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Guidelines for the Detection and Identification of Group B *Streptococcus*

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Laura Filkins, PhD, D(ABMM), Jocelyn Hauser PhD, MLS(ASCP)^{CM},
Barbara Robinson-Dunn, PhD, D(ABMM), FAAM, Robert Tibbetts, PhD, D(ABMM), F(CCM),
Bobby Boyanton, MD, Paula Revell PhD, D(ABMM)
on behalf of the American Society for Microbiology Clinical and Public Health Microbiology Committee, Subcommittee on Laboratory Practices

Summary of Updates since Initial Posting

- For laboratories inoculating both broth and agar plates from the primary specimen, the prior recommendation to inoculate broth first has been removed.
- Clarification was added that laboratories should follow the manufacturer's instructions for incubating differential agar media, with emphasis on determining if the inoculated media should be incubated anaerobically.
- The term "chromogenic" was corrected to "differential" when referring to pigment-production-based color changes.
- Options for vancomycin reporting were added to the antimicrobial susceptibility testing.

Introduction

Streptococcus agalactiae or Group B *Streptococcus* (GBS) has long been a leading cause of neonatal infection. In the United States in the 1970s, GBS emerged as the primary cause of infection of infants in the first week of life, defined as early-onset disease (EOD); with case-fatality rates as high as 50% (1, 2). Maternal colonization with GBS was shown to be the primary risk factor. The CDC's Active Bacterial Core surveillance system reported an incidence of GBS disease of 1.8 cases per 1,000 live births in 1990 (3). Studies showed that intrapartum antibiotic prophylaxis reduced vertical transmission of GBS (4). The recommendation to screen all pregnant women for GBS colonization between 35 and 37 weeks gestation was first released in 1996 by American College of Obstetricians and Gynecologists (ACOG), and was quickly followed by Centers for Disease Control and Prevention (CDC) and American Academy of Pediatrics (AAP) (5–7). As a result of universal screening and intrapartum prophylaxis, GBS EOD has been reduced to an incidence of 0.23 infants per 1,000 live births as of 2015 (8).

The CDC has published recommendations for GBS screening in collaboration with several professional societies since 1996. In 2019, the stewardship of these guidelines was transferred to three professional organizations. ACOG and AAP are now responsible for curation of the guidelines for prophylaxis and treatment of GBS infection in pregnant women and newborns, and the American Society for Microbiology (ASM) is responsible for maintaining and updating guidelines for standard laboratory practices related to detection and identification of GBS (9, 10).

The critical components of preventing early-onset GBS neonatal disease still include universal screening and appropriate intrapartum antibiotic prophylaxis. Noteworthy changes in the most recent ACOG guidelines include the recommendation for antepartum screening for GBS at 36 0/7 to 37 6/7

weeks of gestation (9). This is a change from the recommendation of 35-37 weeks of gestation from the 2010 CDC consensus guidelines (6). ACOG provides complete guidelines for GBS prophylaxis during pre-term delivery and women with unknown GBS status (9).

These laboratory guidelines are intended to provide specific recommendations for optimal specimen collection, storage and transport, organism detection and identification, antimicrobial susceptibility testing (AST), and communication of results. A summary of laboratory testing recommendations is provided in Table 1 and detailed as follows.

Patient Testing

Recommendation: *Antenatal screening is recommended for all pregnant women at 36 0/7 to 37 6/7 weeks of gestation unless intrapartum GBS prophylaxis is already recommended due to identified risk factors.*

The recommended screening interval has changed from 35-37 weeks (per CDC 2010 guidelines) to 36 0/7 to 37 6/7 weeks (ACOG 2019 recommendations) (6, 9). GBS cultures most accurately predict GBS colonization status at birth if GBS screening specimens are collected within 5 weeks prior to delivery. The predictive value of prenatal cultures for GBS decreases significantly when collected more than 5 weeks before delivery. However, approximately 7% of births in the United States occur after 41 weeks of gestation (11). This new recommendation of waiting until at least 36 0/7 weeks of gestation extends the predictive window for screening culture results up to 41 0/7 weeks.

GBS Prophylaxis Recommendations

GBS prophylaxis recommendations are provided by ACOG in their 2019 guidelines (9). These recommendations state that intrapartum prophylaxis is not needed for women who present in labor with unknown GBS colonization status if they have a negative intrapartum GBS nucleic acid amplification testing (NAAT) result and are absent clinical risk factors.
Intrapartum NAAT without enrichment has an unacceptably high false negative rate, ranging from 6.3% to 22% (12–14). As such we do not recommend the use of intrapartum NAAT without enrichment to rule out the need for prophylaxis.

Specimen Collection, Storage and Transport

Recommendation: *Use a single swab to obtain a screening specimen first from the lower vagina and then from the rectum without use of a speculum.*

Colonization with GBS often occurs in low bacterial cell concentrations. Vaginal-rectal swabs have been reported to provide high bacterial yields (15–18). To maximize the likelihood of GBS recovery, a single swab is used to obtain the specimen from the vagina and rectum. Without using a speculum, collect the specimen first from the vagina (near the introitus) by inserting the swab about two centimeters and then from the rectum by inserting the same swab one centimeter through the anal sphincter. Culturing both the lower vagina and rectum, either with two individual swabs or, preferably, using a single swab to sequentially sample both sites, increases the culture yield substantially compared with either sampling the cervix or vagina alone without a rectal culture (16, 17, 19–21). Cervical, perianal, perirectal or perineal specimens are not acceptable, and a speculum should not be used for culture collection (6).

Recommendation: *Collect vaginal-rectal specimens using a flocked swab and place in a liquid-based transport medium such as Amies transport media.*

Traditional fiber wrapped swabs such as Rayon, Dacron, and cotton swabs, have been used for vaginal and or rectal specimens for culturing GBS. However, these types of swabs prevent release of microorganisms which reduces GBS recovery (22). Flocked swabs release microorganisms more efficiently than traditional fiber swabs (23). The applicator tip of flocked swabs is composed of fibers that protrude perpendicularly to the swab shaft optimizing collection and minimizing entrapment of specimens (24).

Optimal recovery of viable organisms from swabs (including flocked swabs, Dacron, or Rayon) is achieved when the swab is preserved using non-nutritive transport media, such as Amies transport medium (6, 25, 26). Amies medium, a modification of Stuart's transport medium, contains an inorganic phosphate buffer and may also contain charcoal, which absorbs inhibitory substances released in the medium during transport of sample (22, 24, 27, 28). Transport systems such as the Eswab (Copan Diagnostics, Murrieta, CA), significantly increase the recovery of GBS compared to traditional fiber swabs (22, 23, 29, 30). Using the Eswab, recovery of GBS by enriched culture has been reported to be as high as 100% (22). Additionally, some transport systems using a flocked swab and Amies liquid transport medium to support efficient recovery of GBS after inoculation into broth culture, are also approved for use with FDA-cleared molecular diagnostic assays, and are compatible with automated specimen processors enabling standardization between methods and simplifying specimen collection for caregivers (22, 23).

Recommendation: *Transport vaginal-rectal specimens to the testing laboratory within 24 hours.*

Immediately after specimen collection, swabs should be inserted into Amies transport medium or equivalent and transported to the clinical laboratory within 24 hours (24, 31). If transport and/or culturing are delayed, refrigerate specimens in Amies transport medium at 4-8°C or store specimens collected in Eswabs at room temperature (6, 24). Prolonged storage and storage at temperatures at or above 21°C are suboptimal for specimens collected with standard fiber swabs as GBS viability decreases after 24 hours. GBS recovery from Eswabs is highest when stored at 4°C and room temperature, with room temperature (21-24°C) being the optimal temperature (24). Although GBS remains viable in Eswab transport systems for up to 6 days in some patients, we recommend all GBS screening specimens be transported to the clinical laboratory within 24 hours to prevent decrease in GBS viability and expedite reporting of screen results. Culturing specimens greater than 24 hours after collection may yield false-negative results and is strongly discouraged. If delay beyond 24 hours occurs, specimen rejection and request for recollection are recommended.

DETECTION

Recommendation: *Incubate GBS screening specimens in selective enrichment broth prior to agar media plating or NAAT.*

The sensitivity of GBS detection is strongly impacted by culture enrichment. Incubation in broth media prior to plating increases sensitivity of screening methods by about two-fold compared to direct specimen plating (17, 20, 32). Likewise NAAT sensitivity is increased following culture enrichment.

After consensus or discrepancy analysis, NAATs are reported to provide enhanced sensitivity for detection of GBS from enrichment broth for screening (33–35). NAAT sensitivity from enrichment broth culture varies by specific test, but is typically >96% compared to gold standard culture methods. Whether culture or NAAT are selected as the primary method of GBS detection, enrichment broth culture must first be performed. Recommended workflows are summarized in Figure 1. Importantly, commercial NAATs performed directly from specimen (without enrichment) are available, but their use is not recommended at this time due to high false negative rates of 6.3% to 22% (12–14).

To achieve increased detection, we recommend all vaginal-rectal swabs be inoculated into a selective enrichment broth media and incubated for 18-24 hours at 35-37°C in ambient or 5% CO_2 conditions. If using Eswab transport system, vortex the specimen briefly and inoculate 1:10-1:20 volumetric ratio of specimen to enrichment broth medium; then, incubate as described above. Several enrichment broths are available, including non-selective, selective, and differential broths. Selective enrichment broth incubation is reported to increase GBS detection compared to non-selective broths resulting in up to 2.5-fold increased frequency of GBS-positive screening (36). Selective broths include components to inhibit or suppress the growth of enteric organisms and some Gram positive bacteria such as *Staphylococcus*. When non-selective broth is used, these organisms can overgrow GBS making detection difficult. Acceptable selective enrichment broths include: Todd-Hewitt broth with gentamicin (8 μg/ml) and nalidixic acid (15 μg/ml) (known as Trans-Vag Broth), Todd-Hewitt broth with colistin (10 μg/ml) and nalidixic acid (15 μg/ml) (known as Lim broth) (36–38). Modified formulations of these selective media are available from commercial vendors and are generally acceptable. The addition of 5% defibrinated sheep's blood to Trans-Vag Broth was shown to enhance detection of GBS (39). However, evidence supporting supplementation of selective enrichment broth to improve GBS detection is limited. Therefore, use of selective enrichment broth without blood is acceptable.

Differential enrichment broths allow for GBS propagation and detection in a single step. Differential broths include Carrot Broth, Granada Liquid Biphasic broth, and others. These media support pigment production by hemolytic strains of GBS. An advantage of using differential tube media for primary specimen inoculation is the ability to report positive GBS detection directly from broth results in as quickly as 10 hours, with the majority of specimens reported within 24 hours (40–42). When an orange-red color pigment is observed in these broths, the specimen can be reported as positive for GBS. Compared to selective broth enrichment followed by subculture, pigment-production-based differential broth is highly specific for GBS and is sensitive for hemolytic strains (43). However, non-hemolytic strains are not detected by this method (40, 43). Therefore, subculture of all pigment-negative broth cultures to agar plates is recommended. A NAAT may alternatively be performed from broth cultures if approved by the NAAT manufacturer. Similarly, when AST is performed on isolates from positive specimens, subculture from broth is required. The requirement to subculture most, if not all, broth cultures limits the advantages of pigment-based broths over non-differential, selective enrichment broths, but is an acceptable alternative.

Infrequently, the growth of other microorganisms found in vaginal-rectal samples may decrease recovery of GBS after enrichment broth incubation. In particular, abundant quantities of *Enterococcus faecalis* can competitively suppress the growth of GBS during enrichment broth incubation leading to false negative GBS detection (15, 44). Laboratories may choose to directly inoculate agar culture plates in addition to enrichment broth inoculation. When positive, direct plating decreases time-to-detection of GBS compared to enrichment broth incubation followed by plating to agar media.

However, direct specimen plating alone, in the absence of enrichment broth culture, is unacceptable and should not be performed.

Recommendation: *Culture media and GBS isolation methods should detect both hemolytic and non-hemolytic strains.*

After enrichment in broth medium, plating the enriched broth culture to an agar plate medium is recommended. Acceptable agar media include: Tryptic Soy Agar with 5% Sheep's Blood or Columbia Agar with 5% Sheep's Blood (referred to as 'blood agar plates'), Columbia Agar with colistin and nalidixic acid (CNA), and some differential or chromogenic media (6). Use of plates with selective agents may reduce growth of other normal flora enabling easier isolation of GBS. Non-selective blood agar plates are acceptable but may require a higher number of candidate isolate screening. When blood agar plates or CNA agar plates are used, candidate GBS isolates are large (>0.5mm after 24 hours), grey to white, translucent colonies with a narrow zone of beta-hemolysis or non-hemolytic. Non-hemolytic strains compose about 5-6% of GBS in screening specimens (45–47), therefore careful scrutiny for non-hemolytic isolates is recommended. Alternatively, hemolysis-enhancing media, such as GBS Detect (Hardy Diagnostics), is used to detect isolates that would be non-hemolytic on standard blood agar plates. Candidate isolates from any of these described media should be identified by an acceptable method described below. If no candidate isolates are observed after 24 hours of incubation, continue incubating and re-examine culture plates at 48 hours. After 48 hours, samples may be reported as negative for GBS if no candidate isolates are seen.

Chromogenic and differential media used in place of or in addition to agar media described above can facilitate GBS detection. These media use colony color changes to enable quick detection of candidate GBS colonies, but the mechanism of pigment production or color change differs between media. Some agars, such as Granada medium, utilize GBS's natural red-orange pigment production. However, these media do not detect non-hemolytic strains of GBS. A correlation between pigment production and hemolysis by GBS has been recognized for over 80 years (48–50). More recently, the *cyl* operon was determined to be responsible for production of pigment/hemolysin (51–54). Further, these two phenotypes (hemolysis and pigment production) are likely due to a single ornithine rhamnolipid molecule (55). Therefore, laboratory reagents exploiting pigment production for detection of GBS lack sensitivity for non-hemolytic strains and are not recommended unless non-pigmented colonies are also screened. For differential media, refer to the manufacturer's instructions for optimal growth conditions; in particular, an anaerobic growth environment is recommended for many pigment-production-based differential media.

Pigment-production-independent, chromogenic agars are acceptable for GBS screening. Examples of these media include: Brilliance GBS (Thermo Fisher Scientific), ChromID StrepB (bioMérieux), CHROMagar StrepB (CHROMagar Microbiology, Paris, France), and StrepB*Select* (Bio-Rad, Hercules, CA). If a commercially available chromogenic medium is used, the manufacturer's instruction for use should be followed. Incorrect incubation conditions and duration can alter visual interpretation of colonies growing on chromogenic media. Equivalent or even enhanced detection of GBS is reported using these media compared to blood agar plates or CNA (56–58). Specificity can be low and depends on the enrichment broth and specific chromogenic agar selected, with studies reporting up to 31% of target-color isolates identified as non-GBS (46, 59). Therefore, identification of candidate isolates is recommended from chromogenic agars.

While some studies report modestly enhanced GBS detection using one agar medium over another, overall, detection of GBS is similar (57, 60). The choice of a non-selective medium, selective medium, differential medium, or chromogenic medium can be determined by individual labs. Cost, work-flow, labor, and downstream identification of candidate isolates should be evaluated when choosing a plated agar medium.

Recommendation: *Report GBS in any quantity from urine cultures from pregnant women during all trimesters.*

Urine cultures may be collected anytime during pregnancy. Infectious Diseases Society of America recommends collecting a urine culture early in pregnancy for evaluation of asymptomatic bacteriuria (GBS or other organism) (61). In addition to standard of care culture work-up for potential causes of urinary tract infection, ACOG recommends urine culture screening and reporting for GBS in any quantity from pregnant women (9). Treatment of significant quantities (≥100,000 CFU/ml) of GBS in symptomatic patients is recommended and can reduce risk of pyelonephritis, low birth weight, and preterm birth in asymptomatic mothers (9, 62). Even low quantities of GBS in urine specimens correlate with anogenital colonization, increased risk of intrapartum colonization, and increased risk of neonatal EOD (9, 63–65). While treatment is not recommended for lower concentrations of asymptomatic GBS bacteriuria, any quantity is an indication for intrapartum prophylaxis.

Isolating and identifying GBS in any quantity from all urine cultures is labor intensive and will result in increased laboratory costs. Further, reporting low abundance GBS bacteriuria is not clinically indicated for most patients (e.g. most males and non-pregnant females). Specimens collected from pregnant women that require reporting of GBS in any quantity should be labeled or otherwise indicated during the ordering or collection process. Discussion with physicians is recommended to determine an optimal approach to communicating to the laboratory which specimens are collected from pregnant females. Unique test codes for ordering obstetric urine cultures, order indications or selection options built into an order form, or order comments are examples of approaches that may be used. Alternatively, GBS reporting on all urine cultures collected from females of reproductive age is acceptable. If the latter approach is used, communication with providers and reporting results with interpretative comments is recommended to acknowledge that treatment is not needed in many cases.

IDENTIFICATION

Recommendation: *Acceptable phenotypic and proteomic methods of identification of candidate isolates include CAMP test, latex agglutination, and mass spectrometry*.

Candidate isolates detected on agar media can be identified by several acceptable phenotypic methods. Previously, candidate isolates were presumptively identified as GBS if they were catalase negative and produced a positive Christie, Atkins, and Munch-Peterson (CAMP) factor reaction (66). This method is still acceptable practice but increases time-to-results due to an extra 18-24hr aerobic incubation for the CAMP factor test (67). Modified CAMP tests improve time-to-results using preparations of beta-lysin from *Staphylococcus aureus* and well isolated candidate colonies of GBS. For example, rapid spot-CAMP methods are performed in less than an hour and have demonstrated high specificity for GBS (68).

Latex agglutination reagents detect group-specific carbohydrate antigens. *Streptococcus* species are grouped according to carbohydrate antigens, also known as Lancefield typing (69). Detecting Group B antigens is consistent with identification of *Streptococcus agalactiae* and is an acceptable detection method. However, these reagents also react with cell wall components of some strains of *S. pseudoporcinus* and *Streptococcus halichoeri*, yielding a positive interpretation for presence of Group B *Streptococcus*. The role of *S. pseudoporcinus* and *S. halichoeri* is discussed below.When using latex agglutination reactions for detection of GBS, performing pyrrolidonyl arylamidase (PYR) is recommended. *S. agalactiae* is PYR test reaction-negative. Isolates yielding positive Group B antigen agglutination and positive PYR are consistent with *S. halichoeri* or *S. pseudoporcinus* (70–72). However, a negative PYR reaction does not rule-out *S. pseudoporcinus* (72).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI) is a specific method to identify *S. agalactiae*. This method also enables differentiation between *S. agalactiae* and *S. halichoeri* or *S. pseudoporcinus* (73).

Identification of GBS is possible directly from enrichment broth, without sub-culturing to agar plated medium. FDA-approved or -cleared molecular assays are commercially available for detection of GBS from enrichment broth (LIM or other) and are acceptable methods for GBS screening.

Recommendation: *Nucleic acid amplification-based identification of GBS from enrichment broth is acceptable, but not sufficient for all patients.*

Identification of GBS is possible directly from enrichment broth culture, without sub-culturing to agar plate media. FDA-cleared molecular assays are commercially available for detection of GBS from enrichment broth (LIM or other) and are acceptable methods for GBS screening. We do not recommend direct from specimen NAAT (without enrichment). While direct NAAT is more rapid than enrichment culture followed by NAAT, direct from specimen amplification remains fraught with low sensitivity and high rates of false negative results (12, 13).

NAAT sensitivity from enrichment broth culture varies by specific test but is typically >96% compared to gold standard culture methods. Specificity of molecular methods is relatively low (~88-96%) compared to culture (33–35, 74). These additional positives may be true positives due to increased sensitivity of NAAT compared to culture, false-positive detection due to cross-reaction of primers or probes, or a combination of both. After consensus or discrepancy analysis, NAATs demonstrate improved detection of GBS from enrichment broth culture of screening specimens (33–35). Molecular assays typically require less than 5 minutes of hands-on-time for a single sample and sample batching increases efficiency (75). Some high-throughput assays, such as the NeuMoDX 288, can support testing of >100 samples at once, enabling improved workflow for high-volume screening laboratories and faster results compared to culture (76).

The greatest limitation of NAAT for detection and identification of GBS is the lack of organism isolation for AST. Specifically, pregnant women who cannot receive penicillin or cefazolin for intrapartum prophylaxis, typically due to severe penicillin allergy, require GBS AST and therefore NAAT, alone, is not sufficient. In these women, clindamycin is the preferred antibiotic for prophylaxis, and intravenous vancomycin is a less desirable alternative. However, due to increasing rates of clindamycin resistance, confirmation of isolate susceptibility by laboratory testing is needed

prior to clindamycin use. Therefore, laboratories utilizing a NAAT as the primary detection/identification method should reflex positive specimens requiring AST for subculture to agar media.

Recommendation: *Latex agglutination directly from enrichment broth and direct-from-specimen immunoassays are unacceptable methods for GBS detection.*

In addition to NAAT, other culture-independent methods of detection and identification of GBS from enrichment broth have been evaluated, but are not recommended. Latex agglutination performed on enrichment broth culture is reported to have high specificity (>99%), but variable sensitivity ranging from 65-99% (20, 44, 77). The high sensitivity reported in some studies suggests direct latex agglutination testing on enrichment broth may be a less expensive alternative to NAAT. However, due to the variable performance reported we do not recommend this method. Optical immunoassays and immunochromatography methods used on primary specimen and from enrichment broth were also explored. These approaches demonstrated unacceptable performance direct-from-specimen and reduced sensitivity from enrichment broth compared to culture (78–81). Previously available commercial immunoassays are largely discontinued and remaining methods are not recommended.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Recommendation: *Perform antimicrobial susceptibility testing on all GBS isolates from pregnant women with severe penicillin allergy.*

Intravenous penicillin remains the preferred agent for intrapartum prophylaxis and is recommended for non-penicillin-allergic women who meet criteria for intrapartum GBS prophylaxis. Additionally, ACOG 2019 guidelines recommend women with non-severe penicillin allergies may be treated with first generation cephalosporins, including cefazolin (9). *Streptococcus agalactiae* and other beta-hemolytic streptococci remain predictably susceptible to penicillin and cefazolin (82). Therefore, routine AST is not required prior to administration of these agents.

Clindamycin is the recommended agent for prophylaxis of women with severe penicillin allergy (9). Rates of resistance to both erythromycin and clindamycin are increasing with recent studies reporting 15%->40% clindamycin-resistant isolates (8, 83–85). Erythromycin and clindamycin resistance testing is recommended by Clinical and Laboratory Standards Institute (CLSI). Testing methods include disk diffusion or broth microdilution CLSI reference methods, an FDA-cleared automated AST platform, or FDA-cleared agar gradient diffusion methods (82). Laboratories should perform AST for both erythromycin and clindamycin in cases of severe penicillin allergy, but, of the two antimicrobials, only clindamycin should be reported in the laboratory results. Erythromycin is used as screening tool for possible inducible clindamycin resistance. Specifically, isolates demonstrating erythromycin resistance and clindamycin-susceptible or clindamycin-intermediate results must be further tested for inducible clindamycin resistance. Acceptable methods of inducible clindamycin resistance testing include disk diffusion D-zone test, broth microdilution, or inducible clindamycin screening methods incorporated in some FDA-cleared AST platforms (82, 86, 87). Inducible resistance testing can be performed from subculture plates after confirmation of candidate isolates or from subculture after AST (“purity plates”). Inducible clindamycin resistance has been reported in about a third of all clindamycin-resistant isolates (8).

AST is recommended for all *S. agalactiae* isolates from pregnant women with high risk penicillin allergy. When AST is performed to guide intrapartum prophylaxis for severely penicillin-allergic patients, we recommend reporting results for the following agents: clindamycin (including inducible clindamycin) and vancomycin. Reporting of vancomycin may be considered if the isolate is not clindamycin susceptible and if the patient is penicillin allergic. A laboratory may also choose to add a comment to the vancomycin report stating that vancomycin may be an acceptable alternative if the isolate displays resistance to clindamycin. Additional or alternative agent reporting may be considered at individual institutions, but is not broadly recommended. Laboratories must develop methods to ensure AST is performed for all at-risk patients. A laboratory may consider performing AST on all GBS from all positive pre-natal and intrapartum screening tests. This approach does not rely on a penicillin allergic woman being properly identified by the ordering physician and improves laboratory epidemiology of GBS antimicrobial resistance. However, testing of all GBS isolates may not be feasible in many locations due to workflow, resource, and financial considerations. If AST is not automatically performed on all GBS screening isolates, penicillin allergic patients with severe allergies must be identified in laboratory requisitions for screening cultures. We recommend GBS screening culture orders include indication of penicillin allergy as a mandatory field in electronic orders.

Urine culture isolates from penicillin-allergic pregnant women should, similarly, be tested for clindamycin resistance, with the understanding that clindamycin is inappropriate for treatment of a urinary tract infection, but, rather, could be used as intrapartum prophylaxis. GBS in this situation is a marker of high level of colonization of the vaginal tract, rather than a marker of a urinary tract infection. Identifying specimens collected from pregnant women is more difficult for urine cultures. AST on all GBS isolates from urine collected from women of reproductive age is acceptable. Communication with physicians and a reporting comment are recommended to emphasize that clindamycin is not an appropriate drug for treating urinary tract infections and is only reported for guidance of intrapartum prophylaxis (9). Alternatively, urine cultures may incorporate two clinical indications on laboratory requisitions: pregnancy status and penicillin allergy. Some institutions offer obstetric urine cultures to be ordered for pregnant women in place of a routine urine culture. The decision of how to identify samples requiring AST on GBS isolates from urine should be made based on the needs and population of each institution.

DISCUSSION

Despite strong recommendations for screening and prophylactic treatment for GBS, neonatal disease remains a leading cause of infection and death in the United States (6). Despite universal GBS screening recommendations for all pregnant women without risk-based indication for intrapartum prophylaxis, approximately 81% of neonatal GBS disease are from mothers who had negative screening cultures, suggesting inadequate sensitivity of culture or late onset changes in the mother's GBS colonization (88). The 2010 CDC guidelines and our recommendations, above, include provisions for using NAAT for GBS screening from enrichment broth, potentially with increased sensitivity compared to culture-only methods (6). There are several FDA-cleared molecular testing platforms that vary considerably in specificity but do have sensitivities often greater than 96% (33). While culture remains the gold standard, recent studies report highly variable culture sensitivities compared to NAAT, indicating sensitivity of culture may be as low as 53 to 70% (33, 74, 89).

NAAT for GBS detection and identification is sensitive, but also has limitations. Genotypic amplification assays use primers and probes to hybridize specific locations within the genome of the organism of interest. Infrequently, target GBS may contain sequence diversity, mutations, or deletions at the primer or probe target site causing false-negative results (90). In these cases, culture detects a low proportion of isolates missed by molecular detection. Molecular assays also may yield invalid results due to inhibition of the amplification reaction or other cause of test failure (74). Repeat molecular testing or reflex to culture is recommended for samples with invalid or indeterminate results. However, if NAAT is used as the primary laboratory method of detection and identification of GBS, reflex to culture is not required for every negative specimen.

NAATs are rapid, requiring approximately 1-2 hours depending on the platform. However, all FDA-cleared NAATs still require enrichment broth culture, except the Cepheid Xpert® GBS test (for intrapartum use) (34). Despite the continued need for the enrichment broth step, NAATs enable faster turn-around-time for GBS detection when compared to culture-only workflows which typically require 24-48 hours for growth and identification after enrichment broth. Intrapartum direct-from-specimen is used in some institutions and potential positive impacts are reported. For example, Babu et al. compared intrapartum, direct-from-specimen NAAT to culture in women presenting early in labor and without preexisting indications for intrapartum antibiotic prophylaxis. Of the 158 women included, NAAT was positive in 27/30 culture-positive cases (90% sensitivity) and 81% of patients with positive GBS screen by a NAAT did not receive intrapartum antibiotics. Further, 81% of women with a positive GBS screen delivered their baby >3 hours after specimen collection; enough time for a NAAT to have been performed (91). This study highlights the potential impact of using a rapid NAAT to detect GBS colonization at time of delivery in women who would not otherwise have received prophylaxis. In our current recommendations, we do not recommend use of direct-from-specimen, intrapartum NAAT due to poor sensitivity, low negative predictive value, and insufficient supporting evidence to date. However, this is an evolving area for GBS screening that may provide benefit if testing is performed cautiously and results are interpreted conservatively. Evidence-based studies are needed to evaluate when, how, and if a direct-from-specimen NAAT should be used.

Regarding cost, culture is about 10 times less expensive than PCR and detection rates are similar to culture (92). Despite the increase in cost of testing, El Helali et al. determined that using an intrapartum NAAT resulted in significantly reduced likelihood of neonatal GBS infection and, thus, reduction of severe cases, in their population. They attributed this to high sensitivity and negative predictive values of NAAT resulting in decreased costs to treat GBS-infected newborns from $146,057 to $25,433 over the study period (92). While NAAT is an effective method for detecting GBS, the primary limitation is the inability to determine antimicrobial susceptibilities of the bacteria, which is particularly important in women with penicillin allergies or in geographic areas in which the susceptibility of GBS to the macrolides and lincosamides has substantially decreased. Therefore, culture remains a critical component of laboratory testing for GBS.

As an important component of GBS detection in culture, we recommend isolation and detection of non-hemolytic GBS strains. However, it should be noted that the role of non-hemolytic strains in invasive disease is debated. Hemolysis is associated with virulence but is not the sole determinant of virulence (54, 55, 93–95). Rodriguez-Granger and colleagues reported only 1% of invasive GBS isolates from EOD were non-hemolytic in their study. The CDC reported 4% of total isolates from invasive EOD were non-hemolytic during a study period from 2006-2008 (6). Both reports are lower than the typically reported 5% frequency of non-hemolytic strains isolated from maternal GBS

screening. However, neither study specifically compared the proportion of non-hemolytic isolates detected in maternal colonization screening for their same geographic population. While these data suggest the proportion of non-hemolytic isolates causing invasive disease may be reduced compared to the overall frequency of non-hemolytic strains, we cannot definitively make this conclusion. Importantly, both studies identified non-hemolytic strains from cases of invasive EOD. In contrast, Adler et al reported 7% of strains from neonatal/peripartum infections to be non-hemolytic, a disproportionately high fraction compared to the 0% of non-hemolytic strains detected from vaginal swabs in this study (96). Another possibility is that hemolytic strains are responsible for initial infection and become non-hemolytic while in the neonatal host. Sigge et al. described a single case of non-hemolytic and hemolytic strain heterogeneity in a GBS isolate from a newborn with EOD and whose mother was colonized with only a hemolytic strain, suggesting loss of hemolysis may occur after neonatal invasion (97). Taken together, we concluded that the risk of invasive EOD may be lower when mothers are colonized with non-hemolytic strains compared to mothers colonized with hemolytic strains of GBS. However, non-hemolytic strains likely cause a proportion of EOD. Therefore, we recommend GBS screening methods include detection of non-hemolytic strains.

The majority of GBS strains isolated in human specimens are *S. agalactiae* and "GBS" is often used synonymously with *S. agalactiae*. However, bacterial strains from two additional species of *Streptococcus* positively react with immunoglobulin against the Lancefield Group B antigen leading to their identification as GBS (98). In the absence of distinct biochemical reactions, identification by MALDI, or 16S rRNA gene sequencing, group B-positive *S. agalactiae*, *S. pseudoporcinus* (previously part of the *S. porcinus* species), and *S. halichoeri* are difficult to distinguish. Recent reports indicate these non-*agalactiae* streptococcal species may each account for about 2.5% of beta-hemolytic, group B antigen-positive strains in prenatal screening cultures (72, 99). Colonization rates of *S. pseudoporcinus* detected in GBS screening specimens are typically 1-2%, but can vary in different populations (73, 99). Stoner et al reported 5.4% of non-pregnant women of reproductive were vaginally or rectally colonized with *S. pseudoporcinus*. Notably, colonization rates with GBS were also higher in this study than typically reported (54.8%) (100). The clinical significance of detecting and identifying *S. pseudoporcinus* and *S. halichoeri* in pre-natal screening specimens is not well understood. Increased rates of pre-term rupture of membranes and spontaneous preterm birth are reported in mothers colonized with *S. pseudoporcinus* compared to GBS-negative mothers or mothers colonized with *S. agalactiae* (99, 101). *S. halichoeri* was reported as the etiologic agent in cases of cellulitis and empyema, indicating its potential as a rare pathogen (102, 103). However, the relevance of *S. halichoeri* detection during pregnancy is not established.

Differentiation of GBS is most easily performed from cultured isolates. *S. halichoeri* tests positive for PYR, enabling rapid biochemical differentiation from *S. agalactiae*, while *S. pseudoporcinus* produces variable PYR reactions (71–73). MALDI and gene sequencing can distinguish PYR negative, group B-reacting *S. pseudoporcinus* from *S. agalactiae*. Detection or exclusion of *S. pseudoporcinus* and *S. halichoeri* is not thoroughly evaluated for most commercial NAATs. Accurate identification and reporting of group B-positive isolates will aid future investigations toward elucidating the clinical role of non-*agalactiae* GBS. Currently, there is insufficient evidence for the role of non-*agalactiae* GBS in pregnancy and neonatal health. Therefore, differentiation and reporting of *S. pseudoporcinus*, *S. halichoeri*, and *S. agalactiae* are not strongly recommended for all laboratories at this time, but may be considered by individual laboratories.

CONCLUSIONS

In 2019, ASM accepted the responsibility of updating the best practice clinical laboratory guidelines for GBS screening in pregnancy, previously published in 2010. Clinical and laboratory studies over the past decade support continued application of prior procedures for GBS screening specimen collection, organism detection, and identification. Laboratory methods for GBS screening have not changed substantially over the past decade and culture remains the gold standard method. In the current guidelines, we acknowledge molecular methods of GBS detection and identification as techniques that are routinely used today. NAAT from enrichment broth culture shortens time to detection compared to culture-alone and MALDI-TOF MS is the principal method of bacterial identification in many laboratories. These advancements improve the turn-around-time of GBS screening assays and simplify laboratory workflow. However, there are currently insufficient data to recommend direct-from-specimen testing by NAAT and further studies and evidence-based reviews are required to determine optimal use of NAAT for GBS testing. Finally, we emphasize the importance of culture remaining as hallmark steps in GBS screening, including the need for broth enrichment from all specimens and cultivation of GBS for susceptibility testing.

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Table 1: Summary of recommendations for GBS laboratory testing from pregnant women

Topic	Recommendations	Examples/Key points
Patient testing	Antenatal screening is recommended for all pregnant women at 36 0/7 to 37 6/7 weeks of gestation unless intrapartum GBS prophylaxis is already recommended due to identified risk factors.	GBS prophylaxis is recommended according to ACOG 2019 guidelines (9)
Specimen Collection, Storage, and Transport	1. Use a single swab to obtain a screening specimen first from the lower vagina and then from the rectum without use of a speculum. 2. Collect vaginal-rectal specimens using a flocked swab and place in a liquid-based transport medium such as Amies transport media. 3. Transport vaginal-rectal specimens to the testing laboratory within 24hrs.	**Acceptable rectovaginal collection devices**: Flocked swab **Acceptable transport media:** Amies media+/- Charcoal E-swab (Copan)
Laboratory Detection of GBS	1. Incubate GBS screening specimens in selective enrichment broth prior to agar media plating or NAAT. 2. Culture media and GBS isolation methods should detect both hemolytic and non-hemolytic strains 3. Report GBS in any quantity from urine cultures from pregnant women during all trimesters.	**Acceptable selective broths for enrichment include:** Todd Hewitt Broth with gentamicin (8 µg/ml) and nalidixic acid (15 µg/ml) Lim Broth Carrot Broth, Granada Liquid Biphasic broth **Acceptable media for GBS culture and isolation include**: Tryptic Soy Agar with 5% Sheep's Blood Columbia Agar with 5% Sheep's Blood Columbia Agar with 5% Sheep's Blood, colistin, and nalidixic acid Brilliance GBS ChromID StrepB ChroMagar StrepB*Select*
Laboratory Identification	1. Acceptable phenotypic and proteomic methods of identification of candidate isolates include CAMP test, latex agglutination, and MALDI. 2. Nucleic acid amplification-based identification of GBS from enrichment broth is acceptable, but not sufficient for all patients. 3. Latex agglutination directly from enrichment broth and direct-from-specimen immunoassays are unacceptable methods for GBS detection.	**Key biochemical reactions for Presumptive GBS identification**: Catalase: negative CAMP: positive PYR: negative **Acceptable molecular methods for GBS identification**: MALDI NAAT from enrichment broth
Susceptibility Testing	Perform antimicrobial susceptibility testing on all GBS isolates from pregnant women with penicillin allergy	**Available methods for susceptibility testing include (but not limited to):** Disk diffusion Broth microdilution **Susceptibility testing and reporting should be performed (in case of severe penicillin allergy) for**: Erythromycin (perform but do not report) Clindamycin (including inducible resistance) Vancomycin **Acceptable test methods for inducible clindamycin resistance** D-zone test Broth microdilution Automated methods

Abbreviations: CAMP, Christie, Atkins, and Munch-Peterson; MALDI, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NAAT, nucleic acid amplification testing; PYR, pyrrolidonyl aminopeptidase.

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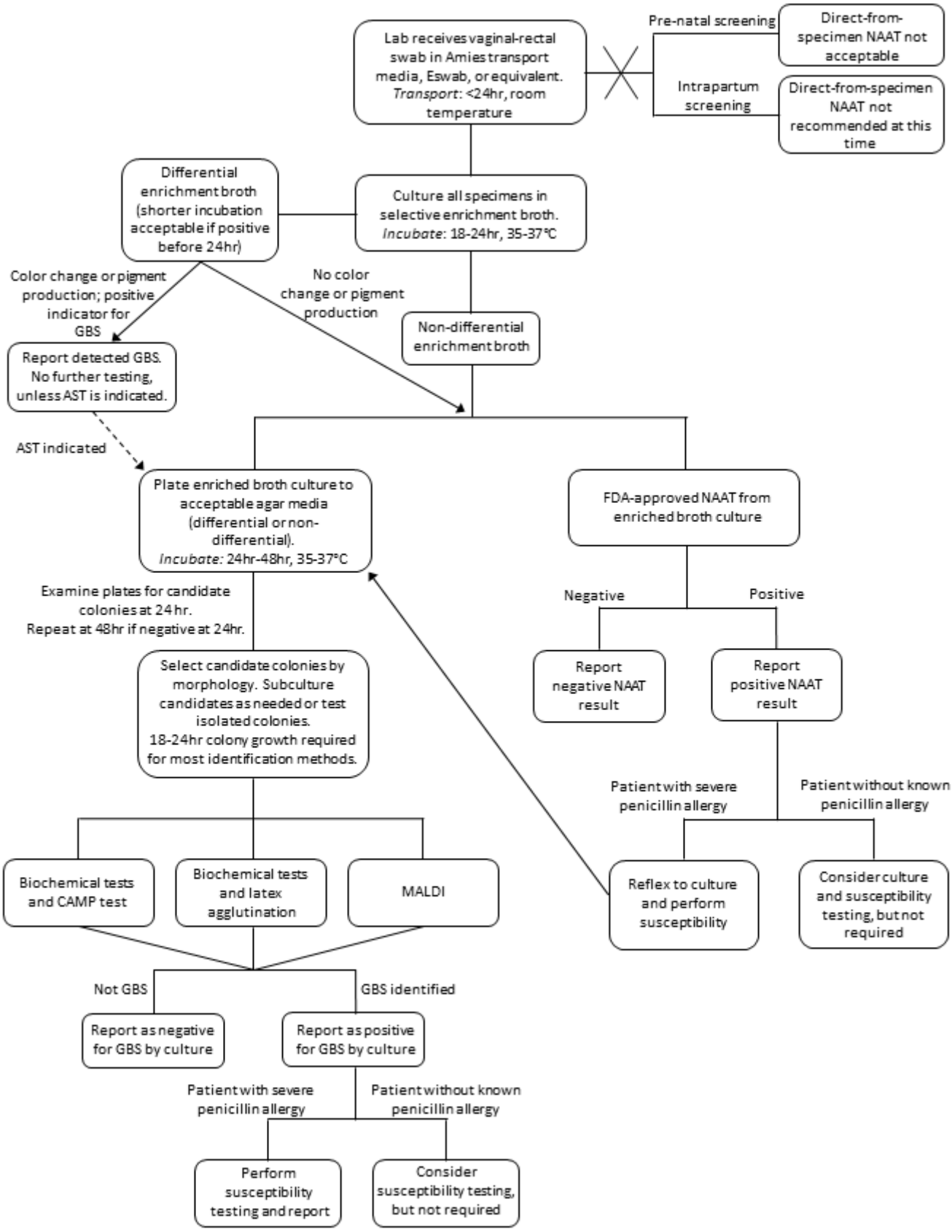

Figure 1: Laboratory workflow for GBS testing in pregnant women.