-luxuriant
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant

Key: *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°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 (5,6).

Reference

- 1. Brewer J. H., 1940, J. Bacteriol., 39:10.
- 2. Hansen P. A., Price K. E. and Clements M. F., 1952, J. Bacteriol., 64:772.
- 3. Vera H. D., 1944, J. Bacteriol., 47:59-70.
- 4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.1 William and Wilkins, Baltimore.
- 5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
- 6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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