Investigation of Apparent False-Positive Urine Latex Particle Agglutination Tests for the Detection of Group B Streptococcus Antigen

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In our nursery, we identified neonates with positive urine latex particle agglutination (LPA) tests for group B streptococcus (GBS) antigen who did not have corroborating cultural evidence of infection. To investigate the mechanisms underlying these apparent false-positive reactions, we examined the urine LPA test in an unselected population of neonates suspected of sepsis. Urine specimens for LPA testing and culture and simultaneous perirectal cultures were obtained from 134 neonates with suspected sepsis. Six infants had positive blood cultures for GBS; four of the six were positive by LPA testing. An additional 20 infants had positive LPA tests but negative blood cultures; of these, 13 had mothers who received antibiotic treatment prior to delivery. Two infants with positive LPA results and negative blood cultures had bacteria isolated from urine cultures obtained in a nonsterile fashion (GBS, Escherichia coli). GBS was not isolated from perirectal swabs of infants with positive LPA tests and negative blood cultures. In conclusion, (i) a high proportion of neonates evaluated for sepsis gave positive LPA tests and negative blood cultures, (ii) local contamination of the perirectal skin or urinary tract with GBS was an unlikely source of false-positive LPA reactions, and (iii) maternal antibiotic pretreatment during labor may represent an important cause of apparent false-positive LPA reactions.

Despite recent advances in intensive care for the newborn and the use of broad-spectrum antimicrobial agents, group B streptococcus (GBS) sepsis remains a major cause of morbidity and mortality during the newborn period (2, 8, 11). Early-onset streptococcal disease is difficult to distinguish from other noninfectious causes of neonatal respiratory distress and frequently occurs with fulminant sepsis and cardiovascular collapse. Several authors, therefore, have advocated the use of a commercially available latex particle agglutination (LPA) test for the rapid detection of GBS antigen in body fluids (3, 7, 12, 13).

A positive urine LPA test should reflect antigen excretion via the urinary tract after GBS bacteremia. Recently, however, in our nursery and others, infants with positive agglutination reactions in the absence of identifiable bacteremia have been identified (17). The significance of these positive urine LPA test results, however, has not been determined.

This study investigated the possible mechanisms underlying apparent false-positive urine LPA reactions in an unselected population of neonates suspected of sepsis. Our purpose was (i) to determine the frequency with which neonates suspected of sepsis have positive LPA reactions in the absence of GBS bacteremia, (ii) to determine whether microbial contamination of urine specimens with GBS or other bacteria caused false-positive LPA reactions, and (iii) to examine the effect of maternal antibiotic pretreatment on the frequency of positive LPA reactions.

MATERIALS AND METHODS

Patient population. The study population consisted of 134 infants admitted to the intensive care or transitional nursery at the Hospital of the University of Pennsylvania who were evaluated for sepsis during the first week of life. Clinical characteristics of the study population are shown in Table 1. Prior to delivery, 50% of the mothers received antibiotics to reduce the incidence of neonatal GBS colonization and disease (5). Since the Hospital of the University of Pennsylvania is a referral perinatal center with a high incidence of maternal GBS colonization and neonatal sepsis, intrapartum antibiotics are frequently administered.

All decisions to initiate an evaluation for sepsis were made by the pediatric resident-attending neonatologist, and no attempt was made to alter existing clinical practice. Infants strongly suspected of sepsis were immediately given antimicrobial therapy pending culture results and additional laboratory information. This study was performed in accordance with the guidelines of the Human Investigation Committee at the Hospital of the University of Pennsylvania. Informed parental consent was waived.

Study design. During the evaluation for sepsis, which included a complete blood count and blood and cerebrospinal fluid cultures, urine samples were obtained for the identification of GBS by LPA test. Urine samples for LPA testing were initially collected in a nonsterile manner; however, additional sterile urine samples were obtained by a rigorous aseptic technique. Nonsterile urine samples were collected without prior skin disinfection, while sterile samples were obtained after betadine preparation of the skin with a sterile urine bag. Perirectal swabs were also evaluated to determine whether local skin colonization with GBS caused microbial contamination of urine LPA specimens.

According to the study design, the first nonsterile urine bag was placed on the infant for LPA testing immediately after completion of the evaluation for sepsis (Fig. 1). This nonsterile urine specimen was also processed for culture. A perirectal swab was obtained at the same time. If the initial urine LPA test was negative for GBS, a second nonsterile

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specimen was sent to the laboratory for antigen testing and culture. If the initial urine was latex positive, a sterile urine specimen was sent for LPA testing and urine culture, along with another perirectal swab. In the few cases where the first urine was negative by LPA assay and the second specimen was positive, a third sample was obtained by the sterile collection method. Urine specimens were obtained from study infants by a mean age of 36 h. This method was designed to determine whether skin colonization or urine contamination with GBS or other microorganisms caused false-positive urine latex specimens.

Experimental methods. (i) Cultures. Blood cultures were performed with Difco TSB/Thiol Bottles (Difco Laboratories, Detroit, Mich.). Cerebrospinal fluid samples were subjected to centrifugation, and the sediment was Gram stained and cultured. Urine cultures were performed by semiquantitative culture methods. After the urine was received in the laboratory, a 0.01-ml portion of well-mixed urine was plated on Trypticase soy sheep blood agar (BBL Microbiology Systems, Cockeysville, Md.) and MacConkey agar and was incubated for 48 h at 37°C. This method allowed the detection of at least 100 CFU/ml. All organisms were identified by standard procedures. For the first 3 months of study, perirectal swabs were plated on sheep blood agar and colonies were identified. After this period, to enhance the recovery of GBS, the specimens were first incubated overnight in selective broth medium containing Todd-Hewitt broth, 5% sheep blood, 15 µg of nalidixic acid per ml, and 8 µg of gentamicin sulfate per ml (1). On the following day, the broths were subcultured to blood agar.

![Diagram](http://jcm.asm.org/)

**FIG. 1.** Study design, as described in Materials and Methods. +, Perirectal swab obtained at these branch points.

### TABLE 1. Summary of characteristics seen in the study population (n = 134)

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>% or mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td></td>
</tr>
<tr>
<td>Rupture of membranes &gt;18 h</td>
<td>25%</td>
</tr>
<tr>
<td>Temp ≥38°C</td>
<td>49%</td>
</tr>
<tr>
<td>Antibiotic pretreatment</td>
<td>50%</td>
</tr>
<tr>
<td>GBS colonization</td>
<td>26%</td>
</tr>
<tr>
<td>Neontal</td>
<td></td>
</tr>
<tr>
<td>Birth wt (g)</td>
<td>3.186 ± 691</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>Symptoms*</td>
<td>46%</td>
</tr>
</tbody>
</table>

* A total of 44 mothers did not have peripartum GBS cultures. & Neonatal symptoms included respiratory distress, temperature instability, apnea and bradycardia, hypotonia, lethargy, seizures, and hypoglycemia.

(ii) Antigen testing. GBS antigen was detected with the Wellcogen Group B Latex Test (Wellcome Diagnostics, Research Triangle Park, N.C.) according to the instructions of the manufacturer. All urine specimens were initially tested unconcentrated and, if negative, concentrated 25- to 50-fold with Amicon filters (Amicon Corp., Lexington, Mass.) and retested. Urine specimens that tested positive were subsequently boiled, centrifuged, and retested to eliminate nonspecific positive reactions.

(iii) Statistics. Clinical characteristics of the study population were determined by using means and standard deviations of the mean. Yates-corrected chi-square was used to determine differences between infant groups (20).

### RESULTS

During the 9-month study period, nine infants with bacterial sepsis were identified. Six demonstrated positive blood cultures for GBS (one infant also had GBS meningitis), while the remaining three infants had cultures positive for alpha-hemolytic streptococcus (cerebrospinal fluid), *Streptococcus viridans* (blood), and *Hemophilus influenzae* (blood).

Of 134 study infants, 24 had one or more positive urine LPA tests (Table 2). Six infants born to mothers who received no antimicrobial pretreatment had positive blood cultures for GBS, whereas none of the infants whose mothers received antibiotic pretreatment were blood culture positive (*P* = 0.032). A total of 20 infants had positive LPA tests without corroborating cultural evidence of infection. These infants were initially considered to have apparent false-positive LPA reactions. A total of 108 infants had both negative blood cultures and negative LPA reactions.

**Infants whose mothers did not receive antibiotic pretreatment.** Among those who did not receive antibiotic pretreatment, all mothers whose infants had a positive blood culture were colonized with GBS, as were 80% of the mothers whose infants had negative blood cultures but positive LPA tests. Overall, 55% of the untreated mothers carried GBS at cervical or rectal sites.

Both nonsterile and sterile urine specimens were evaluated to determine the role of skin and urine contamination as a cause of false-positive antigen tests. All four infants with positive blood cultures for GBS had positive LPA reactions from nonsterile and sterile urine specimens. Among the seven infants with positive LPA tests and negative blood cultures, three had both nonsterile and sterile LPA specimens which were positive. The remaining four infants had positive LPA reactions in nonsterile urine specimens only. Bacteria (GBS, *Escherichia coli*) were isolated in nonsterile urine cultures from two of these four infants. However, sterile urine cultures from these infants showed no growth.
GBS was not isolated from perirectal swabs of any of the seven infants with positive LPA reactions and negative blood cultures.

**Infants whose mothers received antibiotic pretreatment.**

Among those who received antibiotic pretreatment, 70% of the mothers whose infants had negative blood cultures but positive LPA tests were colonized with GBS. Overall, 19% of the treated mothers carried GBS at cervical or rectal sites.

Among the 13 infants with positive LPA tests and negative blood cultures, six had both nonsterile and sterile LPA specimens which were positive. The remaining seven infants had positive LPA reactions in nonsterile urine samples only. None of these nonsterile urine specimens was culture positive. All 13 infants also had perirectal cultures which were negative for GBS.

**DISCUSSION**

This study demonstrates that a high proportion of neonates evaluated for sepsis (18%) demonstrate a positive LPA test, both in the presence and absence of identifiable bacteremia. Local contamination of the perirectal skin or urinary tract with GBS is an unlikely source of false-positive LPA reactions. In addition, neonates born to mothers who received antibiotic pretreatment were more likely to have negative blood cultures, making the LPA test uninterpretable in this group.

Previous studies have demonstrated the usefulness of the Wellcogen Strep B Latex Particle Agglutination test as a rapid, commercially available screen for the detection of GBS (3, 7, 9, 10, 12, 13). Most investigators have found the LPA test to be both sensitive (92 to 100%) and specific (84 to 100%) when evaluated in newborn infants. Several recent studies, however, have reported positive LPA tests in the absence of identifiable bacteremia (9, 13, 15, 17). Rabalais found false-positive urine LPA test results in patients with sepsis caused by other organisms and positive LPA tests of uncertain clinical importance in asymptomatic infants without positive blood cultures (17). In contrast, using clinical and laboratory criteria, Nelson et al. concluded that blood cultures may underestimate the true attack rate of GBS disease (15). Admittedly, blood cultures may identify only 82% of neonates with actual bacterial infection (18).

Prior to the inception of this study, we considered several possibilities to explain the positive urine LPA tests which could not be verified by blood culture. First, some infants may have false-negative blood cultures despite true GBS infection (18). This is especially likely to occur in infants born to mothers who were pretreated with antibiotics. Alternatively, infants may demonstrate false-positive LPA reactions secondary to (i) systemic infection with other organisms, (ii) contamination of the urine with bacterial species other than GBS which demonstrate cross-reactivity, (iii) local contamination of the perirectal skin or urinary tract with GBS, or (iv) nonspecific substances in the urine causing agglutination.

In contrast to Rabalais and Ingram, we did not demonstrate positive LPA tests after systemic infection with other organisms. However, the urine culture obtained in a nonsterile fashion from one infant with a positive, nonsterile LPA screen and a negative blood culture grew *E. coli*. In this case, it is conceivable that urine contamination may have produced the false-positive reaction. Although we did not inoculate sterile urine with this organism to test for cross-reactivity, previous studies have demonstrated false-positive agglutination reactions with *Streptococcus pneumoniae* type 14, group G streptococcus, and *Proteus mirabilis* (13).

Other authors have suggested that urogenital colonization with GBS may cause false-positive reactions and the detection of spurious antigenuria (17). The current study demonstrates, however, that contamination of the perirectal skin or urinary tract with GBS is an unlikely source of false-positive LPA tests. Only one infant with a negative blood culture and a positive LPA test grew GBS from the urine, and none of the infants had GBS isolated from perirectal swabs. Previous studies have also shown that the majority of infants become colonized with GBS at rectal sites by 72 h of life (16). In our study, therefore, rectal colonization may not have occurred by 36 h of age, when cultures were completed. It is unproven whether urine latex tests obtained after 72 h are affected by urogenital contamination with GBS.

In infants with GBS sepsis, Baker et al. previously demonstrated that the mean duration of antigenuria is 5.2 days once antimicrobial therapy is initiated (4). In this study, nine infants with positive LPA specimens and negative blood cultures had both nonsterile and sterile LPA tests which were positive. We believe it is less probable that specimens from these infants represented false-positive LPA reactions. In addition, we tested five urine samples from this group of nine infants with an independent monoclonal antibody sandwich enzyme assay (14), of which four samples were also positive. In the situation of a positive LPA test and negative blood culture, therefore, a second sterile urine LPA specimen might provide additional, useful information.

Recent studies have investigated the efficacy of maternal antibiotic treatment during labor to reduce morbidity and mortality from GBS disease in high-risk neonates (5). These studies have demonstrated significant antibiotic levels in amniotic fluid 30 to 60 min after treatment, decreased maternal and infant GBS colonization, and a diminished incidence of neonatal GBS sepsis (6, 19). As a consequence, this treatment strategy is used in many high-risk perinatal centers for the prevention of GBS disease. In this study, none of the infants born to mothers pretreated with antibiotics had a positive blood culture, whereas six infants with positive blood cultures were born of mothers who did not receive pretreatment. From this data, we infer that another reason for the apparent false-positive LPA tests might be maternal antibiotic treatment during labor. Since the blood samples from the infants may be sterilized by this therapy, the urine LPA test is uninterpretable in these situations.

Previous studies have shown that the Wellcogen urine LPA test may provide a rapid presumptive diagnosis of GBS infection before culture results are available. In this study, a high proportion of neonates demonstrated positive LPA tests in the absence of a positive blood culture. When faced with a positive LPA reaction and negative blood culture, we recommend obtaining a second urine LPA test in a sterile manner if possible, since a second positive LPA reaction is more likely to represent true antigenuria. The clinician, then, can use the available clinical and laboratory parameters to best determine the use and duration of antibiotic therapy.

**LITERATURE CITED**

