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

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 all Carrot Broth which changed from colourless to orange were screened directly with latex agglutination testing (Prolex, Prolab Diagnostics, Richmond Hill, Ont.) for GBS. If additional susceptibility testing of GBS is required, 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 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 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°C CO2 for 24 h. 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 colonies. Positive bile esculin colonies 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.

Results

- 55/200 (28%) swabs were positive for GBS with Carrot Broth.
- 49/200 (25%) swabs were positive using Selective Strep Broth.
- 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.
- In this evaluation Hardy Strep B Carrot Broth demonstrated 100% sensitivity and 100% specificity compared to the conventional method.
- Hardy Strep B Carrot Broth was 34 h and 24 h, respectively.
- The mean and median turnaround time for detecting GBS using Hardy Strep B Carrot Broth was 34 h and 24 h, respectively.
- 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.

Materials and Methods

- Swab 2 was inoculated into GBS Broth (PML Microbiologicals, Mississauga, Ont.) using our standard method. The selective Strep Broth was incubated at 35°C 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 (Oxoid Company, Nepean, Ont.). Positive 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.

Conclusions

- All Carrot Broths that turned orange or slightly orange confirmed as being positive for GBS on subculture to CNA. 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.
- In this evaluation Hardy Strep B Carrot Broth demonstrated 100% sensitivity and 100% specificity compared to the conventional method.
- Sensitivity 100%* 91%*
- Specificity 100% 100%*

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th>Negative</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot Broth</td>
<td>55 (28%)</td>
<td>145 (73%)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Strep Broth</td>
<td>49 (25%)</td>
<td>157 (76%)</td>
<td>91%*</td>
<td>100%*</td>
</tr>
</tbody>
</table>

*testing algorithm includes subculture to solid agar media