

### BOX 3. Procedures for clindamycin and erythromycin susceptibility testing of group B streptococcal (GBS) isolates, when ordered for penicillin-allergic patients

- The Clinical and Laboratory Standards Institute (CLSI) recommends disk diffusion or broth microdilution testing for susceptibility testing of GBS.\* Commercial systems that have been cleared or approved for testing of streptococci other than *S. pneumoniae* also may be used.
- To ensure accurate results, laboratories should include a test for detection of inducible clindamycin resistance. The double-disk diffusion method (D-zone test) is recommended for testing erythromycin-resistant and clindamycin-susceptible GBS.† Other validated tests to detect inducible clindamycin resistance in GBS may be used in place of the D-zone test.
- Use a cotton swab to make a suspension from 18–24 hour growth of the organism in saline or Mueller-Hinton broth equal to a 0.5 McFarland turbidity standard.
- Within 15 minutes of adjusting the turbidity at room temperature, 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 plate of Mueller-Hinton agar with 5% sheep blood.

After the plate is dry, use sterile forceps to place a clindamycin (2 µg) disk and an erythromycin (15 µg) disk 12 mm apart for D-zone testing (Note: This differs from recommended 15–26 mm for staphylococci and a disk dispenser cannot be used to place disks on the plate for streptococci testing).

- Incubate inoculated agar plate at 35°C in 5% CO<sub>2</sub> for 20–24 hours.
- Isolates with blunting of the inhibition zone around the clindamycin disk adjacent to the erythromycin disk (D-zone positive) should be considered to have inducible clindamycin resistance and are presumed to be resistant. (Note: Other validated tests to detect GBS with inducible clindamycin resistance may be used.)
- The following comment could be included in patient reports for isolates that show inducible clindamycin resistance: “This isolate is presumed to be resistant on the basis of detection of inducible clindamycin resistance. Clindamycin still might be effective clinically in some cases.”

\* **Source:** Clinical and Laboratory Standards Institute. Performance standard for antimicrobial susceptibility testing, M100-S20, Table 2H-1, Wayne, Pa: Clinical and Laboratory Standards Institute; 2010. CLSI 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. Interpret according to CLSI guidelines for *Streptococcus* spp. Beta-hemolytic Group (2010 breakpoints for disk-diffusion: for clindamycin: ≥19 mm = susceptible, 16–18 mm = intermediate, and ≤15 mm = resistant; for erythromycin: ≥21 mm = susceptible, 16–20 mm = intermediate, and ≤15 = resistant; for broth microdilution: clindamycin: ≤0.25 µg/ml = susceptible, 0.5 µg/ml = intermediate, and ≥1.0 µg/ml = resistant; and for erythromycin: ≤0.25 µg/ml = susceptible, 0.5 µg/ml = intermediate, and ≥1.0 µg/ml = resistant).

† **Sources:** Tang P, Ng P, Lum M, et al. Use of the Vitek-1 and Vitek-2 systems for detection of constitutive and inducible macrolide resistance in Group B streptococci. J Clin Microbiol 2004;42:2282–4. Richter SS, Howard WJ, Weinstein MP, et al. Multicenter evaluation of the BD Phoenix automated microbiology system for antimicrobial susceptibility testing of Streptococcus species. J Clin Microbiol 2008;45:2863–71.