Department of Microbiology
Strep B Carrot Broth™ Test Procedure

I. Purpose and Test Principle

The use of a selective enrichment broth is recommended by the CDC to aid in the detection of group B streptococcus (GBS) in vaginal/rectal specimens from pre-partum women for the purpose of preventing subsequent transmission to newborns. Strep B Carrot Broth™ is a selective medium that also incorporates chromogenic pigments to permit the detection of GBS. The production of orange pigment in Strep B Carrot Broth™ is a unique characteristic of hemolytic GBS due to reaction with substrates such as starch, peptone, serum, and folate pathway inhibitors. GBS detection with this medium is only possible with strains that produce beta-hemolytic colonies. Beta-hemolysis and pigment production occurs with > 95% of all GBS strains isolated from clinical specimens. A positive result, as indicated by orange pigment production, may occur in as little as 6 h and does not require subculture or additional testing for confirmation. However, since non-hemolytic strains are not detected, all negative Strep B Carrot Broth™ cultures must be subcultured for further evaluation. Since non-hemolytic strains of GBS may be difficult to find in cultures on routine blood agar, a special medium called GBS Detect™ is used. This medium contains selective agents and hemolysis enhancers that enable non-hemolytic GBS strains to appear hemolytic, so they can be easily recognized and identified within 24 h.

II. Specimen Information

A. Collection

A vaginal/rectal sample should be submitted in BBL CultureSwab™ Plus (with Amies).

1. Wipe away excessive amount of secretion or discharge from the vaginal area.
2. Carefully insert the swab into the lower one-third part of the vagina, and sample secretions from the mucosa.
3. Carefully insert the same swab approximately 2.5 cm beyond the anal sphincter, and gently rotate to sample anal crypts.
4. Replace swab in its container.
5. Label the container with patient identification, date and time.

B. Transport

Specimens should be kept at 2-30° C during transport. Protect against freezing or exposure to excessive heat. Specimens processed within 24 h may be kept at room temperature. If transport will delay processing more than 24 h, specimens should be refrigerated.

III. Reagents & Equipment

- Strep B Carrot Broth™

  Broth should appear hazy to cloudy, colorless; may or may not have a white precipitate at the bottom of the tube.

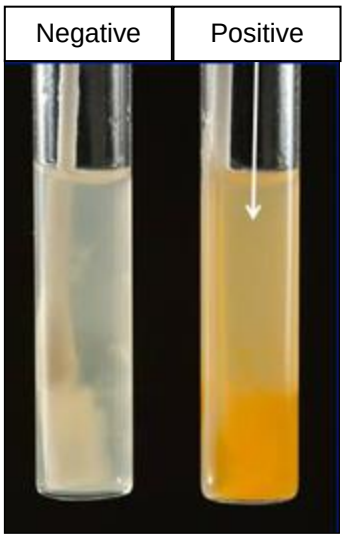
- Strep B Carrot Broth™ Tiles (contain growth and pigment factors). Tiles are 1/4 x 1/2 inch filter paper rectangles, and should appear light yellow in color.
  Storage of broth and tiles: Products are temperature sensitive. Upon receipt, store media at 2-8° C and protect from excessive heat, moisture, and freezing. Products are extremely light sensitive. Protect against damage from excessive illumination and store away from any direct light source. Do not use media after the expiration date.
- Aerobic incubator at 35 ± 2°C

IV. Procedure

A. A white precipitate may be present in the tube. Prior to inoculation, invert the tube once to re-suspend any precipitate.
B. Using sterile forceps, aseptically drop one Strep B Carrot Broth™ Tile into one tube of Strep B Carrot Broth™. Ensure tile remains submerged within the broth.
C. Insert the specimen swab into the Strep B Carrot Broth™ tube. If using a gel-based transport medium, rotate the swab in the broth to emulsify the gel in the broth. Do not vortex. Carefully break the swab shaft, leaving the swab in the tube. Replace the tube cap and screw down **tightly**. It is important that the caps be tightly sealed in order to create the necessary anaerobic conditions at the bottom of the tube.
D. Incubate the inoculated Strep B Carrot Broth™ tube with swab and tile for at least 18 to 24 h at 35 ± 2° C unless broth changes color in less time. Color change may occur in as little as 6 h.
E. Examine tubes for a pale peach, orange, or orange-red color change and/or spots typical of group B streptococci. See "Interpretation of Results" section below.
F. If no orange color is present, subculture the specimen to a GBS Detect™ plate. Incubate plates for 24 h at 35 ± 2° C.

V. Interpretation of Results

A. Positive Test
   Beta-hemolytic GBS turn the Strep B Carrot Broth™ orange within 6 - 24 h. The color will be most intense in the lower portion of the tube. Visualization of any orange pigment in the broth confirms the presence of beta-hemolytic GBS in the specimen. In cases where the GBS count is low in the specimen, development of small orange spots on the swab or at the bottom of the tube can be observed rather than the entire tube turning orange. These should also be considered positive.
B. Negative Test
   No color change in the broth medium indicates that no beta-hemolytic GBS are present in the sample. The broth must be subcultured to GBS Detect™ agar to rule out the presence of a non-hemolytic strain of GBS.



VI. Quality Control

A. Inspection of Media
   Each new lot or shipment of media should be examined for product deterioration and tested with the following control strains.

B. Quality Control Testing
   1. Prepare a 0.5 McFarland suspension of each test strain.
   2. Dilute *S. agalactiae* ATCC 12386 1:100.
   3. Dilute *E. coli* ATCC 25922 and *P. mirabilis* ATCC 35659 1:10. Combine suspensions into one mixture.
   4. Use a 0.01 mL calibrated loop to inoculate two separate broths.
   5. Incubate tubes overnight at 35 ± 2°C in an aerobic atmosphere.
   6. Observe tubes for growth and color change.

Control strain	Expected Results
S. agalactiae ATCC 12386	Growth; bright orange color change
E. coli ATCC 25922 and *P. mirabilis* ATCC 35659	Partial to complete inhibition; no color change

VII. Limitations

A. Although uncommon, a small percentage of GBS do not produce beta-hemolysis. GBS detection with the Strep B Carrot Broth™ is only possible with beta-hemolytic colonies. It is recommended to subculture negative Strep B Carrot Broth™ tubes to a GBS Detect™ plate. This agar will cause non-hemolytic strains to appear hemolytic, making them more easily recognizable.

B. Since positive reactions can appear on the swab tip, the use of swabs containing charcoal is not suitable.

C. When using gel-based transport media, emulsify the swab in the broth to ensure that organisms are not trapped in the gel. Do not vortex.

D. Failure to place the Strep B Carrot Broth™ Tile into the Strep B Carrot Broth™ tube before incubation will prevent color development and will produce erroneous results.

VIII. Verification of Test Method

To evaluate the performance of Strep B Carrot Broth™, simulated specimens were created using aliquots of a diluted stool specimen seeded with clinical and control strains. A stool specimen submitted to the laboratory was selected to serve as a specimen matrix. This sample was diluted 1:100 with sterile saline. A swab was dipped into the diluted sample and used to inoculate a tube of LIM broth, a blood agar plate (BAP), and a Strep B Carrot Broth™. After overnight incubation, the LIM broth was tested and confirmed negative for GBS by PCR. The Strep B Carrot Broth™ did not have any orange pigment. The BAP subculture grew a mixture of organisms including coliforms and *Enterococcus* spp.

In order to evaluate a lower threshold of detection, 3 clinical strains of beta-hemolytic GBS were used. The isolates were grown on BAP and used to

make suspensions in sterile saline equivalent to a 0.5 McFarland (~10^8 CFU/mL). These suspensions were diluted to ~10^5 and 10^4 CFU/mL; 100 µL of each dilution was loaded onto separate swabs that were used to inoculate individual Strep B Carrot Broth™ tubes. Each tube of Strep B Carrot Broth™ contains 4 mL of broth. This produced cultures with ~2,500 CFU/mL and 250 CFU/mL. The tubes were incubated overnight and examined the following day for pigment production. All 3 isolates produced readily visible pigment with the lower concentration of inoculum. This agrees with claims from previous studies cited by the manufacturer. These studies found a high degree of sensitivity at concentrations as low as 100-200 CFU/tube.

Thirty-three additional test strains were used to further evaluate the performance of Strep B Carrot Broth™. This included 30 additional clinical strains of beta-hemolytic GBS, 1 clinical non-hemolytic strain of GBS, 1 beta-hemolytic group A strep, and *S. agalactiae* ATCC 13813 (non-hemolytic). All of the clinical isolates were recovered from vaginal or vaginal/rectal specimens. Each test strain was diluted and seeded into the stool matrix to achieve approximate concentrations of 10^4 CFU/mL as outlined above. Individual swabs were inoculated with 100 µL of each simulated specimen suspension and used to inoculate separate Strep B Carrot Broth™ tubes. The tubes were incubated for 24 h and examined for pigment production. All 33 (100%) of the beta-hemolytic GBS strains produced orange pigment, including the 3 strains previously used for establishing a lower dilution of inoculum. The tubes showed varying degrees of pigment. Most cultures were distinctly orange while a few of the broths were more of a pale peach color. The non-hemolytic GBS clinical strain and the ATCC strain both failed to produce any pigment. The beta-hemolytic group A strep isolate also did not produce any pigment. The Strep B Carrot Broth™ cultures were used to inoculate GBS Detect™ plates to verify the performance of that product.

While the supply cost associated with Strep B Carrot Broth™ and GBS Detect™ was essentially equivalent to the LIM broth/BAP method, there was substantial labor savings associated with the conversion. The labor savings in this cost analysis produced data similar to that previously published in an evaluation of Strep B Carrot Broth™ versus LIM broth.[D]

IX. References

A. Package insert: Hardy Diagnostics Strep B Carrot Broth™, 102010ha.
B. www.hardydiagnostics.com
C. www.cdc.gov
D. Church, D.L., Baxter, H., Lloyd, T., Miller, B., and Elsayed, S. 2008. Evaluation of StrepB Carrot Broth versus Lim Broth for Detection of Group B Streptococcus Colonization Status of Near-Term Pregnant Women. J. Clin. Microbiol. p. 2780-2782 Vol. 46, No. 8.

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