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Evaluation of the New Brilliance GBS Chromogenic Medium for Screening of *Streptococcus agalactiae* Vaginal Colonization in Pregnant Women

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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 medium (bioMérieux), 100% and 90.3% for Brilliance GBS medium (Thermo Fisher Scientific), and 100% and 98.8% for ChromID STRB medium (bioMérieux), respectively.

Streptococcus agalactiae, usually termed 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 1,000 live births in developing countries (1). Guidelines recommend screening vaginal or rectovaginal GBS colonization in pregnant women at 35 to 37 weeks of gestation (3, 4). The prevalence of rectovaginal colonization varies from 6.5 to 36% in European countries (5). For 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 has been 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 preenrichment step, recommended in some countries (3, 4), increases the sensitivity of the GBS screening (8, 11), mostly in cases of low bacterial loads (11, 12). Enriched and direct cultures were shown to have similar performances (9), although failure to perform direct culture can decrease sensitivity due to overgrowth of competing organisms in enrichment broth (13). Finally, nucleic acid amplification tests (NAATs), notably those including the extraction step, reduce the delay in results and could be used at the admission of pregnant women in active labor in order to administer adequate antibiotic prophylaxis (14, 15). NAATs offer similar performances compared to direct selective culture (16), but controversial results were reported after comparison with enriched culture (7, 9, 17, 18). Recent guidelines advised the use of NAATs in selected circumstances, such as for term gestation women with unknown colonization status and no risk factors (3, 4).

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

From February to April 2013, 760 vaginal swabs (ESwab 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 by using the swab and spread with a sterile loop. Plates were incubated under anaerobic conditions at 36°C and read after 24 and 48 h of incubation following the manufacturer's recommendations. Orange colonies were considered *S. agalactiae* without confirmation of identification. The prevalence of *S. agalactiae* vaginal colonization was 16.7% (127/760).

From this panel, 200 nonconsecutive 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 by 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 media and under anaerobic conditions for Granada medium, as recommended by each manufacturer. All the plates were read after 22 to 24 h of incubation. Granada medium plates were read after 48 h in the routine workflow. Based on colony color, presumptive GBS colonies isolated on each chromogenic medium were systematically identified at the species level by MALDI-TOF MS (Microflex LT; Bruker) following the manufacturer's recommendations. Non-target-colored 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 Table 1. The two GBS-positive samples missed by the Granada medium were found positive for nonhemolytic *S. agalactiae*. The 33 posi-

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TABLE 1 Performances of Granada, Brilliance GBS, and ChromID STRB chromogenic media with 200 nonconsecutive vaginal swabs (including 35 positive ones by at least two culture methods) taken from women 35 to 37 weeks pregnant[e]

Medium	No. of samples				Se (%)	Sp (%)	PPV (%)	NPV (%)
	Target color[a]		Nontarget color					
	GBS	Other sp.	GBS	Other sp.				
Granada	33	0	2[b]	165	94.3	100	100	98.8
Brilliance GBS	35	16[c]	0	149	100	90.3	68.6	100
ChromID STRB	35	2[d]	0	163	100	98.8	94.6	100

[a] Target-colored colonies were orange on Granada medium, pink on Brilliance GBS medium, and pink to red on ChromID STRB medium.
[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*.
[e] Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; GBS, group B *Streptococcus*; sp, species.

tive samples on Granada medium were detected within 22 to 24 h of incubation; an extended incubation of 48 h failed to detect any further GBS-positive samples.

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) (Fig. 1A). A few samples yielding faintly colored GBS colonies could not be reliably quantified from Brilliance GBS ($n = 3$), Granada ($n = 5$), and ChromID ($n = 4$) medium plates. After exclusion of these samples, the bacterial loads were available for the three media in 29 specimens, and these values ranged from 1.3 to 8.4 log CFU/ml with a strong correlation between media (Fig. 1B); the medians of the bacterial loads were 4.56, 4.82, and 4.57 log CFU/ml for Brilliance GBS, Granada, and ChromID

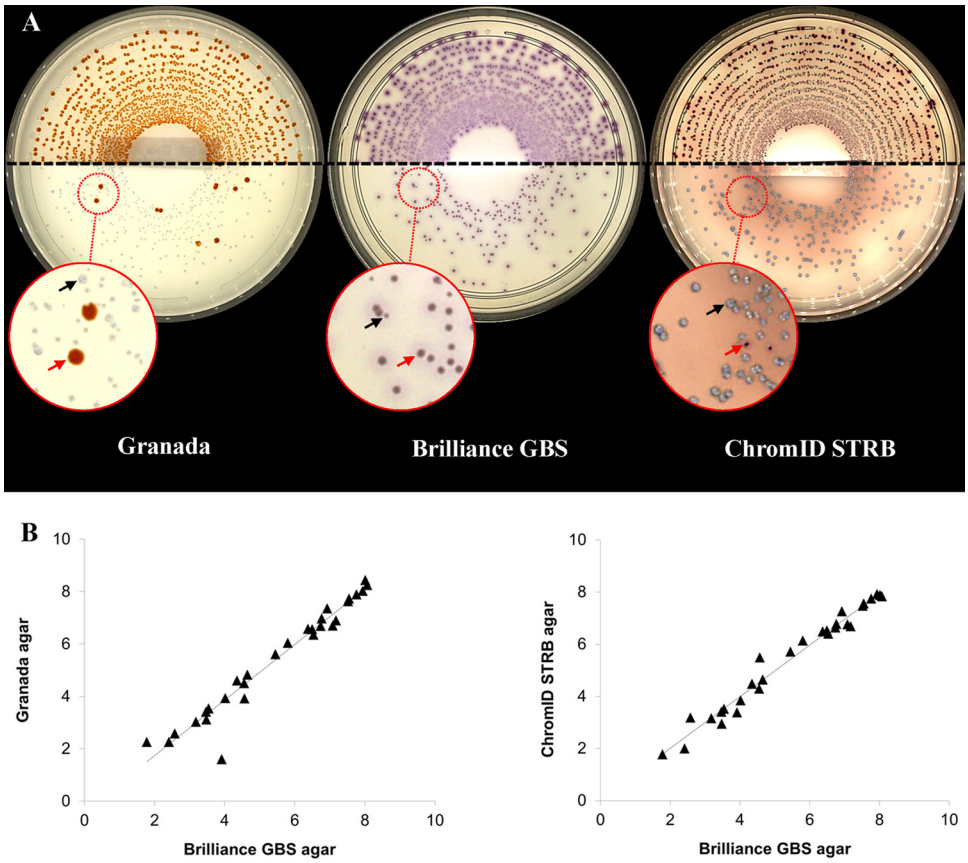

FIG 1 Comparison of three chromogenic media for GBS screening. (A) Pictures of Granada (left), Brilliance GBS (middle), and ChromID STRB (right) agar plates inoculated with two samples yielding averages of 6.42×10^7 CFU/ml (top) and 7.70×10^2 CFU/ml (bottom). In the enlarged parts of the picture, red arrows indicate target-colored colonies of *S. agalactiae* and black arrows non-GBS colonies. Agar plates were inoculated using the EasySpiral Dilute instrument and photographed with the Scan 1200 reader (see the text for details). (B) Correlations 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, $P < 0.0001$).

STRB media, respectively (no significant difference by the Kruskal-Wallis test). Although it seems intuitive that a high level of GBS colonization in the vagina could favor neonatal infection, to our knowledge, the clinical significance of this parameter has not been evaluated prospectively. This evaluation 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 on the basis of French recommendations. Although the three tested chromogenic media showed similar performances, the practicabilities differed greatly from one medium to another. The Granada medium failed to recognize 2 of the 35 GBS isolates that were shown to be nonhemolytic GBS (20). Plates were very easy to read because of the absence of atypical-colored colonies. The ChromID STRB medium showed excellent specificity and sensitivity but needed a high level of technical expertise due to the difficulty of recognizing the target colonies among all the colored colonies. The Brilliance GBS medium showed an excellent sensitivity and was easier to read than the ChromID STRB 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 colored 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 labor. In contrast, false-positive results could lead to unnecessary administration of antibiotics during labor in a few women, which does not have a major adverse impact on mother or baby but leads 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 to avoid false-positive results with other species of streptococci.

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