Validation of Strep B Carrot Broth™ Using A Prospective Double Swab Comparison Method

M. Miller, L. Tomalty, R. Liao and D. Zoutman

The Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario

Pathology and Molecular Medicine, Queen's University

Kingston General Hospital

Contact: millerm1@kgh.kari.net

Abstract

Objective: Screening for Group B Streptococci (GBS) in prenatal women at 35-37 weeks continues to be a workload and procedural issue for clinical microbiology laboratories. We set out to conduct a prospective comparative study to screen for GBS using Strep B Carrot Broth (Hardy Diagnostics, Inverness Medical, Ottawa, Ont.) and GBS Broth (PML Microbiologicals, Mississauga, Ont.) **Methods:** 200 combined vaginal/rectal swabs were collected from patients at 35-37 weeks gestation. Specimen collection was performed in parallel using a double headed swab. Swab # 1 was inoculated into Strep B Carrot Broth. Swab #2 was inoculated into GBS Broth. The GBS Broth was incubated for 24 h and then plated to Colistin Naladixic Acid agar (CNA). After overnight incubation any Carrot Broth which changed from colourless to orange was screened directly with latex agglutination testing (Prolex, Prolab Diagnostics, Richmond Hill, Ont.) for Group B. After the initial overnight incubation all Carrot Broths were subcultured to CNA to confirm the agglutination testing results and to detect false negatives. Nonhemolytic GBS do not produce the caratenoid pigment that converts the Carrot Broth to the orange colour indicating GBS positivity. **Results:** 55/200 (28%) were positive with Carrot Broth, 49/200 (25%) were positive using the conventional GBS Broth method. The Carrot Broth captured 6/200 (3%) of positives which were missed using the standard culture method. All Carrot Broths that turned orange were confirmed as being positive for GBS. 47/200 (24%) of our samples were direct latex agglutination positive for Group B from the Carrot Broth; providing results within 24 h. The average TAT for the Carrot Broth was 24-48 hr compared to our standard culture method which requires 48-96 hr. Carrot Broths subcultured to CNA for confirmation and exclusion of nonhemolytic GBS produced bacterial growth on CNA that was easier to read due to inhibition of commensal bacteria. **Conclusion**: Testing with Carrot Broth will decrease workload since 24% of samples were completed on day 1 without subculture. A continued limitation of GBS testing remains the requirement for subculture to solid agar media. Strep B Carrot Broth does not detect nonhemolytic GBS and in this study 48 of 55 (87%) GBS detected using Strep B Carrot Broths were hemolytic and produced the colour change to orange indicating positivity. This product shows increased sensitivity, specificity and a marked decrease in TAT compared to conventional testing.

Introduction

Since the 1970s *Streptococcus agalactiae* (GBS) has been increasingly reported as the cause of invasive neonatal infections. Neonatal infections present as two different clinical entities: early onset neonatal disease, characterized by sepsis and pneumonia within the first 7 days of life: and late-onset disease with meningitis and sepsis between 7 days and 3 months of age. The most important risk factor for the development of invasive neonatal disease is the colonization of the maternal urogenital or gastrointestinal tract by GBS which is found in 10 to 30% of pregnant women. Prevention of early-onset neonatal infections can be achieved in the majority of cases by administration of intrapartum antibiotic prophylaxis starting at least 4 h before delivery. Screening for GBS colonization at 35-37 weeks gestation continues to be the optimal testing approach recommended by the Society of Obstetricians and Gynecologists of Canada. New procedural methods for the detection of GBS colonization have been recently manufactured which are intended to reduce laboratory costs and decrease turnaround time. GBS screening continues to be a workload and procedural issue for many clinical microbiology laboratories across the country. The purpose of this study was to perform a prospective comparative study using Strep B Carrot Broth (Hardy Diagnostics, Inverness Medical, Ottawa, Ont.) in combination with our current method.

Materials and Methods

200 combined vaginal/rectal swab samples were collected at 35-37 weeks gestation and submitted to the laboratory for GBS culture. Single site collection sampling using a double headed swab (Stuart liquid Transystem 2x rayon CA 139C, Copan) allowed for true parallel testing (Fig. 1).

Swab number 1 was inoculated into Strep B Carrot Broth (Hardy Diagnostics, Inverness Medical, Ottawa, Ont.). The Carrot Broth was incubated at 35° O_2 overnight. After incubation any orange or slightly orange Carrot Broth (Fig. 3) was screened directly using 2 drops of the broth and a Prolex Diagnostics latex agglutination test (Prolab Diagnostics, Richmond Hill, Ont.). Latex reagent specific for the streptococcal Lancefield antigen grouping B sera was used to detect GBS. Latex reagent for antigen grouping D was used as the negative control. After overnight incubation all Carrot Broths regardless of colour were subcultured to CNA agar to confirm positive Group B latex agglutination results and to permit culture of any nonhemolytic GBS. Beta-hemolytic colonies were tested with latex agglutination. Non-hemolytic grey colonies were first screened with Bile Esculin Agar (Oxoid Company, Nepean, Ont.). Positive bile esculin colonies were determined to be negative for GBS. Negative bile esculin colonies were then tested for GBS using latex agglutination for streptococcal Lancefield antigen grouping. CNA plates were evaluated at 24 h for GBS and negative plates were reincubated and read at 48 h. All Group B positive isolates were confirmed with Gram stain and catalase.

Swab number 2 was inoculated into Group B selective Strep Broth (PML Microbiologicals, Mississauga, Ont.) using our standard method. The selective Strep Broth was incubated at 35° CO2 for 24 h and then plated to CNA. Beta-hemolytic colonies were tested with latex agglutination. Non-hemolytic grey colonies were first screened with Bile Esculin Agar. Positive bile esculin colonies were determined to be negative for GBS. Negative bile esculin colonies were then tested for GBS using latex agglutination for streptococcal Lancefield antigen grouping. The CNA plate was evaluated at 24 h for GBS and if not present was reincubated and read at 48 h.

Figure 1. Copan Liquid Stuart Swab 139C (double plastic swab)

Results

- All Carrot Broths that turned orange or slightly orange confirmed as being positive for GBS on subculture to CNA.
- 55/200 (28%) swabs were positive for GBS with Carrot Broth.
- 49/200 (25%) swabs were positive using Selective Strep Broth.
- Carrot Broth identified 6/200 (3%) GBS positive swabs which were determined to be negative for GBS using conventional testing.
- 47/200 (24%) were direct latex agglutination positive for GBS from the Carrot Broth providing direct results within 24 h.
- 7 Carrot Broths did not turn orange but grew GBS on subculture. These broths were subsequently tested with latex agglutination and were Lancefield group B-positive, and group D-negative.
- 4 orange Carrot Broths were latex agglutination B and D positive; on subculture to CNA all 4 orange broths were GBS positive.
- The mean and median turnaround times for all Carrot Broth positive swabs including subculture and identification of nonhemolytic GBS were 34 and 24 h, respectively.
- The mean and median turnaround times for Selective Strep Broth positive swabs including subculture and identification of nonhemolytic GBS were 54 and 48 h, respectively.
- There were no false positive samples using either Carrot Broth or Selective Strep Broth.
- CNA plates used to subculture Carrot Broth tubes were simpler and faster to evaluate due to the reduction in growth of some commensal organisms.
- Hardy Carrot Broth demonstrated 100% sensitivity and 100% specificity for all GBS. The standard 24 h incubation period and subculture to solid agar media must be included in order to identify all serogroups of GBS.
- Selective Strep Broth demonstrated 91% sensitivity and 100% specificity for all GBS.

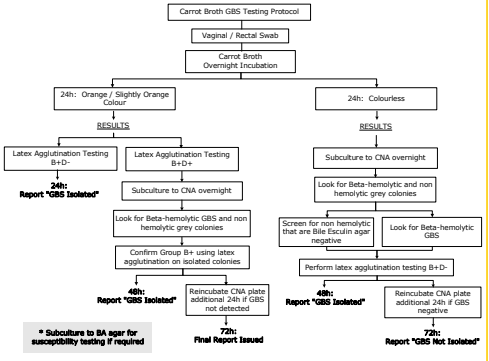

Figure 2. Algorithm for Surveillance Testing of Swabs for GBS incorporating Carrot Broth Culture into Routine Testing

Conclusions

- The Hardy Strep B Carrot Broth used in conjunction with latex agglutination and CNA subculture is a rapid, sensitive, specific, and easy to use test for the detection of GBS.
- In this evaluation Hardy Strep B Carrot Broth demonstrated 100% sensitivity and 100% specificity compared to the conventional method.
- 49/200 (25%) swabs were positive using Selective Strep Broth.
- The mean and median turnaround time for detecting GBS using Hardy Strep B Carrot Broth was 34 h and 24 h, respectively.
- TAT for GBS screening is decreased as 24% of specimens were completed at 24 h without the need for subculture.
- Nonhemolytic GBS do not produce the caratenoid pigment that changes the Carrot Broth from colourless to orange or light orange indicating the presence of GBS. Subculture to solid agar media is required to detect nonhemolytic strains of GBS.
- If additional susceptibility testing of GBS is required, Carrot Broth positive samples can be subcultured to sheep blood agar for Clindamycin and Erythromycin testing. Alternatively positive Carrot Broth tubes can be held at room temperature. GBS has been shown to be viable in Carrot Broth for up to 51 days.

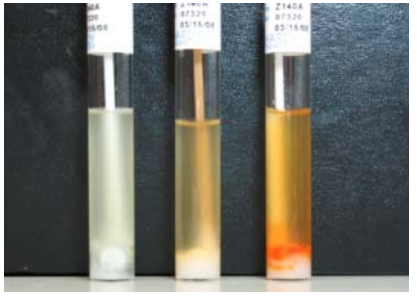

Figure 3. Culture of surveillance swabs in Carrot Broth after 24 h. Results from left to right: GBS negative, GBS positive (slightly orange), GBS positive (orange).

Table 1. Performance of Carrot Broth and Strep Broth testing for the detection of GBS using 200 swabs

	Carrot Broth	Strep Broth
Positive	55 (28%)	49 (25%)
Negative	145 (73%)	157 (76%)
Sensitivity	100%*	91%*
Specificity	100%	100%

*testing algorithm includes subculture to solid agar media