BBL™ Thioglycollate Medium, Enriched (with Vitamin K_1 and Hemin), with Calcium Carbonate

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Enriched Thioglycollate Medium with Calcium Carbonate is a general-purpose medium for the cultivation of fastidious and nonfastidious microorganisms and for the maintenance of stock cultures.

II PERFORMANCE TEST PROCEDURE

1. Reduce tubes of the medium by boiling* with caps loosened. After boiling, tighten caps immediately and allow tubes to cool to room temperature.

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

   Use a 48- to 72-h culture of Enriched Thioglycollate Medium, Chopped Meat Medium, or colonies from a CDC Anaerobe 5% Sheep Blood Agar plate which have been transferred to a pre-reduced tube of Enriched Thioglycollate Medium, and adjust to a turbidity comparable to a 0.5 McFarland standard.
3. Using a sterile 0.01 mL calibrated loop, inoculate tubes from the standardized inoculum for each organism.
4. Incubate tubes at 35 ± 2°C in an aerobic atmosphere with tightened caps.
5. Examine tubes after 18 – 24 and 42 – 48 h for growth.
6. Expected Results

Organisms	ATCC™	Recovery
**Peptostreptococcus anaerobius*	27337	Growth
**Bacteroides vulgatus*	8482	Growth
**Clostridium perfringens*	13124	Growth
Clostridium novyi A	7659	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

Enriched Thioglycollate Medium with Calcium Carbonate is a general-purpose medium used in qualitative procedures for the cultivation of fastidious as well as nonfastidious microorganisms, including aerobic and anaerobic bacteria, from a variety of clinical and nonclinical materials. It is also recommended for the maintenance of stock cultures.

V SUMMARY AND EXPLANATION

Enriched Thioglycollate Medium is **BBL** Thioglycollate Medium without Indicator-135C supplemented with vitamin K_1 and hemin.[1-3] The enriched broth medium is recommended for use in the isolation and cultivation of fastidious or slow growing, obligately anaerobic microorganisms present in clinical materials.[4,5] It is also recommended for the isolation and cultivation of a wide variety of aerobic and facultatively anaerobic microorganisms. The medium is prepared with an anaerobic head space and is provided in screw-capped tubes in accordance with CDC recommendations.[4] Vitamin K_1 and hemin have been shown to be required by certain anaerobes for growth.[6,7]

Calcium carbonate enhances the maintenance of stock cultures by neutralizing acids produced during growth.[8]

The isolation of microorganisms from clinical materials frequently requires the use of enriched broth media in addition to the selective, differential and nonselective plated media normally used for primary isolation. The use of liquid "back up" media reduces the possibility of completely missing an etiological agent that is present in low numbers, slow growing on plated media, susceptible to selective agents, or sensitive to unfavorable incubation conditions; i.e., insufficient anaerobiosis for optimal growth of obligate anaerobes.

VI PRINCIPLES OF THE PROCEDURE

Casein and soy peptones provide amino acids and other nitrogenous substances to support bacterial growth. Yeast extract provides the B-complex vitamins. Sodium chloride provides essential ions. Dextrose is source of energy.

The reducing action provided by sodium thioglycollate and sodium sulfite binds molecular oxygen, thereby removing it from the medium to maintain a low Eh.[9] A small amount of agar is added to retard the absorption of oxygen by reducing convection currents in the medium.[9]

Vitamin K_1 is a growth requirement for some strains of *Prevotella melaninogenica*[6] and is reported to enhance the growth of some strains of *Bacteroides* species and gram-positive nonsporeformers.[10] Hemin is the source of the X factor which stimulates the growth of many microorganisms.

The incorporation of calcium carbonate is recommended because otherwise fastidious organisms may grow and then die off rapidly; it serves to neutralize acid produced during growth.[8,11]

VII REAGENTS

Enriched Thioglycollate Medium with Calcium Carbonate

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	12.0 g	Agar	0.7 g
Papaic Digest of Soybean Meal	3.0 g	L-Cystine	0.25 g
Dextrose	6.0 g	Sodium Sulfite	0.1 g
Yeast Extract	5.0 g	Hemin	0.005 g
Sodium Chloride	2.5 g	Vitamin K_1	0.001 g
Sodium Thioglycollate	0.5 g	Marble Chip	1 per tube

*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"[12-15] 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 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.[16,17] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Enriched Thioglycollate Medium with Calcium Carbonate

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 condition 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 media of choice as soon as it arrives 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 such as Enriched Thioglycollate Medium with Calcium Carbonate 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.

Broth cultures should be held at least 1 week before discarding as negative.[8]

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

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

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, such as Enriched Thioglycollate Medium with Calcium Carbonate, is demonstrated by the appearance of turbidity when compared to an uninoculated control.

If growth is detected, cultures should be examined by Gram staining and subcultured onto selective and nonselective plated media. If anaerobes are suspected, subcultures should also be made to appropriate anaerobic plated media.

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.[8]

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.[16-18]

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 Enriched Thioglycollate Medium with Calcium Carbonate are tested for performance characteristics. Before inoculation, representative samples of the lot are reduced by boiling in a water bath for a minimum of 10 minutes and cooled. Using a 0.01 mL calibrated loop, tubes are inoculated with cultures that have been adjusted to a 0.5 McFarland turbidity standard. The inocula for *Porphyromonas levii* (ATCC 29147), *Clostridium perfringens* (ATCC 13124) and *Peptostreptococcus anaerobius* (ATCC 27337) are prepared from colonies grown on CDC Anaerobe 5% Sheep Blood Agar plates and adjusted to the correct inoculum concentration in pre-reduced Thioglycollate Medium, Enriched. The inoculum for *Bacteroides vulgatus* (ATCC 8482) is taken from Thioglycollate Medium, Enriched and the inoculum for *C. novyi* (ATCC 7659) is taken from Chopped Meat Glucose Broth, PR II. Tubes are inoculated below the surface of the broths as deeply into the medium as possible. The caps are tightened immediately after inoculation and the tubes are incubated aerobically 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
297264	**BBL™** Enriched Thioglycollate Medium with Calcium Carbonate, Ctn. of 100 size K tubes

XIV REFERENCES

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