THIOGLYCOLLATE MEDIUM ALTERNATIVE

Dehydrated culture medium

1 - INTENDED USE

General purpose liquid medium for the cultivation of aerobic, anaerobic, microaerophilic bacteria. Suitable for the bacterial sterility test of turbid and viscous products.

2- COMPOSITION

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Tryptone	15.0 g
Yeast extract	5.0 g
Glucose	5.5 g
Sodium chloride	2.5 g
L-cystine	0.5 g
Sodium thioglycollate	0.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Brewer in 1940[1] formulated Fluid Thioglycollate Medium, subsequently to previous studies by Quastel and Stephenson in 1926[2] and by Falk, Bucca and Simmons[3] in 1939, focused on formulations that allowed microbial growth starting from low inocula and the growth of anaerobic bacteria in liquid media containing a low concentration of agar and reducing compounds.
Thioglycollate Medium Alternative, also known as NIH Thioglycollate Broth and USP Alternate Thioglycollate Medium, is an alternative formulation prepared without agar and resazurin. This formula conforms to the specifications given in the U.S. Pharmacopeia and the National Formulary[4,5]. The medium is suitable for testing the sterility of turbid and viscous products and for washing articles on which sterility of the lumen is tested, when the lumen is very small. The medium may be used for the cultivation of aerobic and facultative anaerobic bacteria and, when incubated under anaerobic conditions, for the cultivation of strict anaerobes.
Cystine and sodium thioglycollate, at a concentration with a low toxicity for microorganisms, act as reducing substances by reacting with and removing molecular oxygen from the medium and preventing accumulation of peroxides, which may be lethal to some aerobic and anaerobic microorganisms.[6] Sulfhydryl groups (SH) of the two compounds inactivate arsenic, mercury and other heavy metal compounds, maintaining a low redox potential and ensuring anaerobic conditions.[6] A ratio of 15 mL of liquid medium and 3 mL of inoculum is sufficient to inactivate the mercurial preservatives present at a concentration not greater than 0.08% in the sample. For the neutralisation of other preservatives, different from mercurial compounds, adequate dilutions of the sample are required.
Tryptone and yeast extract are sources of nitrogen, carbon, vitamins and minerals for microbial growth, glucose is a source of carbon and energy, sodium chloride maintains osmotic equilibrium.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 29 g in 1000 mL of cold purified water, heat to boiling with frequent agitation, distribute and sterilise by autoclaving at 121 °C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, clear
Final pH at 20-25 °C	7.1 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Thioglycollate Medium Alternative	Dehydrated medium	4021352	500 g (17.2 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, incubator and laboratory equipment as required, sterile loops and swabs, tubes, flasks, Erlenmeyer flasks, ancillary culture media and reagents.

8 - SPECIMENS

For collection and handling of samples intended for sterility test consult the appropriate reference.[5]

9 - TEST PROCEDURE

For general use, inoculate specimens directly into the medium and incubate tubes for up to 7 days at 35 ± 2 °C.
For specific applications, incubate at the temperature and for the time provided by Laboratory procedures and according to the cultivated microorganisms. To perform the sterility test with Thioglycollate Medium Alternative, USP recommendations should be followed.[5]

10 - READING AND INTERPRETATION

After incubation, the presence of bacterial growth is evidenced by the presence of turbidity compared to an un-inoculated control.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus	ATCC	25293	35°C / 72h /A	good growth
P. aeruginosa	ATCC	9027	35°C / 72h / A	good growth
B. subtilis	ATCC	6633	35°C / 72h / A	good growth
C. perfringens	ATCC	13124	35°C / 72 h / AN	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Thioglycollate Medium Alternative is tested for productivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of organisms in test tubes and incubating at 35°C for 72 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *C. perfringens* ATCC 13124, *C. sporogenes* ATCC 3584, *S. pyogenes* ATCC 12384, *S. pneumoniae* ATCC 6303, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *P. aeruginosa* ATCC 27853, *S. cerevisiae* ATCC 9763. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.

13 - LIMITATIONS OF THE METHOD

- It is recommended that medium be boiled prior to its use to enhance recovery rate. However, do not heat tubes more than once to drive off absorbed oxygen.[6]
- Fast-growing facultative anaerobic bacteria can grow in excess and mask the growth of strict anaerobes.
- Some anaerobes can be inhibited by the metabolic products or acids formed during the growth of fast-growing facultative anaerobic bacteria.
- Rapid death of bacteria may occur especially with Gram-negative cocci, *S.pneumoniae*, *C.perfringens* and other acid-sensitive organisms; if the subculture from tubes to plated media does not reveal microbial growth, perform a Gram staining from the broth culture.[6]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (tubes/bottles) and the storage method (temperature and packaging). According to MacFaddin the prepared tubes/bottles must be freshly prepared, boiled and cooled within 4 hours of use.[6]

16 - REFERENCES

1. Brewer JH. Clear liquid medium for the "aerobe" cultivation of anaerobes. J Am Med Assoc 1940; 115:598-600
2. Falk CR, Bucca HB, Simmons MP. A comparative study of the use of varying concentrations of agar in the test medium used to detect contaminants in biologic products. J Bacteriol 1939; 37:121-131.
3. Quastel JH, Stephenson M. Experiments on "strict" anaerobes: the relationship of B. sporogenes to oxygen. J Biochem 1926; 20:1125-1137.
4. N.I.H. Memorandum, Culture Media for Sterility Tests, 4th Revision (1955)
5. The United States Pharmacopeia/National Formulary, current edition
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

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TABLE OF APPLICABLE SYMBOLS

REF or **REF** Catalogue number	**LOT** Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/12

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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