Comparison of Group B Streptococcus (GBS) Detection Methods Using a New Pre and Post Broth Inoculated Chromogenic Plate, Direct Broth Agglutination and an Enhanced Blood Agar Plate Method

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Introduction

Group B Streptococcus (GBS) continues to be a leading cause of neonatal sepsis and meningitis. Approximately 10-30 % of pregnant women are asymptomatic carriers of GBS which represents a risk to the newborn.

For maximum sensitivity of GBS detection, the CDC recommends the inoculation of a selective enrichment broth incubated 18-24 hrs. This broth is then subcultured to a blood agar plate from which typical and atypical colonies of GBS are tested for. Furthermore, if colonies of GBS are absent, reexamination is recommended after an additional 18-24 hrs. This traditional method has been viewed as costly and time consuming (5). The purpose of this study was to determine the efficacy of the following enhanced GBS detection methods in facilitating labor, cost and time.

- SBS-1 and SBS-2 Method: Utilization of a new chromogenic GBS agar (Bio-Rad Strep B Select) to compare recovery on pre (SBS-1) and post (SBS-2) broth inoculated plates.
- DB Method: Utilization of a group B latex Direct Broth agglutination test (PathoDx) with an 18-24 hr. incubated broth .
- CNA Method: Utilization of CNA (Oxoid) directly pre-broth inoculated.
- TSA Method: Utilization of standard broth sub-culture to TSA +5% sheep blood (Oxoid) with a 10 µg gentamicin disc applied to the second quadrant.
- Effect of extended 48 hr. incubation times on recovery using these methods.

Routine blood medias vary in their ability to demonstrate GBS hemolysis and growth patterns (8). Plate quality and technologist experience are paramount in GBS detection, two factors which were taken into consideration with this study.

Method

A total of 500 vaginal/rectal screening samples received at Gamma-Dynacare Medical Laboratories were included in this study. These swabs were alternatively inoculated to each of one CNA plate and one SBS-1. Each swab was then immersed into a GBS modified Todd-Hewitt broth with 5%sheep blood (Oxoid) containing 8µg/ml gentamicin and 15 µg/ml. nalidixic acid.

After overnight incubation at 35 C in air, CNA and SBS-1 plates were examined and tested for GBS using the PathoDx Group B latex agglutination direct method. GBS appears as turquoise to sky-blue colonies on SBS. PYR, catalase, and CAMP tests were used as required. These plates were re-incubated and read again at 48 hrs.

The broths were tested directly with PathoDx Gp. B latex by mixing one drop of broth and one drop of the latex test. A positive result was observed if agglutination occurred within 30 seconds.

The broths were sub-cultured to a TSA plate and another SBS (-2) plate after their initial 18-24 hr. incubation. A 10 µg gentamicin disc was applied to the second quadrant of each TSA plate for enhanced hemolytic and non-hemolytic GBS detection and E. faecalis differentiation. Both of these plates were then re-read at 48 hrs incubation for any further GBS colony break-outs not found at 24 hrs.

All tests were set-up, incubated and examined in accordance with manufacturer's instructions. Through rotation, four well experienced technologists read and interpreted results.

Results

A total of 152 positives were identified by any one or combination of testing methods. This is a 30% positivity rate which is 9% higher than the current 21% provincial average.

- Four of the positives were non-hemolytic on TSA but were the characteristic sky blue color on SBS.
- Four samples were direct broth latex positive but negative upon sub-culture. Since no visible colonies of GBS were seen, these were considered false positives. No further tests were available to verify this.
- 2 SBS-1 exclusive GBS growth only plates exhibited a 3+ growth of Enterococcus faecalis on TSA and SBS-2, a previously witnessed phenomenon. (3,5,6,8).
- Groups A, B, C and G Beta hemolytic Streptococci all appear as blue colonies on SBS. There is a noticeable variation of the blue hue between S. agalactiae and the other species.
- Enterococcus species appear lavender on SBS.
- Gentamicin discs were useful on TSA plates as GBS colonies grow up to the disc whereas Enterococci and most other interfering flora are susceptible to this antibiotic and exhibit a zone of no growth around the disc. (Fig. 1)
- No additional GBS were recovered at 48 hrs with TSA and SBS-2. An additional 7 % were picked up on SBS-1 and 3% with CNA at 48 hrs.

Summary of Results

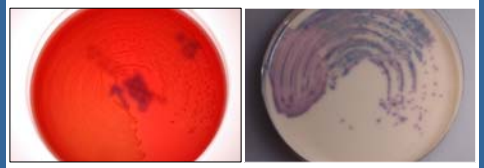
	CNA		SBS-1		DB	TSA genta		SBS-2	
Time	24	48	24	48	24	24	48	24	48
Total Positives (152)	106	109	122	131	147	144	144	146	146
False Negatives	46	43	30	21	5	8	8	6	6
Sensitivity	70%	72%	80%	87%	97%	95%	95%	96%	96%

SBS-1 only	2
SBS-2 only	1
TSA/SBS-2 only	2
DB only	4

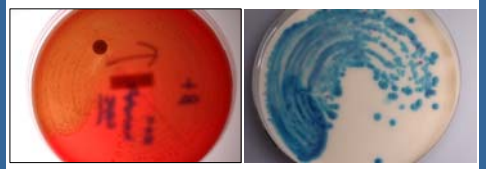
Exclusive Recoveries

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

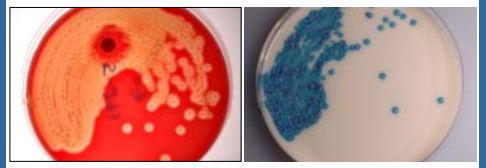
Results



Sample #0288 with non-hemolytic GBS and E. faecalis on TSA (left) and on SBS-2 (right). Note: Blue colonies on SBS-2 are GBS.



Sample #0138 with non-hemolytic GBS and E. faecalis on TSA (left) and on SBS-2 (right). Note: Blue colonies on SBS-2 are GBS.



Sample #0273 with GBS and group G Streptococcus on TSA (left) and on SBS-2 (right).

Discussion

The enhanced methods of GBS detection demonstrated excellent sensitivity and positivity rates.

- Pre-broth inoculation of SBS plates gave fair sensitivity rates (87%) and is an acceptable method as per manufacturer.
- Technologists preferred the ease of use of the new SBS plates as blue colonies were easier to detect than the typical vague hemolytic zones of GBS on a blood agar. Non-hemolytic GBS also appeared blue on SBS.
- TSA with gentamicin discs applied gave very good sensitivity results and this is an inexpensive enhanced alternative method.
- Direct broth agglutination using PathoDx had the highest sensitivity, lowest cost and turn-around-time.
- Enterococcus faecalis may demonstrate an antagonistic effect with GBS and can mask its presence, especially on post broth inoculated plates.

Discussion

- All blue colonies on SBS require a confirmatory test for GBS such as latex agglutination, PCR, or CAMP tests.
- CNA is useful for testing and obtaining colonies for susceptibility testing 24 hrs. in advance of the usual broth subcultures. This could be used in conjunction with a broth sub method in that only negative CNA accompanying broths need sub-culturing. Approximately 70% of positives are detected at 24 hrs. with this method.
- All GBS were recovered by subculture of broth within 24 hrs. using these enhanced methods.
- Additional GBS can be recovered using a pre-broth inoculated plate such as SBS.

Conclusions

These are sensitive, inexpensive, time saving and enhanced methods of detecting typical and atypical GBS colonies. Any one of these methods may ultimately improve recovery of GBS, save costs and improve turn-around-time (TAT) in the clinical laboratory.

In summary:

Ease of use (easy→difficult): SBS-1 =DB>SBS-2>TSA+gent

Best TAT (best→worst): DB=SBS-1>SBS-2=TSA+gent

Expense (least→most): DB>TSA+gent>SBS-1>SBS-2

Sensitivity (highest→lowest): DB>SBS-2>TSA+gent>SBS-1>CNA

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