BD™ Fluid Thioglycollate Medium (FTM)

INTENDED USE

BD Fluid Thioglycollate Medium is a general purpose liquid enrichment medium used in qualitative procedures for the sterility test and for the isolation and cultivation of aerobes, anaerobes and microaerophiles that are not excessively fastidious. In clinical microbiology, it may be used as an enrichment medium for clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Fluid Thioglycollate Medium was designed by Brewer for rapid cultivation of anaerobes as well as aerobes.[1] Incorporation of casein peptone was introduced by Vera in 1944.[2]

This medium is capable of supporting good growth of a great variety of organisms, including strict anaerobes, without incubation in an anaerobic atmosphere. 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. Because of its capacity for growth promotion, this formulation was adopted by The United States Pharmacopeia (USP), the AOAC, and the European Pharmacopeia (EP) as a sterility test and enrichment medium.[3-6] Fluid Thioglycollate Medium is also widely used as an enrichment medium in clinical microbiology, especially for specimens from primarily sterile body sites.[7]

Due to its low oxidation-reduction potential, **BD Fluid Thioglycollate Medium** is not the medium of choice for strict aerobes, such as nonfermenters, *Micrococcus*, and similar organisms. For such organisms, Tryptic Soy Broth or Brain Heart Infusion Broth should be used.

In **BD Fluid Thioglycollate Medium**, glucose, peptone, and yeast extract provide the growth factors necessary for bacterial growth. Sodium thioglycollate and L-cystine are reducing agent that prevent 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.[8] Due to its agar content, Fluid Thioglycollate Medium often appears slightly opaque.

The ready-to-use medium described in this document is filled under a stream of nitrogen gas, resulting in a decoloration of the reazurin 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. If oxidation has proceeded further, the broth may be reheated once in steam or boiling water, cooled and used.

The USP permits the presence of water in glucose (=dextrose) and therefore lists 5.5 grams in the formulation.[3] The presence of moisture being undesirable in dehydrated medium, the equivalent amount of glucose is incorporated in the anhydrous form.

REAGENTS

BD Fluid Thioglycollate Medium (FTM)

Formula* Per Liter Purified Water

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

pH 7.1 ± 0.2

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

PRECAUTIONS

IVD . For professional use only.
Do not use vials if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.
Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store vials in the dark at 2 to 8° C until just prior to use. Avoid freezing and overheating. The vials may be inoculated up to the expiration date (see container or package label) and incubated for the recommended incubation times.
Vials from opened packages can be used up to the expiration date. Opened vials must be used immediately.

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 10 to 100 cfu per container must be used.[3-5] According to the European Pharmacopeia, incubate the containers at 30 to 35°C for a maximum of 3 days in normal air. For optimal growth of strict aerobes, vials should be vented during incubation. This may be achieved by slightly loosening the caps.

Test Strain	Expected Growth Results (=Turbidity)*
Staphylococcus aureus ATCC™ 6538	+++ or higher
Micrococcus luteus ATCC 9341	++ or higher
Bacillus subtilis ATCC 6633	++ or higher
Pseudomonas aeruginosa ATCC 9027	+++ or higher
Clostridium sporogenes ATCC 19404	+++ or higher
Clostridium sporogenes ATCC 11437	+++ or higher
Bacteroides vulgatus ATCC 8482	+++ or higher
Uninoculated	Clear to slightly opalescent, light amber, top layer may be pink

* ++++ = opaque, heavy +++ = opalescent, heavy ++ = opalescent + = slight haze

PROCEDURE

Materials Provided

BD Fluid Thioglycollate Medium provided in vials (see **PACKAGING/AVAILABILITY** for details).

STERILE

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

BD Fluid Thioglycollate Medium can be used as an enrichment medium for all types of clinical and nonclinical specimens. 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. If such organisms are likely to be encountered, the medium can be supplemented with 10 mg hemin hydrochloride and 1 mg vitamin K1 (menadione) per liter.

Test Procedure

BD Fluid Thioglycollate Medium can usually be used without further pretreatment. If more than 30% of the medium has been oxidized before use (as is indicated by a pink color of the resazurin indicator), the medium may be reheated once for 5 min with caps slightly loosened in steam or boiling water, cooled and used. Since the medium is filled under a stream of nitrogen gas, venting of the containers during incubation will improve the growth of strictly aerobic bacteria. This may be achieved by slightly loosening the caps.

For use in clinical microbiology, 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. Supplementation is not necessary if a sufficient amount of blood or serum from the specimen is introduced into the vial during inoculation. Inoculate specimens directly into the medium and incubate tubes for up to 7 days at 35 ± 2°C. Note that 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. Since **BD Fluid Thioglycollate Medium** is not optimal for enrichment of certain strict aerobes, a second liquid enrichment medium, e.g., Brain Heart Infusion Broth, may be inoculated with the specimen.
For sterility testing, recommendations of the United States Pharmacopeia (USP) or European Pharmacopeia and various control agencies should be followed.[3-6] 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. If the test sample renders the medium so turbid that microbial growth cannot be easily recognized, transfers should be made to fresh medium.

Results
After incubation, growth is evidenced by the presence of turbidity in the tubes. In case of doubt, appropriate samples should subcultured onto plated media. This procedure should also be followed if the isolated organism(s) shall be further identified. If vented before or during incubation, obligate anaerobes will grow only in that portion of the broth below the oxidized (pink) top layer.
When used for the isolation of pathogens from clinical specimens, subculture a 10 to 50 µl portion of the incubated medium onto **BD Columbia Agar with 5% Sheep Blood** or **BD Trypticase Soy Agar II with 5% Sheep Blood** for the aerobes, and on **BD Schaedler Agar with Vitamin K1 and 5% Sheep Blood** or **BD CDC Anaerobe Agar with 5% sheep blood** for strict anaerobes. Note that appropriate atmospheric conditions must be provided for these subcultures.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE
BD Fluid Thioglycollate Medium is used as an enrichment medium in many nonclinical and clinical applications.[3-5,7,8] Due to its strongly reducing properties, it provides anaerobiosis without incubating in an anaerobic atmosphere. For optimal recovery of fastidious anaerobes, e.g., *Prevotella* spp., the medium should be supplemented with hemin and vitamin K1 (see **Test Procedure**).

Although most obligate aerobes (e.g. *Micrococcus, Pseudomonas* and related genera, and strictly aerobic sporeforming rods) will grow in this medium if the vials are vented during incubation (usually they grow as a thin film near its surface), Fluid Thioglycollate Medium is not the optimal medium for recovery of strict aerobes. For their recovery, Tryptic Soy Broth or Brain Heart Infusion Broth should be used.

Once reheated, FTM should not be heated again as this may reduce the microbiological performance of the medium.

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

REFERENCES
1. Brewer, J.H. 1940. Clear liquid medium for the "aerobic" cultivation of anaerobes. J. Am. Med. Assoc. *115*:598-600.

2. Vera, H.D. 1944. A comparative study of materials suitable for the cultivation of clostridia. J. Bacteriol. *47*:59-70.
3. U.S. Pharmacopeial Convention, Inc. 1999. The U.S. Pharmacopeia 24/The national formulary 19--2000. U.S. Pharmacopeial Convention, Inc., Rockville, Md
4. Council of Europe, 1996. European Pharmacopoeia, 3rd edition. European Pharmacopoeia Secretariat. Strasbourg/France.
5. Council of Europe, 2000. European Pharmacopoeia, Supplement 2001. European Pharmacopoeia Secretariat. Strasbourg/France.
6. Cunniff, P. (ed.). 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Gaithersburg, Md.
7. Thomson, R.B., and J.M. Miller. 2003. Specimen collection, transport, and processing: bacteriology. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
8. MacFaddin, J.F. 1985. Media for isolation-cultivation- identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.

PACKAGING/AVAILABILITY

BD Fluid Thioglycollate Medium

Cat. no. 257144 Ready-to-use Bottled Medium cpu 50; 20 ml in a 30 ml screw cap vial

FURTHER INFORMATION

For further information please contact your local BD representative.

BD Diagnostic Systems
Tullastrasse 8 – 12
D-69126 Heidelberg/Germany
Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16
Reception_Germany@europe.bd.com

BD Diagnostic Systems Europe
Becton Dickinson France SA
11 rue Aristide Bergès
38800 Le Pont de Claix/France
Tel: +33-476 68 3636 Fax: +33-476 68 3292 http://www.bd.com