Evaluation of GBS Detect™ and PNA FISH™ for the Detection of Group B Streptococci in Subcultures of LIM Broth
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Introduction
At least 2% of GBS isolates are non-hemolytic. The Hardy GBS Detect™ (Hardy Diagnostics, CA) plate allows for the detection of non-hemolytic strains and beta-hemolytic colonies masked by other non-GBS organisms. The goal of this study was to compare conventional subculture from LIM broth to BAP and GBS Detect™ with the PNA FISH™ test. A total of 45 vaginal-rectal specimen culture swabs were inoculated to a BAP and LIM broth. After 24 hours of incubation, the LIM broth was subcultured to a BAP and GBS Detect™ media and a slide was prepared for PNA FISH™ testing. Sixteen specimens were positive for GBS using one or more methods (35.6%). One specimen was positive for beta-hemolytic GBS after 48 hours of incubation, but by all other methods. Another specimen was positive on the GBS Detect™ method. In conclusion, use of the GBS Detect™ medium provides increased sensitivity for the detection of GBS compared to the other methods, and especially for the detection of non-beta-hemolytic GBS.

Materials And Methods
Study duration
This study was conducted between April 2010 and July 2010.

Sample collection
Vaginal and rectal swabs from pregnant women at 35 to 37 weeks of gestation were collected. Both swabs were used to inoculate the broth. A total of 45 vaginal-rectal specimen culture swabs were inoculated to a BAP and LIM broth. After 24 hours of incubation, the LIM broth was subcultured to a BAP and a GBS Detect™ plate. A slide was prepared for PNA FISH™ testing. Sixteen specimens were positive for GBS using one or more methods (35.6%). One specimen was positive for beta-hemolytic GBS after 48 hours of incubation, but by all other methods. Another specimen was positive on the GBS Detect™ method. In conclusion, use of the GBS Detect™ medium provides increased sensitivity for the detection of GBS compared to the other methods, and especially for the detection of non-beta-hemolytic GBS.

Microbiological Analysis
LIM Broth
For this study, subculture of the LIM broth was performed even when GBS was not detected on the primary BAP. All LIM Broths incubated for 18 to 24 hours at 37 °C to 5 °C, with appropriate controls.

GBS Detection
GBS-positive media (PNA) fluorescent in-situ hybridization (FISH) assay
A slide was prepared using 10 to 50 µL of the incubated LIM broth samples mixed with one drop of PNA FISH™ working solution and allowed to dry.

RESULTS
Of the 45 samples positive for GBS, 44 (97.8%) were identified as hemolytic on both GBS Detect™ and conventional culture methods. One non-beta-hemolytic strain was identified using the GBS Detect™ only. One isolate did not appear beta-hemolytic on the BAP subculture, but appeared beta hemolytic on the primary BAP after 48 hours of incubation. The specimen was positive using the GBS Detect™ and PNA FISH™ methods. A total of 45 vaginal-rectal specimen culture swabs were inoculated to a BAP and LIM broth. After 24 hours of incubation, the LIM broth was subcultured to a BAP and GBS Detect™ media and a slide was prepared for PNA FISH™ testing. Sixteen specimens were positive for GBS using one or more methods (35.6%). One specimen was positive for beta-hemolytic GBS after 48 hours of incubation, but by all other methods. Another specimen was positive on the GBS Detect™ method. In conclusion, use of the GBS Detect™ medium provides increased sensitivity for the detection of GBS compared to the other methods, especially for the detection of non-beta-hemolytic GBS.

Positive for GBS

Overall 35.6% of specimens included in the study were positive for GBS. Among the positive samples, one non-hemolytic GBS isolate was detected by GBS Detect™ only.

Discussion
Of the 45 samples positive for GBS, 44 (97.8%) were identified as hemolytic on both GBS Detect™ and conventional culture methods. One non-beta-hemolytic strain was identified using the GBS Detect™ only. One isolate did not appear beta-hemolytic on the BAP subculture, but appeared beta hemolytic on the primary BAP after 48 hours of incubation. The specimen was positive using the GBS Detect™ and PNA FISH™ methods. A total of 45 vaginal-rectal specimen culture swabs were inoculated to a BAP and LIM broth. After 24 hours of incubation, the LIM broth was subcultured to a BAP and GBS Detect™ media and a slide was prepared for PNA FISH™ testing. Sixteen specimens were positive for GBS using one or more methods (35.6%). One specimen was positive for beta-hemolytic GBS after 48 hours of incubation, but by all other methods. Another specimen was positive on the GBS Detect™ method. In conclusion, use of the GBS Detect™ medium provides increased sensitivity for the detection of GBS compared to the other methods, especially for the detection of non-beta-hemolytic GBS.

References
1. AdvanDx, Inc. GBS PNA FISH® Streptococcus agalactiae Culture Identification Kit Package Insert. May 15, 2009 Rev B.

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Sheepthoda agalactiae ATCC® 19613 growing on GBS Detect™

Conclusion
Based on the results of this study, GBS Detect™ is a reliable method to increase the sensitivity of traditional culture methods for GBS detection, especially for non-hemolytic strains. The GBS Detect™ method is significantly less expensive and much less labor-intensive than the PNA FISH™ method. Since GBS screening is recommended at 35-37 weeks of gestation, and not at the time of delivery, a rapid test is not necessary for patient management.