

# Comparison of Culture Media and Methods Used for the Detection of Group B Streptococcus from Pre-Natal Vaginal/Rectal Specimens

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## Introduction

Group B Streptococcus (GBS) is the most frequent cause of systemic infections in neonates less than one week old. The acquisition of GBS is from the mother's vagina upon birth. Colonization occurs in 10-30% of pregnant women in the vaginal/rectal area. Laboratory testing of GBS, at the Center for Disease Control (CDC) recommended 35-37 week gestation screening time, is critical for the prevention of neonatal GBS disease. The current laboratory gold standard is the CDC recommended selective broth method (Todd-Hewitt GBS or LIM broths) with a sub-culture to a blood agar plate. Several alternative microbiological assays have been employed to detect GBS with varying success including PCR, FISH and the beta hemolytic GBS detecting Granada/Carrot broths media. The CDC's August 2002 revised guideline states: "hemolysis may be difficult to observe so typical colonies without hemolysis should also be further tested". In light of that statement, this study was undertaken to develop a method to aid in the detection of hemolytic and non-hemolytic GBS while minimizing turn-around time (TAT) strategies and expense. The study included evaluations of:

1. A modified, more selective Todd Hewitt broth. Preliminary studies showed that an increase in gentamicin to 14 µg/ml from the original 8 µg/ml may effectively reduce the diverse normal flora including Enterococcus faecalis – a troublesome organism that may resemble and inhibit GBS by its inherent bacteriocins. (3,5,6)
2. The use of PathoDx to group the Lancefield B antigens directly from broth. This method eliminates the reliance on hemolysis detection as an identification method and decreases TAT.
3. The variability of the hemolysis effect (the key colonial attribute by which GBS is commonly recognized) from one common blood agar to another (TSA and Columbia) and from one manufacturer to another (Oxoid and PML).
4. The additional benefit of adding a 10 µg gentamicin disc to broth subcultures to differentiate GBS from enterococci and other normal flora by virtue of its resistance to this antibiotic. (3,4)

Because requests for susceptibility testing of GBS isolates are not always relayed to the lab via the requisition, the lab must be prepared to incorporate this additional test in its workflow while striving for minimal TAT. Because of this, our workflow process includes an initial CNA plate along with the selective broth inoculation. Even though this step has a lower efficiency rate of recovery of GBS (approximately 75%), it does reduce TAT by 1 day for earlier detected positives.

## Method

A total of 218 vaginal/rectal screening samples received at Gamma-Dynacare Medical Laboratories were included in this survey. The swabs were immersed in 0.5 ml of saline and vortexed for 10 seconds. 50 µl were inoculated to each of: 1 CNA plate, 1 standard Oxoid Todd-Hewitt GBS broth with 5% sheep blood (STHB) containing 8 µg/ml of gentamicin and 15 µg/ml of nalidixic acid and 1 modified TH broth with 5% sheep blood (MTHB) containing 14 µg/ml gentamicin and 15 µg/ml nalidixic acid.

After overnight incubation at 35°C in air, CNA plates and broths were examined and tested for GBS directly using PathoDx latex agglutination. PYR, catalase, and CAMP test were used as required. Each broth was sub-cultured to both TSA and Columbia with 5% sheep blood (Oxoid). Gentamicin discs (10µg.BBL) were added to the 2<sup>nd</sup> quadrant of each broth sub-culture. Positive GBS broths, determined by agglutination directly from broth or from sub-cultures demonstrating typical narrow band hemolytic grey colonies, were further sub-cultured to PML TSA and Columbia blood agars. All tests were set-up, incubated and read in accordance with manufacturer's instructions. Four well experienced technologists read and interpreted results.

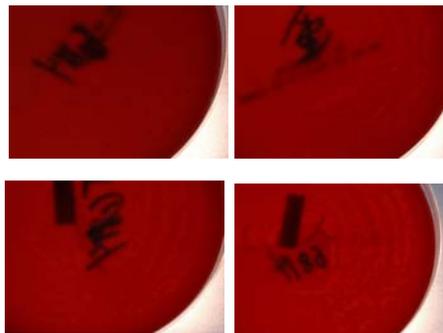
## Results

88 positives were detected by any one or combination of testing methods. This is a 40% positivity rate which is higher than the average 10-30% reported in the literature.

- Oxoid TSA /Columbia and PML TSA presented hemolytic activity of GBS equally well. Oxoid TSA demonstrated hemolytic intensity slightly better than Oxoid Columbia and PML TSA but much better than PML Columbia.
- 1 GBS isolate exhibited no hemolysis in all 4 blood media.
- 3 Lancefield Group G Streptococci and 1 Gp. C yielded 3+ growth on subs from STHB. These same 4 organism isolates, except for a +/- growth in one, failed to grow from MTHB subs. Because of its exceptional hemolytic activity, one of the Gp. G masked a 3+ growth of GBS.
- 9 broths were STHB positive and MTHB negative on sub-culture. 15 broths were MTHB positive and STHB negative. 92% of these showed no growth on initial CNA. The remaining 8% were +/- growths on initial CNA, likely indicating low inocula of GBS.
- MTHB reduced the growth of Enterococcus sp. compared to STHB. 2/6 noted cases of 3+ growth of Enterococcus were MTHB positive for GBS and STHB negative.
- PathoDx direct grouping matched broth subs in all but 2 cases using STHB and in all but 8 cases using MTHB. Each of these cases showed no growth on initial CNA, indicating a likely initial low level GBS inocula.
- Gentamicin discs were useful for GBS grown on PML Columbia and increased recognition and recovery by 20%. Oxoid TSA and Columbia increased by 1% and PML TSA increased by 3%.

Detection Test	Positives (N=88 total)	Sensitivity
Initial CNA screen	83	78%
STHB Oxoid Col/TSA	77	89%
MTHB Oxoid Col/TSA	83	95%
PML TSA	74	86%
PML Columbia	61	77%
Direct STHB PdX	86	98%
Direct MTHB PdX	80	91%

Table 1 GBS Recovery Rates.



Sample #6814 on PML Columbia (upper left), on PML TSA (upper right), on Oxoid Columbia (lower left) and on Oxoid TSA (lower right).

## Results

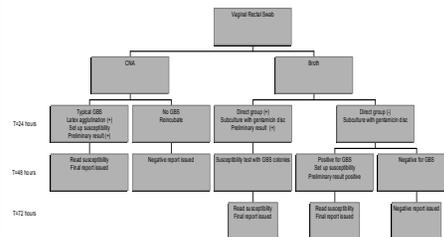


Sample #6971 STHB sub (left) and MTHB sub (right). Note predominance and masking effect of group G Streptococcus on GBS from STHB sub.



Image of normal flora with GBS. Note GBS growth up to the edge of gentamicin disc.

Sample #6838 with E. faecalis and non-hemolytic GBS. Note E. faecalis zone of inhibition around disc.



Suggested process algorithm for GBS screening.

## Discussion

Optimal results, including minimum TAT and benchwork, incorporated the use of initial CNA, direct PathoDx grouping from broth, and when subculture is required, the use of a quality blood plate for hemolytic detection of GBS. If a sub-optimal plate, in terms of hemolysis demonstration is used, an inexpensive gentamicin disc could be added to increase the recovery of non-hemolytic strains of GBS.

## Discussion

- Not all standard blood media demonstrate hemolytic activity equally. Hemolytic intensity of media was Oxoid TSA > Oxoid Columbia = PML TSA > >PML Columbia.
- PathoDx has very good sensitivity for direct detection of GBS from broths, particularly STHB.
- MTHB demonstrated slightly better sub-culture recovery than STHB. It was also slightly better at eliminating or reducing growth of normal flora including E. faecalis. This could be important because E. faecalis demonstrated an inhibitory effect on GBS in 2 of the samples. MTHB also successfully eliminated the more hemolytic Gp. G and C beta streps which tend to mask GBS when using STHB.
- Addition of 5% blood in a broth culture can aid in detection of hemolytic organisms such as GBS.
- TSA medias give the visual impression of reduced normal flora when compared to Columbia. Colonies are typically smaller but GBS hemolytic activity is better represented, making it easier to detect GBS.
- GBS resistance to gentamicin (growth up to the disc) is more clearly depicted on PML Columbia than the other medias. This is also where it is especially helpful.
- A high 40% positivity rate may suggest a regional or seasonal phenomenon or may be indicative of more sensitive testing methods.

## Conclusion

The proper choice of blood medias, PathoDx direct broth grouping, use of a gentamicin disc on sub-culture and use of a good broth including the possibility of the more selective MTHB, are effective, inexpensive and simple methods to enhance the recovery of hemolytic and non-hemolytic GBS. These methods also allow for better adherence to CDC's recommendation of testing for hemolytic and non-hemolytic GBS colonies.

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## Acknowledgements

The authors wish to thank the staff of the Ottawa microbiology department at GDML for their dedicated assistance.