



Note

Prepartum vaginal/anorectal Group B *Streptococcus* screening: Improvement of the enrichment step by the broth additive RambaQUICK StrepB

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ABSTRACT

We used RambaQUICK StrepB in enrichment broth with samples taken from 31 pregnant women and compared its performance to that of standard enrichment media. RambaQUICK StrepB shortened the required incubation time and raised the sensitivity of streptococcal screening by 1.6 fold.

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The Centres for Disease Control and Prevention guidelines for the prevention of perinatal Group B *Streptococcal* (GBS) infections recommend that pregnant women be tested at 35 to 37 weeks of pregnancy for GBS carriage by selective enrichment broth of vaginal/anorectal swab samples, followed by subculture on blood agar plates (Schrag et al., 2002) (CDC – Centers for Diseases Control and Prevention, 2010).

Van Dyke et al. examined records from 2003 to 2004 for 254 births in which the infant had developed a GBS infection. Missed screening accounted for 13.4% of them. A total of 61.4% of the infants with GBS infections (116/189) were born to women who had tested negative for GBS prior to delivery (Van Dyke et al., 2009).

A pregnant women's GBS status can change in the period between screening and delivery. El Helali et al. evaluated this conversion in a cohort of 968 women: 115 tested positive in the prepartum screening and 818 tested negative among whom 65 turned positive in the intrapartum screening (El Helali et al., 2009). This may suggest that either GBS carriage is intermittent or the sensitivity of prepartum screening method requires improvement.

The use of blood agar may account for false negative results since it is not a selective medium and the anorectal flora usually consists of a heavy load of fast-growing bacteria, among which GBS are most often present at relatively low levels (Donati et al., 2009). The use of selective enrichment broths can help ameliorate this issue.

Specifically, Todd–Hewitt broth, modified with addition of 15 µg/ml nalidixic acid and 10 µg/ml colistin, to suppress the growth of Gram-negative intestinal flora has been used, and is referred to as LIM broth.

The CDC guidelines also recommended the development of media with a reliable colour indicator to signal the presence of GBS (Schrag et al., 2002) (CDC, 2010). Several media possessing both chromogenic and selective properties have been formulated and are commercially available. The selective properties of these media can suppress the growth of Gram negative bacilli, staphylococci, yeasts and anaerobic bacteria, leading some researchers to suggest that their use obviates the need for selective enrichment broths (El Aila et al., 2010). However, a likely source of false negative results still remains since the selective formulas are not dedicated to the inhibition of *Enterococci*, which are actually part of the anorectal flora. Chromogenic agars help differentiate *Enterococci* from GBS by producing colonies of a distinct colour, but they remain a risk as high levels can lead to growth that may obscure GBS colonies. The presence of *Enterococci* on chromogenic media plates was reported by authors, on up to 60% of plates used (Tazi et al., 2008) (El Aila et al., 2010) (Poisson et al., 2011). Moreover, *Enterococci* are able to hinder the growth of GBS in broths by competition (Dunne and Holland-Staley, 1998). *Enterococci* growth properties are very similar to the GBS ones and the few differentiating susceptibilities are not favourable to GBS growth.

RambaQUICK StrepB is a selective enrichment additive designed to inhibit *Enterococci*, while allowing growth of GBS. We hypothesized that RambaQUICK StrepB would facilitate recovery of GBS in

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samples containing high loads of *Enterococci*. The purpose of this study was to evaluate the RambaQUICK StrepB as an additive for an enrichment broth, first *in vitro* and second in clinical specimens taken from pregnant women for their GBS prepertum screening.

RambaQUICK StrepB was supplied by the manufacturer and used according to the manufacturer's recommendations. In brief, the product is supplied in vials as a sterile powder to be solubilized in sterile water/alcohol (50/50) and added to the culture media at a level of 10 µl/ml. The solution expiry date is 1 year at +4 °C.

Every involved microbiological method has been extensively described recently. Briefly, plates were incubated at 37 °C in aerobic conditions. Cultures were examined on Day 1 and Day 2 for presumptive colonies which were defined as pink opaque colonies, 1–2 mm in diameter on Day 1, and over 2 mm on Day 2. Typical colonies were confirmed as GBS using a commercially available latex agglutination kit (Streptex, Oxoid) following the manufacturer's recommendations (Poisson et al., 2010).

In initial experiments, RambaQUICK StrepB had been included into CHROMagar™ StrepB agar plates (both supplied by CHROMagar, Paris, France) and used for GBS screening. During the incubation period, GBS colonies would appear first but the appearance of *Enterococci* colonies would only be delayed and after two days of incubation their inhibition was often not apparent (unpublished data).

We conducted a first *in vitro* experiment that was designed to test the hypothesis that the inhibitory effect of RambaQUICK StrepB would significantly decrease through an adaptation of *Enterococci* during the 2-day incubation period necessary to achieve the highest sensitivity in GBS isolation. Clinical isolates of GBS and *Enterococci* were selected at random from the routine activity in the laboratory. Growth curves of these isolates were generated using 5 ml tubes of LIM broth (Becton, Dickinson, Sparks, USA). Each isolate was allowed to grow in parallel in two tubes, one with and one without RambaQUICK StrepB. After inoculation tubes were vortexed and subcultured (10 µl) on CHROMagar™ StrepB agar plates (time zero), and incubated aerobically at 37 °C. Subcultures were obtained from broths by the same method after 2, 4, 6, 9, and 20 h. Fig. 1 displays the results of one of these experiments. The inhibitory effect observed against the *Enterococcus* isolate often disappeared after complete overnight incubation (20 h). However, a GBS-favourable window began at 4 h and was at its maximum between 6 h and 9 h. Incubation times between 9 h and 20 h were not evaluated because this time period is not easily compatible with the routine activity of most clinical laboratories.

We conducted a second *in vitro* experiment. The intended clinical use required that the differential inhibitory effect would remain effective when *Enterococci* and GBS isolates were incubated in the same culture. Therefore, the previous experiment was repeated using several mixed cultures of *Enterococci* and GBS isolates selected at random from the routine activity in the laboratory. Following this second set of experiments, it was determined that the inhibitory effect was optimal between 6 and 9 h.

This process was then tested in a clinical experiment. Between January and February 2011, all women who presented for prepertum GBS screening were invited to participate. They received exhaustive information about the difference between US and French Guidelines. After informed consent was received, 31 women were included in the study. Vaginal/anorectal specimens were collected as prescribed in the US Guidelines (Schrag et al., 2002) (CDC, 2010), and a second vaginal swab was also collected but kept separate. Both samples were transported to the laboratory in Port-A-Cul tubes (Becton, Dickinson, and Company, Sparks, USA).

The isolated vaginal swabs were tested separately, by direct plating on CHROMagar™ StrepB Agar plates, according to the recommendations available in France (ANAES, 2001).

Tubes with the vaginal/anorectal swabs were received in the laboratory where they were kept at room temperature and processed every morning before 9 am. For samples taken from each patient the two swabs were swirled and vortexed together for 1 min in 1 ml LIM broth. This mixture was immediately isolated on CHROMagar™ StrepB Agar plates (10 µl) and equally distributed between two 5 ml LIM broth tubes, one with and one without RambaQUICK StrepB. Both tubes were incubated at 37 °C and isolated in parallel on CHROMagar™ StrepB Agar plates (10 µl) at 12 am (3 h), 3 pm (6 h), 6 pm (9 h) and on the following morning at 9 am (24 h). Plates were noted as positive or negative according to the presence, or not, of at least one latex confirmed GBS colony.

According to guidelines available in France, only the results of the isolated vaginal swabs indicated candidates for antibiotic prophylaxis. The results of the vaginal/anorectal swabs analysis were given to the obstetricians only as supplemental data regarding the patient's GBS-status.

The results are presented in Fig. 2 and show that only two women tested positive using vaginal swabs. This rate was low but not significantly different from our usual rate concerning vaginal carriage: 11.5% (Poisson et al., 2010).

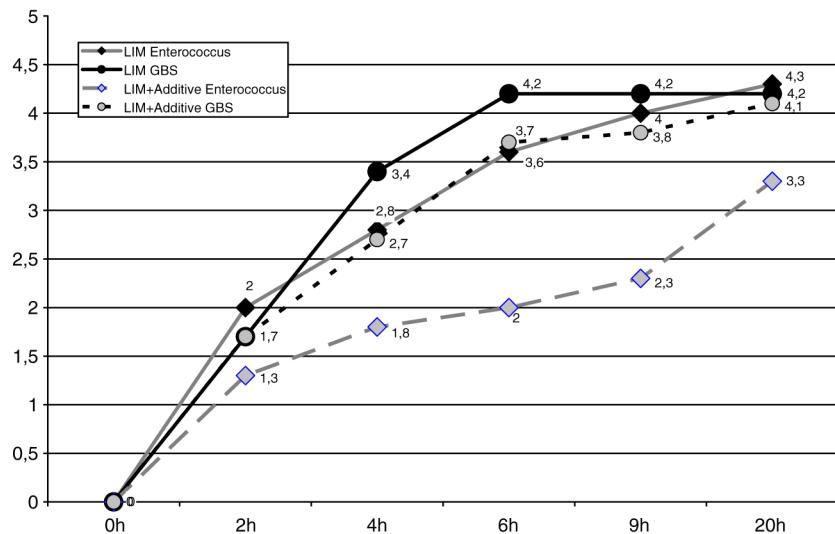


Fig. 1. Comparative growth curves of an *Enterococcus* and a Group B Streptococcus (GBS) in LIM broths with and without RambaQUICK StrepB. (Y axis:log₁₀ of the ratio:population/initial inoculum – X axis:hours of incubation). The additive hardly affects GBS growth, whereas it inhibits *Enterococci* from 4 h on. After several identical experiments (data not shown) the divergence of curves was considered maximal and steady between 6 h and 9 h.

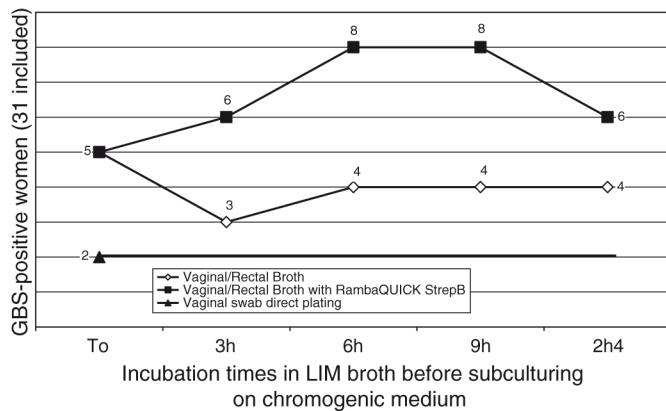


Fig. 2. Change in GBS positive subcultures after different incubation times of vaginal/anorectal swabs in LIM broths with and without RambaQUICK StrepB (Total cohort = 31 women). The favourable window was between 6 and 9 h. The results of parallel separate vaginal swabs, directly inoculated on CHROMagar™ StrepB Agar plates without any enrichment, in accord with French guidelines (ANAES, 2001), are presented for information.

With regards to the vaginal/anorectal swabs processed without RambaQUICK StrepB, interestingly, 5 women tested positive immediately for their time zero broths, but for 2 of them already the 3 h-plates were falsely negative due to overgrowth of *Enterococci*. One of these 2 women remained testing falsely negative for the following plates. The overnight enrichment without additive did not produce a better sensitivity than the time zero culture, a figure that was recently observed by others (El Aila et al., 2010). The overall positive rate was 5/31 for the initial samples and 4/31 after enrichment. Both results are somewhat lower than reported results achieving 30–35% in the USA (Craven et al., 2010) and 29.5% in our usual population (Poisson et al., 2011).

Concerning the modified LIM broths, the effect of the RambaQUICK StrepB additive was the clearest at 6 h and 9 h achieving a two fold increase compared to the unmodified broth and providing a GBS-positive rate of 8/31. At the 24 h time point, 2 tests among the 8 positives had turned negative due to *Enterococci* overgrowth. The adaptation of the *Enterococci* population to the additive was demonstrated by re-inoculating their 24 h-additive-containing-broth in new additive containing tubes: *Enterococci* could not be inhibited any more and GBS did not emerge again.

The RambaQUICK StrepB produced a potential 2 fold increase of the GBS recovery (8/4) when compared to the LIM broth enrichment and a 1.6 fold (8/5) increase when compared to the direct plating of initial samples. In order to achieve this shortened incubation samples must be kept unprocessed in the laboratory until the following morning but this does not lead to delayed results since broths are transferred to plates on the evening of the same day. For the routine laboratory, this schedule is compatible with inoculations of broths carried out on arrival of the laboratory staff in the morning and inoculation of plates before the staff leaves at the end of the working time.

The weakness of the negative predictive value of prepartum screening leads obstetricians to welcome a last intrapartum test for women who still test negative at the beginning of labour and have no other indication for prophylaxis. At the point of delivery, microbiological

techniques stumble against the need for a quick result. Molecular techniques are currently evaluated to address this last-ditch screening. Their results can be available in less than 75 min, but they are still expensive, require a skilled staff, do not allow antibiotic testing and suffer from technical limits:PCR-inhibitors or significant presence of mucus (CDC, 2010) (El Helali et al., 2009).

RambaQUICK StrepB is simple to use with LIM Broth and potentially able to raise the sensitivity of the prepartum screening method. The 6 to 9 hour incubation period for enrichment is compatible with the usual times of work in routine clinical laboratories. It should reduce the need for an intrapartum molecular screening method and allows for antibiotic susceptibility testing, another recommendation of the guidelines (CDC, 2010).

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