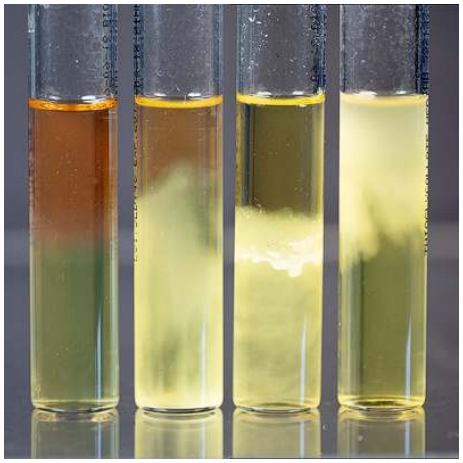
INSTRUCTIONS FOR USE

THIOGLYCOLLATE MEDIUM

Ready-to-use tubes



Thioglycollate Medium. From left: un-inoculated tube, facultative anaerobe (*S.aureus*), anaerobe (*B.fragilis*), strict aerobe (*P.aeruginosa*)

1 - INTENDED USE

In vitro diagnostic. General purpose liquid medium for the cultivation of aerobic, anaerobic, microaerophilic bacteria from clinical specimens and other materials. Suitable for the bacterial sterility test according to the harmonized method EP, USP, JP.

2 - COMPOSITION -TYPICAL FORMULA *

Tryptone	15.000 g
Glucose	5.500 g
Yeast extract	5.000 g
Sodium chloride	2.500 g
L-cystine	0.500 g
Sodium thioglycollate	0.500 g
Agar	0.750 g
Resazurin	0.001 g
Purified water	1000 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Thioglycollate Medium, also known as Fluid Thioglycollate Medium, is a liquid medium formulated by Brewer in 1940[1], 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 is prepared according to the formula specified in EP, USP, JP harmonized method[4] for sterility test of pharmaceutical products.

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.[5] Sulfhydryl groups (SH) of the two compounds inactivate arsenic, mercury and other heavy metal compounds, maintaining a low redox potential and ensuring anaerobic conditions.[5]

Agar, included at a concentration of 0.75%, aids in initialization of the growth of anaerobes and allows their growth from low inocula; it also retards the dispersion of CO_2, diffusion of oxygen and reducing substances; the small concentration reduces convection currents within the medium to enhance anaerobic condition in the lower portion of the tubed medium.[5] Resazurin is an oxidation-reduction indicator, being pink when oxidized and colourless when reduced, replacing the methylene blue present in Brewer's original formula. Casein peptone 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 - PHYSICAL CHARACTERISTICS

Medium appearance	pale yellow, clear when hot, slightly turbid at room temperature, with a pink ring
Final pH at 20-25°C	7.1 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Thioglycollate Medium	Ready-to-use tubes	552137	20 x 10 mL glass tubes, 17x125 mm, flat bottom, aluminium screw-cap. Packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Thioglycollate Medium may be used for the bacteriological processing of clinical specimens such as tissues, purulent exudates, wounds and abscess,[5,6] Collect clinical specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied. For samples collection and handling intended for sterility test consult the appropriate reference.[4]

8 - TEST PROCEDURE

Allow the tubes to come to room temperature.

Before use, if the tubes or bottles show a pink color (indicative of aerobic conditions) or the solution contains aggregates due to the presence of agar, it is necessary to partially unscrew the caps and place the tubes or bottles in boiling water for about 5 minutes until the medium has reduced (colorless) and the aggregates have dissolved.

Cool quickly, screwing the caps tightly, to avoid introducing non-sterile air inside.

For general use, inoculate specimens directly into the medium and incubate tubes for up to 7 days at 35 ± 2 °C.

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For specific applications, incubate at the temperature and for the time provided by Laboratory procedures and according to the cultivated microorganisms. For sterility testing, recommendations of EP[4] should be followed.

9 - READING AND INTERPRETATION

After incubation, the presence of bacterial growth is evidenced by the presence of turbidity compared to an un-inoculated control.
Obligate aerobes tend to grow on the upper portion of the broth in the oxidized pink layer, while anaerobes grow only in the lower, oxygen -deficient and pink-free portion of tubed broth. Micro-aerophilic grow in the middle portion of the broth.

10 - 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, the end user can perform its own Quality Control 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.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
B.fragilis **	ATCC	25285	35-37°C / 48h - A	good growth
*S.aureus***	ATCC	25293	35-37°C / 48 h -A	good growth
C.sporogenes *	ATCC	19404	35-37°C / 72h - A	good growth
P.aeruginosa *	ATCC	9027	35-37°C / 72h - A	good growth
S.aureus *	ATCC	6538	35-37°C / 72h - A	good growth
*B.subtilis**	ATCC	6633	35-37°C / 72h - A	good growth
C.perfringens ***	ATCC	13124	35-37°C / 18-24 h - A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection
* EP[4]; ** CLSI[8]; *** ISO 11133:2014/Amd 1:2018[9]

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready-to-use tubes of Thioglycollate Medium and of the raw material used for the production of prepared tubes (dehydrated Thioglycollate Medium REF 402137) 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, incubating at 35-37°C for 18-72 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.sporogenes* ATCC 19404, *P.aeruginosa* ATCC 9027, *S.aureus* ATCC 6538, *S.aureus* ATCC 25923, *B.subtilis* ATCC 6633, *C.albicans* ATCC 10231, *S.pyogenes* ATCC 12384, *C.perfringens* ATCC 13124, *B.fragilis* ATCC 25285. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.

12 - LIMITATIONS OF THE METHOD

- Do not boil media more than once, as frequent boiling may lead to toxic products forming in the medium.[5]
- 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 in Thioglycollate Medium 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.[5]
- Thioglycollate Medium should not be used for fermentation tests because it contains yeast extract which is high in carbohydrates content.[5]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use tubes 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each tube is for single use only; do not transfer or subdivide the tube content in other containers.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Ready-to-use tubes are subject to terminal sterilization by autoclaving.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the tubes inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostics.
- 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store tubes in their original pack at 2-25°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. After opening the box, the tubes can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g. microbial contamination, atypical colour, precipitate).

15 – REFERENCES

1. Brewer JH. Clear liquid medium for the “aerobe” cultivation of anaerobes. J Am Med Assoc 1940; 115:598-600
2. Falk Bucca and Simmons. J Bacteriol 1939; 37:121
3. Quastel and Stephenson. J Biochem 1926; 20:1125
4. European Pharmacopoeia, current edition.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. Baron EJ, Specimen Collection, Transport and Processing:Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015.
7. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve:World Health Organization
8. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.
9. ISO 11133:2014/Amd.1:2018 Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media. Amendment 1

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Do not reuse	Recyclable pack This side up
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2021/03
Revision 2	Removal of obsolete classification	2023/04
Revision 3	Update of analysis procedure and storage temperature in accordance with EP	2025/02

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