GBS DETECT™

Cat. no. A300	GBS Detect™, 15x100mm Plate, 17ml	10 plates/bag
Cat. no. A300BX	GBS Detect™, 15x100mm Plate, 17ml	100 plates/box

INTENDED USE

Hardy Diagnostics GBS Detect™ is recommended for the isolation and detection of gamma-hemolytic (non-hemolytic) Group B Streptococcus by inducing beta-hemolysis on sheep blood agar upon subculture from enrichment broth procedures; such as Strep B Carrot Broth™ and LIM Broth.

SUMMARY

Approximately 10-35% of women are asymptomatic carriers of group B streptococci (GBS) in the genital and gastrointestinal tracts.[7] 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.[8] The case-fatality ratios are now declining due to prompt recognition and proper treatment.[9]

The Centers for Disease Control and Prevention (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 plate (Cat. no. A10) or other appropriate media.[10] The use of a selective enrichment broth that incorporates chromogenic pigments, such as Strep B Carrot Broth™, has recently been included in CDC's Recommendations for the Prevention of Group B Streptococcal Disease.[27] Strep B Carrot Broth™ demonstrates increased sensitivity and specificity, reduced incubation time, reduced need for additional plated media, and elimination of the need to confirm positives with additional testing.[11-15,20-23]

A small percentage of GBS may not produce beta-hemolysis. GBS detection with the Strep B Carrot Broth™ Kit is only possible with beta-hemolytic colonies. There is evidence of a direct genetic linkage between pigment production in Strep B Carrot Broth™ and hemolysin production by GBS bacteria. Beta-hemolytic, pigment producing GBS occurs with 95.3 to 99.5% of all GBS strains isolated from clinical specimens.[17-19]

Subcultures of enrichment broths may contain non-hemolytic or gamma strains of GBS that may be missed by normal plating procedures, because non-hemolytic GBS is not readily distinguishable from other small non-hemolytic colonies. Therefore all LIM Broth cultures and negative Strep B Carrot Broth™ cultures should be subcultured to GBS Detect™ plates for detection of gamma-hemolytic GBS. GBS Detect plates contain special supplements that cause otherwise non-hemolytic strains of GBS to appear as beta-hemolytic, thus increasing the sensitivity of detection methods used to detect GBS colonization in pregnant women. Selective agents are added to suppress coliforms, staphylococci and other organisms that might be present as normal flora.

GBS Detect™ eliminates needless steps in screening for non-hemolytic group B streptococci and makes Strep B Carrot Broth™ a more sensitive method for all strains of GBS.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	15.0gm
Peptic Digest of Soybean Meal	5.0gm
Sodium Chloride	5.0gm

Nucleic Acid	3.0gm
Selective Agents	15.3gm
Hemolysis Inducing Agents	20.0ml
Sheep Blood	50.0ml
Agar	15.0gm

Final pH 7.3 +/- 0.2 at 25 degrees C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8 degrees C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date applies to the product in its intact packaging when stored as directed.

This product has the following shelf life from the date of manufacture:

70 Days:	A300	GBS Detect
	A300BX	GBS Detect

Refer to the keyword "Storage", in the Hardy Diagnostics software program HUGO™, for more information on storing culture media.

PRECAUTIONS

This product is for *in vitro* diagnostic use only and 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". The "Guideline for Isolation Precautions" is available from the Centers of Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

Refer to www.HardyDiagnostics.com for the Material Safety Data Sheet (MSDS) for this product.

PROCEDURE

Method of Use: Medium should be brought to room temperature prior to inoculation. Inoculate according to standard microbiological procedures.

1. Using a vaginal-rectal specimen, inoculate and incubate either LIM Broth (Cat. no. L57) or Strep B Carrot Broth™ (Cat. no. Z140) according to the procedures in the technical information sheets.

2. Subculture all LIM Broth or color negative Strep B Carrot Broth™ to a GBS Detect™ plate. Streak inoculum in four quadrants to obtain isolated colonies. Note: Isolated colonies must be obtained.

3. Incubate the GBS Detect™ plate for 18-24 hours at 35 +/- 2 degrees C. in an aerobic atmosphere.

4. After 18-24 hours observe for growth of beta-hemolytic gram-positive, catalase-negative colonies. GBS will produce large, tranparent zones of hemolysis, with a soft edge. Ignore small, incomplete or weak zones of hemolysis (most likely *E. faecalis*).

5. Using isolated colonies from the GBS Detect™ plate described in step 4, perform latex particle agglutination test (StrepPRO™ Grouping Kit, Cat. no. PL030HD) or other tests recommended for the detection of group B streptococci antigen following the procedure

specified by the manufacturer.

LIMITATIONS

It is recommended that biochemical and/or serological tests be performed on colonies from pure culture for complete identification.

Organisms other than GBS can produce faint or incomplete zones of hemolysis.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, LIM Broth (Cat. no. L57), Strep B Carrot Broth™ (Cat. no. Z140), StrepPRO™ Grouping Kit (Cat. no. PL030HD), other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method	Incubation			Results
		Time	Temperature	Atmosphere	
Streptococcus agalactiae ATCC® 13813	A	24hr	35°C	Aerobic	Growth; beta-hemolysis
Streptococcus agalactiae Clinical Strain	A	24hr	35°C	Aerobic	Growth; beta-hemolysis
Enterococcus faecalis ATCC® 29212	A	24hr	35°C	Aerobic	Partial inhibition

USER QUALITY CONTROL

Check for signs of contamination and deterioration. Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate growth or a positive reaction; and at least one organism to demonstrate inhibition or a negative reaction (where applicable).

PHYSICAL APPEARANCE

GBS Detect™ should appear opaque, and cherry red in color.

Streptococcus agalactiae (ATCC® 13813) colonies growing on GBS Detect™ (Cat. no. A300) showing beta-hemolytic colonies. This strain is not hemolytic on a regular blood agar plate. Incubated aerobically for 24 hours at 35 deg. C.

REFERENCES

1. Anderson, N.L., et al. 2005. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory,* Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Murray, P.R., et al. 2011. *Manual of Clinical Microbiology,* 10th ed. American Society for Microbiology, Washington, D.C.

3. Forbes, B.A., et al. 2007. *Bailey and Scott's Diagnostic Microbiology,* 12th ed. C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. 2006. *Color Atlas and Textbook of Diagnostic Microbiology,* 6th ed. J.B. Lippincott Company, Philadelphia, PA.

6. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

7. 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.

8. 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.

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

10. Schrag, S., Gorwitz, R., Fultz-Butts, K., Schuchat, A. 2002. *Prevention of perinatal group B streptococcal disease.* Revised guidelines from CDC: MMWR Recommendations and Reports / 6;51(RR-11):1-22.

11. Overman, S.B., D.D. Eley, B.E. Jacobs, J.A. Ribes. 2002. *Evaluation of methods to increase the sensitivity and timeliness of detection of Streptococcus agalactiae in pregnant women.* J. Clin. Microbiol.; 40(11):4329-31.

12. de la Rosa, M., M. Perez, C. Carazo, L. Pareja, J.I. Peis and F. Hernandez. 1992. *New Granada Medium for detection and identification of group B streptococci.* J. Clin. Microbiol.; 30:1019-1021.

13. Garcia Gil, E., M.C. Rodriguez, R. Bartolome, B. Berjano, L. Cabero and A. Andreu. 1999. *Evaluation of the Granada Agar plate for detection of vaginal and rectal group B streptococci in pregnant women.* J. Clin. Microbiol.; 37:2648-2651.

14. Rosa-Fraile Manuel, J. Rodriguez-Granger, M. Cueto-Lopez, A. Sampedro, E. Biel Gaye, J.M. Haro and A. Andreu. 1999. *Use of Granada Medium to detect group B streptococcal colonization in pregnant women.* J. Clin. Microbiol.; 37:2674-2677.

15. Rosa-Fraile, Manuel, A. Sampedro, J. Varela, M. Garcia-Pena, and G. Gimenez-Gallego. 1999. *Identification of a peptide pigment from mammal albumins responsible for enhanced pigment production by group B streptococci.* Clin. Diag. Lab. Imm.; 6:425-426.

16. B. Spellerberg, B. Pohl, G. Haase, S. Martin, J. Weber-Heynemann and R. Lütticken. 1999. *Identification of Genetic Determinants for the Hemolytic Activity of Streptococcus agalactiae by ISS1 Transposition.* J. Bacteriol.; 181: 3212-3219.

17. Young, Uh, et al. 1998. *Serotypes and Biochemical Reaction Patterns of Group B Streptococci.* Korean J. Clin. Path.; 18:386-390.

18. Merrit, K. and Jacobs, N. 1978. *Characterization and Incidence of Pigment Production by Human Clinical Group B Streptococci.* J. Clin. Microbiol.; Vol. 8, No. 1, p. 105-107.

19. Noble, M., Bent, J., West, A. 1983. *Detection and identification of group B streptococci by use of pigment production.* J. Clin. Path.; 36:350-352.

20. da Gloria Carvalho, M., R. Facklam, D. Jackson, B. Beall, and L. McGee. 2009. *Evaluation of Three Commercial Broth Media for Pigment Detection and Identification of a Group B Streptococcus (Streptococcus agalactiae).* J. Clin. Microbiol.; Vol. 47, No. 12, p.4161-4163.

21. Block, T., E. Munson, A. Culver, K. Vaughan, and J. Hryciuk. 2008. *Comparison of Carrot Broth- and Selective Todd-Hewitt Broth-Enhanced PCR Protocols for Real-Time Detection of Streptococcus agalactiae in Prenatal Vaginal/Anorectal Specimens.* J. Clin. Microbiol.; Vol. 46, No. 11, p.3615-3620.

22. Church, D.L., H. Baxter, T. Lloyd, B. Miller, and S. Elsayed. 2008. *Evaluation of Strep B Carrot Broth™ versus Lim Broth for Detection of Group B Streptococcus Colonization Status of Near-Term Pregnant Women.* J. Clin. Microbiol.; Vol. 46, No. 8, p.2780-

2782.

23. Czerepuszko, D.J., and M.J. Lewis. 2010. *Comparison of LIM Broth with PNA FISH to Carrot Broth with PNA FISH for Identification of Group B Streptococcus in Prenatal Vaginal/Rectal Specimens.* A poster presentation at American Society for Microbiology, San Diego, CA.

110514hh

HARDY DIAGNOSTICS
1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: www.HardyDiagnostics.com
Email: TechService@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida

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