**MICRO.CULT.24.0 VAGINAL RECTAL SCREEN FOR GROUP B BETA STREP**

**PRINCIPLE**

Approximately 10-35% of women are asymptomatic carriers of Group B Streptococci (GBS) in the genital and gastrointestinal tracts. 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. 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 or other appropriate media.

The production of light orange to red-orange pigment is a unique characteristic of hemolytic GBS due to reaction with substrates such as starch, peptone, serum, and folate pathway inhibitors. Hardy Diagnostics Strep B Carrot Broth™ One-Step contains these substrates necessary for pigment detection of beta-hemolytic GBS. The advantage to this medium is that it will produce positive results in as little as sixteen hours and does not require subculture to a blood agar plate or other appropriate media unless the results are negative. Strep B Carrot Broth™ One-Step is used to detect beta-hemolytic GBS without the need for further testing and, as an enrichment broth, provides greater sensitivity. Enrichment broth procedures are known to be more sensitive than plate methods in their ability to detect GBS colonization.

A small percentage of GBS may not produce beta-hemolysis. Beta-hemolytic, pigment producing GBS occurs with 95.3 to 99.5% of all GBS strains isolated from clinical specimens. GBS detection with Strep B Carrot Broth™ One-Step is only possible with beta-hemolytic strains, but assists in the isolation and identification of the non-hemolytic strains of GBS (which will comprise 0.5 to 4.7% of the total GBS isolates), when used in conjunction with GBS Detect™ agar. The GBS Detect™ plate is used when subculturing the negative Strep B Carrot Broth™ One-Step cultures. It contains selective agents and 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.

**OWNERS**

Microbiology Manager/Lead Techs

**SPECIMEN**

| CRITERIA | |
|---|---|
| Preferred specimen type | Using a single swab (preferred) or two separate dacron swabs, swab the distal vagina (vaginal introitus) and the rectum (through the anal sphincter). |
| Volume | One swab in appropriate bacterial transport medium |
| Collection container | Bacterial culture transport swab with:<br>• Liquid Stuart's (clear or red top) medium, or<br>• Amies (blue top) medium |
| Timing | Collect specimen at 35-37 weeks gestation |
| Stability and Storage Requirements | • Room temperature: ≤24 hours<br>• Refrigerated ($2^o$ – 8 $^oC$): 72 hours<br>• Frozen: Not acceptable |
| Unacceptable specimens | • Specimens other than vaginal/rectal<br>• Expired transport medium<br>• Frozen or dry swabs |

**REAGENTS**

A. Strep B Carrot Broth™ One-Step. Store at 2-8$^oC$, shielded from light.
B. GBS Detect™ Store at 2 - 8$^oC$, shielded from light.
C. PathoDX Strep typing kit
D. 3% Hydrogen Peroxide
E. Gram stain reagents

**EQUIPMENT**

A. Inoculating loop
B. Incubator 35 - 37$^oC$
C. Test tube 12 X 75

**QUALITY CONTROL**

QC should be performed on each new lot/shipment of Strep B Carrot Broth™ One-Step and GBS Detect plates prior to reporting patient results. If QC yields results other than the expected results, notify lead or manager and do not use for patient testing.

**Strep B Carrot Broth**

| Test Organisms | Inoculation Method* | Incubation | | | Expected Results |
|---|---|---|---|---|---|
| | | Temp | Atmosphere | Time | |
| *Streptococcus agalactiae* ATCC® 12386 | A | 35°C | Aerobic | 16-24hr | Growth; bright orange to red color change |
| *Proteus mirabilis* ATCC® 12453 | B | 35°C | Aerobic | 24hr | Partial to complete inhibition; no color change |

**GBS Detect™ Media**

| Test Organisms | Inoculation Method* | Incubation | | | Expected Results |
|---|---|---|---|---|---|
| | | Temp | Atmosphere | Time | |
| *Streptococcus agalactiae* ATCC® 13813 | A | 35°C | Aerobic | 24hr | Growth; beta-hemolysis |
| *Enterococcus faecalis* ATCC® 29212 | A | 35°C | Aerobic | 24hr | Growth; gamma hemolysis |

***Inoculation Method**

**Method A** (to test nutritive capacity)**:** Using a fresh isolate (18 to 24 hours of growth) of the organism, suspend three to five isolated colonies in a small volume of Tryptic Soy Broth (TSB) and incubate for 4 to 5 hours. Adjust the turbidity to match that of a 0.5 McFarland standard. Dilute the cell suspension to 1:100 in TSB or normal saline. Inoculate the test media with a 10uL calibrated loop of the diluted suspension. This will provide approximately $10^3$ to $10^4$ CFU per tube.

**Method B** (to test inhibitory capacity): Using a fresh isolate (18 to 24 hours of growth) of the organism, suspend three to five isolated colonies in a small volume of Tryptic Soy Broth (TSB) and incubate for 4 to 5 hours. Adjust the turbidity to match that of a 0.5 McFarland standard. Dilute the cell suspension to 1:10 in Tryptic Soy Broth (TSB). Inoculate the test media with a 10uL calibrated loop of the diluted suspension. This will provide approximately $10^4$ to $10^5$ CFU per tube. A non-inhibitory plate is also inoculated at the same time, to serve as a positive control.

**PROCEDURE**

| Step | Action |
|---|---|
| 1. | Allow a Strep B Carrot Broth™ One-Step tube to come to room temperature. |
| 2. | Insert the specimen swab into the Strep B Carrot Broth™ One-Step tube. If using a gel-based transport swab, rotate the swab in the broth to emulsify the gel in the broth.<br>*Do not shake, agitate, or vortex*<br>Carefully break the swab shaft, leaving the swab in the tube. |
| 3. | Replace the tube cap and screw down **tightly**. It is important that the caps are tightly sealed in order to create the necessary anaerobic condition at the bottom of the tube. |
| 4. | Incubate the inoculated Strep B Carrot Broth™ One-Step tube at 35°C. |
| 5. | Examine tubes after 16-24 hours for a light orange to red-orange color change and/or spots typical of Group B Streptococci. |

**INTERPERTATION**

| If | Then |
|---|---|
| Light orange to red-orange color change in broth, small orange to red spots or streaks (colonies) in broth, or streaks of orange on the swab. | Result as positive for Group B Strep. |
| No orange color is present | Allow GBS Detect™ plate to come to room temperature. Subculture the negative broth to a GBS Detect™ plate and streak for isolation (Invert Carrot Broth tube prior to subculture). Issue a preliminary report and incubate for 18-24hrs at 35 ± 2°C in an aerobic atmosphere. |

| If | Then |
|---|---|
| Beta hemolytic colonies on GBS Detect | Perform group specific latex and catalase. |
| No Beta hemolytic colonies on GBS Detect | Result as negative. |

| If | Then Report | Preliminary | | Final | |
|---|---|---|---|---|---|
| | | Sunquest | QLS | Sunquest | QLS |
| Negative for Group B Streptococcus | No Beta Strep Group B Isolated | CPR (Culture in Progress) | RESULT:XB | NBSB (key 8) | RESULT: XB |
| Group B Streptococcus isolated | Beta Hemolytic Streptococci, Group B | N/A | N/A | BSBI (key 7) | RESULT: LB ISO1:#STRB |

**REPORTING RESULTS**

| If | Then |
|---|---|
| GBS exhibits suspicious colonies that are:<br>• Positive for B strep using the PathoDx strep grouping kit and catalase negative | • Issue a final report Beta Hemolytic Streptococci, Group B.<br>• Charge IDS1<br>• Retain GBS plate for 7 days. |
| GBS does not exhibit suspicious colonies that meet the criteria listed above after 18 - 24hrs of incubation. | • Issue a final report of No Beta Strep Group B<br>• Retain GBS plate for 7 days |

Note: The presence or absence of normal flora need not be reported.

**PROCEDURE NOTES**

Susceptibility testing for penicillin allergic patients may be required. This can be requested as an internal comment noted on the requisition or by ordering test code 51317 Reflex to GBS Susceptibility. The standard antimicrobial susceptibility panel for Beta Streptococcus is tested and reported.

**LIMITATIONS**

Although rare, a small percentage of GBS may not produce beta-hemolysis. GBS detection with the Strep B Carrot Broth™ One-Step is only possible with beta-hemolytic colonies. Beta-hemolytic, pigment producing GBS occurs with 95.3-99.5% of all GBS strains isolated from clinical specimens. For this reason, **do not** use *S. agalactiae* ATCC® 13813 for quality control purposes because it will not produce the characteristic orange pigment.

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

Failure to properly emulsify gel-based transport medium, as outlined in the procedure, may inhibit proper color development and recovery of Group B Streptococci.

Some Strains of *E. faecalis,* when at concentrations above $10^5$ CFU/mL in the vaginorectal swab specimen, have been shown to be inhibitory to the detection and recovery of GBS strains.

Organisms other than GBS can produce faint or incomplete zones of hemolysis on GBS Detect™ agar.

Color-blind individuals may encounter difficulty in distinguishing color differences in Strep B Carrot Broth™ One-Step.

**REFERENCES**


A. Garcia, Lynn et al., Clinical Microbiology Procedures Handbook 3rd ed., Vol 1, sec 3.9.2. American Society for Microbiology. ASM Press, Washington DC, 2012
B. Prevention of Perinatal Group B Streptococcal Disease, Revised Guidelines from CDC. MMWR Vol 59, No RR-10. 2010.
C. Strep B Carrot Broth™ package insert.
D. GBS Detect™ Agar package insert.