

BOX 2. Procedures for processing clinical specimens for culture of group B *Streptococcus* (GBS) (see Figure 7)

- Remove swab(s) from transport medium.* Inoculate swab(s) into a recommended selective broth medium, such as Todd-Hewitt broth supplemented with either gentamicin (8 µg/ml) and nalidixic acid (15 µg/ml) [TransVag broth], or with colistin (10 µg/ml) and nalidixic acid (15 µg/ml) [Lim broth]. TransVag broth may be supplemented with 5% defibrinated sheep blood to increase the recovery of GBS.† As an alternative, swabs may be inoculated into selective enrichment broth that incorporates chromogenic pigments for the detection of beta-hemolytic GBS using color detection. Examples of appropriate commercially available options include StrepB carrot broth or Granada Biphasic broth.§
- Incubate inoculated selective broth for 18–24 hours 35°–37°C in ambient air or 5% CO₂.
- For TransVag or Lim broth, subculture the incubated broth to an appropriate agar plate (e.g., tryptic soy agar with 5% defibrinated sheep blood, Columbia agar with colistin and nalidixic acid, or a commercial chromogenic agar). For chromogenic broth, monitor for color change indicative of GBS per product instructions. GBS detection using chromogenic broth is possible only for beta-hemolytic strains,¶ and therefore all broths that are negative (i.e., no color detection) should be subcultured to a sheep blood agar plate with 5% sheep blood or tested for GBS antigen or by DNA probe to further identify nonhemolytic GBS strains.
- Inspect agar plates and identify organisms suggestive of GBS (e.g., narrow zone of beta hemolysis on blood agar, gram-positive cocci, catalase-negative, and/or hippurate-positive). Note that hemolysis can be difficult to observe, so typical colonies without hemolysis should also be further tested. If GBS is not identified after incubation for 18–24 hours, then reincubate plates overnight and examine for suspected GBS colonies.
- Various streptococcal grouping latex agglutination tests or other tests for GBS detection (e.g., GBS Accuprobe) may be used for specific identification, or the CAMP test can be employed for presumptive identification.
- Optional direct broth testing:** Detection of GBS can be determined directly from broth media using latex agglutination, probes or nucleic acid amplification tests (NAAT) such as PCR.

* Before the inoculation step, laboratories may choose to roll the vaginal-rectal swab(s) on a blood agar plate with or without colistin and nalidixic acid or commercially available chromogenic agar (appropriate recommendations include chromID Strepto B [which might detect both hemolytic and nonhemolytic GBS] or Granada Agar [which detects hemolytic GBS]). **Source:** Tazi A, Réglier-Poupet H, Dautezac F, Raymond J, Poyart C. Comparative evaluation of Strepto B ID chromogenic medium and Granada media for the detection of group B *Streptococcus* from vaginal samples of pregnant women. *J Microbiol Methods* 2008;73:263–5). This approach should be taken only in addition to, and not instead of, inoculation into selective broth. The directly inoculated blood agar plate should be streaked for isolation, incubated at 35°–37°C in ambient air or 5% CO₂ for 18–24 hours and inspected for organisms suggestive of GBS as described above. If suspected colonies are confirmed as GBS, the selective broth can be discarded, thus shortening the time to obtaining culture results. The directly inoculated chromogenic agar should be streaked for isolation and incubated at 35°–37°C for 18–24 hours. Hemolytic GBS isolates are identified by colored colonies as directed by specific manufacturers' instructions, and selective broth can be discarded if GBS positive.

† **Source:** Fenton LJ, Harper MH. Evaluation of colistin and nalidixic acid in Todd-Hewitt broth for selective isolation of group B streptococci. *J Clin Microbiol* 1979;9:167–9. Although Trans-Vag medium often is available without sheep blood, direct comparison of medium with and without sheep blood has shown higher yield when blood is added. Lim broth also might benefit from the addition of sheep blood, although the improvement in yield is smaller, and sufficient data are not yet available to support a recommendation.

§ **Sources:** Church DL, Baxter H, Lloyd T, Miller B, Elsayed S. Evaluation of StrepB carrot broth versus Lim broth for detection of group B *Streptococcus* colonization status of near-term pregnant women. *J Clin Microbiol* 2008;46:2780–2. Martinho F, Prieto E, Pinto D, Castro RM, Morais AM, Salgado L, Exposto Fda L. Evaluation of liquid biphasic Granada medium and instant liquid biphasic Granada medium for group B *Streptococcus* detection. *Enferm Infecc Microbiol Clin* 2008;26:69–71.

¶ **Source:** de la Rosa M, Perez M, Carazo C, Pareja L, Peis JJ, Hernandez F. New Granada medium for detection and identification of group B streptococci. *J Clin Microbiol* 1992;30:1019–21.

** Direct latex agglutination, probe detection or nucleic acid amplification testing on enriched selective broth is an additional option. **Sources:** Guerrero C, Martinez J, Menasalvas A, Blazquez R, Rodriguez, Segovia M. Use of direct latex agglutination testing of selective broth in the detection of group B streptococcal carriage in pregnant women. *Eur J Clin Microbiol Infect Dis* 2004;23:61–2. Goodrich JS, Miller MB. Comprison of culture and 2 real-time polymerase chain reaction assays to detect group B *Streptococcus* during antepartum screening. *Diagn Microbiol Infect Dis* 2007;59:17–22. Block T, Munson E, Culver A, Vaughan K, Hryciuk JE. Comparison of carrot broth- and selective Todd-Hewitt broth-enhanced PCR protocols for real-time detection of *Streptococcus agalactiae* in prenatal vaginal/anorectal specimens. *J Clin Microbiol* 2008;46:3615–20. Montague NS, Cleary TJ, Martinez OV, Procop GW. Detection of group B streptococci in Lim broth by use of group B *Streptococcus* peptide nucleic acid fluorescent in situ hybridization and selective and nonselective agars. *J Clin Microbiol* 2008 46:3470–2. If a rapid test performed on enriched broth yields positive results and antimicrobial susceptibility testing is recommended (for penicillin-allergic women at high risk for anaphylaxis), the enriched broth should be subcultured to obtain an isolate.