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LETTER TO THE EDITOR

Suggested Modifications To Improve the Sensitivity and Specificity of the 2010 CDC-Recommended Routine *Streptococcus agalactiae* Screening Culture for Pregnant Women

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In 2010, the Centers for Disease Control and Prevention (CDC) recommended routine screening of pregnant women for *Streptococcus agalactiae* to avoid neonatal *S. agalactiae* infections in the first week of life (1): rectovaginal samples should be incubated in a selective broth such as LIM (Todd-Hewitt broth containing colistin [10 μ/ml] and nalidixic acid [15 μ/ml]) for 16 to 24 h and then subcultured on a solid medium such as tryptic soy agar containing 5% sheep blood (both BD BBL, Franklin Lakes, NJ). The CDC recommended that the identity of beta-hemolytic colonies be confirmed by Gram stain (positive cocci in pairs and chains), negative catalase, and agglutination with antisera against the Lancefield group B antigen. The American College of Obstetricians and Gynecologists supported these recommendations for routine screening (2). In 2010, it was known that *S. agalactiae* beta-hemolysis could be difficult to detect or absent (1). However, it was not appreciated that other streptococcal species, including *Streptococcus pseudoporcinus* on sheep blood agar plates and *S. halichoeri* on GBS Detect agar (GBSDA; Hardy Diagnostics, Santa Maria, CA) could fulfill the CDC criteria for *S. agalactiae*. Unlike *S. agalactiae*, *S. halichoeri is* pyrrolidonyl arylamidase (PYR) positive, and *S. pseudoporcinus* exhibits variable PYR reactions (3–6). Here, we report results that led us to change our solid medium to enhance the detection of hemolysis, followed by confirmatory testing to ensure the accuracy of identification.

Detroit Medical Center University Laboratories (DMCUL) participated in a four-center comparison between a molecular technique and CDC-recommended screening cultures for *S. agalactiae*: two laboratories missed more than 10% of the *S. agalactiae* compared to molecular analysis. In response, DMCUL changed to GBSDA, a sheep-blood agar containing proprietary additives enhancing selectivity and beta-hemolysis, enlarging zones of hemolysis and facilitating detection of nonhemolytic *S. agalactiae* (7). In a week, our laboratory received rectovaginal samples from two penicillin-allergic, pregnant women (aged 22 and 28 years). Incubation in LIM broth and subculture to GBSDA yielded beta-hemolytic, catalase-negative colonies, reacting with antibody to the group B antigen (PathoDx Strep B grouping latex; Thermo Scientific, Waltham, MA). For antibiotic susceptibility determinations, the organisms were analyzed on the BD Phoenix SMIC/ID2 101 panel, which provided susceptibilities but did not identify either isolate. Phoenix reported both to be PYR positive, suggesting that they were not *S. agalactiae* (3–6). Matrix-assisted laser desorption/ionization time-of-flight mass spec-

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troscopy (MALDI-TOF MS; Bruker Biotyper, BD) identified both isolates as *S. halichoeri* (scores 2.1 and 2.3; RUO Software). Neither isolate was identified as *S. agalactiae* by the BD Max GBS assay following the manufacturer's procedure. Using an off-label method, the *S. halichoeri* isolates were also inoculated into blood culture bottles and gave negative results for *S. agalactiae* when tested on Verigene Gram-positive blood culture identification panels (Luminex, Austin, TX).

We began confirming positive screens by MALDI-TOF MS, as suggested by Suwantarat et al. (6), since antibiotic prophylaxis for organisms other than *S. agalactiae*, conveys risks of anaphylaxis (1) and *Clostridium difficile* infection (8) without known benefits. Although *S. halichoeri* is beta-hemolytic on GBSDA agar, it is nonhemolytic on blood agar, so susceptibility interpretation criteria intended for the *Streptococcus* spp. beta-hemolytic group should not be used for *S. halichoeri* (9).

We performed 717 consecutive *S. agalactiae* screening cultures on clinical specimens received from pregnant women. Of these, 260 (36.3%), were beta-hemolytic and group B antigen positive. These apparent positives were subjected to MALDI-TOF MS, and 248 (95.4% of 260; 34.6% of 717) were confirmed as *S. agalactiae*, while 6 were identified as *S. halichoeri* and 6 were identified as *S. pseudoporcinus*. Without MALDI-TOF MS, 12 (4.6%) of the 260 screen-positive organisms would have been falsely identified as *S. agalactiae*.

Available U.S. Food and Drug Administration-cleared molecular techniques for routine *S. agalactiae* screening require initial overnight LIM broth incubation. Molecular methods are significantly more expensive than culture, but they reduce the turnaround time by 1 to 2 days, although false-negative results may occur due to alterations in the target sequence (10). MALDI-TOF MS is costly, but each test takes minutes, has a very low incremental cost, and reduces false-positive screening results for *S. agalactiae*. We recommend using a more sensitive culture medium, such as GBSDA, followed by the routine use of MALDI-TOF MS confirmation techniques in *S. agalactiae* screening culture identification. In the absence of MALDI-TOF MS, a PYR test may be helpful but will not eliminate reporting of PYR-negative *S. pseudoporcinus* as *S. agalactiae* (4–6).

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