



## QUALITY CONTROL PROCEDURES

## I INTRODUCTION

Todd Hewitt Broth with Gentamicin and Nalidixic Acid is used for the selective enrichment of group B streptococci (*Streptococcus agalactiae*).

## II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with the cultures listed below.
  - Using sterile 1.0 mL pipettes, inoculate tubes with 1.0 mL of dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures. The dilution used should contain 1,000 or less CFU/mL for *S. agalactiae* and  $1.0 \times 10^5$  CFU/mL for *E. coli*.
  - Incubate tubes with loosened caps at  $35 \pm 2$  °C in an aerobic atmosphere.
- Examine tubes for up to 3 days for growth.
- Expected Results

Organisms	ATCC®	Recovery
* <i>Streptococcus agalactiae</i>	12386	Growth
* <i>Escherichia coli</i>	25922	No growth

\*Recommended organism strain for User Quality Control.

## III ADDITIONAL QUALITY CONTROL

- Examine tubes as described under "Product Deterioration."
- Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification of  $7.8 \pm 0.3$ .
- 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

Todd Hewitt Broth with Gentamicin and Nalidixic Acid is used for the selective enrichment of group B streptococci (*Streptococcus agalactiae*), especially from genital specimens.

## V SUMMARY AND EXPLANATION

Since its emergence in the 1970s, neonatal group B streptococcal disease has become the major infectious cause of illness and death among newborns. Prior to 1994, an estimated 7,600 episodes of invasive group B streptococcal disease, primarily sepsis and meningitis, occurred in newborns each year in the United States, with approximately 80% of those episodes representing early-onset disease occurring within the first week of life.<sup>1</sup> The disease is spread to newborns through vertical transmission from a mother who carries group B streptococci in her anorectum or genital tract.

The Centers for Disease Control and Prevention (CDC) has proposed guidelines for screening and use of intrapartum chemoprophylaxis for prevention of neonatal group B streptococcal disease.<sup>2</sup> The use of Todd Hewitt Broth with Gentamicin and Nalidixic Acid (or Lim Broth) is recommended to maximize the likelihood of recovering group B streptococci upon plating on sheep blood agar.<sup>2,3</sup>

Group B streptococci have also been found in cases of sepsis in nonparturient women and in men, and in joint infections, osteomyelitis, urinary tract infections and wound infections. They are associated with endocarditis, pneumonia, and skin and soft tissue infections in compromised patients.<sup>4</sup>

## VI PRINCIPLES OF THE PROCEDURE

Todd Hewitt Broth is a general-purpose medium primarily used for the cultivation of  $\beta$ -hemolytic streptococci, especially for serologic studies.<sup>5,6</sup> The medium is highly nutritious due to its content of peptones, dextrose and salts. Dextrose stimulates hemolysin production. Sodium phosphate and sodium carbonate provide buffering action to counteract the acidity produced during fermentation of dextrose, thereby protecting the hemolysin from inactivation by the acid.<sup>7</sup>

Selectivity for group B streptococci is obtained by the inclusion of gentamicin and nalidixic acid in the medium. Selective enrichment broths include the advantages of both enrichment and selection by providing conditions conducive to the growth of group B streptococci while inhibiting the growth of contaminants.

## VII REAGENTS

## Todd Hewitt Broth with Gentamicin and Nalidixic Acid

Approximate Formula\* Per Liter Purified Water

Heart Infusion from (solids) .....	3.1 g	Sodium Phosphate .....	0.4 g
Peptone .....	20.0 g	Sodium Carbonate .....	2.5 g
Dextrose .....	2.0 g	Gentamicin .....	8.0 mg
Sodium Chloride .....	2.0 g	Nalidixic Acid .....	15.0 mg

\*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"<sup>8-11</sup> 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.<sup>4,12</sup> Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

## IX PROCEDURE

**Material Provided:** Todd Hewitt Broth with Gentamicin and Nalidixic Acid

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

**Test Procedure:** Observe aseptic techniques.

Inoculate tubes and incubate with loosened caps at 35 ± 2 °C for 18–24 h in an aerobic atmosphere with or without added carbon dioxide.

If turbidity is observed, subculture from the broth culture to a sheep blood agar plate; otherwise, incubate an additional 24 h before discarding.<sup>3,4,13</sup>

**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 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 medium is indicated by the presence of turbidity compared to an uninoculated control.

Subculture to a **Trypticase** Soy Agar with 5% Sheep Blood (TSA II) plate and incubate for 18–24 h, or up to 48 h if necessary. Identify organisms suggestive of group B streptococci (β- or non-hemolytic, gram-positive and catalase negative). Specific identification may be performed; e.g., using streptococcal grouping sera, the CAMP test or other procedures.

## XI LIMITATIONS OF THE PROCEDURE

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.<sup>4,12,14</sup>

## XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Todd Hewitt Broth with Gentamicin and Nalidixic Acid are tested for performance characteristics. Representative samples of the lot are inoculated with 1.0 mL of *Streptococcus agalactiae* (ATCC 12386) diluted to contain 1,000 or less colony-forming units (CFU) per mL and 1.0 mL of *Escherichia coli* (ATCC 25922) diluted to contain 10<sup>5</sup> CFU/mL. The tubes with loosened caps are incubated at 35 ± 2 °C. *S. agalactiae* shows moderate to heavy growth within 3 days while *E. coli* is inhibited after 3 days incubation.

## XIII AVAILABILITY

Cat. No.	Description
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299486	BD BBL™ Todd Hewitt Broth with Gentamicin and Nalidixic Acid, Ctn. of 100 size K tubes
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## XIV REFERENCES

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