Evaluation of the new Brilliance GBS chromogenic medium
for the screening of *Streptococcus agalactiae* vaginal colonization
in pregnant women

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Running title: Evaluation of chromogenic media for GBS screening

Keys words: *Streptococcus agalactiae*, group B streptococcus, microbiological techniques,
chromogenic medium, vaginal colonization, pregnancy, human.

Abstract word count: 47

Main text word count: 1119

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ABSTRACT

Three commercial chromogenic agar media were evaluated for *Streptococcus agalactiae* screening in 200 vaginal swabs from pregnant women. The sensitivity and specificity were 94.3% and 100% for Granada (bioMérieux), 100% and 90.3% for Brilliance GBS (Thermo Fisher Scientific) and 100% and 98.2% for ChromID STRB (bioMérieux), respectively.

MAIN TEXT

*Streptococcus agalactiae*, usually termed as Group B streptococcus (GBS), is one of the most important causes of early onset neonatal infection (1, 2). The incidence of neonatal GBS infection ranges from 0.80 to 3.06 per 1000 live births in developing countries (1). Guidelines recommend screening vaginal or recto-vaginal GBS colonization in pregnant women at 35-37 weeks of gestation (3, 4). The prevalence of recto-vaginal colonization varies from 6.5 to 36% in European countries (5). In pregnant women colonized by GBS, intrapartum administration of antibiotics is recommended to prevent GBS transmission to the newborn during delivery (3, 4).

The performance of microbiological methods for GBS screening was greatly improved by the use of selective chromogenic media (6–9), together with rapid bacterial identification using matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (10). The overnight broth pre-enrichment step, recommended in some countries (3, 4), increases the sensitivity of the GBS screening (8, 11), mostly in cases of low bacterial load (11, 12). Enriched and direct cultures were shown to have close performances (9) although failure to perform direct culture can decrease sensitivity due to overgrowth of competing...
organisms in enrichment broth (13). Finally, nucleic acid amplifications tests (NAATs), notably those including the extraction step, reduce the delay of result and could be used at the admission of pregnant women in active labour for administering adequate antibiotic prophylaxis (14, 15). NAATs offer close performances compared to direct selective culture (16) but controversial results were reported after comparison with enriched culture (7, 9, 17, 18). Recent guidelines advised to use NAATs in selected circumstances such as at term gestation women with unknown colonisation status and no risk factor (3, 4).

The aim of this study was to assess the performances of the new Brilliance GBS chromogenic medium (Thermo Fisher Scientific, Dardilly, France) for the detection of *S. agalactiae* compared to two commercially available chromogenic media selective for this bacterium, ChromID STRB and Granada (bioMérieux, Marcy l’Etoile, France).

From February to April 2013, 760 vaginal swabs (eswab ref: 480CE, Copan, Brescia, Italy) from pregnant women were sent to the microbiology department of the University Hospital of Saint-Etienne, France. The routine screening of GBS in pregnant women was performed by direct culture following French recommendations (19). Approximately 50 µl of eswab medium was deposited on Granada medium using the swab and spread with a sterile loop. Plates were incubated under anaerobic conditions at 36°C and read after 24 and 48 hours of incubation following the manufacturer’s recommendations. Orange colonies were considered as *S. agalactiae* without confirmation of identification. The prevalence of *S. agalactiae* vaginal colonization was 16.7% (127/760).

From this panel, 200 non-consecutive samples were included prospectively in the study and plated onto Brilliance GBS, Granada and ChromID STRB agar plates. Briefly, a 50 µl-volume of eswab medium was plated onto each chromogenic plate using the EasySpiral Dilute instrument (Interscience, Saint Nom la Bretêche, France) and samples were stored at 4°C. Plates were incubated at 36°C under aerobic conditions for GBS Brilliance and ChromID
STRB, and under anaerobic conditions for Granada, as recommended by each manufacturer. All the plates were read after 22-24h of incubation. Granada plates were read after 48 hours in the routine workflow. Based on colony colour, presumptive GBS colonies isolated on each chromogenic medium were systematically identified at the species level with MALDI-TOF MS (Microflex LT, Bruker) following manufacturer’s recommendations. Non-target coloured colonies were also identified by MALDI-TOF MS if at least one chromogenic medium yielded GBS; otherwise no MALDI-TOF MS identification was performed. Thirty-five (17.5%) of the 200 vaginal swabs were found positive for *S. agalactiae* by at least two chromogenic agar plates. No sample was found positive by only one medium. The performances of the chromogenic media are depicted in the Table. The two GBS positive samples missed by the Granada medium were found positive for non-haemolytic *S. agalactiae*. The 33 positive samples on Granada were detected within 22 to 24 hours of incubation; an extended incubation of 48 hours failed to detect any more GBS positive sample.

Additionally, the *S. agalactiae* load obtained on chromogenic media inoculated with the EasySpiral Dilute instrument was determined by automated reading using the Scan 1200 colony counter (Interscience) (Figure 1A). A few samples yielding faint coloured GBS colonies could not be reliably quantified from Brilliance GBS (n=3), Granada (n=5) and ChromID (n=4) plates. After exclusion of these samples, the bacterial load was available for the three media in 29 specimens, ranging from 1.3 to 8.4 logCFU/ml with a strong correlation between media (Figure 1B); the median of bacterial load was of 4.56, 4.82 and 4.57 logCFU/ml for Brilliance GBS, Granada and ChromID STRB, respectively (not significant difference by Kruskal-Wallis test). Although it seems intuitive that a high level of GBS colonisation in vagina could favour neonatal infection, to our knowledge, the clinical
significance of this parameter has not been evaluated prospectively; this goal could be achieved by using a chromogenic agar medium.

This study evaluated the new Brilliance GBS medium for screening GBS in vaginal samples of pregnant women based on French recommendations. Although the three tested chromogenic media showed close performances, the practicability varied greatly from one medium to another. The Granada medium failed to recognize 2 of the 35 GBS isolates that were shown to be non-haemolytic GBS (20). Plates were very easy to read because of the absence of atypical coloured colonies. The ChromID STRB medium showed excellent specificity and sensitivity but needed a high technical expertise due to the difficulty of recognizing the target colonies among all the coloured colonies. The Brilliance GBS medium showed an excellent sensitivity and was easier to read than the former medium; however, it exhibited a lower specificity than the two other media because of the occurrence of false positive results with other species of streptococci, highlighting the need to perform an additional identification step (agglutination test or MALDI-TOF MS) on coloured colonies.

Unlike Granada medium, Brilliance GBS and ChromID STRB media do not require an anaerobic atmosphere, which improves the laboratory workflow. From a clinical point of view, false negative results could increase the risk of neonatal GBS infection due to the absence of antibiotic prophylaxis during labour. By contrast, false positive results could lead to unnecessary administration of antibiotics during labour in a few women, which has no major adverse impact on both mother and baby but lead to an overuse of antibiotics.

In conclusion, the new Brilliance GBS medium is a highly sensitive medium for GBS screening; it does not require anaerobic incubation. The positive colonies are easily recognized but the identification of *S. agalactiae* at the species level would need an additional step for avoiding false positive results with other species of streptococci.
REFERENCES


CONFLICTS OF INTEREST

The authors declare having no conflict of interest related to this study.

ACKNOWLEDGMENTS

This study was funded through a partnership with Thermo Fisher Scientific. Emma Scopes is acknowledged for helpful discussion.
Performances of Granada, GBS Brilliance and ChromID STRB chromogenic media from 200 non-consecutive vaginal swabs (including 35 positive ones by at least two culture methods) taken in 35-37 weeks pregnant women.

<table>
<thead>
<tr>
<th>Media</th>
<th>Number of samples</th>
<th>Target colour a</th>
<th>Non-target colour</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GBS</td>
<td>Other sp.</td>
<td>GBS</td>
<td>Other sp.</td>
<td></td>
<td></td>
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<tr>
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<td>33</td>
<td>0</td>
<td>2 b</td>
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<td>100</td>
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<tr>
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<td>16 c</td>
<td>0</td>
<td>149</td>
<td></td>
<td>100</td>
<td>90.3</td>
</tr>
<tr>
<td>ChromID STRB</td>
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<td>2 d</td>
<td>0</td>
<td>163</td>
<td></td>
<td>100</td>
<td>98.8</td>
</tr>
</tbody>
</table>


a Target coloured colonies were orange on Granada medium, pink on Brilliance GBS medium and pink to red on ChromID STRB.

b Growth of white colonies.

c Six strains of Streptococcus pneumoniae, six strains of Streptococcus mitis/oralis, four strains of Streptococcus salivarius and one strain of Streptococcus parasanguinis. One sample yielded pink colonies for both S. pneumoniae and S. salivarius.

d One strain of S. pneumoniae and one strain of Enterococcus faecium.
**Figure**. Comparison of three chromogenic media for GBS screening. **A.** Pictures of Granada (left), Brilliance GBS (middle) and ChromID STRB (right) agar plates inoculated with two samples yielding an average of $6.42 \times 10^7$ CFU/ml (top) and $7.70 \times 10^2$ CFU/ml (bottom), respectively. In the enlarged parts of the picture, red arrows indicate target coloured colonies of *S. agalactiae* and black arrows non-GBS colonies. Agar plates were inoculated using EasySpiral Dilute instrument and photographed with Scan 1200 reader (see main text for details). **B.** Correlation of GBS loads in 29 samples recovered on Granada and Brilliance GBS media (left graph) (Pearson coefficient of 0.97, $P < 0.0001$) and on ChromID STRB and Brilliance GBS media (right graph) (Pearson coefficient of 0.98, $P < 0.0001$).