Recommendations for Collection and Culture of Clinical Specimens from Pregnant Women for Group B Streptococcus

Rationale

Group B streptococcal (GBS) disease is the leading bacterial infection associated with illness and death among newborns in the United States. GBS (*Streptococcus agalactiae*) is transmitted vertically from colonized women to their infants prior to, or during birth. Early-onset GBS infection (infection occurring at less than 7 days of life) can result in severe, potentially fatal infection in newborns that can include sepsis, meningitis and pneumonia. Infected infants can suffer neurological sequelae and require prolonged hospitalization. Major risk factors for early-onset neonatal disease include maternal GBS colonization, prolonged rupture of membranes, intrapartum fever, labor at less than 37 weeks of gestation, GBS bacteriuria during pregnancy, and previous delivery of an infant with GBS disease. GBS can also cause disease in pregnant women. Maternal GBS infections include asymptomatic bacteriuria, endometritis, amnionitis, premature delivery, stillbirth, and rarely endocarditis.

In 1996, the Centers for Disease Control and Prevention (CDC), in collaboration with the American College of Obstetricians and Gynecologists (ACOG), and the American Academy of Pediatrics (AAP), developed guidelines to address prevention of perinatal GBS disease. The guidelines included the choice of either a screening or non-screening based approach. In 2002, the CDC developed revised guidelines. Published on August 16, 2002 the guidelines recommend a universal screening-based approach to perinatal GBS disease prevention. Using the screening approach, pregnant women are screened for GBS between 35 and 37 weeks of gestation. Because the gastrointestinal tract is a primary source of this organism, the appropriate specimens for culture are a vaginal swab and a rectal swab. The specimens should be cultured using a selective enrichment broth.

A substantial number of GBS infections can be prevented with preterm screening and subsequent intrapartum antimicrobial prophylaxis (antibiotics given after the onset of labor or membrane rupture, but before delivery). In general, penicillin G is the preferred agent for prophylaxis specific for GBS colonization because of its empiric efficacy against GBS, and its narrow spectrum of activity (it is less likely than other agents to select for antibiotic resistant organisms).

Specimen Collection

Rayon or dacron swabs, with non-nutritive transport media, preserve the viability of the organism by providing moisture, and buffering to maintain the pH. Suggested example include:

- **Cultureswab Transport System^{R} (Liquid Amies or Liquid Stuarts) Becton Dickinson, Sparks, MD (800) 638-8663**

The most sensitive method to detect prenatal colonization with GBS requires the use of two swabs. One swab is used to obtain a specimen from the vaginal introitus. The second swab is used to collect a specimen from the anorectum. It may be preferable to use separate swabs for each site to eliminate the potential for iatrogenic transfer of infectious agents such as human papillomavirus (HPV).

Culture of the cervix, and use of a speculum result in the lowest sensitivity and are not recommended.

Immediately after specimen collection, swabs are inserted into the transport media. If separate swabs are collected, both swabs can be placed in the same transport media. When an appropriate transport media is used, GBS from rectovaginal swabs can remain viable up to 96 hours at room or refrigerator temperature, permitting transport to a central microbiology laboratory for processing. All specimens should be labeled with the explicit source (vaginal or rectal).

Specimens should be identified for the laboratory as *specifically for group B streptococcus*. Failure to specify the specimen as a screen for GBS may result in alternate processing of the swab, compromising the sensitivity of detection of group B *streptococcus*. If susceptibility testing is ordered for penicillin-allergic women, specimen labels should identify the patient as penicillin allergic and specify antibiotic susceptibility testing for clindamycin and erythromycin if GBS is isolated.

Selective Enrichment Broths

Extensive studies have shown that optimum recovery of GBS is accomplished using selective enrichment broths as the primary detection method. These broths may consist of Todd Hewitt broth, which provides an excellent nutritional base for streptococci, and specific antibiotic combinations to suppress the growth of saprophytic gram negative organisms. Examples of these selective enrichment broths include:

- **LIM Broth (Todd Hewitt broth with 10 µg/ml colistin and 15 µg/ml nalidixic acid)**
- **Trans-Vag Broth (Todd Hewitt broth with 8 µg/ml gentamicin and 15 µg/ml nalidixic acid) (requires the addition of 5% defibrinated sheep blood for increased recovery of GBS)**

The above selective enrichment broths are available from:

- **Remel Microbiology Products, Lenexa, KS (800) 255-6730**
- **Becton Dickinson, Sparks, MD (800) 638-8663.**

Culture Procedure

Always consult manufacturers product insert for procedure updates.

Step 1: Upon receipt of the specimen into the laboratory, prepare the selective enrichment broth tube by labeling appropriately. If using Trans-Vag broth, add 0.25 ml of sterile, defibrinated sheep blood to each tube. Sheep blood is not added to LIM broth.

Step 2: Primary solid media with and/or without selective agents may be inoculated before the broth. This should be done in addition to, and not instead of inoculation into selective broth. Remove the swab(s) from the transport system and inoculate the plate(s). Streak for isolation and incubate at 35-37^{o} C in ambient air or CO_2 for 18-24 hours.

Step 3: Insert the swab(s) into the selective enrichment broth using aseptic technique. If both a vaginal and rectal swab is submitted from one patient, both swabs may be placed into one enrichment broth. Agitate the swab(s) and express excess liquid against the wall of the tube. The swab(s) may be broken off into the tube or returned to the transport system to retain, as per individual laboratory protocol. Loosely screw the cap onto the inoculated tube.

Step 4: Incubate broth and plates aerobically at 35° C with or without CO_2 for 24 hours. At 24 hours, observe the broth for evidence of bacterial growth (turbidity). If there is no turbidity, reincubate the broth for an additional 24 hours. Even if there is no visible turbidity at 48 hours, the broth may contain low numbers of organisms. Proceed to step 5.

Step 5: If growth is present at 24 hours, or the broth has been incubated for 48 hours, subculture the broth onto one or two nonselective 5% sheep blood agar plates. Incubate subculture plates at 35° C aerobically.

Step 6: Observe subculture growth after a full 24 hours of incubation, for the presence of beta or non hemolytic streptococci. If negative at 24 hours, reincubate an additional 24 hours before reporting a negative result.

Laboratory Identification of Group B Beta Streptococcus

Currently, the most reliable method to ascertain the presence of GBS is identification from colonial growth. On sheep blood agar, group B *streptococcus* produce translucent to opaque, whitish gray, soft, smooth colonies. Most, but not all strains produce a relatively narrow zone of beta hemolysis. Occasional strains do not produce beta hemolysis.

Suspicious colonies (catalase negative, gram positive cocci) should be identified with routine, standardized methods for identification of group B streptococci including:

- Streptococcal serologic grouping, such as slide agglutination is an accurate and reliable methodology to identify GBS. If growth is insufficient or heavily mixed, subculture to a fresh plate.
- GBS can be identified using an algorithm of conventional biochemical tests such as positive CAMP test, positive hippurate hydrolysis, negative bile esculin, negative PYR, negative VP and susceptibility to a 30 µg vancomycin disk. The presumptive identification of GBS is a satisfactory method of identification, but not as accurate as the serologic grouping.
- Fluorescent antibody or genetic probe are useful options and may be used to identify beta hemolytic colonies.
- Automated methods and kits, including Microscan gram positive panels, Vitek cards and API strips, can identify GBS with a high degree of accuracy, although at greater cost.

Currently, direct (from swab) antigen detection methods such as optical immunoassay and enzyme immunoassay are highly specific, but not sufficiently sensitive to detect moderate or low levels of GBS. Therefore, use of direct detection methods for GBS screening is NOT recommended except in highly unusual circumstances.

Suggested Reporting

When GBS is recovered, report as: **Group B streptococcus isolated**

If no GBS is recovered, report as: **No Group B streptococcus isolated**

All results, whether positive or negative, should be reported to the ordering clinician or department.

If a culture from an inappropriate source (any source other than vaginal and rectal) is received, the

provider should be notified.

Any amount of GBS isolated in a urine culture from a pregnant woman should be reported.

Continuous access for clinicians (24 hours a day, 7 days a week) is optimal.

Quality Control

Manufacturers' recommendations for user quality control must be performed on each lot of selective enrichment broth. See NCCLS document M22-A2, December 1996 *Quality Assurance for Commercially Prepared Microbiological Culture Media - Second Edition; Approved Standard,* for comprehensive guidelines.

If media are found to be deficient, it should be documented by the laboratory and the manufacturer should be notified. Appropriate corrective action should be established and noted in the laboratory records. Media quality control failure should lead to additional testing of the media until the cause of the deficiency can be identified and corrected.

***Note: Do not use media that appears compromised**
Always consult manufacturers' product insert for procedure updates

Suggested quality control measures include:

A. **Visual Examination** (Examine each shipment of each lot for physical defects or deterioration)

1. Cracked or broken tubes or caps
2. Evidence of freezing
3. Contamination
4. Spillage

B. **Broth Test Procedure**

Suggested QC organisms and expected results include:

- *Streptococcus agalactiae* ATCC 12386 (moderate to heavy growth)
- *Escherichia coli* ATCC 25922 (partial to complete inhibition)

1. If using Trans-Vag Broth, aseptically add 0.25 ml sterile defibrinated sheep blood to each tube.

2. Inoculate separate broths with the above QC organisms:

   a. Using sterile pipettes, inoculate tubes with 1.0 ml dilutions of 18-24h trypticase soy broth cultures. The dilution used should contain 1000 or less CFU/ml. For *S. agalactiae* and 1.0×10^5 CFU/ml for *E. coli*.

   b. Incubate tubes with loosened caps at 35 ± 2^{o} C in an aerobic atmosphere.

3. Examine tubes for up to 3 days for growth.

Antibiotic Susceptibility Testing

- Use a cotton swab to make a suspension from 18-24 hour growth of the organism in saline or Mueller-Hinton broth to match a 0.5 McFarland turbidity standard.

- Within 15 minutes of adjusting the turbidity, dip a sterile cotton swab into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. Use the swab to inoculate the entire surface of a Mueller-Hinton sheep blood agar plate. After the plate is dry, use sterile forceps to place the clindamycin (2µg) disk onto half of the plate and an erythromycin (15µg) disk onto the other half.

- Incubate at 35^{o} C in 5% CO_2 for 20-24 hours.

- Measure the diameter of the zone of inhibition using a ruler or calipers. Interpret according to NCCLS guidelines for *Streptococcus* species other than *S. pneumoniae.*

2002 Breakpoints*

Clindamycin:	≥ 19 mm = susceptible 16-18 = intermediate ≤ 15 = resistant
Erythromycin:	≥ 21 mm = susceptible 16-20 = intermediate ≤ 15 = resistant

* **Source:** NCCLS. Performance standard for antimicrobial susceptibility testing, M100-S12, Table 2H, Wayne, Pa.: NCCLS, 2002. NCCLS recommends disk diffusion (M-2) or broth microdilution testing (M-7) for susceptibility testing of GBS. Commercial systems that have been cleared or approved for testing of streptococci other than *S. pneumoniae* may also be used. Penicillin susceptibility testing is not routinely recommended for GBS because penicillin-resistant isolates have not been confirmed to date.

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References

ASM Manual of Clinical Microbiology. Seventh edition. 1999. p. 285.

Committee on Obstetric Practice, American College of Obstetricians and Gynecologists. Prevention of early onset group B streptococcal disease in newborns. Washington, DC; American College of Obstetricians and Gynecologists. 1996.

Committee on Infectious Diseases/Committee on Fetus and Newborn, American Academy of Pediatrics. Revised guidelines for prevention of early-onset group B streptococcal infection. Pediatrics 1997; 99:489-96.

Clinical Microbiology Procedures Handbook. 1992. Vol 2: 1.20.12-23.

ASM Manual of Clinical Microbiology. Sixth edition. 1995. p. 300-305.

Bailey and Scott's Diagnostic Microbiology. 1994. Ninth edition. p 340-342.

Prevention of Perinatal Group B Streptococcal Disease: A Public Health Perspective. CDC MMWR. May 31, 1996. Vol. 45. No. RR-7.

Prevention of Perinatal Group B Streptococcal Disease: Revised Guidelines from CDC. CDC MMWR. August 16, 2002. Vol. 51. http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5111a1.htm

Hillier, S.L., A. Schuchat.1997. Preventing neonatal group B streptococcal disease: the role of the clinical microbiology laboratory. Clinical Microbiology Newsletter Vol 19:113-16.

Whitney, C.G., B. Schwartz, K. Deaver, W. Gozansky, J. Wenger, A. Schuchat. 1995. Institutionalizing prevention of perinatal group B streptococcal (GBS) Infections. ICAAC Abstract K192: p. 322.

Recommendations for Collecting and Culturing Clinical Specimens from Pregnant Women for Group B Streptococcus. Nov. 1997. Connecticut Department of Public Health.

Baker, C.J. 1996. Inadequacy of rapid immunoassays for intrapartum detection of Group B streptococcal carriers. Obstetrics and Gynecology. Vol 33. No. 1.

NCCLS. 1996. Quality Assurance for Commercially Prepared Media - Second Edition: Approved Standard. M22-A2. Vol 16.

Philipson, E.H., D.A. Palermino, A. Robinson. 1995. Enhanced antenatal detectionof group B streptococcus colonization. Obstetrics and Gynecology. 85:437-9.

Platt, M.W., J.C. McLaughlin, G.J. Gilson, M.F. Wellhoner, L.J. Nims. 1996. Increased recovery of group B streptococcus by the inclusion of rectal culturing and enrichment. Diagnostic Microbiology Infectious Disease. 21:65-8.

Todd Hewitt Broth w/ Gentamicin and Nalidixic Acid (Trans-Vag Broth) Technical Information. 1993. Remel Microbiology Products.

LIM Broth Quality Control / Product Information. 2001. BBL Quality Control and Product Information Manual for Tubed Media.