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Comparison of LIM Broth with PNA FISH to Carrot Broth with PNA FISH for Identification of Group B Streptococcus in Prenatal Vaginal/Rectal Specimens

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Abstract

Background: Screening of pregnant women for Group B Streptococcus (GBS) colonization with antibiotic administration for colonized women has significantly reduced the incidence of early-onset neonatal sepsis. The most common method for identification of colonized women is vaginal/rectal culture in LIM broth with subculture onto blood agar and phenotypic identification of GBS colonies. This process can be time consuming and require two or more days to complete. Another broth option is Carrot Broth (Hardy Diagnostics Santa Maria, CA), which can identify hemolytic GBS in 24 hours or less. This broth still requires subculture of negative samples for the possibility of non-hemolytic strains of GBS. With either broth, non-hemolytic strains may be difficult to identify and isolate in cultures of mixed vaginal/rectal flora. GBS PNA FISH (AdvanDx, Inc. Woburn, MA) has been FDA cleared for use with LIM broth. This protocol requires that PNA FISH be performed on all samples. The purpose of this study was to validate the use of GBS PNA FISH with Carrot broth. This protocol would require performance of PNA FISH only on Carrot broth negative samples and provide a one day turn around time to sample result.

Method: 99 random prenatal vaginal/rectal samples received in The Hospital of Central Connecticut Laboratory on dual swab collection containers were selected for inclusion in this study. One swab from each collection was inoculated into LIM broth and the other into Carrot broth. All samples were both sub-cultured to blood agar and tested for GBS using PNA FISH. All GBS culture positive plates had the GBS identified using BBL Streptocard (Becton, Dickinson and Company, Sparks MD).

Results: Of the 99 samples tested, 18 were found to be positive by either LIM or Carrot broth (CB) methods and 81 were negative by both methods (Fig 1). 2 of the positives were only identified by Carrot broth, and not LIM broth. 17 GBS isolates were hemolytic strains (HGBS) and one isolate was a non-hemolytic GBS (NGBS) strain (Fig. 2) recovered by PNA FISH and subculture from both broth methods but as expected was Carrot broth negative. 18/18 culture positive samples were PNA FISH positive from both LIM and Carrot broth. 1 was positive by Carrot/PNA FISH and negative with both LIM subculture and PNA FISH. 1 sample was positive with Carrot/PNA FISH and LIM/PNA FISH but was not recovered from LIM or Carrot broth subculture (Fig. 4). 15/18 of the samples were positive by both the LIM/PNA FISH and Carrot/PNA FISH methods (Fig. 3).

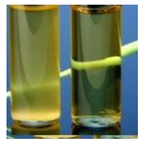
Conclusions:
Carrot broth compares favorably with LIM broth for isolation of Group B Streptococcus in conjunction with PNA FISH.
PNA FISH in conjunction with either LIM or Carrot broth identified more Group B Streptococci than broth culture alone.

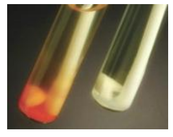
Discussion: In this study, all samples that were subculture positive were GBS PNA FISH positive indicating that PNA FISH can be successfully performed from Carrot broth cultures. One sample was a non-hemolytic strain that would be difficult to discern on a blood agar plate subculture and would not be Carrot broth positive. This isolate was PNA FISH positive and so identified without lengthy subculture. One GBS was only recovered from Carrot broth, indicating that this broth may be a better medium for GBS recovery than LIM broth. All but three culture positive samples were Carrot broth positive, requiring PNA FISH testing only on broth negative cultures. The combination of Carrot broth and PNA FISH minimizes labor and maximizes recovery of within a 24 hour time frame from prenatal vaginal/rectal culture screen samples.

Methods

Random prenatal vaginal/rectal samples collected in dual swab collection containers (Becton, Dickinson and Company, Sparks, MD)

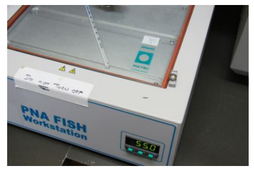
One swab was placed in LIM Broth (Becton, Dickinson and Company, Sparks, MD) and the other into Carrot Broth (Hardy Diagnostics, Santa Maria, CA). Both were incubated 15-18 hours at 35ºC in ambient air.



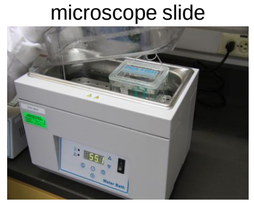


All broth samples were sub-cultured to sheep blood agar and tested for GBS with GBS PNA FISH (AdvanDx, Inc., Woburn, MA.

One drop of Fixation Solution and 10µL broth culture added to microscope slide



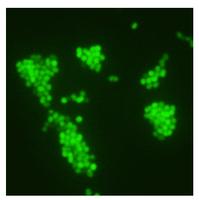
Incubate for 30 min. with one drop of GBS PNA at 55ºC



Incubate in Wash Solution for 30 min. in 55ºC water bath

Cover slip and examine slide for fluorescence using dual band Texas red filter at 100x magnification

GBS appear as fluorescent green cells on a black background with FITC/Texas Red filter



Streptococcus algalactae

Results

Fig. 1

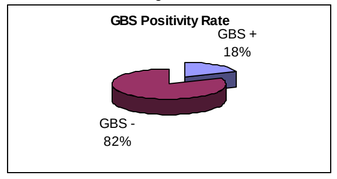
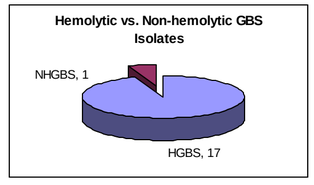


Fig. 2



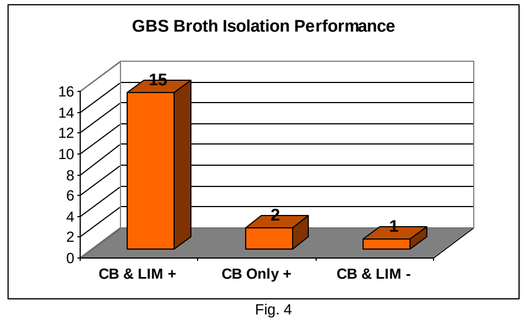


Fig. 4

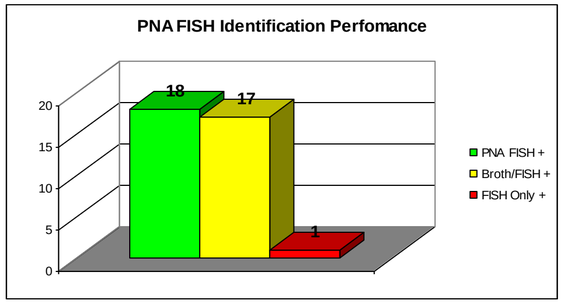


Fig. 3

Conclusions

CDC recommends vaginal/rectal culture of pregnant women at 35–37 weeks gestation using supplemented Todd-Hewitt broth. Another available medium option is Carrot broth. This broth has the advantage of rapid identification of GBS without subculture by development of orange color in the presence of hemolytic GBS reducing labor and cost. Isolation is equal to, or better than LIM broth[1,2]. Negative cultures do, however, require subculture. Recently, PNA FISH reagents for use in conjunction with LIM broth became available. This study was designed to determine the efficacy of using Carrot broth with GBS PNA FISH. All Carrot broth samples that were positive for GBS were also GBS PNA FISH positive, indicating that PNA FISH can be performed in conjunction with Carrot broth. This process eliminates the need for broth subculture reducing labor and culture turn around time.

References

1. D.L. Church, Heather Baxter, Tracie Lloyd, Beverly Miller, and Sameer Elsayed, 2008, Evaluation of StrepB Carrot Broth versus Lim Broth for Detection of Group B Streptococcus Colonization Status of Near-Term Pregnant Women, J Clin Microbiol 46:2780-2.
2. Timothy Block, Erik Munson, Anne Culver, Katharine Vaughan, Jeanne E. Hryciuk, Comparison of Carrot Broth- and Selective Todd-Hewitt Broth-Enhanced PCR Protocols for Real-Time Detection of *Streptococcus agalactiae* in Prenatal Vaginal/Anorectal Specimens, J Clin Microbiol 46:3615-20.