Detection of Group B Streptococcus in under One Hour by a Modification of a Previously Described Rapid Diagnostic Test

Jonathan Faro, Karen Bishop, Gerald Riddle, Allan Katz, Sebastian Faro
UT Health Science Center at Houston, Obstetrics, Gynecology and Reproductive Sciences, Houston, TX

INTRODUCTION

GBS exposure during delivery may lead to significant neonatal morbidity.

In conditions such as preterm labor, universal screening is often performed.

Routine culture requires at least 2-3 days to identify GBS, and is therefore not suitable as an intrapartum test.

A method is needed for the rapid determination of GBS.

ABSTRACT

Introduction: Current screening guidelines for Group B streptococcus (GBS) recommend that all pregnant patients be tested between 35-37 weeks gestation. Traditional culture technique requires 2-3 days before GBS may be identified. Rapid diagnostic tests for GBS have relied on polymerase chain reaction (PCR) technology, and are cumbersome, expensive, and often require specialized training. A rapid test that is inexpensive and easy to perform would allow for intrapartum screening for GBS.

Materials and Methods: Nitrocellulose (NC) membranes were cut into 1.5 cm squares, and then coated with 20 microliters of a 1:40 dilution of a mouse monoclonal antibody against rabbit IgG and allowed to air dry. NC membranes were then blocked with milk diluent and again air dried. Membranes were either next stored at 4 degrees Celsius or inoculated with the sample specimen as follows: A 0.5 McFarland of GBS was set up (GBS was purchased from ATCC), and the bacteria was serial diluted out, starting at 10^6 bacteria per milliliter. To each dilution, a 1:30 dilution of horse-radish peroxidase rabbit polyclonal antibody against GBS was added to bring the final volume up to 100 microliters. The sample was spun at 3,000 rpm in a microcentrifuge and washed with phosphate buffered saline (PBS) three times. The pellet was resuspended in PBS and 20 microliters was added to the NC membrane. After washing with PBS three times, bound GBS was detected with diaminobenzide.

Results: GBS was detected reliably at 10^-6 bacteria per milliliter. Minimal background was observed, and no binding was observed when Enterococcus or Staphylococcus aureus were substituted for GBS. All tests were performed in triplicate.

Conclusion: GBS may be detected in as little as 30 minutes by this antibody-based immunoblot, and shows no cross-reactivity with Staphylococcus aureus or Enterococcus.

METHODS

Immunoblots were prepared by substituting antibodies for those used in the assay described previously by our group:

- General assay consists of capture antibody, sample, followed by detection antibody
- This assay requires an incubation period, after the sample is added
- Our aim was to develop a test in which this incubation time could be either greatly reduced or eliminated

RESULTS

Serial Dilutions of GBS in LIM broth, with HRP–conjugated polyclonal anti–GBS antibody. NCM pre–coated with monoclonal anti–rabbit antibody.

CONCLUSIONS

- This modification of a previously described assay allows for GBS to be detected in under one hour
- GBS may be detected at 10^5 bacteria/ml, without the requirement of an incubation step

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REFERENCES