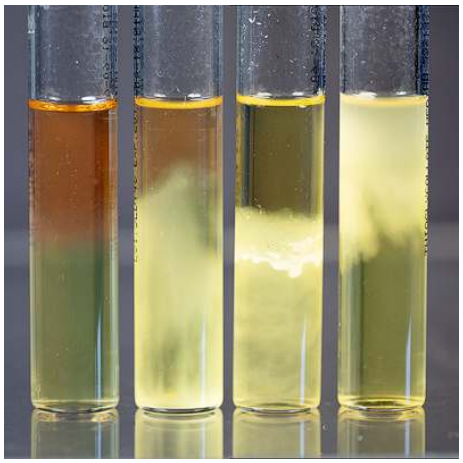
INSTRUCTIONS FOR USE

THIOGLYCOLLATE MEDIUM

Dehydrated culture medium



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 (AFTER RECONSTITUTION WITH 1 L OF WATER) *

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

*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; small concentration reduces convection currents within the medium to enhance condition in lower portion of 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. Thioglycollate Medium can be enriched with various compounds, including Vitamin K to stimulate the growth of some strains of *Bacteroides* and *Prevotella* and hemin, as a source of factor X, to increase microbial growth.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 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. Cool rapidly and store in the dark at room temperature. If necessary, heat in a water bath before use.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, clear when hot, slightly turbid at room temperature, with a pink ring indicating medium oxidation on top
Final pH at 20-25 °C	7.1 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Thioglycollate Medium	Dehydrated medium	4021372	500 g (16.7) CND: W0104010101; EDMA:14.01.01.01; RDM: 1875761/R
		4021374	5 kg (167) CND: W0104010101; EDMA:14.01.01.01; RDM: 1875770/R

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 for the identification of the bacteria.

8 - 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]

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9 - TEST PROCEDURE

Allow the tubes to come to room temperature.
Check the upper portion of each tubed medium prior to inoculation. If greater than one third of fluid column is oxidized indicated by a pink colour, discard the tube. If one-third or less is pink loosen the caps and boil in a water bath, without agitation, 5 minutes to drive off absorbed oxygen; tighten caps immediately after removing from heat and cool to room temperature before use. Do not boil more than once.
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. For sterility testing, recommendations of EP[4] should be followed.

10 - 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. Microaerobes grow in the middle portion of the broth.

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.

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]

12 - PERFORMANCES CHARACTERISTICS

Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of organisms in test tubes and incubating at 35-37°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.

13 - LIMITATIONS OF THE METHOD

- 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.
- If the tubes of Thioglycollate Medium are used for the preservation of bacteria, add approximately 0.1 g of calcium carbonate to each tube which neutralizes the acids produced during microbial growth, promotes bacterial vitality and the formation of spores, thus improving the shelf life of acid-sensitive bacteria.
- 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.

14 - 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.
- 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.
- 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 tubes 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 were damaged or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

16 - 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	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2020/07

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

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