BD BBL™ Fluid Thioglycollate Medium (FTM) • BD Fluid Thioglycollate Medium (FTM), Special • BD Fluid Thioglycollate Medium (FTM), Double Wrapped • BD Fluid Thioglycollate Medium (FTM), ETO • BD Fluid Thioglycollate Medium with 1% Polysorbate 80• BD Fluid Thioglycollate Medium with 0.5% Polysorbate 80, Sterile Pack

INTENDED USE

Fluid Thioglycollate Medium (FTM) is used for the sterility testing of biologics[1-3] and for the enrichment and cultivation of anaerobes, aerobes and microaerophiles. **Fluid Thioglycollate Medium, double wrapped,** and **Fluid Thioglycollate Medium, ETO**, are used for testing in sterile filling rooms. **Fluid Thioglycollate Medium with 1% Polysorbate 80** and **Fluid Thioglycollate Medium with 0.5% Polysorbate 80, Sterile Pack** are used for testing oils or materials containing lecithin in sterile fill rooms.

Note that this document is valid for all catalogue numbers of the products mentioned above.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Fluid Thioglycollate Medium was designed by Brewer for rapid cultivation of anaerobes as well as aerobes.[4] Incorporation of casein peptone was introduced by Vera in 1944.[5]
This medium is capable of supporting good growth of a great variety of fastidious organisms, of both pathogenic and nonpathogenic species. A feature of sodium thioglycollate, in addition to lowering the oxidation-reduction potential, is its ability to neutralize the antibacterial activity of mercurial compounds. These characteristics make FTM particularly useful for determining the presence of contamination in biological and other materials.

Glucose, peptone, L-cystine and yeast extract provide the growth factors necessary for bacterial replication. Sodium thioglycollate is a reducing agent that prevents the accumulation of peroxides which are lethal to some microorganisms. Resazurin is an oxidation-reduction indicator, being pink when oxidized and colorless when reduced. The small amount of agar assists in the maintenance of a low redox potential by stabilizing the medium against convection currents, thereby maintaining anaerobiosis in the lower depths of the medium. Due to its agar content, Fluid Thioglycollate Medium often appears slightly opaque. **Fluid Thioglycollate Medium (FTM), Special**, and all other FTM products except catalog numbers 257143, 257144, 257176 and 257206 are produced from a medium containing a special quality of agar, resulting in a higher clarity of the prepared medium. In **Fluid Thioglycollate Medium with 1% Polysorbate** and **Fluid Thioglycollate Medium with 0.5% Polysorbate 80, Sterile Pack**, Polysorbate 80 [= Polyoxyethylene (80) sorbitan monooleate] is included for testing oils or materials containing lecithin.[1-3]

The USP permits the presence of water in glucose (=dextrose) and therefore lists 5.5 grams in the formulation.[1] The presence of moisture being undesirable in dehydrated medium, the equivalent amount of glucose is incorporated in the anhydrous form.

The ready-to-use media described in this document are all filled under a stream of nitrogen gas, resulting in a decoloration of the resazurin indicator. However, the media may be used until approximately 30% of the medium (top layer) has been oxidized, as indicated by a pink color of the resazurin near the surface.

REAGENTS

Fluid Thioglycollate Medium (FTM) and **Fluid Thioglycollate Medium (FTM), Special**
Approximate Formula* Per Liter Purified Water

Yeast Extract	5.0 g	Agar	0.75
Pancreatic Digest of Casein	15.0	Resazurin	0.001
Glucose (anhydrous)	5.0	pH 7.1 ± 0.2	
L-Cystine	0.5		
Sodium Chloride	2.5		
Sodium Thioglycollate	0.5		

*Adjusted and/or supplemented as required to meet performance criteria

Fluid Thioglycollate Medium with 1% Polysorbate 80, in addition to the ingredients mentioned above, contains contains 10 g Polysorbate 80 [=Polyoxyethylene (80) sorbitan monooleate] per liter. **Fluid Thioglycollate Medium with 0.5% Polysorbate 80, Sterile Pack**, in addition to the ingredients listed above, contains 5 g Polysorbate 80 per liter.

Packaging and Sterility Information
The products mentioned in this document are sterilized by autoclaving in their final primary containers. For many of these products, a sterility claim is available on the Certificate of Analysis (**http:// reqdocs.bd.com** or **http://www.bd.com/europe/regulatory/**).

Each container of **Fluid Thioglycollate Medium, Double Wrapped, Fluid Thioglycollate Medium with 1% Polysorbate,** and **Fluid Thioglycollate Medium with 0.5% Polysorbate 80, Sterile Pack** is packaged in two Stericlin® bags before autoclaving, providing a sterile outer surface of the containers.
Fluid Thioglycollate Medium, ETO: after autoclaving the medium in the containers, the whole package unit is sealed in a Stericlin bag and is sterilized by ethylene oxide (=ETO) treatment, providing a sterile outer surface of the containers and package.
The multiple bags in these products allow the introduction of the containers into clean rooms from a non-sterile to a sterile area without the risk of contamination.

PRECAUTIONS

For laboratory use only
Do not use containers if they show evidence of microbial contamination e.g. turbidity, discoloration, drying, cracking, leakage or other signs of deterioration.
If Wide Mouth jars (closed with Twist-off screw caps) are opened, a popping sound must be perceived. Jars without this popping sound upon opening must be discarded.
The medium has a pale yellow to light amber appearance. The media may be used until approximately 30% of the medium (top layer) has been oxidized, as indicated by a pink color of the resazurin near the surface. Media that are completely pink or media with a brown discoloration must not be used.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store containers in the dark as stated on the label until just prior to use. Avoid freezing and overheating. The containers may be inoculated up to the expiration date and incubated for the recommended incubation times.

USER QUALITY CONTROL

Test samples with the organisms mentioned in the Table below. In order to achieve the USP and EP requirements, an inoculum of < 100 cfu per container must be used. Venting of the containers during incubation is mandatory to provide satisfactory growth of aerobes. Use venting needles (Blunt Filter needle, BD cat. no. 305211 or equivalent). Venting does not reduce the recovery of strict anaerobes since the small amount of agar and the reducing agents cystin and thioglycollate included in the medium provide a low redox potential and anaerobiosis

in the depth of the medium. Incubate the containers at 32.5 ± 2.5°C for a maximum of 3 days in air.
Risk of secondary contamination: Before inoculation, the outer surface of the bottles, especially the lid, cap, and/or stopper should be disinfected using a sporocidal disinfectant . Twist-off screw caps of Wide Mouth jars must be opened in a Laminar Airflow cabinet. Wear gloves that have been disinfected before opening the lid!

Test Strain	**Growth Results***	
Staphylococcus aureus ATCC 6538	Growth	
Pseudomonas aeruginosa ATCC 9027	Growth	
Clostridium sporogenes ATCC 19404	Growth	
Clostridium sporogenes ATCC 11437	Growth	
Appearance of uninoculated media:	Fluid Thioglycollate Medium (cat.nos. 257143, 257176 and 257206)	Slightly opalescent, pale to light amber, top layer (<10%) may be pink. Media containing Polysorbate 80 may have a slightly hazy appearance
	Fluid Thioglycollate Medium (cat. nos. 257293, 257485, 257422, 257246)	These products may contain inert particulate that is an inherent part of the cap liner.
	Fluid Thioglycollate Medium (all other cat. nos.)	Clear to very slightly opalescent, pale yellow to light amber, top layer (<10%) may be pink. Media containing Polysorbate 80 may have a very slightly hazy appearance

*Growth of bacteria depends on their relation to oxygen: strictly aerobic bacteria tend to grow near the surface of the medium, while facultative anaerobes will grow throughout the medium, and strict anaerobes will only grow in the deeper layers.

PROCEDURE

Materials Provided

BBL™ Fluid Thioglycollate Medium (FTM),
Fluid Thioglycollate Medium (FTM), Special,
Fluid Thioglycollate Medium (FTM), Double Wrapped,
Fluid Thioglycollate Medium (FTM), ETO,
Fluid Thioglycollate Medium with 1% Polysorbate,
Fluid Thioglycollate Medium with 0.5% Polysorbate 80, Sterile Pack

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Test Procedure

Risk of secondary contamination: Before inoculation, the outer surface of the bottles, especially the lid, cap, and/or stopper should be disinfected using a sporocidal disinfectant . Twist-off screw caps of Wide Mouth jars must be opened in a Laminar Airflow cabinet. Wear gloves that have been disinfected before opening the lid!

The FTM media described in this document can be used without further pretreatment. Since they are filled under a stream of nitrogen gas, venting of the containers during incubation is mandatory to provide satisfactory growth of aerobes. Use venting needles (BD Blunt Filter needle, cat. no. 305211 or equivalent).
Venting does not reduce the recovery of strict anaerobes since the small amount of agar and the reducing agents cystine and thioglycollate included in the medium provide a low redox potential and anaerobiosis in the depth of the medium. According to the Pharmacopeias, incubate the containers at 32.5 ± 2.5°C for a maximum of 3 days in air.

For sterility testing, recommendations of the United States Pharmacopeia (USP)[1] and various control agencies must be followed.[1-4] These reference sources specify the ratio of medium to product that should be utilized in sterility tests as well as details of sampling and test result interpretation. For sterility testing purposes, it is important that the medium in the test vessels is

not completely oxidized to ensure the growth of obligate anaerobes and microaerophilic organisms in the deeper layers of the broth. When used with automated filtration units, the pump speed should not be set to >50% since a higher pump speed may introduce too much oxygen into the medium; this may occasionally result in (chemical) precipitations in the medium and, possibly, in reduced recovery of anaerobes. Also, excessive shaking of the containers must be avoided.
If the test sample renders the medium so turbid that microbial growth cannot be easily recognized, transfers should be made to fresh medium and appropriate subcultures to solid media should be performed.
For general use, inoculate specimens or samples directly into the medium and incubate tubes for up to 7 days at 35 ± 2°C or at the optimal temperature of the organisms recovered.

Use with clinical specimens: only FTM media provided in small containers (e.g. cat. no. 257144) should be used with clinical specimens. Consider the specimen to broth ratio! If small specimen volumes are added into excessively large media volumes the detection may be delayed!
Usually, enrichment cultures should only be inoculated if specimens are derived from primarily sterile body sites. Note that some strict anaerobes require hemin and vitamin K for optimal growth. The medium may be supplemented with 10 mg of hemin hydrochloride per liter (prepare a tenfold stock solution in 0.1 N NaOH, filter sterilize, and add the appropriate amount to the vial) and 1 mg vitamin K1 per liter (prepare a tenfold stock solution in absolute ethanol, filter sterilize, and add the appropriate amount to the vial). Hemin and vitamin K stock solutions may be kept refrigerated for 4 weeks in the dark. Inoculate specimens directly into the medium and incubate tubes for up to 7 days at 35 ± 2°C. Note that clinical specimens should also be inoculated directly onto solid media, such as **BD Columbia Agar with 5% Sheep Blood** or **BD Trypticase Soy Agar II with 5% Sheep Blood** and, eventually, on additional selective and nonselective media. For the isolation and cultivation of strict anaerobes, **BD Schaedler Agar with Vitamin K1 and 5% Sheep Blood** should be used.

Results
After incubation, growth is evidenced by the presence of turbidity in the containers. In case of doubt, appropriate samples should be taken and subcultured onto plated media. This procedure must also be followed if the isolated organism(s) shall be further identified. Appropriate plated media and atmospheric conditions must be applied when subcultures for aerobes or anaerobes are set up.

LIMITATIONS OF THE PROCEDURE
Although most obligate aerobes (e.g. *Micrococcus, Pseudomonas* and related genera and strictly aerobic *Bacillus* species) will grow in the media described in this document (usually they grow near the surface), Fluid Thioglycollate Medium is not the optimal medium for recovery of strict aerobes, including aerobic fungi. For their recovery, Tryptic Soy Broth should be used.

For cultivation of strict aerobes, continuous venting during incubation is mandatory. Continuous venting will not reduce the cultivation of strict anaerobes if excessive shaking and mixing is avoided (see also below).

The capacity of Fluid Thioglycollate Medium to absorb and neutralize oxygen is limited. Repeated or strong oxidation by excessive shaking, mixing or fast pumping may exhaust the redox system of the vented medium. This may result in an irreversible brownish coloration which may show a reduced microbiological performance.

Strict anaerobes can be overgrown by more rapidly growing facultative organisms. Some strict anaerobes may be inhibited by acids or other metabolic products produced from more rapidly growing facultative organisms. Never rely on broth cultures exclusively for isolation of anaerobes.

Growth obtained in this medium must be subcultured onto suitable solid media to obtain pure cultures which afterwards can be identified with methods appropriate for the isolate(s).

REFERENCES

1. U.S. Pharmacopeial Convention, Inc. The U.S. Pharmacopeia /The national formulary *Current edition*. U.S. Pharmacopeial Convention, Inc., Rockville, Md
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4. Brewer, J.H. 1940. Clear liquid medium for the "aerobic" cultivation of anaerobes. J. Am. Med. Assoc. 115:598-600.
5. Vera, H.D. 1944. A comparative study of materials suitable for the cultivation of clostridia. J. Bacteriol. 47:59-70.
6. MacFaddin, J.F. 1985. Media for isolation-cultivation- identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.

PACKAGING/AVAILABILITY

For container types, fill volumes, package sizes, and for availability of these products, please contact your local BD representative.

FURTHER INFORMATION

For further information please contact your local BD representative.

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