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Note

Evaluation of CHROMagar™ StrepB agar, an aerobic chromogenic medium for prepartum vaginal/rectal Group B *Streptococcus* screening

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ABSTRACT

An aerobic chromogenic medium, CHROMagar™ StrepB agar, designed for isolation of group B *Streptococci*, was evaluated on 285 prepartum vaginal/rectal swabs from pregnant women. After overnight enrichment in Todd-Hewitt broth containing 15 μg/ml nalidixic acid and 10 μg/ml colistin, sensitivities were respectively 79% on day 1 and 92% on day 2, and significantly higher than those achieved by blood agar (40% and 58%) and colimycin-nalidixic-acid agar (82% on day 2).

The Centre for Diseases Control and Prevention guidelines for prevention of perinatal group B streptococci (GBS) infections recommended that pregnant women be tested at 35 to 37 weeks of pregnancy for GBS carriage by a selective enrichment broth using vaginal/anorectal swabs, followed by subculture on blood agar plates. The guidelines also recommended the development of media with a reliable colour indicator to signal the presence of GBS and the surveillance of GBS antibiotic-susceptibility (Schrag et al., 2002; CDC, 2010). Aerobic chromogenic media agar plates have been developed yielding GBS-colonies with a unique predictive colour. They were evaluated favourably for genital specimens (Tazi et al., 2008; Poisson et al., 2010). Though this screening is currently routinely carried out (Phares et al., 2008), data remain scant about their efficacy when rectal swabs are involved (El Aila et al., 2010). Actually, bacteria are far more numerous and florid in the rectum. Gram negative bacilli and anaerobes can be easily inhibited, but *Enterococci* remain challenging (Dunne and Holland-Staley, 1998; Tazi et al., 2008). Therefore the sensitivity and predictivity of a medium designed for GBS detection in rectal specimens cannot be extrapolated from results including a majority of vaginal specimens. We have recently reported results supporting the use of CHROMagar™ StrepB for GBS detection from diverse perinatal specimens, but no rectal specimens had been included (Poisson et al., 2010).

The purpose of this study was to evaluate CHROMagar™ StrepB following rigorously the CDC's Guidelines, meaning exclusively vaginal/rectal swabs with enrichment step and subcultures, with comparison to blood agar plates.

Between April and June 2010, all pregnant women who presented for prepartum GBS detection were informed that the Hospital of Orléans was complying with the CDC's Guidelines. They received exhaustive information about the difference with the French Guidelines. After informed consent they were included in the study. Vaginal/rectal specimens were collected as prescribed in the Guidelines (Schrag et al., 2002; CDC, 2010) and transported to the laboratory in a Port-A-Cul tube (Becton, Dickinson, and Company, Sparks, USA). Swabs were swirled and vortexed for 1 min in a 5 ml tube of Todd-Hewitt broth containing yeast extract, 15 μg/ml nalidixic acid and 10 μg/ml colistin (LIM broth) (Becton, Dickinson, Sparks, USA). LIM broths were placed at 37 °C. After overnight incubation they were vortexed and subcultured (10 μl) on a CHROMagar™ StrepB agar plate (CHROMagar, Paris, France), a Columbia Blood Agar plate (COH) (BioMérieux, France) and a Colimycin Nalidixic Agar plate (CNA) (BioMérieux, France). The subsequently used methods and computations have been extensively described recently (Poisson et al., 2010). *Enterococci* were noted for CHROMagar™ StrepB at the threshold of one latex-confirmed colony per plate.

Results are presented in Table 1. The study allowed isolation of 84 GBS, from 285 women, achieving a prevalence of 29.5%, which is not statistically different from the most recently issued results: 81 GBS in 250 samples ($p = 0.55$) (Craven et al., 2010).

CHROMagar™ StrepB yielded 78 GBS, 67 of which (85%) were noticed on day 1 cultures. Six False Negative CHROMagar™ StrepB plates were observed, which all originated from paucimicrobial samples (<5 colonies on the parallel positive plates).

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Table 1
Recovery of *Streptococcus agalactiae* (GBS) from 285 vaginal/rectal specimens on CHROMagar™ StrepB (CHROM-B), Columbia Horse Blood Agar (COH) and Colimycin Nalidixic Agar (CNA). Total GBS: 84. Prevalence: 29.5%.

Medium	Day	True Positives	False Negatives	False Positives	Sensitivity[a] (%)	Predictivity (%)
CHROM-B	d1	67	17	3	79	96
	d2	78	6	4	92	95
CNA[b]	d2	69	15	6	82	92
COH	d1	34	50	3	40	92
	d2	49	35	5	58	91

[a] CHROM-B was significantly more sensitive than COH on day 1 and day 2, and than CNA on day 2.
[b] In our routine the day 1-predictivity of the anaerobically-incubated CNA plates is really low and their reading is not performed before day 2.

When compared with COH, CHROMagar™ StrepB was significantly more sensitive: increasing the sensitivity 1.97 fold on day 1 ($p=0.001$) and 1.59 on day 2 ($p=0.005$). False Negative COH plates ($n=35$) were strongly associated with Gram-negative bacilli and yeast overgrowths.

Enterococci produced their expectedly blue colonies and were noted on 61% of the CHROMagar™ StrepB plates on day 1 and 63% on day 2. No False Negative CHROMagar™ StrepB plates were associated with their presence.

CHROMagar™ StrepB was significantly more sensitive than CNA ($p<0.05$). False Negative CNA plates ($n=15$) originated in abundant cultures of anaerobes.

As in our previous study, COH underperformed due to heavy growth of rectal flora.

The vaginal dominant flora is composed of slow-growing bacteria, while the rectal flora consists of a much heavier load of fast-growing bacteria. Inside both sites, GBS are most often present in low counts and will grow little colonies (Donati et al., 2010). LIM broth contains colistin and nalidixic acid, two broad spectrum antibiotics aiming at Gram negative digestive bacteria. CHROMagar™ StrepB agar is selective against Gram-negative bacilli, *Staphylococci* and yeasts. It allows growth of all GBS as mauve opaque colonies whatever their haemolytic properties (Poisson et al., 2010).

Now, only *Enterococci* remain as potentially obstructing bacteria. Their growth properties are very similar to the GBS ones. They can hinder the growth of GBS by competition in selective broths (Dunne and Holland-Staley, 1998). Their prevalence is rarely reported, but several authors present photographs describing the differentiation between colonies of *Enterococci* and those of GBS, therefore supporting the reality of their presence (Tazi et al., 2008; El Aila et al., 2010). *Enterococci* were expectedly more prevalent in the present study than in the previous one which involved vaginal specimens: 63% vs 12%, but did not lead to a decrease of CHROMagar™ StrepB sensitivities (Poisson et al., 2010).

Chromogenic Islam base-derived media have been evaluated under several forms, firstly a broth and an agar inoculated under a coverslide, both of which provided no immediately disposable colonies for antibiotic-susceptibility testing (Votava et al., 2001), and the Granada formula (Rosa-Fraile et al., 2005), which is strictly dependent upon the minimum duration of its incubation time and upon GBS haemolytic properties (Poisson et al., 2010).

Blood-based selective formulas yielded interesting results. A direct comparison of Neomycin Nalidixic Agar (NNA) to a chromogenic medium without an enrichment step revealed a higher sensitivity for the chromogenic medium (Craven et al., 2010).

Enrichment and subcultures require a minimum of two days and might be unsuitable for women presenting in labour with unknown GBS status. These women might beneficiate from molecular techniques. Unfortunately, most of them require an enrichment step to achieve a similar sensitivity (Scicchitano and Bourbeau, 2009; Craven et al., 2010). Otherwise, the enrichment step may become useless since two other recently evaluated chromogenic media have achieved almost identical sensitivities with or without it (El Aila et al., 2010).

CHROMagar™ StrepB is suitable for the 35–37 weeks vaginal/rectal screening. It is sensitive, predictive and provides pure colonies allowing antibiotics susceptibility testing, another important recommendation of the Guidelines (Schrag et al., 2002; CDC, 2010).

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CHROMagar™ StrepB pre-poured plates were provided free of charges by CHROMagar, Paris, France.

References

Centers for Disease Control and Prevention, 2010. Prevention of Perinatal Group B Streptococcal Disease. Revised Guidelines from CDC, 2010. MMWR 59, 1–32 RR-10.
Craven, R.R., Weber, C.J., Jennemann, R.A., Dunne, W.M., 2010. Evaluation of a chromogenic Agar for detection of group B Streptococcus in pregnant women. J. Clin. Microbiol. 48, 3370–3371.
Donati, L., Di Vico, A., Nucci, M., Quagliozzi, L., Spagnuolo, T., Labianca, A., et al., 2010. Vaginal microbial flora and outcome of pregnancy. Arch. Gynecol. Obstet. 281, 589–600.
Dunne, W.M., Holland-Staley, C.A., 1998. Comparison of NNA agar culture and selective broth culture for detection of group B streptococcal colonization in women. J. Clin. Microbiol. 36, 2298–2300.
El Aila, N.A., Tency, I., Claeys, G., Saerens, B., Cools, P., Verstraelen, H., et al., 2010. Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women. BMC Infect. Dis. 10, 285–292.
Phares, C.R., Lynfield, R., Farley, M.M., Mohle-Boetani, J., Harrison, L.H., Petit, S., et al., 2008. Epidemiology of invasive group B streptococcal disease in the United States, 1999–2005. JAMA 299, 2056–2065.
Poisson, D.M., Chandemerle, M., Guinard, J., Evrard, M.L., Naydenova, D., Mesnard, L., 2010. Evaluation of CHROMagar™ StrepB: a new chromogenic agar medium for aerobic detection of Group B Streptococci in perinatal samples. J. Microbiol. Meth. 82, 238–242.
Rosa-Fraile, M., Rodriguez-Granger, J., Camacho-Munoz, E., Sampedro, A., 2005. Use of unmodified starches and partial removal of serum to improve Granada medium stability. J. Clin. Microbiol. 43, 1989–1991.
Schrag, S.J., Gorwitz, R., Fultz-Butts, K., Schuchat, A., 2002. Prevention of Perinatal Group B Streptococcal disease. Revised guidelines from CDC. MMWR 51, 1–22.
Scicchitano, L.M., Bourbeau, P., 2009. Comparative evaluation of the AccuProbe group B Streptococcus Culture Test, the BD GeneOhm Strep B Assay, and culture for detection of group B Streptococci in pregnant women. J. Clin. Microbiol. 47, 3013–3023.
Tazi, A., Reglier-Poupet, H., Dautezac, F., Raymond, J., Poyart, C., 2008. Comparative evaluation of strepto B ID chromogenic medium and Granada media for the detection of group B streptococcus from vaginal samples of pregnant women. J. Microbiol. Meth. 73, 263–265.
Votava, M., Tejkalova, M., Drabkova, M., Unzeitig, V., Braveny, I., 2001. Use of GBS media for rapid detection of group B streptococci in vaginal and rectal swabs from women in labor. Eur. J. Clin. Microbiol. Infect. Dis. 20, 120–122.