Identification of group B streptococcus in thirty minutes, and use of FC-fragment to eliminate interference caused by staphylococcus

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ABSTRACT

Objectives: A 6.5-hour diagnostic test for detection of group B streptococcus has recently been described: Through conversion of this test to an ELISA format, we show that purified GBS may be reliably identified in 30 minutes.

Methods: Microtiter wells were coated with polyclonal rabbit antibody against GBS (Virostat 1521) at a 1:100 dilution, 100 microliters/well. After incubating overnight at 4°C and then washing, wells were washed with phosphate buffered saline (PBS). Wells were then blocked with either 5% milk or StartingBlock (Pierce) for 1 hour at room temperature, and then washed with PBS. GBS (10^7 bacteria/well) was added with one of the following commonly isolated co-colonizers: Staphylococcus aureus, Enterococcus faecalis, E. coli, Neisseria gonorrhea, Candida albicans, or Groups A, C, F, or G streptococcus. GBS was diluted serially as the additional co-colonizer was held constant at 10^7 bacteria/well. After incubating for 20 minutes at 37°C, wells were washed and then HRP-conjugated anti-GBS polyclonal antibody was added, and bound GBS was detected after adding tetramethylene blue (TMB) solution. In order to decrease interference due to S. aureus, Triclosan, Protein A and Fc antibody fragment were used to coat separate wells prior to incubating the bacterial supernatants. The bacterial suspensions were then allowed to stand in these coated wells at room temperature for 30 minutes prior to being placed in the antibody-coated wells. The remainder of assay proceeded as described above.

Results: GBS was reliably detected in under thirty minutes, with significant interference due to staphylococcus aureus noted. This interference was completely abrogated by placing the suspensions in Fc-coated wells prior to incubating in antibody-coated wells. The use of Protein A or Triclosan did not show any benefit.

Conclusions: GBS may be detected in 30 minutes, after allowing the bacteria to stand in Fc-coated wells to remove interference by S. aureus. No interference was observed when other commonly isolated co-colonizers were examined.

Learning Objective: Learners will be able to review CDC screening guidelines for prevention of GBS, and to demonstrate methods for reduction of interference in an immunoassay.

INTRODUCTION

•Screening for GBS is recommended between 35-37 weeks gestation[1]

•Routine culture requires 24-48 hours, with determination of antimicrobial susceptibility requiring an additional 24-48 hours[2]

•Per 2010 CDC Guidelines, Identification of GBS by polymerase chain reaction (PCR) may be done after an enrichment step, allowing for a more rapid identification of GBS[3]

•In order for a test to be utilized in intrapartum patients on the labor and delivery unit, an accurate result must be able to be obtained in under 30 minutes[3]

METHODS

Microtiter wells were prepared based on the method previously described in which antibody-coated nitrocellulose membranes served as the binding substrate.[4] Wells were coated with either antibody specific to GBS, or to other bacteria/fungi, and included antibody to Enterococcus, Candida albicans, and E. coli as negative controls (not shown).

After wells were coated and washed with PBS, bacterial suspensions were added in serial dilutions, starting at 10^7 bacteria/well, and diluting out to 10^1 bacteria per well. The optical density was read at 450 nm.

In order to determine specificity, GBS in increasing degrees of dilution was added in the presence of either Staphylococcus aureus, Enterococcus faecalis, E. coli, Neisseria gonorrhea, Candida albicans, or Groups A, C, F, or G streptococcus, each held steady at 10^7 bacteria per well. After incubating at 37°C for 20 minutes, bound antibody was detected by the addition of HRP-conjugated anti-GBS antibody, and results were obtained once TMB was added and allowed to react with HRP-conjugated antibody.

In order to eliminate the high level of interference observed when S. aureus was added, bacterial suspensions were first allowed to stand in tubes coated with varying concentrations of either Protein A, Triclosan, or Fc-Fragment. Aliquots were then removed from these tubes and added to the antibody-coated wells, and the assay proceeded as described above.

ACKNOWLEDGEMENTS

❖ Partial funding for this research was provided by Nanologix, Inc.

REFERENCES

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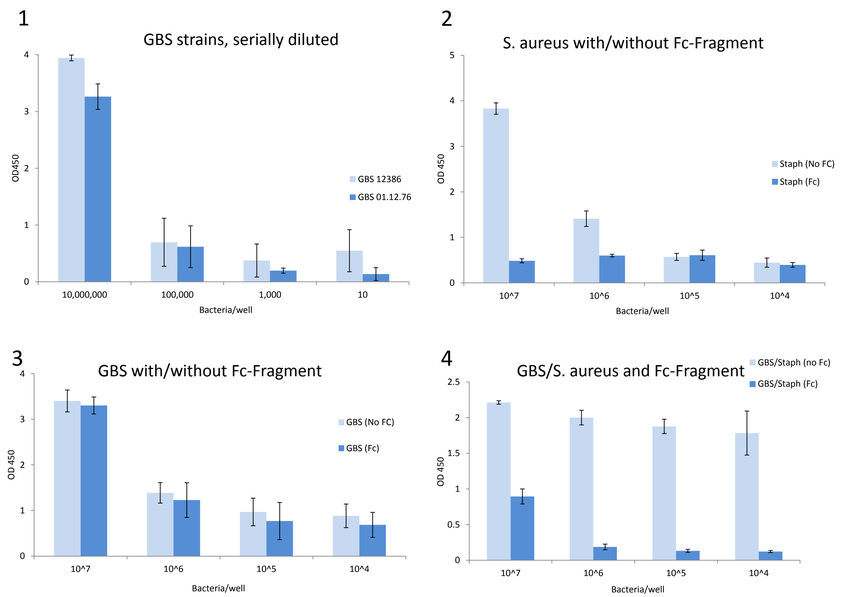
RESULTS



Figures 1-4: Interference caused by binding to S. aureus is completely abrogated by first incubating the bacterial mixture in the presence of Fc-Fragment. Fig 1 shows two unique GBS isolates identified by ELISA. Fig 2 shows that S. aureus displays a high degree of interference when not incubated first in Fc-Fragment. Fig 3 illustrates that Fc-Fragment does not interfere with GBS binding. Fig 4 demonstrates that interference caused by S. aureus is completely abrogated when a mixture of GBS and S. aureus is first incubated in the presence of Fc-Fragment.

CONCLUSIONS

•Purified GBS may be identified in 30 minutes by this ELISA-based assay

•Of the commonly isolated co-colonizers tested, S. aureus binds to anti-GBS antibody coated wells presumably by binding to the non-antigen binding site

•Interference due to S. aureus may be completely eliminated by allowing the bacterial suspension to first stand in tubes coated with purified Fc-fragment