QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

Thioglycollate Medium without Indicator-135C is a general-purpose medium for the cultivation of microorganisms, especially obligate anaerobes.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Loosen the caps and place tubes in boiling water* for 2–5 min. Tighten the caps immediately after removing from the heat and allow the medium to cool to room temperature prior to use.
      ***NOTE:** Use of a microwave oven is not recommended.
   b. Using sterile 1.0 mL pipettes, inoculate tubes of Thioglycollate Medium with 1.0 mL of dilutions of 18- to 24-h broth cultures. Use Chopped Meat Carbohydrate Broth for *Bacteroides fragilis* and **Trypticase™** Soy Broth for *Staphylococcus aureus*. The dilution used for *S. aureus* should be 1,000 or less CFU/mL; the dilution for B. fragilis should contain 105–106 CFU/mL.
   c. Incubate tubes with tightened caps at 35 ± 2 °C in an aerobic atmosphere.
2. Examine tubes at 18–24 and 48 h for growth.
3. Expected Results

Organisms	ATCC®	Recovery
**Bacteroides fragilis*	25285	Growth
**Staphylococcus aureus*	25923	Growth

*Recommended organism strain for User Quality Control.

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 7.0 ± 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

Thioglycollate Medium without Indicator-135C is an enriched general-purpose medium for the recovery of a wide variety of microorganisms, particularly obligate anaerobes, from clinical specimens and other materials.

V SUMMARY AND EXPLANATION

Thioglycollate Medium was originally described by Brewer[1] as a medium favoring the growth of obligately anaerobic as well as aerobic organisms. The original thioglycollate medium was modified to have the nutritional quality of **Trypticase** Soy Broth. As a result the improved formula, 135C, has a broad growth spectrum of both pathogenic and nonpathogenic fastidious microorganisms.[2] The original formula also contained methylene blue, but no Eh indicator is now used. This avoids any possible toxicity of the indicator and also facilitates early detection of growth.

Thioglycollate Medium-135C is characterized by its superior ability to support growth, from minimal inocula, of a wide variety of aerobic and anaerobic organisms. The more strictly aerobic species grow at the top, while anaerobic types grow in the depths of the medium.

Thioglycollate Medium-135C is, therefore, recommended for use as a general utility medium, and for examination of blood cultures and all other materials in which the presence of a variety of aerobic, facultative or anaerobic organisms is possible.

The incorporation of the casein and soy peptones makes possible the growth of certain aerobic organisms, such as members of the genus *Brucella*, which do not grow readily in Fluid Thioglycollate Medium. Both media support growth of strictly anaerobic species, such as *Clostridium novyi*, *C. acetobutylicum*, *Actinomyces bovis* and *Bacteroides*, as well as facultative pneumococci, streptococci, lactobacilli and other bacteria.

The broth may be used with 10% added serum for cultivation of *Trichomonas vaginalis*, the Reiter spirochete and other organisms.

It is for this reason that the medium is satisfactorily used as an enrichment culture for various types of specimens and also as a transportation medium. When used for such purposes, it is recommended that $CaCO_3$ be incorporated, because otherwise fastidious organisms may grow and then die off rapidly; the $CaCO_3$ serves to neutralize acid produced during growth. Rapid growth and death, in the absence of $CaCO_3$ may occur, for example, with cultures of pneumococci, gram-negative cocci, *C. perfringens* and other acid-sensitive bacteria.

VI PRINCIPLES OF THE PROCEDURE

The casein and soybean meal peptones, dextrose and cystine supply nitrogenous and carbonaceous compounds, fermentable carbohydrate and trace ingredients. Sodium chloride provides essential ions. Sulfur is provided by sodium sulfite. Sodium thioglycollate, a reducing agent, lowers the Eh potential, thus enabling obligate anaerobic organisms to grow in the depths of the medium. The relatively small amount of agar aids in the prevention of convection currents in the medium and thus contributes to the maintenance of anaerobiosis.[3]

VII REAGENTS

Thioglycollate Medium without Indicator-135C

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	17.0 g	Sodium Thioglycollate	0.5 g
Papaic Digest of Soybean Meal	3.0 g	Agar	0.7 g
Dextrose	6.0 g	L-Cystine	0.25 g
Sodium Chloride	2.5 g	Sodium Sulfite	0.1 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"[4-7] 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 at 2–8 °C. Avoid freezing and overheating. Do not open until eady 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.[3,8] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Thioglycollate Medium without Indicator-135C

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

Test Procedure: Observe aseptic techniques.

Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18–24 h prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of the **BD GasPak™** EZ anaerobic system. Alternatively, liquid media may be reduced immediately prior to use by boiling*, with caps loosened, and cooling, with tightened caps, to room temperature before inoculation.

Inoculate the specimen into the medium as soon as possible after it is received in the laboratory. With liquid specimens, tubed media should be inoculated with one or two drops of the specimen. Tissue specimens should be minced and ground in sterile, reduced broth for the cultivation of microorganisms. Inoculation is then performed as for liquid specimens. Swab specimens may be inserted into the broth after inoculation of plated media. Alternatively, the swab may be "scrubbed" in a small volume of sterile, reduced broth and the broth used to inoculate media as performed with liquid specimens.

Specimens known or suspected to contain obligate anaerobes should be inoculated near the bottom of the tube.

Incubate tubes with tight caps aerobically at 35 ± 2°C, or other appropriate temperature depending on the type of organism being cultured, and inspect daily for up to 7 days before discarding as negative, unless special circumstances exist that warrant longer incubation.[3,9]

***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.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed media. The tip of the electrode should be placed below the surface of broth media.

X RESULTS

Growth in broth tubes is indicated by the presence of turbidity compared to an uninoculated control. Subcultures to appropriate solid media should be made to obtain pure cultures of isolates which can then be further tested and identified.

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.[9]

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.[3,8,9]

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.

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Thioglycollate Medium without Indicator-135C are tested for performance characteristics. Before inoculation, representative samples of the lot are reduced by boiling in a water bath for 2–5 min. After cooling, the tubes are inoculated with cultures of *Bacteroides fragilis* (ATCC 25285), *Clostridium novyi* (ATCC 7659) and *Staphylococcus aureus* (ATCC 25923). The inoculum (1 mL) for *S. aureus* is taken from a broth culture adjusted to contain 1,000 or less colony-forming units (CFU) per mL. The inoculum (1 mL) for *B. fragilis* is taken from a broth culture adjusted to contain 10^5–10^6 CFU per mL. The inoculum (0.01 mL loop) for *C. novyi* is taken from an undiluted broth culture; the inoculum is placed in the bottom of the tube. The caps are tightened immediately after inoculation and the tubes are incubated at 35 ± 2 °C. Tubes are read for the amount of growth after 18–24 h and 42–48 h. All organisms show trace to heavy growth after 48 h.

XIII AVAILABILITY

Cat. No.	Description
221199	**BD BBL™** Thioglycollate Medium without Indicator-135C, 8 mL, Pkg. of 10 size K tubes
221200	**BD BBL™** Thioglycollate Medium without Indicator-135C, 8 mL, Ctn. of 100 size K tubes U
221047	**BD BBL™** Thioglycollate Medium without Indicator-135C, 20 mL, Ctn. of 100 size A tubes

XIV REFERENCES

1. Brewer, J.H. 1940. A clear liquid medium for the “aerobic” cultivation of anaerobes. J. Bacteriol. *39*:10.
2. Vera, H.D. 1944. Comparative study of materials suitable for the cultivation of clostridia. J. Bacteriol. *47*:59-65.
3. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey & Scott’s diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis.
4. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
5. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. *17*:53-80.
6. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.
7. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
8. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
9. Reischelderfer, C., and J.I. Mangels. 1994. Culture media for anaerobes, p. 2.3.1-2.3.8. *In* H.D. Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.

Becton, Dickinson and Company
7 Loveton Circle
Sparks, MD 21152 USA

EC REP Benex Limited
Pottery Road, Dun Laoghaire
Co. Dublin, Ireland