Department of Microbiology
GBS Detect™ Agar Procedure

I. Purpose and Test Principle

A small percentage of group B streptococcus isolates (GBS) do not produce beta-hemolysis. Non-hemolytic or gamma-hemolytic strains of GBS may be missed by conventional procedures using blood agar because non-hemolytic GBS is not readily distinguishable from other small non-hemolytic colonies. Additionally, detection of GBS in StrepB Carrot Broth™ is only possible with beta-hemolytic strains. GBS Detect™ plates contain special supplements that cause otherwise non-hemolytic strains of GBS to appear beta-hemolytic, thus increasing the sensitivity of detection methods for 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 steps in screening for non-hemolytic GBS and makes StrepB Carrot Broth™ a more sensitive method for detection of all strains of GBS.

II. Specimen Information

Vaginal/rectal swab specimens should be inoculated in StrepB Carrot Broth™ and incubated for up to 24 h. If negative, StrepB Carrot Broth™ cultures should be subcultured to GBS Detect™.

III. Reagents & Equipment

- Negative StrepB Carrot Broth™ cultures
- GBS Detect™
  Storage: Upon receipt, store at 2-8° C away from direct light. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.
- Sterile swabs and inoculating loop
- Aerobic incubator set at 35 ± 2°C

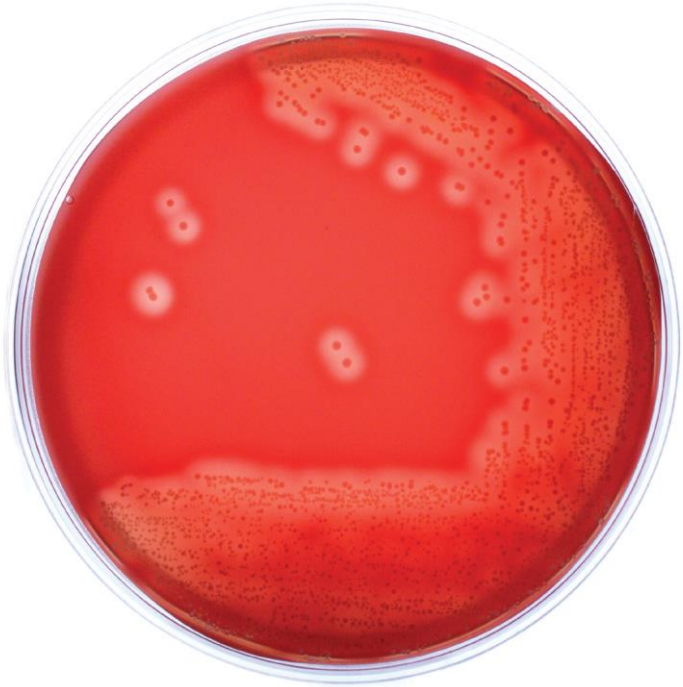
IV. Procedure

A. Subculture all pigment-negative StrepB Carrot Broth™ to a GBS Detect™ plate.
B. Streak inoculum in four quadrants to obtain isolated colonies.
C. Incubate the GBS Detect™ plate for 18-24 h at 35 ± 2° C in an aerobic atmosphere.
D. After 18-24 h, observe for growth of beta-hemolytic gram-positive, catalase-negative colonies. GBS will produce large, transparent zones of hemolysis, with a soft edge. Ignore small, incomplete or weak zones of hemolysis (most likely *E. faecalis*).
E. Using isolated suspect colonies from the GBS Detect™ plate, perform latex particle agglutination test for group B antigen.

V. Interpretation of Results

A. Hemolytic colonies on GBS Detect™ agar that produce positive latex agglutination for B antigen are identified as group B strep.
B. Isolates that are negative for B antigen should be tested for A antigen to rule out the possibility of *S. pyogenes*.

***S. agalactiae* growing on GBS Detect™**



VI. Quality Control

A. Inspection of Media
   Each new lot or shipment of media should be examined for product deterioration. 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.
B. Quality Control Testing
   1. Each new lot or shipment should be tested with control strains.
   2. Prepare a 0.5 McFarland suspension of each test strain and dilute 1:100 with sterile saline.
   3. Use a 0.01 mL calibrated loop to inoculate the media.
   4. Incubate plates at 35 ± 2°C in an aerobic atmosphere.

Control strain	Expected Results
S. agalactiae ATCC 13813	Growth; beta-hemolysis
E. faecalis ATCC 29212	Partial to complete inhibition

VII. Limitations

A. It is recommended that biochemical and/or serological tests be performed on colonies from pure culture for complete identification.
B. Organisms other than GBS can produce faint or incomplete zones of hemolysis.

VIII. Verification of Test Method

A total of 36 test strains were used to evaluate the GBS Detect™ product. This included 33 beta-hemolytic clinical strains of GBS, 1 non-hemolytic GBS clinical strain, *S. agalactiae* ATCC13813 (non-hemolytic), and 1 beta-hemolytic group A strep clinical isolate. These test strains were seeded into a diluted stool matrix and inoculated into Strep B Carrot Broth™ tubes as outlined in the verification study summarized in the Strep B Carrot Broth™ procedure. After 24 h incubation, all Strep B Carrot Broth™ cultures were subcultured to GBS Detect™ plates and incubated aerobically for 24 h at 35 ± 2° C. After incubation, the plates were examined for beta-hemolytic colonies. All GBS isolates, including the 2 non-hemolytic strains, produced easily recognizable colonies with distinct beta hemolysis. A Strep B Carrot Broth™ culture of the diluted stool matrix was also subcultured to GBS Detect™ and did not produce any beta-hemolytic colonies. The growth on all of the subcultures was almost exclusively GBS. Very few colonies of flora grew on the GBS Detect™ as compared to a BAP subculture of the diluted stool matrix. The group A strep clinical isolate failed to grow on the GBS Detect™ subculture. However, a subculture of *S. pyogenes* ATCC 19615 produced growth of beta-hemolytic colonies.

IX. References

A. Package insert: Hardy Diagnostics GBS Detect™, 071708mg.
B. www.hardydiagnostics.com
C. Clasen, R., et al. 2008. Evaluation of GBS Detect™: a New Medium for the Detection of Non-Hemolytic Group B Strep in Subcultures of Carrot Broth™ and LIM Broth. Results of a Multi-Center Trial. Poster presentation at American Society for Microbiology, 108th General Meeting, Boston, Massachusetts.

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