Implementation of the Great Basin Group B Streptococcus (GBS) Assay using Hardy Carrot Broth in a Community Hospital Laboratory

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ABSTRACT

Introduction: *Streptococcus agalactiae* or Group B Streptococcus (GBS) is a Gram positive bacterium that remains a leading cause of serious illness and death in newborn populations. Approximately 10 – 30% of all pregnant women are colonized with GBS in the gentourinary or gastrointestinal tract, and during labor transmission may infect the newborn leading to neonatal sepsis and meningitis. Screening for GBS colonization in antepartum women between 35 and 37 weeks' gestation, followed by intrapartum antibiotic treatment for women with positive colonization status has proven to be an effective mechanism for prevention of perinatal GBS disease. Here we describe the implementation of a polymerase chain reaction (PCR) based GBS Assay (Great Basin Scientific), a qualitative *in vitro* diagnostic test (IVD) for the detection of GBS DNA from vaginal/rectal swabs from antepartum women.

Methods: Samples included 20 known GBS, and 20 known E. faecalis QC organisms performed over 20 separate days, and 26 patient specimens run concurrently with the Illumigene Pro GBS Assay (Meridian Bioscience) which was the procedure in place. All samples were initially cultured in Hardy Carrot broth (Hardy Diagnostics #Z140) which uses the Granada medium reaction and contains the necessary components for pigment detection of beta-hemolytic GBS and produces positive results in as little as 6 hours. Following incubation in enrichment broth (utilizing Hardy Carrot broth as the enrichment broth rather than LIM broth) for 18 - 29 hours, samples underwent automated sample preparation and PCR on the PA500 Portrait Analyzer System to amplify a *cfb* gene sequence specific to the GBS genome which is detected by hybridization probes immobilized on a silica chip surface according to manufacturer's instructions.

Results: Of the 20 known positive and 20 negative control specimens tested, all yielded the expected result. Of the 26 patient specimens, 19 were negative by both Illumigene and Great Basin GBS. Of the 7 positive specimens, 3 were positive by pigment change in Carrot broth, 2 were positive by culture, CAMP and/or Vitek GP ID, and 2 were positive by Illumigene.

Conclusions: The Great Basin GBS assay demonstrated 100% concordance with expected results over 66 specimens and three methodologies. The Carrot broth has proven an effective enrichment media for the assay. In our current workflow, negative Carrot broth specimens are tested using the Great Basin GBS Assay to detect non-hemolytic strains of GBS.

INTRODUCTION

Streptococcus agalactiae or Group B Streptococcus (GBS) is a Gram positive bacterium that remains a leading cause of serious illness and death in newborn populations. Approximately 10 – 30% of all pregnant women are asymptomatically colonized with GBS in the genitourinary or gastrointestinal tract, and during labor transmission may infect the newborn. This happens most commonly when GBS ascends the vagina to contact amniotic fluid after membrane rupture, but may also occurs through intact membranes by aspiration or by mucous membrane exposure during passage through the birth canal, leading to neonatal sepsis and meningitis.

Screening for GBS colonization in antepartum women between 35 and 37 weeks gestation, followed by intrapartum antibiotic treatment for women with positive colonization status has proven to be an effective mechanism for prevention of perinatal GBS disease. As colonization may be transient, intermittent or persistent throughout pregnancy, screening is most effective when performed on specimens collected no more than 5 weeks prior to delivery—35-37 weeks gestation, and after enrichment with selective broth medium.

The 2010 CDC GBS Prevention Guidelines (Figure 1) recommend a vaginal-rectal swab incubated 18-24 hours in pigmented or non-pigmented enrichment broth. For pigmented broth a positive color indicator is reported as GBS positive. Because the pigmented broth will not detect non-hemolytic strains of GBS, negative pigmented broth specimens are further tested using culture methods, latex agglutination, DNA probe or nucleic acid amplification test (NAAT). Hardy Diagnostics' Strep B Carrot Broth was already in use in our laboratory, first in a culture only test, and more recently in combination with the Meridian Illumigene Group B strep assay. In an effort to streamline the process and control costs we wanted to continue using the initial pigmented enrichment broth to screen out the first round of positive specimens, which would not require further testing.

OBJECTIVE

Implementation of a polymerase chain reaction (PCR) based GBS Assay (Great Basin Scientific), a qualitative *in vitro* diagnostic test (IVD) for the detection of GBS DNA from vaginal/rectal swabs from antepartum women.

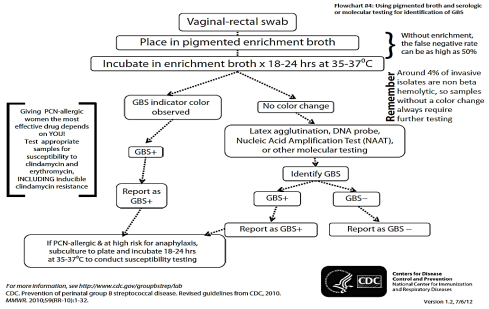

Figure 1. 2010 CDC GBS Prevention Guidelines

METHODS

All samples were incubated for 16-24 hours in Hardy diagnostics Strep B Carrot broth as the enrichment broth as opposed to Great Basin's validated LIM broth. The broth specimens were split and used for testing on both the Great Basin and Illumigene platforms.

Samples included 20 known S. agalactiae (GBS) and 20 known E. faecalis QC organisms incubated in Strep B Carrot broth, tested over 20 separate days by multiple technologists, and 26 patient specimens run concurrently with the Illumigene Pro GBS Assay (Meridian Bioscience) which was the procedure in place.

Following incubation in broth for 18 - 24 hours, samples underwent automated sample preparation and PCR on the PA500 Portrait Analyzer System to amplify a *cfb* gene sequence specific to the GBS genome. The Portrait GBS assay is fully automated from sample to result, and uses hot-start PCR for gene amplification in a completely closed system, single use cassette.

A. Hardy Diagnostics Strep B Carrot Broth

B. Great Basin Group B Strep test cassette

C. Great Basin Portrait Analyzer

Figure 2. A. Carrot broth with color change indicating hemolytic (orange) and non-hemolytic strains of GBS; B. Great Basin test cassette; C. Great Basin Analyzer

RESULTS

Of the 20 known positive and 20 negative control specimens tested, all yielded the expected result. Of the 26 patient specimens, 19 were negative first by the Strep B Carrot broth, then by both the Illumigene GBS assay and Great Basin GBS. Of the 7 positive specimens, 3 were also positive by pigment change in Hardy Strep B Carrot broth. Of the 4 that were negative by Strep B Carrot broth, 2 were positive by culture, CAMP and/or Vitek GP ID (not tested by Illumigene), and 2 were positive by Illumigene (not tested by culture).

	GREAT BASIN	CARROT	ILLUMIGENE	CULTURE	QC	TOTAL
POS	27	3	2	2	20	27
NEG	39		19		20	39
TOTAL	**66**					**66**

CONCLUSIONS

The Great Basin GBS assay demonstrated 100% concordance with expected results over 66 specimens and three methodologies.

The Hardy Diagnostics Strep B Carrot broth has proven an effective enrichment media for the Great Basin GBS assay and is compatible with the test cartridge and components.

In our current workflow, negative Strep B Carrot broth specimens are further tested using the Great Basin GBS Assay to detect non-hemolytic strains of GBS. Strep B Carrot broth positive specimens are reported as Positive for Group B Streptococcus. Positive broths from patients who are allergic to Penicillin are subcultured for susceptibility testing.

REFERENCES

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