A comparison of four commercial chromogenic media and blood agar for the isolation and preliminary identification of *Streptococcus agalactiae* from vaginal swabs

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Abstract

Aim: To evaluate the performance of four commercial chromogenic media for the detection of GBS in vaginal swabs from pregnant women. The media was compared with blood agar and was evaluated in terms of sensitivity, specificity, ease of use and cost.

Methods: 100 vaginal swabs, collected during April and May 2011, were included in the study. The swabs were pre-enriched in LIM broth for 16-24 hours, before a 10µL loop of broth was sub-cultured onto blood agar and each of the chromogenic media. CHROMagar™ StrepB, bioMérieux ChromID, Oxoid Granada, and Bio-Rad StrepB Select, were compared with 5% Columbia blood agar and routine laboratory detection.

Incubation of plates and interpretation of bacterial growth was in accordance with the manufacturers’ guidelines.

Results: Bio-Rad StrepB Select and bioMérieux ChromID were the best performing media with 100% and 96% sensitivity and 91% and 92% specificity, respectively. CHROMagar™ StrepB and Oxoid Granada were less sensitive (88% and 72%), with no improved performance over blood agar.

Conclusion: bioMérieux ChromID was chosen for introduction into routine use, due to its excellent sensitivity and easier differentiation of false positive growth.

Keywords: *Streptococcus agalactiae*, Group B Strep, vaginal swab, LIM broth, Bio-Rad StrepB Select, bioMérieux ChromID, CHROMagar™ StrepB, Oxoid Granada.


Introduction

*Streptococcus agalactiae* (Group B Strep (GBS)) remains a leading cause of invasive disease in neonates. GBS can cause septicaemia, meningitis and pneumonia in the neonate, with resulting high morbidity and mortality rates. Neonates are infected by perinatal transmission and can become ill within hours of becoming infected, or take up to 5 days before symptoms start to show (1).

*S. agalactiae* is a Gram positive coccus that is weakly beta haemolytic with a dull grey colonial appearance on 5% Columbia sheep blood agar. Most colony sizes are 1-2mm and colonies can be hard to detect if there is heavy growth of other commensal bacteria present (2,3,4). Normal vaginal flora includes *Staphylococcus species*, lactobacillus, *Enterococcus species*, alpha-haemolytic, beta-haemolytic and non-haemolytic streptococcus.

Laboratory confirmation of suspect GBS colonies is commonly performed by Lancefield grouping, whereby *Streptococcus species* are classified into serologic groups based on their cell wall antigenic properties. *S. agalactiae* belongs to Lancefield Group B.

Asymptomatic GBS vaginal or rectal colonisation occurs in approximately 10-40% of women. Pregnant women colonised with GBS risk maternal transmission during labour. It is in this group of women that antibiotic prophylaxis is most effective (1).

The United States Centers for Disease Control and Prevention (CDC) recommend for women between 35-37 weeks of gestation to be screened for GBS carriage. However, the New Zealand College of Midwives advocate a risk-based approach, whereby only women deemed to have high risk factors, such as premature rupture of the membranes and preterm delivery, should be screened or given antibiotic prophylaxis. For screening purposes, high vaginal swabs (HVS) or rectal swabs are used, with collection usually carried out by either a registered nurse or a midwife.

Common problems with the laboratory detection of GBS include low colony forming units of GBS in some samples, overgrowth of normal vaginal flora and non-haemolytic GBS colonies. In addition, if GBS is present with non-typical colony morphology it can be difficult to detect or missed, particularly on blood agar (2,3,4). To detect low numbers of organisms, swabs should be pre-enriched in either Todd-Hewitt broth or LIM broth, before agar plate culture (5). Specific GBS selective chromogenic media have been developed by various companies in order to overcome some of the detection problems and to improve isolation rates.

The aim of this study was to evaluate the performance of four chromogenic media for the detection of GBS in 100 high vaginal swabs from pregnant women. The four chromogenic media were compared with blood agar and evaluated in terms of sensitivity, specificity, ease of use, and cost.

Method and materials

100 vaginal swabs, collected from pregnant women during April and May 2011, were included in the study. Swabs were taken during either routine outpatient antenatal screening or pre delivery at Christchurch Hospital. Data on pregnancy gestation was not collected. Swabs were collected into transport media and processed at Canterbury Health Laboratories within 24 hours of collection. Routine laboratory processing was performed independently of the study.

Routine laboratory protocol for HVS analysis consisted of inoculation onto blood agar and Chocolate/Thayer Martin agar, with a wet prep for *Trichomonas vaginalis* and a Gram stain. The swabs were then broken off into LIM broth (Fort Richard Laboratories, Auckland), for the selective enrichment of GBS. The LIM broth is an enriched brain heart infusion base with added nalidixic acid and colistin to suppress the growth of gram negative bacteria (5). LIM broth was incubated for 16-24 hours at 36°C, 5% CO2, before being sub-cultured with a 10µL loop onto blood agar. The blood agar plates were incubated for 16-24 hours at 36°C, in 5% CO2. The plates were then read for GBS, looking for colonies that have typical GBS morphology (4).

Four different chromogenic agar culture plates: CHROMagar™ StrepB, ChromID, Oxoid Granada, and StrepB Select, were compared with 5% Columbia blood agar and routine laboratory detection. All plates were inoculated with a 10µL suspension of LIM broth.
CHROMagar™ StrepB (Fort Richard Laboratories, Auckland) is a selective agar that inhibits most bacteria and allows for differentiation of GBS, which produces a mauve colour (Fig 1). ChromID (bioMérieux, New Zealand) produces colonies that are pink to red in colour. Other bacteria are either inhibited by the media or are blue to green pigmented (Fig 2). Oxoid Granada (Thermofisher Scientific, Auckland) is a clear media, producing orange pigmented colonies in anaerobic conditions (Fig 3). StrepB Select (Bio-Rad New Zealand) produces distinctive blue colonies on an opaque media (Fig 4).

Blood agar plates were incubated for 18 hours at 36°C with 5% CO₂. CHROMagar™ StrepB, bioMérieux ChromID and Bio-Rad StrepB Select were incubated at 36°C in ambient air conditions. Oxoid Granada agar plates were incubated for 18 hours at 36°C in anaerobic conditions. After overnight incubation, all plates were read in accordance with the manufactures’ guidelines.

Typical GBS colonies were confirmed by ProLab Diagnostics Strep latex grouping kit (Ngaio Diagnostics, Nelson). False positive colonies were investigated using standard laboratory methods. The isolation of GBS from any of the media used (chromogenic or blood) was considered to be a positive sample, and failure to detect GBS on any of the other media was considered to be a false negative.

Results
After testing all suspect colonies from the chromogenic agar, there were 25 positive GBS cultures. Bio-Rad StrepB Select detected all 25 isolates, bioMérieux ChromID detected 24 GBS, CHROMagar™ StrepB detected 22 GBS and Oxoid Granada was the least sensitive detecting only 18 GBS. In comparison there were 22 GBS isolated from blood agar but only 20 were detected by routine laboratory testing. Results are listed in Table 1. The population prevalence for GBS in this study is 25%, which is similar to the population prevalence reported by Hickman et al. (1).
Discussion
As expected, blood agar missed several GBS positive cultures. This was mainly due to overgrowth of normal vaginal flora. However, two of the chromogenic agars also lacked sensitivity. Only two of the trial media (bioMérieux ChromID and Bio-Rad StrepB Select) out performed blood agar.

The Bio-Rad StrepB Select media proved to be the most sensitive for GBS at 100% but had a number of false positive results. Colonies were further investigated to reveal the identity of false positive organisms, with staphylococci and streptococci being the major culprits. Results are detailed in Table 2.

Table 1. Media performance

<table>
<thead>
<tr>
<th>Media</th>
<th>True Positives</th>
<th>False Positives</th>
<th>False Negatives</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood agar</td>
<td>22</td>
<td>4</td>
<td>3</td>
<td>88%</td>
<td>95%</td>
</tr>
<tr>
<td>CHROMagar™ StrepB</td>
<td>22</td>
<td>15</td>
<td>3</td>
<td>88%</td>
<td>83%</td>
</tr>
<tr>
<td>bioMérieux ChromID</td>
<td>24</td>
<td>6</td>
<td>1</td>
<td>96%</td>
<td>92%</td>
</tr>
<tr>
<td>Oxoid Granada</td>
<td>18</td>
<td>3</td>
<td>7</td>
<td>72%</td>
<td>96%</td>
</tr>
<tr>
<td>Bio-Rad StrepB Select</td>
<td>25</td>
<td>7</td>
<td>0</td>
<td>100%</td>
<td>91%</td>
</tr>
<tr>
<td>Routine laboratory procedures</td>
<td>20</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

All of the chromogenic media tested gave false positive results. Colonies were further investigated to reveal the identity of false positive organisms, with staphylococci and streptococci being the major culprits. Results are detailed in Table 2.

Table 2. Number of false positive growths

<table>
<thead>
<tr>
<th></th>
<th>Blood agar</th>
<th>CHROMagar™</th>
<th>bioMérieux ChromID</th>
<th>Oxoid Granada</th>
<th>Bio-Rad StrepB Select</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>α-haemolytic streptococcus</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>β-haemolytic streptococcus</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Non-haemolytic streptococcus</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

In terms of specificity, the Oxoid Granada media had the least number of false positive isolates. However this was counteracted by its lack of sensitivity. Bio-Rad StrepB Select and bioMérieux ChromID had a small number of false positive isolates that required further confirmation tests. CHROMagar™ StrepB had a high number of false positive isolates, giving it the lowest specificity. In comparison, blood agar had only 4 false positive isolates.

Conclusion
In this small study we have found that the two best performing chromogenic media were bioMérieux ChromID and Bio-Rad StrepB Select. This was based on a high sensitivity for GBS, with a low false positive rate and similar plate price. The workup required for these media was lower than for the other two media tested. BioMérieux ChromID was chosen for introduction into routine use at Canterbury Health Laboratories due to its excellent sensitivity and reader ability to easily distinguish false positive growth.

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References

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