Fluid Thioglycollate Medium BP

Intended Use
Fluid Thioglycollate Medium BP is a medium used for sterility testing of biologicals and cultivation of aerobes, anaerobes and microaerophiles in compliance with BP.

Summary
Falk, Bucca and Simmons showed the advantage of using small quantities of agar in detecting contaminants during sterility testing of biologicals. Brewer demonstrated that in a liquid medium containing 0.05% agar, anaerobes grew equally well in the presence or absence of sodium thioglycollate and therefore formulated Fluid Thioglycollate Medium for rapid cultivation of aerobes as well as anaerobes by adding a reducing agent and a small amount of agar. Fluid Thioglycollate Medium is recommended by European Pharmacopoeia, United State Pharmacopoeia , British Pharmacopoeia, APHA and the AOAC International sterility testing of antibiotics, biologicals and foods and for determining the phenol coefficient and sporicidal effect of disinfectants. However, it is intended for the examination of clear liquid or water-soluble materials.

Principle
Tryptone, yeast extract and L-cystine provide sources of nitrogen, carbon and other growth factors while dextrose is the carbohydrate source. Sodium chloride provides essential ions and maintains the osmotic balance. Sodium thioglycollate is a reducing agent, which prevents the accumulation of peroxides that is lethal to bacterial growth and neutralizes the antibacterial effect of mercurial preservatives. L-cystine is also a reducing agent, since it contains sulphydryl groups that inactivate heavy metal compounds, which exert a bacteriostatic effect in the materials under examination, and also maintains a low redox potential, thereby maintaining anaerobiosis. Resazurin is the oxidation-reduction indicator; increased oxidation raises the Eh, causing resazurin to change colour to red. The small amount of added agar assists in maintaining a low redox potential by stabilizing the medium, thereby maintaining anaerobiosis in the lower depths of the medium.

Formula*

Ingredients	**g/L**
Pancreatic Digest of Casein	15.0
Dextrose Monohydrate	5.5
Yeast Extract (Water soluble)	5.0
Sodium Chloride	2.5
Sodium Thioglycollate	0.5
L-Cystine	0.5
Resazurin	0.001
Agar	0.75
Final pH (at 25°C)	7.1 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2ºC-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Food and Dairy samples; Pharmaceutical samples

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 29.25 g of (the equivalent weight of dehydrated medium per litre) powder in 1000 mL purified / distilled water & mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Dispense as desired into containers.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
5. Tighten the lids of the container immediately (while still warm) to reduce oxidation.
6. Cool to 25°C and store in cool dark place preferably below 25°C.

Note: If more than the upper one third of the medium is pink prior to use, reheat once (100°C) in a water bath to drive off absorbed oxygen (till pink colour disappears.)

Quality Control

Dehydrated Appearance: Light yellow to yellow coloured, homogeneous, free flowing powder.
Prepared Appearance: Light straw coloured, very slightly opalescent solution with upper portion less than 10% medium turning pink on standing.
Growth Promotion Test: Growth promotion is carried out in accordance with the BP and growth is observed after an incubation at 30°C-35°C for ≤3 days.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time, inoculating 10 - 100 cfu (at 30°C-35°C for ≤ 3 days).

Organism (ATCC)	**Growth**
Staphylococcus aureus subsp. *aureus* (6538)	Good
Pseudomonas aeruginosa (9027)	Good
Kocuria rhizophila Strain PCI 1001 (9341)	Good
Bacteroides vulgatus (8482)	Good
Clostridium sporogenes (11437)	Good
Clostridium sporogenes (19404)	Good
Candida albicans 3147 (10231)	Good
Bacillus spizizenii (6633)	Good
Aspergillus brasiliensis WLRI 034(120) (16404)	Good

Note: Inoculum cfu for Good growth is 10 - 100.

Interpretation of Results

1. After incubation, growth is indicated by the presence of turbidity compared to an un-inoculated control.
2. Strict aerobes tend to grow in a thin layer at the surface of the broth; obligate anaerobes will grow only in the portion of the broth that is below the upper oxidized layer.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. Some dextrose fermenting organisms, which are able to reduce the pH of the medium to a critical level, may not survive in this medium. Early subculture is required to isolate these organisms.
2. In test samples, the proper surface to volume ratio of the medium must be maintained to avoid oxidation of the medium, which is unsuitable for microaerophilic and anaerobic growth.
3. A slight turbidity or haziness may be present due to the small amount of agar present in the medium. When the medium has been boiled, generally it appears clear.
4. Anaerobes can be overgrown by more rapidly growing facultative organisms. Gram stain and examine broth if plating medium reveals no growth.
5. Some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.
6. Do not rely on broth cultures exclusively for isolation of anaerobes.
7. Do not reheat the medium more than once as it may give rise to toxicity.

8. If more than upper one third of the medium has acquired a pink colour, the medium may be restored once by reheating in water bath or in free-flowing steam until the pink colour disappears.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Falk, Bucca and Simmons. 1939, J. Bact; 37:121.
2. Brewer, 1940, J. Am. Mad. Assoc; 115:598.
3. Downes and Ito (ed.) 2001, Compendium of Methods For The Microbiological Examination Of Foods, 4th edition, APHA Washington DC.
4. US Pharmacopeial Convention, Inc. 2001. The United States Pharmacopoeia 25/NF 20-2002. The US Pharmacopeial Convention, Inc; Rockville, Md.
5. British Pharmacopoeia, 2017, The Stationery Office British Pharmacopoeia
6. European Pharmacopoeia, 2012. European Department, for the Quality of Medicines.
7. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201060110500	Dehydrated Culture Media	500 g