

Detection of Group B Streptococcus in Under an Hour by a Newly-Developed Antibody-Based Assay

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ABSTRACT

Introduction: Group B streptococcal (GBS) infection is associated with significant neonatal morbidity, and the Centers for Disease Control has offered guidelines for the prevention of neonatal disease. As culture of GBS requires up to 48 hours, antenatal screening is recommended from 35-37 weeks. Many patients do not receive screening, as they deliver preterm or have inadequate access to healthcare. An inexpensive, rapid diagnostic test is needed which would allow for detection of GBS in laboring patients.

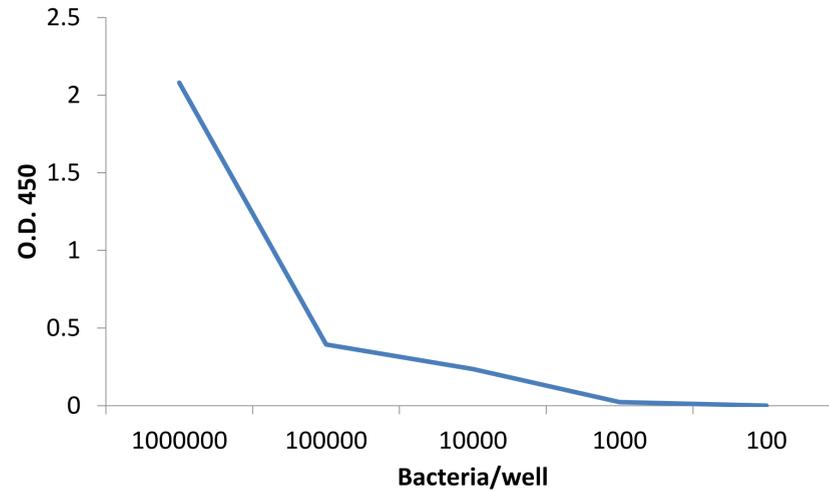
Methods: An enzyme-linked immunosorbent assay was developed in which microtiter wells were coated with a polyclonal antibody against GBS (Virostat 1521) at a 1:100 dilution, 100 microliters/well. After allowing the wells to sit at 4 degrees Celsius overnight, they were washed with saline and blocked with 5% milk for 1 hour at room temperature. Wells were again washed with saline, and dilutions of GBS with or without competing organisms, including *Staphylococcus aureus*, Enterococcus, Lactobacillus, and/or E. coli were added to the wells. After incubation at 37 degrees Celsius for 15 minutes in LIM broth, wells were washed with saline, and horseradish peroxidase conjugated anti-GBS polyclonal antibody was added. This was subsequently washed following a 15 minute incubation, and bound antibody was detected by adding tetramethylene blue substrate and reading at OD 450. GBS was purchased from ATCC, strain 12386. All studies were performed in triplicate.

Results: GBS was reliably detected after 15 minutes of incubation at 37 degrees Celsius, at 10^6 bacteria/well, without interference from competing organisms. Serial dilutions of GBS, with competing organisms maintained at 10^6 showed decreased binding. Dilution experiments showed that GBS may be detected at 10^3 bacteria/well, with ODs consistently above 0.2 nm.

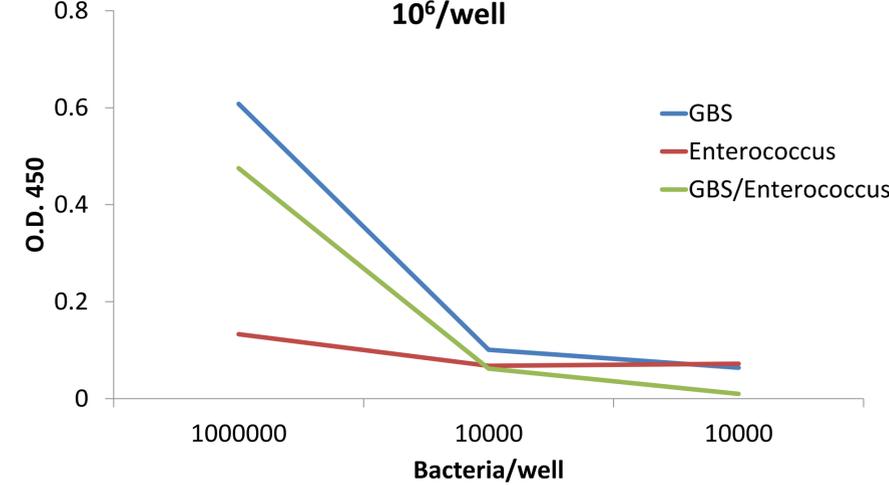
Conclusion: This antibody-based test allows for detection of GBS in the presence of selected competing organisms. Application of this test to patient samples awaits further studies.

RESULTS

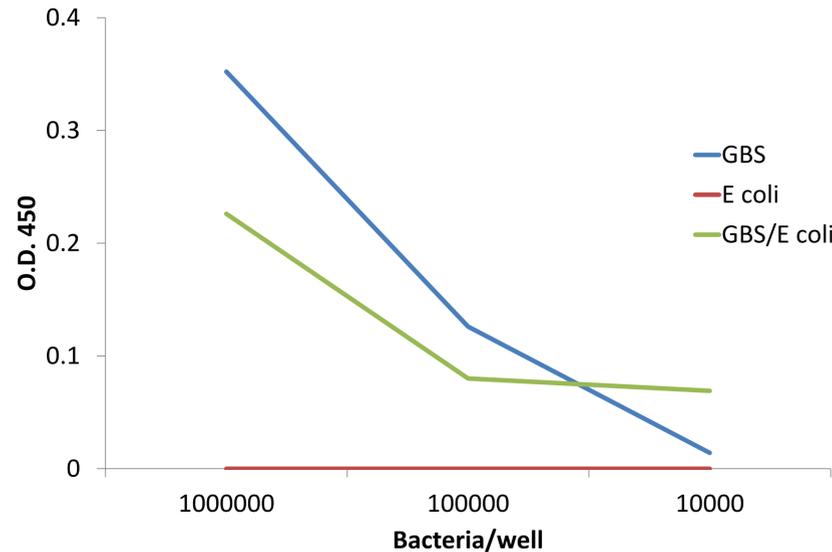
Dilutions of GBS detected after thirty-minute incubation



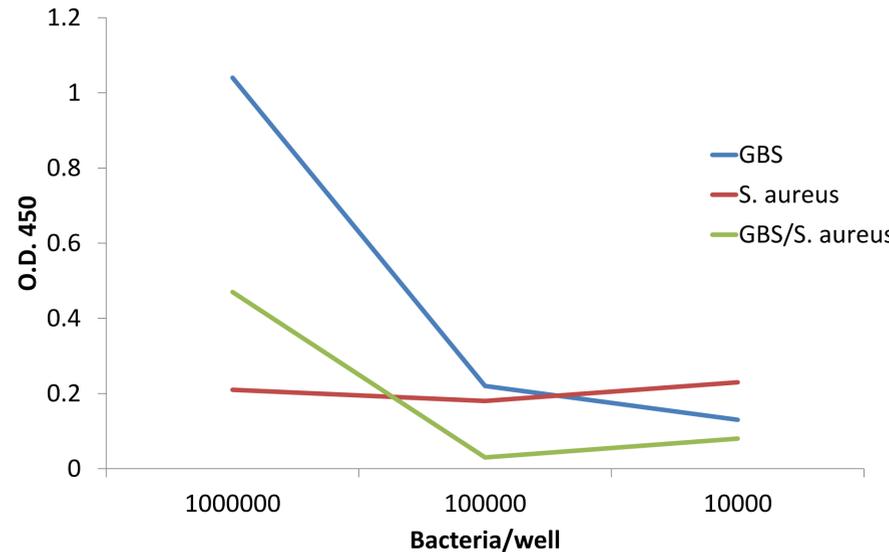
Dilutions of GBS detected after thirty-minute incubation with Enterococcus maintained at 10^6 /well



Dilutions of GBS after thirty-minute incubation with E. coli maintained at 10^6 /well



Dilutions of GBS detected after thirty-minute incubation with S. aureus maintained at 10^6 /well



CONCLUSIONS

- ❖ Once wells are prepared by coating and blocking, storage at 4 degrees Celsius would allow for them to be shipped and then used for detection of GBS.
- ❖ This assay therefore allows for the detection of GBS in under one hour
- ❖ Little to no interference by selected organisms was observed, allowing for a high degree of specificity
- ❖ GBS was detected up to 10^3 dilution, allowing for a high degree of sensitivity
- ❖ Application of this test may be applied directly to patient vaginal-rectal specimens, but this awaits further testing
- ❖ Application of antibiotic susceptibility profiling to this assay awaits further testing, but based on preliminary results, both the identification of GBS and its antibiotic susceptibility profile may possibly be attained in under one hour.

ACKNOWLEDGEMENTS

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REFERENCES

1. Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, CDC. Prevention of perinatal group B streptococcal disease—revised guidelines from CDC, 2010. *MMWR Recomm Rep* 2010;59(RR-10):1–36.
2. Faro J, Katz A, Bishop K, Riddle G, Faro S. Rapid diagnostic test for identifying Group B streptococcus. *Am J Perinatol* 2011;28(10):811-4.
3. Faro, JP, Bishop K, Riddle G, Katz A, Faro S. Optimization of a rapid diagnostic test for detection of group B streptococcus from antepartum patients. *Diagn Microbiol Infect Dis* 2012;73(3):236-8.
4. Faro J, Katz A, Raminrez M, Turrentine M, Bishop K, Riddle G, Faro S. Rapid test for growth and determination of antibiotic sensitivity of group B streptococcus (GBS) in antepartum women. *Am J Obstet Gynecol* 2012;206(1):S268.