



Clinical Laboratory Department POLICY AND PROCEDURE

POLICY NUMBER: 1087
VERSION: 3

SUBJECT: Culture to R/O BSB (Beta Strep group B)

Principle:

Group B Streptococci (*Streptococcus agalactiae*) colonize 10-20% of all pregnant women and are frequent causes of neonatal sepsis, meningitis and respiratory distress. Studies have shown that of the women that carry group B strep, 70% pass it to the infant during delivery. 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.

Specimen:

1. Specimens are to be collected according to laboratory policy, properly labeled and delivered to the lab in a timely manner. Specimens should be plated as soon as possible after collection.
2. Specimen should be collected using commercial swab collection kit that contains two (2) swabs. One swab is to be used to culture the **vagina**; the second is to be used to culture the **rectum**. Since group B strep colonizes both the vagina and rectum, it is important that both these areas are sampled. The specimen is ideally taken from the pregnant woman at 35-37 weeks gestation. The two swabs can be transported in the same culturette tube.

Primary inoculation of Media:

1. Supplies:
 - a. Inoculating loop
 - b. GRANADA MEDIA
 - c. LIM Broth
2. Procedure:
 - a. Inoculate the GRANADA media, using BOTH swabs on the 1st quadrant of the plate. Streak for isolation using 4-quadrant streak.
 - b. Inoculate the LIM Broth by immersing and breaking off BOTH swabs into the media. Cap tightly.
3. Incubation:
 - a. Incubate all primary GRANADA plates in an anaerobe bag or anaerobe box.
 - b. Incubate the LIM broth upright in the incubator (CO₂ or ambient, doesn't matter).
 - c. Incubate for 18 -24 hours.

Culture Work-up

1. Examine the GRANADA plates at 18-24 hours.
2. Positive colonies for Group B Strep will be orange in color, with a range from yellow to red.
 - a. Select suspicious colonies; verify Strep status using Gram Stain, and catalase test (Gram positive cocci, catalase negative).
 - b. Test the suspicious colonies using the Streptococcus latex identification kit for reactivity with the "B" latex antibody only.

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- c. If positive, report and finalize.
3. Negative colonies will be white.
 - a. Negative GRANADA plates are incubated in anaerobic conditions for an additional 24 hours.
 - b. The LIM Broth from negative cultures will be subcultured to BAP at 24 hours, (4 quadrant streak). Incubate in CO₂ incubator, aerobically, for 18-24 hours.
 - c. 48 reading: read GRANADA plate at 48 hours; examine for yellow to orange-red colonies. Any positive colonies will be worked up as in 2a, b, c.
 - d. BAP plate from LIM sub; Look for Beta hemolysis characteristic of Group B Strep. Test or subculture for testing any suspicious colonies.
 - e. BAP plate from LIM sub: If no Beta hemolytic colonies are seen, look for colonies that resemble Enterococcus spp. Approximately 5-10% of Group B Streptococci are non-hemolytic. In order to find these organisms, select colonies that resemble Enterococcus and perform a CAMP test on those colonies. See CAMP test in Culture Work Up section.

Reporting:

1. Negative findings: Report as No Streptococcus agalactiae (Group B) isolated using the Microbiology Coded Response Sheet.
2. Positive findings (Positive GRANADA, positive Strep typing, positive CAMP test; Report as: POSITIVE FOR Streptococcus, Beta hemolytic group B using the Microbiology Coded Response Sheet. Quantitation of growth is not indicated.

References:

Substratum: The Microbiology Laboratory: Bacterial Reporting & Resistance. Baron, Ellen Jo; Hindler, Janet F., Sheffield Dawson. 1998.

Bailey and Scott's Diagnostic Microbiology, 9th Edition, Baron, Ellen Jo; Peterson, Lance R.; Finegold, Sydney M.; Mosby. St. Louis, Missouri, 1994.

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