Evaluation of Three Methods (StrepB Carrot Broth™, LIM Broth, and Granada Agar) for Recovery of Group B Streptococci.

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Introduction

Intrapartum colonization of group B Streptococci (GBS) is a major risk factor for early-onset neonatal sepsis and remains as a significant source of morbidity in perinatal care. Numerous studies employing several methods have documented that the accuracy of prenatal screening cultures performed the Granada Agar in sensitivity by one or two dilutions in 12 (15%) instances whereas Granada Agar was superior in 6 (7%) samples. StrepB Carrot Broth™ and Granada agar were not as consistent as the LIM Broth method, as shown in Table 1. All methods presented 100% sensitivity in dilutions 1, 2, and Granada Agar (84%) and Granada Agar (83%). When compared individually, LIM Broth was the most sensitive method, followed by StrepB Carrot Broth™ (74%) and Granada Agar (69%). The most frequent isolates observed in StrepB Carrot Broth™ and Granada agar methods were enterotoxigenic E. coli (52%) samples. All methods presented a 99% sensitivity for StrepB Carrot Broth™ and Granada agar. All tubes that did not turn orange to red color were considered as false-negatives.

Materials & Methods

Isolates

Eighty-one clinical isolates of Group B Streptococci were analyzed in this study. Thirty (n=30) were kindly provided by Fleury – Medical Diagnostics Center, São Paulo, Brazil. The remaining 51 (n=51) were kindly provided by Fleury – Medical Diagnostics Center, São Paulo, Brazil.

Sample Preparation and Inoculation

Serial dilution from 1:10⁰ to 1:10⁶ (CFU/mL) was performed. Colonies with 10³ to 10⁶ CFU/mL were inoculated using a 1:10 dilution. All isolates were tested at least twice for positive samples.

Table 1 Comparison of sensitivity among methods evaluated (StrepB Carrot Broth™, LIM Broth, and Granada Agar).

<table>
<thead>
<tr>
<th>Dilution</th>
<th>StrepB Carrot Broth™</th>
<th>LIM Broth</th>
<th>Granada Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻⁰ CFU/mL</td>
<td>100% (81/81)</td>
<td>100% (81/81)</td>
<td>100% (81/81)</td>
</tr>
<tr>
<td>10⁻¹ CFU/mL</td>
<td>99% (80/81)</td>
<td>94% (76/81)</td>
<td>94% (76/81)</td>
</tr>
<tr>
<td>10⁻² CFU/mL</td>
<td>82% (66/81)</td>
<td>82% (66/81)</td>
<td>82% (66/81)</td>
</tr>
</tbody>
</table>

Discussion

• Positive samples can develop a color change in as early as 6 hours of incubation in StrepB Carrot Broth™ culture medium.

• StrepB Carrot Broth™ and Granada agar were inoculated, incubated, and interpreted according to manufacturer’s instructions.

• Samples that yielded orange to red colonies were considered as positives.

• All isolates that yielded GBS after subculture to blood agar plate were considered as positives.

• StrepB Carrot Broth™ and Granada agar were not used due to negative results.

• All methods presented a 100% sensitivity in concentrations of 10³ to 10⁶ CFU/mL.

• A suspension equivalent to 0.5 McFarland was prepared for each isolate.

• All tubes that did not turn orange to red color were considered as false-negatives.

• Granada Agar

• Samples that yielded orange to red colonies were considered as positives.

• Positive samples were confirmed by sub-culture or testing for positive samples.

• In simulations of low GBS count, StrepB Carrot Broth™ and Granada agar were able to recover GBS in 80 of 81 (99%) of samples followed by StrepB Carrot Broth™ (74%) and Granada agar (69%).

• Samples that yielded orange to red colonies were considered as positives.

• All isolates that yielded GBS after subculture to blood agar plate were considered as positives.

Quality Control

A total of 81 clinical isolates were tested with the three methods at seven dilutions (10⁻⁰ to 10⁻⁷ CFU/mL). All isolates were tested twice for positive samples.

References


