Evaluation of three commercial broth media for pigment detection and identification of group B streptococci (GBS), *Streptococcus agalactiae*.

Maria da Glória Carvalho¹, Richard Facklam¹, Delois Jackson¹, Bernard Beall¹ and Lesley McGee¹*

¹Streptococcus Laboratory, Respiratory Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA. 30333. USA

Running title: GBS identification using pigment producing media

Corresponding author:

* Lesley McGee, PhD

Respiratory Diseases Branch, Mailstop G-03
Centers for Disease Control and Prevention
1600 Clifton Rd
Atlanta AG 30333
Tel: 404-639-0455
Fax: 404-639-2070
Email: lmcgee@cdc.gov
ABSTRACT

Detection of GBS strains at various bacterial concentrations was evaluated using three pigment-producing broth media. At 10^3 CFU/ml, StrepB Carrot Broth™ (SBCB), Granada Instant Liquid Biphasic (IGLB), and Northeast Laboratory GBS Screening Medium (NEL-GBS) showed 100% detection but at the lower bacterial counts SBCB and IGLB were more sensitive than NEL-GBS after 24hrs.
*Streptococcus agalactiae* remains a significant cause of morbidity and mortality among newborns, despite major efforts to detect colonized pregnant women. The current Centers for Disease Control and Prevention guidelines for the prevention of perinatal GBS infections recommend that pregnant mothers be tested at 35 to 37 weeks of pregnancy for carriage of GBS by a selective enrichment method using anorectal/vaginal swabs in LIM or TransVag broth, followed by subculture on blood agar plates (BAP) (2). Although microbiology-based screening techniques have shown to be an effective means of preventing the onset of perinatal GBS disease, this process can be labor-intensive and typically requires 2 days to provide results. In recent years alternative methods have become available that have improved sensitivity, decreased test turnaround time, increased test efficiency and that are easier to perform in the laboratory (1). Such methods include the use of modified media such as Granada medium (GM) (5, 6), which utilizes the unique ability of β-hemolytic strains of *S.agalactiae* to produce a red-orange pigment, thereby allowing detection and identification of GBS in a single step. Most reports describing this medium have included direct plating of swabs onto the agar medium and variable results on the sensitivity of this medium have been reported, with some investigators considering it to be unacceptably low (4, 7, 10, 11). Enrichment broth modified from the original Granada medium have been developed and investigated more recently (1, 3, 8, 9). These broth media have shown good diagnostic performance compared to LIM broth with respect to sensitivity and specificity and have the added advantage of decreasing turnaround time for positive cultures by 24 hours. The formulations of both these solid and liquid media that enhance pigment production have changed considerably over the past few years and comparisons between these new formulations are few. This study evaluated each of three commercial broth
media for the identification of GBS and their ability to produce pigment at various bacterial
concentrations.

Reference strain cultures (n=10) for each of the GBS serotypes and clinical isolates
(n=36) representing various types were selected from CDC’s Active Bacterial Core Surveillance
collection of invasive GBS. E. faecalis ATCC 29212 was used as negative control, and a single
non-hemolytic GBS strain was included. Organisms were supplied in a blinded manner and the
laboratory tests were performed by a single laboratory technician. Each GBS culture was
inoculated onto a BAP and grown overnight. Culture suspensions were prepared in saline to
achieve a 0.5 McFarland density followed by serial dilutions to obtain 10^3, 10^2 and 10^1 colony
forming units (CFU) per ml. Tubes of LIM, SBCB, IGLB, and NEL-GBS broth were each
inoculated with 1.0 ml of each of the 3 concentrations and tests were repeated 3-4 times over a
four-month period. LIM broths were examined for growth as judged by the increase in turbidity
of the broth after 24 and 48 hours. If the broth showed no visible growth after 24 hours of
incubation, the broth was sub-cultured to BAP plates and these plates examined for growth after
overnight incubation at 35 °C. The SBCB, IGLB and NEL-GBS broths were examined for
pigment production at 24 and 48 hours. If a peach, orange, reddish orange or red color was
evident, a positive pigment production was recorded. If no color change was detected after 24h
of incubation, the tubes were sub-cultured to BAP and incubated overnight. If growth of GBS on
BAP was evident it was recorded as positive for growth.

All three pigment-producing broths were positive for all hemolytic GBS isolates (n=46)
tested at the higher concentration of 10^3 CFU/ml. E. faecalis ATCC 29212 used as a negative
control, as well as the single non-hemolytic GBS strain that was tested, did not produce pigment
in any of the media, but did grow on BAP upon subculture. Any pigment production in the
broths, detected by the presence of peach, orange, red-orange or red color regardless of intensity of color, was recorded as positive. This method assumes that growth of GBS is necessary for pigment production. The StrepB Carrot broth was evaluated on 3 separate days and pigment production was detected in 97.8% – 100% of β-hemolytic GBS inoculated into SBCB at 10^2 and 10^1 CFU concentrations after 24 hours incubation (Table 1). Granada broth (IGLB) medium was tested at four separate times and pigment production was observed for all reference and clinical isolates at 10^2 CFU and for 93.5% - 100% of the 10^1 CFU concentrations after 24hrs. NEL-GBS broth were tested on four separate testing dates and pigment production was poorly observed for both 10^2 CFU/ml and 10^1 CFU/ml (80.4% - 93.4%) in NEL-GBS broth, with around 30% of the reactions varying from very weak to no pigment production. At the lower bacterial counts (primarily 10^1 CFU/ml) all three broths had isolates that were not detected by pigment production and were also negative on subculture (Table 1). Pigment production that was either not detected or poorly observed after 24 and 48 hrs at the lower bacterial counts was associated primarily with serotype V and Ib. All three media performed optimally within their commercially assigned shelf life periods and there was no loss in sensitivity of the media over the 4 month test period.

Although laboratory methods for the detection of GBS have evolved over the past decade, there remains a clinical need for further speed and accuracy. A number of previous studies using pigmented broth formulations for the identification of GBS from clinical specimens have shown this method to be sensitive (1, 8, 9), cost effective and can allow for the improvement of diagnostic efficiency by dramatically reducing the number of subcultures that need to be performed (3). Results from this study and others, suggest that these media may be appropriate alternatives to those recommended by the current CDC guidelines and further
evaluations using clinical specimens to compare the current formulations of the three broth media are required. All three media evaluated in this report are reliable for GBS detection when bacterial loads are above $10^2$ CFU/ml, but in simulations of low GBS count, SBCB and IGLB broths were the most sensitive, with a weak or delayed pigment production common in the NEL-GBS broth. A positive reaction for pigment in these media is very specific for GBS, and therefore obviates the need for subsequent subculture or further identification steps in most cases. The requirement for subculturing onto conventional BAP, all broths that do not produce a pigment, allows for a necessary second level of detection for increased sensitivity. Also, in cases where patients report allergies to penicillin, broths will need to be subcultured to obtain GBS cultures to determine antimicrobial susceptibilities.

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REFERENCES


Table 1. Color detection for β-hemolytic Group B Streptococci strains (n=46) using three chromogenic broth media

<table>
<thead>
<tr>
<th></th>
<th>No. strains with pigment after incubation at 24hrs (%)</th>
<th>No. strains with pigment only after incubation at 48hrs (%)</th>
<th>No. isolates negative for pigment production and positive on BAP subculture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^2 CFU/ml</td>
<td>10^3 CFU/ml</td>
<td>10^4 CFU/ml</td>
</tr>
<tr>
<td>Carrot (SBCB)</td>
<td>45 (97.8)</td>
<td>45 (97.8)</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>TD-1</td>
<td>46 (100)</td>
<td>46 (100)</td>
<td>0</td>
</tr>
<tr>
<td>TD-2</td>
<td>46 (100)</td>
<td>45 (97.8)</td>
<td>0</td>
</tr>
<tr>
<td>TD-3</td>
<td>46 (100)</td>
<td>45 (97.8)</td>
<td>0</td>
</tr>
<tr>
<td>Granada (IGLB)</td>
<td>46 (100)</td>
<td>46 (100)</td>
<td>0</td>
</tr>
<tr>
<td>TD-1</td>
<td>46 (100)</td>
<td>45 (97.8)</td>
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<td>0</td>
</tr>
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<td>TD-3</td>
<td>46 (100)</td>
<td>45 (97.8)</td>
<td>0</td>
</tr>
<tr>
<td>TD-4</td>
<td>46 (100)</td>
<td>45 (97.8)</td>
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<td>NEL GBS</td>
<td>37 (80.4)</td>
<td>38 (82.6)</td>
<td>2 (4.3)</td>
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<tr>
<td>TD-1</td>
<td>43 (93.4)</td>
<td>43 (93.4)</td>
<td>4 (8.6)</td>
</tr>
<tr>
<td>TD-2</td>
<td>37 (80.4)</td>
<td>37 (80.4)</td>
<td>4 (8.6)</td>
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<tr>
<td>TD-3</td>
<td>40 (86.9)</td>
<td>39 (84.7)</td>
<td>3 (6.5)</td>
</tr>
</tbody>
</table>
| CFU/ml = colony forming units per ml
TD-1 = Test Date 1; TD-2 = Test Date 2; TD-3 = Test Date 3; TD-4 = Test Date 4
*Subculture to BAP for 1 isolate that was negative after 24hrs in broth was not determined