QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

GN (Gram Negative) Broth is a selective enrichment medium for the cultivation of gram-negative enteric organisms.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Using sterile disposable 0.01 mL calibrated loops, inoculate tubes with 10^{-1} dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures.
   b. Incubate tubes with loosened caps at 35 ± 2 °C in an aerobic atmosphere.
2. After 18–24 h of incubation, subculture all tubes to MacConkey II Agar plates. Incubate plates aerobically at 35 ± 2 °C for 18–24 h and observe for growth.
3. Expected Results

CLSI Organisms	ATCC®	Recovery on MacConkey II Agar Plates
**Salmonella enterica* subsp. *enterica serotype* Typhimurium	14028	Growth
**Shigella sonnei*	9290	Growth
**Escherichia coli*	25922	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. 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

GN Broth is used for the selective enrichment of *Salmonella* and *Shigella*.

V SUMMARY AND EXPLANATION

GN (Gram Negative) Broth was developed by Hajna as an enrichment medium for the recovery of *Salmonella* and *Shigella* from clinical specimens.[1,2] Croft and Miller succeeded in isolating more *Shigella* strains by use of this medium, rather than by direct streaking.[3] Taylor and Schelhart reported that GN Broth enhanced the isolation of enteric pathogens, producing a 53% increase in *Shigella* and 36% increase in *Salmonella* as compared to direct streaking.[4]

GN Broth currently is recommended for use in the microbiological examination of foods.[5]

VI PRINCIPLES OF THE PROCEDURE

Enzymatic digests of casein and animal tissue provide amino acids and other nitrogenous substances to support bacterial growth. Mannitol and dextrose are sources of energy. Mannitol is provided in a higher concentration than dextrose to enhance the growth of mannitol-fermenting species, such as *Salmonella* and *Shigella*, and limit the growth of *Proteus* and other dextrose-fermenting bacteria. Phosphate buffers are incorporated to maintain the pH of the medium. Sodium chloride maintains osmotic equilibrium. Sodium citrate and sodium desoxycholate are added to inhibit gram-positive and some gram-negative bacteria.

VII REAGENTS

GN Broth

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	10.0 g	Sodium Desoxycholate	0.5 g
Peptic Digest of Animal Tissue	10.0 g	Dipotassium Phosphate	4.0 g
Dextrose	1.0 g	Monopotassium Phosphate	1.5 g
D-Mannitol	2.0 g	Sodium Chloride	5.0 g
Sodium Citrate	5.0 g		

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

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

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"[6-9] 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.[10,11] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: GN Broth

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

Test Procedure: Observe aseptic techniques.

Inoculate the broth as soon as possible after the specimens arrive at the laboratory. Swab specimens may be inserted directly into the broth. For stool specimens, use 1 g of feces or 1 mL of liquid stool per tube. Consult appropriate references for information about the processing and inoculation of other clinical specimens or food samples.[5,10-12]

Incubate the tubes with loosened caps at 35 °C and subculture onto selective and differential media after 6–8 h of incubation and again after 18–24 h of incubation.[13]

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

Growth in broth media is indicated by turbidity compared to an uninoculated control. Subculture onto appropriate selective and differential media to isolate pathogens for identification.

XI LIMITATIONS OF THE PROCEDURE

Enrichment broths should not be used as the sole isolation medium. They are to be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens, especially when they may be present in small numbers. Consult texts for detailed information and recommended procedures.[5,10-12]

XII PERFORMANCE CHARACTERISTICS

In a study by Taylor and Schelhart, a comparison of three enrichment broths (GN, Selenite and Silliker's) and three plating media (EMB, SS and XLD) was performed to determine a combination of media that would improve shigellae detection.[14] A total of 1,405 stool specimens were tested during this study, with a distribution of 158 salmonellae and 49 shigellae isolates observed. The enrichment broths provided a two-fold increase in isolation of both salmonellae and shigellae over the plated media. All broths performed equally well for salmonellae detection, but GN and Silliker's broths detected twice as many shigellae isolates as did Selenite broth.

XIII AVAILABILITY

Cat. No.	Description
221729	**BD BBL™** GN Broth, 8 mL
221730	**BD BBL™** GN Broth, 8 mL

XIV REFERENCES

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