IFU

Instructions for Use

# LIM BROTH

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| Cat. no. L57 | LIM Broth, 13x100mm Tube, 5ml | 20 tubes/box |
| Cat. no. R73BX | LIM Broth, 13x80mm Polypropylene Tube, 2ml | 100 tubes/box |

## INTENDED USE

Hardy Diagnostics LIM Broth is recommended for the selective enrichment of group B streptococci (*Streptococcus agalactiae*) from anovaginal specimens from pregnant women.

## SUMMARY

Approximately 10-35% of women are asymptomatic carriers of group B streptococci (GBS) in the genital and gastrointestinal tracts.[1] GBS remains a leading cause of serious illness and death in newborn populations, and therefore, the detection of GBS in the vaginal-anorectal area is critical to the prevention of neonatal GBS disease. Several surveys have been conducted that show the incidence of neonatal sepsis and meningitis due to GBS is currently 0.5-3 cases per 1,000 live births, although there are substantial geographical and racial differences.[2] The case-fatality ratios are now declining due to prompt recognition and proper treatment.[3] Because group B streptococci produce asymptomatic disease in adult women, it is important to identify the carrier state so that effective therapeutic intervention can be implemented.

The Center of Disease Control (CDC) recommends the screening of all pregnant women for vaginal and rectal GBS colonization between 35 and 37 weeks of gestation using an enrichment broth followed by subculture to a Blood Agar.

Szilagy, Lim, and Jones, et al., in 1978, 1982, and 1983-84, respectively, conducted studies using Todd Hewitt Broth with various supplements for use in the rapid identification of maternal carriers and infants colonized with group B streptococci.[8-11] Szilagy, et al., supplemented the broth with 5% sheep blood, gentamicin and nalidixic acid.[8] Lim, et al., added colistin and nalidixic acid while Jones, et al., utilized Todd Hewitt Broth with yeast extract.[9-11]

Hardy Diagnostics LIM Broth is a further modification of Lim's formula. The medium is made from a Todd Hewitt Base made up of beef heart infusion, peptone and yeast extract. The basal medium is formulated to provide the necessary nutrients for the development of streptococci. Carbon and energy are supplied by glucose. Disodium phosphate and sodium carbonate serve as buffers which ultimately protect the hemolysin from the acids produced during the fermentation of the carbohydrate. Colistin and nalidixic acid are added as inhibitory agents toward gram-negative microorganisms.

## FORMULA

Ingredients per liter of deionized water:*

| | |
|---|---|
| Todd Hewitt Broth | 30.0gm |
| Yeast Extract | 10.0gm |

| | |
|---|---|
| Nalidixic Acid | 15.0mg |
| Colistin Sulfate | 10.0mg |

Final pH 7.6 +/- 0.2 at 25ºC.

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

## STORAGE AND SHELF LIFE

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

## PROCEDURE

Specimen Collection: If performing a Strep B screening of pregnant women, obtain one or two swabs of the vaginal/rectal areas. **Cervical cultures are not acceptable.** Vaginal cultures should be collected with a dacron, rayon or calcium alginate swab between 35 and 37 weeks gestation. The swab(s) should be inoculated into an appropriate transport medium and submitted directly to the laboratory for immediate processing. If specimen is to be delayed more than 24 hours, it must be refrigerated at 2-6ºC.[13]

Method of Use:

1. Inoculate swab(s) directly to LIM Broth.

2. Incubate aerobically for 18-24 hours at 35-37ºC.

3. Following incubation, subculture the broth to a Blood Agar plate (Cat. no. A10).

4. Incubate the subculture for 18-24 hours at 35-37ºC. in an aerobic atmosphere.

5. Observe for growth of beta-hemolytic or non-hemolytic, gram-positive, catalase-negative colonies.*

6. Using isolated colonies obtained from the Blood Agar plate, perform a particle agglutination test or tests

recommended for the detection of group B streptococci antigen (e.g. genetic probe or fluorescent antibody) adhering to the manufacturers procedure.

* If group B streptococci is not identified after 18-24 hours incubation of the Blood Agar subculture, reincubate and inspect at 48 hours.

## INTERPRETATION OF RESULTS

Consult the manufacturer's package insert for interpretation of the test method employed for the detection of group B streptococci antigen.

## LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

This media is a selective enrichment broth. Additional tests including subculture to an appropriate agar media as well as biochemical and/or serological tests using pure cultures should be performed for complete identification. For more information, consult appropriate references.

Refer to the document "Limitations of Procedures and Warranty" for more information.

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

| Test Organisms | Inoculation Method* | Incubation | | | Results |
|---|---|---|---|---|---|
| | | Time | Temperature | Atmosphere | |
| *Streptococcus agalactiae* ATCC® 12386 | A | 24hr | 35°C | Aerobic | Growth; beta-hemolytic colonies upon subculture to Blood Agar |
| *Proteus mirabilis* ATCC® 12453 | B | 24hr | 35°C | Aerobic | Partial to complete inhibition |
| *Escherichia coli* ATCC® 25922 | B | 24hr | 35°C | Aerobic | Partial to complete inhibition |
| *Pseudomonas aeruginosa* ATCC® 27853 | B | 24hr | 35°C | Aerobic | Partial to complete inhibition |

* Refer to the document "Inoculation Procedures for Media QC" for more information.

### USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or

a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

## PHYSICAL APPEARANCE

LIM Broth should appear clear, and light amber in color.


**LEFT:**
*Streptococcus agalactiae* (ATCC® 12386) growing in LIM Broth (Cat. no. L57). Incubated aerobically for 24 hours at 35ºC.

**RIGHT:**
*Proteus mirabilis* (ATCC® 12453) growth inhibited in LIM Broth (Cat. no. L57). Incubated aerobically for 24 hours at 35ºC.

## REFERENCES

1. Regan, J.A., Klebanoff, M.A., Nugent, R.P. 1991. *The epidemiology of group B streptococcal colonization in pregnancy*. Vaginal Infections and Pregnancy Study Group. Obstet. Gynecol.; 77:604-10.

2. Schrag, S.J., E.R. Zell, R. Lynfield, et al. 2002. *A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates.* N. Engl. J. Med. 25;347(4):233-9.

3. Schuchat, A. 2001. *Group B streptococcal disease: from trials and tribulations to triumph and trepidation.* Clin. Infect. Dis. 5;33(6):751-6.

4. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

5. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

6. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

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8. Szilagyi, G., et al. 1978. *Journal of Clinical Microbiology*; 8:410-412.

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10. Jones, E.E., et al. 1983. *Journal of Clinical Microbiology*; 18:558-560.

11. Jones, et al. 1984. *Journal of Clinical Microbiology*; 20:438-440.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.



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