



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Todd Hewitt Broth primarily is used for the growth of beta-hemolytic streptococci for use in serological testing.

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.
 - Incubate tubes with loosened caps at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere.
- Examine tubes for up to 3 days for growth.
- Expected Results

Organisms	ATCC™	Recovery
* <i>Streptococcus pyogenes</i>	19615	Growth
* <i>Streptococcus pneumoniae</i>	6305	Growth
* <i>Streptococcus agalactiae</i>	12386	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 ± 0.2 .
- Incubate uninoculated representative tubes at $20\text{--}25^\circ\text{C}$ and $30\text{--}35^\circ\text{C}$ and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Todd Hewitt Broth is a general-purpose medium which primarily is used for the cultivation of beta-hemolytic streptococci, especially for serological studies.

V SUMMARY AND EXPLANATION

Todd Hewitt Broth originally was developed for use in the production of streptococcal hemolysin.¹ The modification of Updyke and Nickle² is used for the growth of beta-hemolytic streptococci for use in fluorescent antibody test procedures³ and for serological typing based on the production of type-specific M protein.⁴

VI PRINCIPLES OF THE PROCEDURE

This medium is highly nutritious due to its content of peptones, dextrose and salts. Dextrose stimulates hemolysin production. Disodium phosphate and sodium carbonate provide buffering action to counteract the acidity produced during the fermentation of the dextrose, thereby protecting the hemolysin from inactivation by the acid.⁴

VII REAGENTS

Todd Hewitt Broth

Approximate Formula* Per Liter Purified Water

Heart Infusion from (solids)	3.1 g	Sodium Chloride	2.0 g
Peptonen	20.0 g	Sodium Phosphate	0.4 g
Dextrose	2.0 g	Sodium Carbonate	2.5 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.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. 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 $2\text{--}25^\circ\text{C}$. Avoid freezing and overheating. Do not open until ready to use. 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. Minimize exposure to light.

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.^{5,6} 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

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

Test Procedure: Observe aseptic techniques.

Incubate throat swabs in loosely capped tubes of Todd Hewitt Broth at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere, with or without added carbon dioxide, for 2–5 h prior to use in fluorescent antibody procedures for the identification of group A streptococci.

Incubation may be continued for approximately 24 h prior to streaking for isolation on blood agar plates. Pure cultures of streptococci may be cultured in Todd Hewitt Broth prior to the preparation of extracts for serological typing.

Consult appropriate references for specific serological test procedures.^{3,7}

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.

X RESULTS

Refer to the appropriate references for the methods of utilization of streptococcal cultures propagated in Todd Hewitt Broth for serological procedures.^{3,7}

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.^{5,6,8}

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 Todd Hewitt Broth are tested for performance characteristics. Representative samples of the lot are inoculated with 1.0 mL of cultures diluted to contain 1,000 or less Colony Forming Units (CFU) per mL of *Streptococcus agalactiae* (ATCC 12386), *S. pneumoniae* (ATCC 6305) and *S. pyogenes* (ATCC 19615). The tubes with loosened caps are incubated at 35 ± 2 °C. All organisms show moderate to heavy growth within 3 days.

XIII AVAILABILITY

Cat. No.	Description
221713	BD BBL™ Todd Hewitt Broth, 5 mL, Pkg. of 10 size K tubes
221714	BD BBL™ Todd Hewitt Broth, 5 mL, Ctn. of 100 size K tubes
297778	BD BBL™ Todd Hewitt Broth, 0.5 mL, Pkg. of 10 size K tubes

XIV REFERENCES

1. Todd, E.W., and L.F. Hewitt. 1932. A new culture medium for the production of antigenic streptococcal haemolysin. J. Pathol. Bacteriol. 35:973-975.
2. Updyke, E.L., and M.I. Nickle. 1954. A dehydrated medium for the preparation of type specific extracts of group A streptococci. Appl. Microbiol. 2:117-118.
3. Jones, G.L., G.A. Hebert, and W.B. Cherry. 1978. Fluorescent antibody techniques and bacterial applications, HEW Publication (CDC) No. 78-364, Center for Disease Control, Atlanta.
4. MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
6. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
7. Facklam, R.R., and J.A. Washington II. 1991. *Streptococcus* and related catalase-negative gram-positive cocci, p. 238-257. In A. Balows, W.J. Hausler, Jr., K.L. Herrmann, H.D. Isenberg, and H.J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
8. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

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