Rapid Diagnostic Test for Identifying Group B Streptococcus

Jonathan Faro, M.D., Ph.D.,1 Allan Katz, M.D.,1 Karen Bishop, B.S.,1 Gerald Riddle, M.S.,1 and Sebastian Faro, MD, Ph.D.1

ABSTRACT

Neonatal infection with Streptococcus agalactiae (group B streptococcus [GBS]) causes significant morbidity and mortality. A truly rapid diagnostic test for identifying GBS would allow for more timely initiation of antibiotic prophylaxis and also reduce the administration of antibiotics for the prevention of early onset neonatal GBS infection. A stock culture was formed from a laboratory reference strain of GBS and was diluted from 107 to 103 bacteria/mL. Specific concentrations were used to inoculate nitrocellulose membranes (NCMs) that had been coated previously with polyclonal rabbit antibody against GBS. After specific times, the NCMs were removed from the sheep blood agar medium, and horseradish-peroxidase conjugate polyclonal antibody against GBS was added. Bound antibody was detected with diaminobenzidine. After 6 hours of incubation, GBS was detected at concentrations from 103 through 105 bacterial/mL. After 4 hours of incubation, GBS was detected at concentrations from 105 through 107 bacteria/mL. GBS was not detected at 2 hours of incubation. Rapid growth and detection of GBS can be performed, and the results can be reliably attained as early as 4 hours. This is in marked contrast to the 48 to 72 hours required by current methods.

KEYWORDS: GBS, rapid diagnostic test, colonization, pregnancy

Group B streptococcal infection in neonates, postpartum women, and adults can cause serious morbidity and mortality (i.e., bacteremia, sepsis, and death).1,2 It is estimated that up to 29% of pregnant women may be colonized with group B streptococcus (GBS), although colonization rates seem to vary geographically.3 The 2010 Centers for Disease Control and Prevention (CDC) guidelines recommend that all pregnant women undergo rectovaginal culture for the detection of GBS for the prevention of GBS transmission from mother to fetus during labor.4 We currently screen patients at 35 to 37 weeks' gestation using a traditional agar-based method of growth and identification of GBS by polymerase chain reaction (PCR). The current methods for culturing GBS may take up to 72 hours.

There has been significant interest in developing a rapid and reliable method of detecting GBS colonization, which would be of great benefit in the preterm population as well as those patients admitted in labor beyond 35 weeks' gestation and not screened in the antepartum period. A truly rapid test to detect GBS that can be performed within hours on a single sample and yields highly sensitive and specific results is needed to assist in determining if antibiotic therapy is indicated.

1Department of Obstetrics, Gynecology and Reproductive Sciences, University of Texas Health Science Center, Houston, Texas. Address for correspondence and reprint requests: Jonathan Faro, M.D., Ph.D., Department Obstetrics, Gynecology and Reproductive Sciences, UT Health Science Center, Houston, TX (e-mail: Jonathan. P.Faro@uth.tmc.edu).


Received: April 6, 2011. Accepted after revision: June 4, 2011. Published online: August 4, 2011. DOI: http://dx.doi.org/10.1055/s-0031-1285099. ISSN 0735-1631.
 METHODS

Reagents
Polyclonal nonconjugated and horseradish peroxidase (HRP)-conjugated rabbit antibodies against GBS were purchased from Virostat (Portland, ME; catalogue numbers 1521 and 1524, respectively). Nitrobind, Cast, pure nitrocellulose 0.45-μm membranes (NCM) were purchased from GE Water and Process Technologies (#EP4HY450FS; Boulder, CO); 3,3’,5,5’-diaminobenzidine tetrahydrochloride hydrate (DAB, #D5637) and Trizma base (#T1503) were purchased from Sigma-Aldrich (St. Louis, MO). Milk diluent (#50-82-01) was purchased from KPL (Gaithersburg, MD). Sheep blood agar (triplicate soy agar w/5% sheep blood) was purchased from Remel (Lenexa, KS). Phosphate was purchased from Fisher Scientific (Pittsburgh, PA; #BP399).

Buffer, Blocking Solution, and DAB Preparation
Tris-saline buffer was prepared by dissolving 0.48 g of Trizma base and 6 g sodium chloride in 200 mL distilled deionized water at a pH of 7.4. Phosphate-buffered saline (PBS) was prepared by making a 10 x concentrated stock solution and diluting 10-fold with distilled deionized water and autoclaving prior to use. To prepare the blocking solution, milk diluent concentrate containing 2% nonfat milk was diluted 1:10 in sterile distilled deionized water. To prepare the chromogenic detection solution, 0.025 g DAB was placed into a capped tube, and immediately prior to use, 7.5 mL Tris-buffered saline was added to the DAB tube and vortexed to dissolve. Aliquots of DAB working solution (1 mL) were prepared by adding 10 μL of 3% hydrogen peroxide at a 1:10 dilution in deionized, distilled water, and 10 μL of 1% nickel chloride solution. This solution was used within 5 minutes of preparation.

NCM Preparation
For the initial experiments, NCM coated with antibody and blocked with milk diluent were supplied courtesy of Nanologix (Nanologix, Inc., Hubbard, OH). Experiments were repeated with NCM prepared in our laboratory in an identical fashion, but cut into squares instead of circles. All NCM were marked with a line at the 12 o’clock position with a pen prior to preparation. Next, NCMs were cut into either 2.5-cm diameter circles or 1.5 x 1.5-cm squares, and a 50 μL aliquot of a 1:10 dilution of polyclonal rabbit anti-GBS antibody in PBS was placed in the center of the NCM. These were allowed to dry for 10 minutes, and then the NCMs were blocked with the blocking solution. The NCMs were allowed to dry for 10 minutes at room temperature, and the bacterial inoculums were prepared next.

Inoculum Preparation and Incubation on NCM
A 0.5 MacFarland stock culture was prepared in PBS. An aliquot was withdrawn from this stock culture to create a dilution of 10^7 bacteria/mL. This stock culture was used to create further dilutions down to 10^1 bacteria/mL. Specific bacterial concentrations, 10^2 to 10^3 bacteria/mL, were used to inoculate onto NCMs. The NCMs were then incubated on sheep blood agar at 37°C for increasing periods. After each time point was reached, the NCMs were removed from the agar and washed with PBS twice for 2 minutes each time. HRP-conjugated rabbit polyclonal antibody was next added at a 1:40 dilution in PBS for 5 minutes at room temperature, followed by a 2-minute wash in PBS with 0.5% Tween-20. DAB was added and the reaction was recorded in 30 seconds. Subsequent experiments were performed in triplicate.

Quantification of Bacterial Inocula
Bacteria were plated out on sheep’s blood agar, and colony counts were performed to accurately ascertain the concentration of bacteria used to inoculate the NCMs. Escherichia coli, Staphylococcus aureus, and Enterococcus faecalis were used as controls and were grown for the maximal amount of allotted time.

RESULTS
Increasing the duration of incubation from 2 to 12 hours on sheep blood agar resulted in greater detection by the HRP-conjugated polyclonal antibody (Fig. 1). Using inoculums of increasing concentration of bacteria resulted in a stronger signal, so that a concentration of 10^7 bacteria/mL consistently yielded greater detection than more dilute inoculums. GBS was detected in as little as 4 hours at this concentration, and signal is faintly observed even at 2-hour incubation. Negative controls consisted of inoculating 10^7 bacteria/mL on NCMs not coated with antibody. Additional negative controls were NCMs coated with antibody and inoculated, separately, with E. coli, S. aureus, and E. faecalis, which were substituted for GBS, each at a concentration of 10^7 bacteria/mL. These controls were incubated for 12 hours, and tests were performed in triplicate as well (negative controls not shown).

The signal given after incubating GBS on sheep blood agar was observed to increase linearly according to the duration that the cultures were allowed to incubate. Although a dilute concentration of 10^6 bacteria/mL was not visible after 4-hour incubation, 8-hour incubation or greater showed consistent positive staining at this bacterial concentration. Furthermore, a heavily concentrated culture was readily detected at a very early time point, so that 10^7 bacteria/mL was detected in as little as 4-hour incubation, and this signal persisted through the
12-hour end point. Very dilute samples were detected after prolonged incubation, so that the $10^3$, $10^5$, and $10^7$ dilutions stained positively at 8, 10, and 12 hours, respectively. Colony counts for these dilutions after growth on blood agar overnight were 2,854, 322, and 34 colony-forming units, respectively. We observed very low background in the negative controls (data not shown).

**DISCUSSION**

A clear association between colonization with GBS during pregnancy and the development of infection in the first week of life has been well established. It has also been documented that GBS causes significant maternal postpartum and adult infection. Current CDC guidelines recommend antepartum screening and the administration of intrapartum prophylactic antibiotics for the prevention of early onset neonatal disease. As a large number of patients present with threatened preterm labor and GBS status is unknown, a significant number of patients are exposed to prophylactic and therapeutic antibiotics. A rapid culture method would reduce not only the number of patients receiving antibiotics but also the duration of antibiotic therapy. Patients admitted for preterm labor are almost universally administered therapeutic regimens of antibiotics preferentially directed against GBS.

The method we report on here is an antibody-based method, and therefore has an inherent specificity that should minimize the possibility of false-positives.
Furthermore, the use of a capture antibody (the coating antibody on the NCM) not only selects for GBS, but the subsequent washing step should theoretically remove any competing bacteria.

The antibodies used in this assay were selected as they were found to bind specifically and with great avidity for GBS. Use of alternative antibodies in both the coating and detection steps can potentially provide for a vast array of organisms to be cultured. Furthermore, multiple antibodies may be used simultaneously on one NCM, spatially oriented, so that several infectious organisms can be identified rapidly. Theoretically, a panel can be created in which a specimen can be collected from a patient and processed and the offending organism can be detected within hours of collecting the specimen.

Although PCR can rapidly detect GBS, it is not cost-effective to perform PCR on one specimen, and specimens are typically performed in batches. Furthermore, many PCR methods require an enrichment process, or overnight incubation, to increase the sensitivity of the test. The NanoLogix BioNanoPore™ (BNP) assay is a culture-based method for detection of bacteria using a chromogenic metabolite and allows for bacteria to be grown and detected in a much shorter period of time than the traditional agar-based methods. Our assay incorporates the BNP method as well as NanoLogix QuickTestTM method of detecting a specified organism with HRP-conjugated antibody. This novel assay allows for the detection of GBS within 4 to 6 hours of receiving the specimen, can be performed on a single specimen, and does not require batching of samples. An additional strength of this assay is that it is easy to use and requires minimal additional equipment, other than an incubator, which would allow the assay to be run in remote regions in which resources are scarce.

ACKNOWLEDGMENTS

This work was supported by Nanologix, Inc., which supplied the reagents used in generating the assay.


REFERENCES