QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

Fluid Thioglycollate Medium is a general-purpose medium for the cultivation of anaerobes, microaerophiles and aerobes, and is recommended as one of the media for the sterility testing of biologics.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Before use, loosen the caps and place the tubes in boiling water* for approximately 5 min until the medium is reduced (colorless). Tighten caps immediately after removing from heat. Allow medium to cool to room temperature.

      ***NOTE:** Use of a microwave oven is not recommended.

   b. From 24- to 48-h **Trypticase™** Soy Broth cultures or Enriched Thioglycollate Medium cultures for the *Bacteroides* and *Clostridium* strains, prepare a dilution containing 100 or less CFU/mL.
   c. Using sterile 1.0 mL pipettes, inoculate tubes with 0.75 mL of the dilutions.
   d. Incubate tubes with loosened caps at 30 – 35 °C in an aerobic atmosphere except for CLSI strains (*Bacteroides fragilis* ATCC™ 25285 and *Staphylococcus aureus* ATCC 25923) which should be incubated with tightened caps.
2. Examine tubes of the CLSI-recommended control strains (tightened caps) at 18 – 24 and 48 h for growth. Examine tubes of the other control strains (*USP*-recommended) for up to 3 days for growth.
3. Expected Results

CLSI Organisms	**ATCC®**	**Recovery**
**Bacteroides fragilis*	25285	Growth
**Staphylococcus aureus*	25923	Growth
Additional Organisms		
(*USP* Growth Promotion Test)		
***Staphylococcus aureus*	6538	Growth
***Pseudomonas aeruginosa*	9027	Growth
***Clostridium sporogenes*	11437	Growth
***Clostridium sporogenes*	19404	Growth
***Bacillus subtilis*	6633	Growth
***Kocuria rhizophila*	9341	Growth
***Bacteroides vulgatus*	8482	Growth

* Recommended organism strain for User Quality Control.

**For verification of growth promotion for use in *USP* Sterility Tests.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under “Product Deterioration.”
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.1 ± 0.2.
4. Incubate uninoculated representative tubes at 20 – 25 °C and 30 – 35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Fluid Thioglycollate Medium conforms with specifications of *The United States Pharmacopeia* (*USP*).

Fluid Thioglycollate Medium (FTM) is used for the sterility testing of biologics and for the cultivation of anaerobes, aerobes and microaerophiles.

V SUMMARY AND EXPLANATION

Fluid Thioglycollate Medium was designed by Brewer for rapid cultivation of anaerobes as well as aerobes.[1] It was first made available in dehydrated form by the Baltimore Biological Laboratory (BBL) in 1940. Incorporation of casein peptone was introduced by Vera in 1944.[2]

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. The BBL formula meets the requirements of the *USP* growth promotion test.[3]

Fluid Thioglycollate Medium may be used after its preparation until approximately 30% of the medium has been oxidized, as indicated by a pink color of the resazurin at the surface. If oxidation has proceeded further, the broth may be reheated once in steam or boiling water, cooled and used.

VI PRINCIPLES OF THE PROCEDURE

Dextrose, peptone, L-cystine and yeast extract provide the growth factors necessary for bacterial replication. Sodium chloride provides essential ions. Sodium thioglycollate is a reducing agent that prevents the accumulation of peroxides which are lethal to some microorganisms. The L-cystine is also a reducing agent, since it contains sulfhydryl groups which inactivate heavy metal compounds and maintain a low redox potential, thereby supporting anaerobiosis. 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.[4] The *USP* lists 5.5 g/L of dextrose in the formulation for Fluid Thioglycollate Medium. The **BBL** formula contains the anhydrous form of dextrose (5.0 g/L).

VII REAGENTS

Fluid Thioglycollate Medium

Approximate Formula* Per Liter Purified Water

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

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Caution should be exercised in reporting direct Gram stain and/or other direct microbiological stain results on tissue specimens processed with this medium due to the possible presence of nonviable organisms in the culture medium.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[5-8] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark per label directions. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[9,10] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Fluid Thioglycollate Medium

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Before use, loosen the caps and place the tubes in boiling water* for approximately 5 min until the medium is reduced (colorless). Tighten caps immediately after removing from heat. Allow medium to cool to room temperature.

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

For sterility testing, recommendations of the *USP3* and various control agencies should be followed.[11] 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 reduced to a sufficient degree to ensure the replication of obligate anaerobes and microaerophilic organisms. If the test sample renders the medium so turbid that microbial growth cannot be easily recognized, transfers should be made to fresh medium.

***NOTE:** Use of a microwave oven is not recommended.

User Quality Control: See "Quality Control Procedures."

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures.

X RESULTS

After incubation, growth is evidenced by the presence of turbidity compared to an uninoculated control. Strict aerobes tend to grow in a thin layer at the surface of the broth; obligate anaerobes will grow only in that portion of the broth below the upper oxidized (pink) layer. By carefully removing liquid from different levels, it is possible to enhance the ability to separate different species in a mixed culture.

XI LIMITATIONS OF THE PROCEDURE

Anaerobes can be overgrown by more rapidly growing facultative organisms. Examine and Gram stain broth if plating medium reveals no growth. Never rely on broth cultures exclusively for isolation of anaerobes. Some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.[12]

Culture media sometimes contain dead organisms derived from medium constituents, which may be visible in smears of culture media. Other sources of dead organisms visible upon Gram staining include staining reagents, immersion oil, glass slides and the specimens used for inoculation. If there is uncertainty about the validity of the Gram stain, the culture should be reincubated for another hour or two and the test repeated before a report is given.

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[9,10,13]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Fluid Thioglycollate Medium are tested for performance characteristics. Before inoculation, representative samples of the lot are reduced by boiling in a water bath for approximately 5 min. After cooling, the tubes are inoculated with 0.75 mL of cultures of *Bacillus subtilis* (ATCC 6633), *Bacteroides fragilis* (ATCC 25285), *B. vulgatus* (ATCC 8482), *Clostridium sporogenes* (ATCC 11437 and 19404), *Kocuria rhizophila* (ATCC 9341), *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538 and ATCC 25923). The inocula for *B. subtilis*, *B. fragilis*, *C. sporogenes*, *K. rhizophila*, *P. aeruginosa* and *S. aureus* are diluted to contain 100 or less colony-forming units (CFU) per mL. The inoculum for *B. vulgatus* is prepared from colonies grown on CDC Anaerobe 5% Sheep Blood Agar plates and adjusted in Thioglycollate Medium without Dextrose and Indicator to obtain 10–100 CFU/mL. The caps are tightened immediately after inoculation for tubes containing *B. fragilis* and *S. aureus*; caps of the remaining tubes are loosened. Tubes are incubated at 35 ± 2 °C. Tubes containing *B. fragilis* and *S. aureus* (ATCC 25923) show trace to heavy growth within 48 h incubation. Remaining organisms show moderate to heavy growth within 3 days incubation.

XIII AVAILABILITY

Cat. No.	Description
221195	**BD BBL™** Fluid Thioglycollate Medium, 8 mL, Pkg. of 10 size K tubes
221196	**BD BBL™** Fluid Thioglycollate Medium, 8 mL, Ctn. of 100 size K tubes
299802	**BD BBL™** Fluid Thioglycollate Medium, 8 mL, Ctn. of 100 size K tubes (ink-jet label)
220888	**BD BBL™** Fluid Thioglycollate Medium, 20 mL, Pkg. of 10 size A tubes
220889	**BD BBL™** Fluid Thioglycollate Medium, 20 mL, Ctn. of 100 size A tubes
299803	**BD BBL™** Fluid Thioglycollate Medium, 20 mL, Ctn. of 100 size A tubes (ink-jet label)

XIV REFERENCES

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