

QUALITY CONTROL PROCEDURES

I INTRODUCTION

Lim Broth is used for the selective enrichment of group B streptococci (*Streptococcus agalactiae*).

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. 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 1.0×10^5 CFU/mL for *E. coli*.
 - b. Incubate tubes with loosened caps at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere.
2. Examine tubes for up to 3 days for growth.
3. Expected Results

Organisms	ATCC™	Recovery
* <i>Streptococcus agalactiae</i>	12386	Growth (moderate to heavy)
* <i>Escherichia coli</i>	25922	Inhibition (partial to complete)

*Recommended organism strain for User Quality Control.

NOTE: This medium is exempt from User QC testing according to CLSI M22-A3.

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 7.8 ± 0.2 .
4. Incubate uninoculated representative tubes at $20 - 25^\circ\text{C}$ and $30 - 35^\circ\text{C}$ and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Lim Broth 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.¹ The disease is spread to newborns through vertical transmission from a mother who carries group B streptococci in her anorectum or genital tract. Lim and colleagues combined the use of an enriched, selective broth medium and slide coagglutination test to rapidly screen such maternity patients.²⁻⁵

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.⁶ The use of Todd Hewitt Broth with colistin and nalidixic acid is a medium recommended to maximize the likelihood of recovering group B streptococci upon plating on sheep blood agar.¹ Lim Broth is prepared from Todd Hewitt Broth by the addition of colistin and nalidixic acid, at the recommended concentrations, plus yeast extract for enhanced growth of group B streptococci.²

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.⁷

VI PRINCIPLES OF THE PROCEDURE

Todd Hewitt Broth base is a general-purpose medium primarily used for the cultivation of β -hemolytic streptococci, especially for serologic studies.⁸

The peptones, dextrose and salts provide an excellent nutritional base for the growth of streptococci. The added yeast extract is a rich source of B-complex vitamins. Dextrose stimulates hemolysin production. Disodium phosphate and sodium carbonate provide buffering action to counteract the acidity produced during the fermentation of the carbohydrate, thereby protecting the hemolysin from inactivation by the acid. Nalidixic acid and colistin suppress growth of gram-negative bacteria.

VII REAGENTS

Lim Broth

Approximate Formula* Per Liter Purified Water

Heart Infusion from (solids)	3.1	g
Peptonen	20.0	g
Dextrose	2.0	g
Sodium Chloride	2.0	g
Disodium Phosphate.....	0.4	g
Sodium Carbonate	2.5	g
Yeast Extract	10.0	g
Colistin.....	0.01	g
Nalidixic Acid.....	0.015	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"⁹⁻¹² 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.^{7,13} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Lim Broth

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 in an aerobic atmosphere with or without added carbon dioxide. If desired, perform a slide coagglutination test for group B streptococci after 5 h of incubation.⁴

If turbidity is observed after 18 – 24 h, subculture from the broth culture to a sheep blood agar plate; otherwise, incubate an additional 24 h before discarding.¹

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 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 cocci and catalase-negative). Specific identification may be performed; e.g., using streptococcal grouping sera, the CAMP test or other procedures.

Jones et al. reported detection of group B streptococci by slide coagglutination after 5 h of incubation when the concentration of organisms in the culture was 10⁷ per mL or greater.⁴

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.^{7,13,14}

XII PERFORMANCE CHARACTERISTICS

Nguyen et al. used Lim Broth in conjunction with **Trypticase** Soy Agar with 5% Sheep Blood (TSA) as the 'gold standard' for the detection of group B streptococcus from the lower genital tract of pregnant women. Of 524 women, 90 had positive cultures in either Lim Broth or TSA. The sensitivity, specificity, positive predictive value, and negative predictive value of Lim Broth were 97% (87/90), 100% (434/434), 100% (87/87), and 97% (434/437), respectively.¹⁵


XIII AVAILABILITY


Cat. No.	Description
292209	BBL™ Lim Broth, 5 mL, Pkg. of 10 size K tubes
296266	BBL™ Lim Broth, 5 mL, Ctn. of 100 size K tubes

XIV REFERENCES

1. Federal Register. 1994. Prevention of group B streptococcal disease: a public health perspective. Fed. Regist. 59:64764-64773.
2. Jones, D.E., E.M. Friedl, K.S. Kanarek, J.K. Williams, and D.V. Lim. 1983. Rapid identification of pregnant women heavily colonized with group B streptococci. J. Clin. Microbiol. 18:558-560.
3. Lim, D.V., K.S. Kanarek, and M.E. Peterson. 1982. Magnitude of colonization and sepsis by group B streptococci in newborn infants. Curr. Microbiol. 7:99-101.
4. Jones, D.E., K.S. Kanarek, and D.V. Lim. 1984. Group B streptococcal colonization patterns in mothers and their infants. J. Clin. Microbiol. 20:438-440.
5. Jones, D.E., K.S. Kanarek, J.L. Angel, and D.V. Lim. 1983. Elimination of multiple reactions of the Phadebact *Streptococcus* coagglutination test. J. Clin. Microbiol. 18:526-528.
6. Centers for Disease Control and Prevention. 2002. Morbid. Mort. Weekly Rep.51 (No. RR-11):1.
7. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
8. Todd, E.W., and L.F. Hewitt. 1932. A new culture medium for the production of antigenic streptococcal haemolysin. J. Pathol. Bacteriol. 35:973-974.
9. National Committee for Clinical Laboratory Standards. 2001. Approved Guideline M29-A2. Protection of laboratory workers from occupationally acquired infections, 2nd ed. NCCLS, Wayne, Pa.

10. 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.
11. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
12. 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.
13. 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.
14. 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.
15. Nguyen, T.M. et al. 1998. Detection of group B *Streptococcus*: comparison of an optical immunoassay with direct plating and broth-enhanced culture methods. *J. Matern. Fetal. Med.* Jul-Aug; 7(4):172-176.

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