Eur J Clin Microbiol Infect Dis (2012) 31:357–363
DOI 10.1007/s10096-011-1317-8

ARTICLE

In vitro evaluation of the performance of Granada selective enrichment broth for the detection of group B streptococcal colonization

R. te Witt · P. M. Oostvogel · R. Yahiaoui · Y. Wu · A. van Belkum · A. E. Muller

Received: 23 May 2011 / Accepted: 4 June 2011 / Published online: 23 June 2011

Abstract A broth for the screening of group B streptococcal (GBS) carriage during pregnancy is about to be introduced. Simulating conditions in everyday practice, we have compared the sensitivity of this Granada tube broth (GT) with that of classical Amies transport medium (AT) in vitro. A total of 1,485 GT and 1,485 AT were tested with 33 well-characterized GBS strains in three different concentrations, five different incubation times, and three different temperatures. After initial incubation at room temperature (RT) or 4°C, GT were placed at 37°C. GT were scored for the presence of orange pigment. GT and AT were subcultured on blood agar (BA). Pigment was observed in 98% of GT incubated at 37°C. GBS could be cultured in 91%, 73%, and 55% of GT incubated at 37°C, RT, or 4°C, respectively. For AT, these percentages were only 20% at 37°C, 52% at RT, and 59% at 4°C. When GT initially incubated at RT or 4°C were subsequently incubated at 37°C, the sensitivity improved significantly. We conclude that GT is a more sensitive GBS transport and culture medium than the conventional method, especially for low inocula and prolonged transport/incubation times. GT does not exclude the presence of GBS, and should always be incubated at 37°C and subcultured on solid agar for optimal sensitivity.

R. te Witt (✉) · Y. Wu · A. van Belkum · A. E. Muller
Department of Medical Microbiology and Infectious Diseases,
Erasmus MC,
's Gravendijkwal 230,
3015 CE, Rotterdam, The Netherlands
e-mail: r.tewitt@erasmusmc.nl

P. M. Oostvogel
Department of Medical Microbiology, MC Haaglanden,
Den Haag, The Netherlands

R. Yahiaoui
Department of Medical Microbiology, Haga Hospital,
Den Haag, The Netherlands

A. van Belkum
Microbiology Research Unit, bioMérieux,
La Balme les Grottes, France

Introduction

Group B streptococci (GBS) has been known as a human pathogen since 1938. In the course of pregnancy and the postpartum period, GBS may cause a variety of serious infections in both the mother and neonate [1, 2]. Neonatal early-onset GBS disease (GBS-EOD) presents in the first week of life and is usually acquired during delivery by neonates born from mothers colonized with GBS in the rectovaginal tract. Up to 35% of pregnant women are colonized with GBS in the rectovaginal tract, most often without symptoms [3–5]. Approximately 1% of neonates born to colonized mothers develop GBS-EOD and up to 40% of the surviving neonates suffer serious sequelae, such as mental retardation or seizures [2]. Mortality is high among preterm infants, with average case–fatality rates of approximately 20% [2]. These rates vary from as high as 30% among those children born before 33 weeks of gestation to 2–3% among full-term infants [2].

Schrag et al. demonstrated that a prevention strategy based on routine screening for GBS carriage prevents more cases of GBS-EOD than an approach based on risk-factor assessment [6]. Therefore, screening for GBS carriage during pregnancy is the key in many guidelines to prevent GBS-EOD [2, 7]. Consequently, sensitive and specific GBS

cultivation is essential and laboratory procedures need to be streamlined. Little attention has been paid to the consequences of transport and transport delay of swabs to the laboratory. Revised Centers for Disease Control and Prevention (CDC) guidelines state specifically that GBS isolates remain viable in Amies transport medium for several days at room temperature. However, the recovery of isolates declines over a period of 1–4 days, particularly at elevated temperatures. Even when appropriate transport media are used, the sensitivity of culture is highest when the specimen is stored at 4°C before culture and processed within 24 h of collection [2, 8–11].

More rapid and, especially, more sensitive methods than the currently available and recommended transport and culture methods would improve the effectiveness of the screening-based approach. A new transport and enrichment broth, called Granada tube broth (GT) (bioMérieux, Marcy l'Etoile, France) is about to be introduced. In this broth, an orange pigment is produced in the presence of GBS [12, 13].

The aim of this study was to investigate whether the use of GT would improve the sensitivity of GBS cultures in comparison with the current gold standard under various culture conditions. We also investigated the reliability of GT after prolonged transport times.

Materials and methods

Bacterial isolates

A subset of 33 colonizing and invasive GBS isolates representing all seven of the important subtypes was obtained from a reference collection (Table 1) [14]. All isolates were previously identified as GBS using both biochemical and molecular methods [14]. Strains were stored at −80°C. Prior to testing, strains were subcultured twice on Columbia III agar with 5% sheep blood (bioMérieux) for 18–24 h at 37°C.

Table 1 Group B streptococci (GBS) isolates used (18)

Serotype	Invasive	Colonizing
Ia	2	3
Ib	0	5
II	0	4
III	3	2
IV	0	4
V	3	2
VI	0	5

Variables and culture

Phase I

In the first phase of the study, we studied the viability of the GBS strains using GT and Amies transport medium (AT) under different circumstances (five different incubation times [1, 2, 3, 4, and 7 days], three different temperatures [37°C, room temperature (RT) (±20°C), and 4°C], and three different inocula). Of each strain, a suspension of 0.5 McFarland (~1.5×10^8 colony-forming units [CFU]/ml) was prepared in sterile saline. This suspension was diluted until three concentrations were obtained: 1.5×10^6, 1.5×10^4, and 1.5×10^2 CFU/ml, respectively. All suspensions were subcultured on blood agar (BA) for purity checking and growth control. For every strain, 45 GT and 45 AT were inoculated with 100 μl of suspension. After incubation, GT were scored for the presence of orange pigment by two individuals and subcultured on BA for the detection of growth. The presence of orange pigment was checked after 1, 2, 3, 4, and 7 days. All changes in color were compared to a negative control tube, which was processed similarly to the inoculated tubes. After incubation, both GT (using 10 μl of broth) and AT were subcultured onto BA using the four-quadrant technique, to allow semi-quantification of the number of GBS colonies. Growth was recorded after 1 and 2 days of incubation at 37°C and was graded as negative (no growth), weakly positive (growth in the first quadrant), or positive (growth in the second, third, or fourth quadrants).

Phase II

Phase II of the study was performed in parallel to phase I. GT initially incubated at 4°C or RT in phase I were subsequently incubated at 37°C for 1 and 2 days and were checked for the presence of orange pigment as described previously. After this second incubation period, BA were inoculated and both GBS growth detection and quantification were performed as described above.

Results

Phase I

Overall, orange pigment was detected in 98% of all GT after incubation at 37°C for 1–7 days, with the lowest

detection rate at day 3 for 10×10^2 CFU/ml (90%). After incubation at RT, orange pigment was observed after 3 days of incubation and in 10–40% of the GT (Fig. 1). At 4°C, in none of the GT was orange pigment detected.

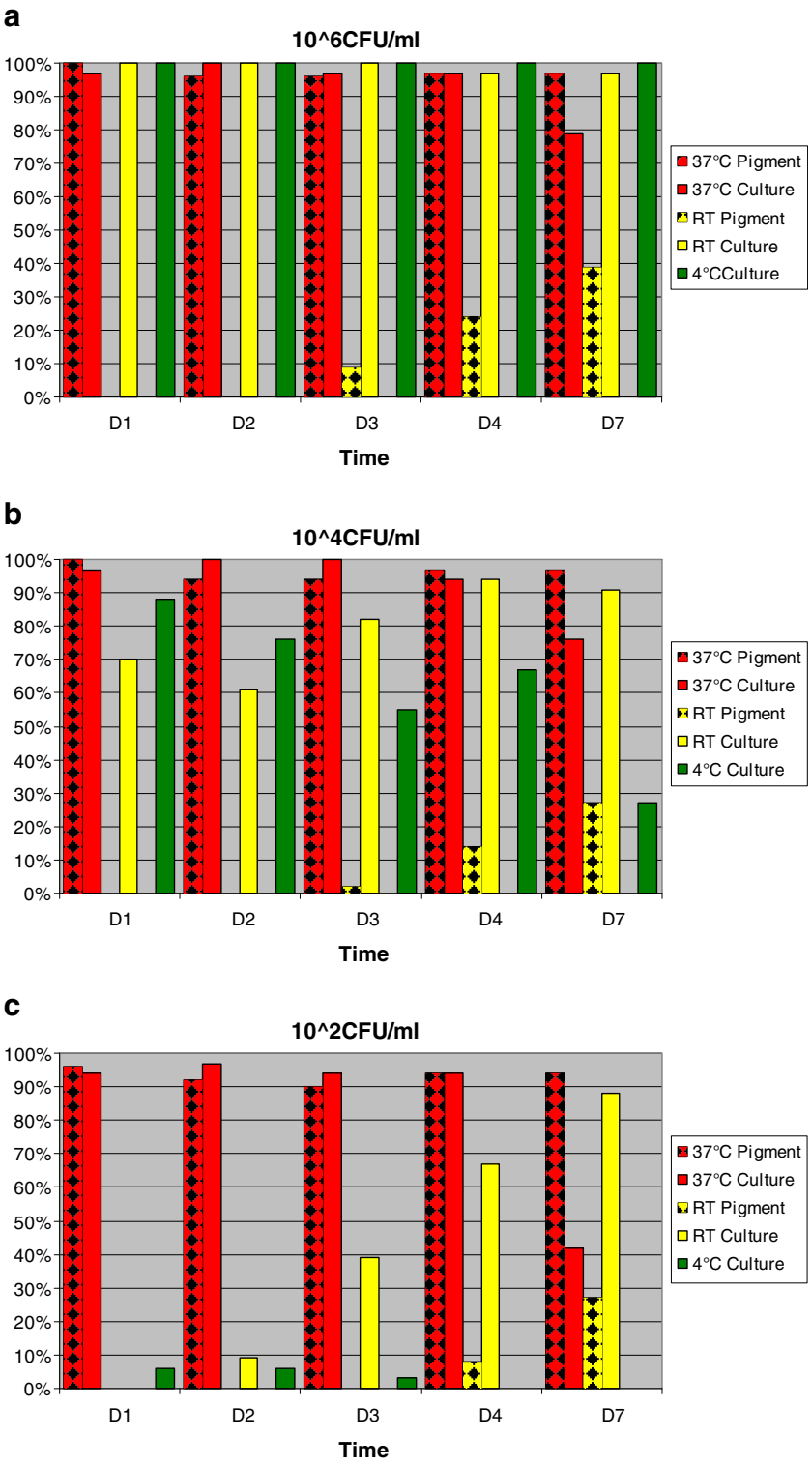

Fig. 1 Detection of orange pigment and group B streptococci (GBS)-positive cultures in Granada tube broth (GT) after incubation for 1, 2, 3, 4, or 7 days at 37°C, room temperature (RT), or 4°C using three different inocula (study phase I). In none of the GT at 4°C was orange pigment detected

Comparisons of GT with AT for growth detection for the different incubation times, different incubation temperatures, and different inocula are shown in Fig. 2 and Table 2. Data for semi-quantification via the four-quadrant technique showed no differences (data not shown).

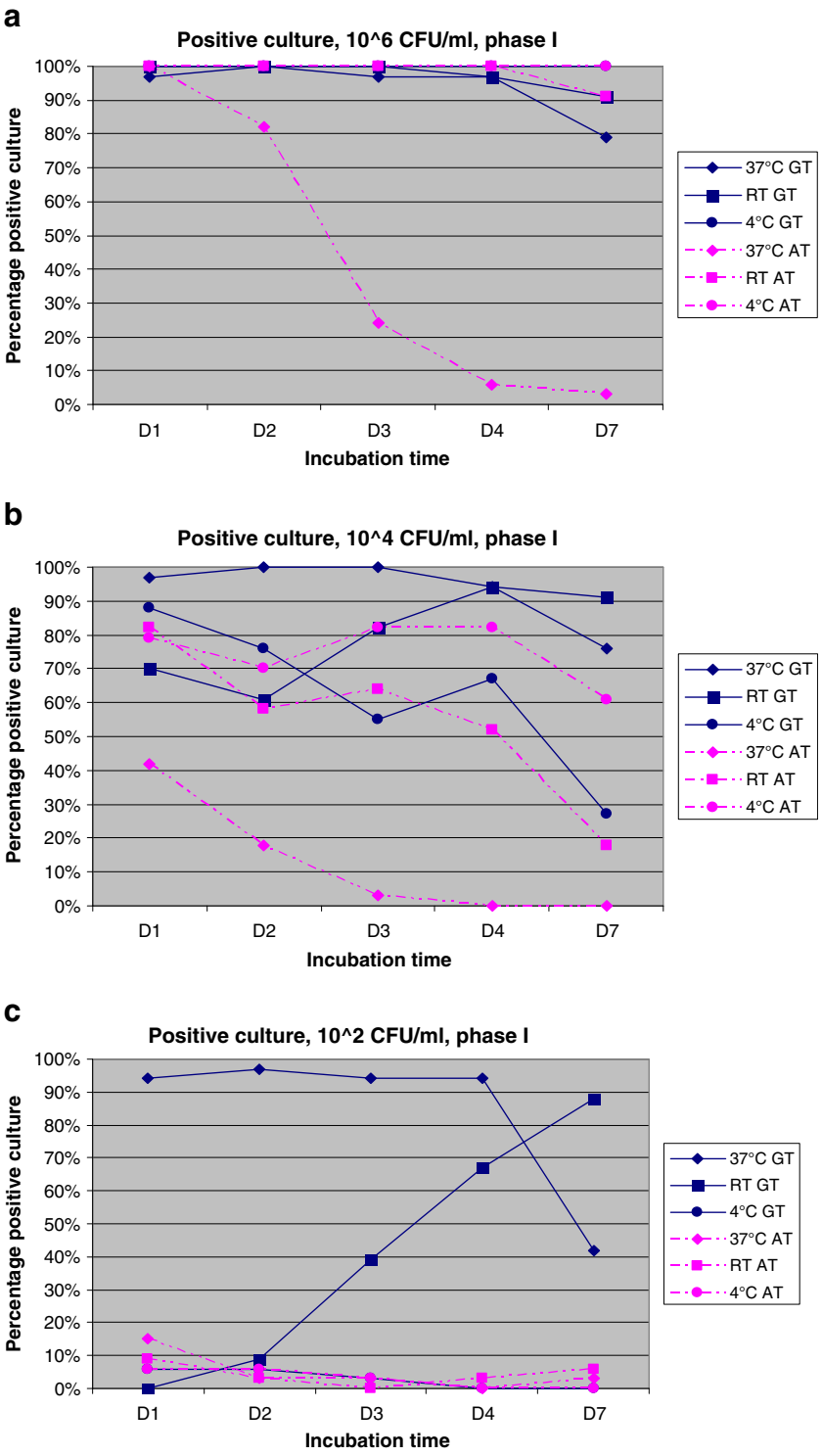

Fig. 2 Detection of GBS after subculture of GT and Amies transport medium (AT) on blood agar (BA) after initial incubation for 1, 2, 3, 4, or 7 days at 37°C, RT, or 4°C using three different inocula (study phase I)

Table 2 Overall detection of GBS after subculture of Granada tube broth (GT) and Amies transport medium (AT) on blood agar (BA), after incubation for 1, 2, 3, 4, or 7 days at 37°C, room temperature (RT), or 4°C using 10^6, 10^4, or 10^2 colony-forming units [CFU]/ml (study phase I). A *p*-value <0.05 is considered to be statistically significant

	GT culture-positive (%)	AT culture-positive (%)	*p*-value
10^6 CFU/ml			
37°C	94	43	<0.0001
RT	98	98	NS
4°C	100	100	NS
10^4 CFU/ml			
37°C	93	13	<0.0001
RT	80	55	NS
4°C	63	75	NS
10^2 CFU/ml			
37°C	84	5	<0.0001
RT	41	4	0.0002
4°C	3	3	NS

Phase II

Data on the observation of orange pigment after subsequent incubation at 37°C and the detection of GBS after subculture on BA are shown in Fig. 3.

No differences were observed in the presence of orange pigment and positive cultures between 1 and 2 days subsequent incubation (data not shown). Furthermore, no differences were observed between the different virulence types or between the different serotypes (data not shown).

Discussion

Granada agar has long been used in Europe to detect GBS in pregnant women. Most strains of beta-hemolytic GBS produce an orange carotenoid pigment on this agar, usually within 24 h of incubation. Variable results on the sensitivity of this culture medium have been reported, with some authors considering it to be unacceptably low [15–18]. However, our results cannot be compared with the results of these studies, as we tested Granada broth instead of agar.

On average, we observed the production of orange pigment in 98% of all GT that were incubated at 37°C for 1–7 days, with the lowest detection rate at day 3 for 10^2 CFU/ml (90%). At room temperature, orange pigment was observed only after 3 days of incubation and in only 10–40% of the GT. At 4°C, in none of the GT was orange pigment detected. As the overall sensitivity for GT for GBS culture was 95%, independent of the incubation temperature and the production of orange pigment, our results show that the absence of orange pigment does not exclude the presence of GBS. Furthermore, because the GBS pigment is linked to hemolysin activity, less or non-hemolytic strains may not be detected with GT [19]. Therefore, it may be reasonable to subculture all GT (both positive and negative) on BA. This allows the recognition of non-hemolytic GBS strains and other GBS that may not produce the orange pigment. This is important for identification, susceptibility testing, and potential subtyping of the cultured strain.

In our simulation of transport conditions, GBS could be cultured only in 20%, 52%, and 59% of AT incubated at 37°C, RT, or 4°C, respectively. These percentages correspond with those found by Rosa-Fraile et al., Stoner et al., and with revised CDC guidelines [2, 10, 11]. The sensitivity of GT was significantly higher than that of AT, especially for low inocula and extended transport/incubation time. This is important, since specimens may be exposed to high temperatures during transport, especially when swabs are obtained outside the hospital.

Direct incubation of GT at 37°C resulted in the highest yield of GBS. No difference in the growth of GBS was observed between transport at RT or at 4°C. Without incubation at 37°C after transport, the growth of GBS was seen in 73% and 55% of GT transported at RT or 4°C, respectively. When GT were subsequently incubated at 37°C, after transport at RT or 4°C, the sensitivity increased to almost 100%.

The study is limited in that it is performed in vitro and that the application of purified isolates of GBS in GT and onto AT may not reflect GBS survival on swabs containing vaginal and/or rectal flora. Nonetheless, we can conclude that GT is a highly sensitive transport and culture medium to detect GBS in pregnant women. The survival of GBS was significantly better in GT when compared to AT.

GT may not be suitable for the direct detection of GBS, as the absence of orange pigment does not conclude in the absence of GBS. Therefore, GT should always be subcultured for optimal use. Furthermore, GT should always be incubated at 37°C to improve its sensitivity.

GT may especially be suited for the transport of swab specimens from general practitioners and midwifery practices to the laboratory, which may take 2–3 days. Specifically, if a woman has low-density GBS colonization,

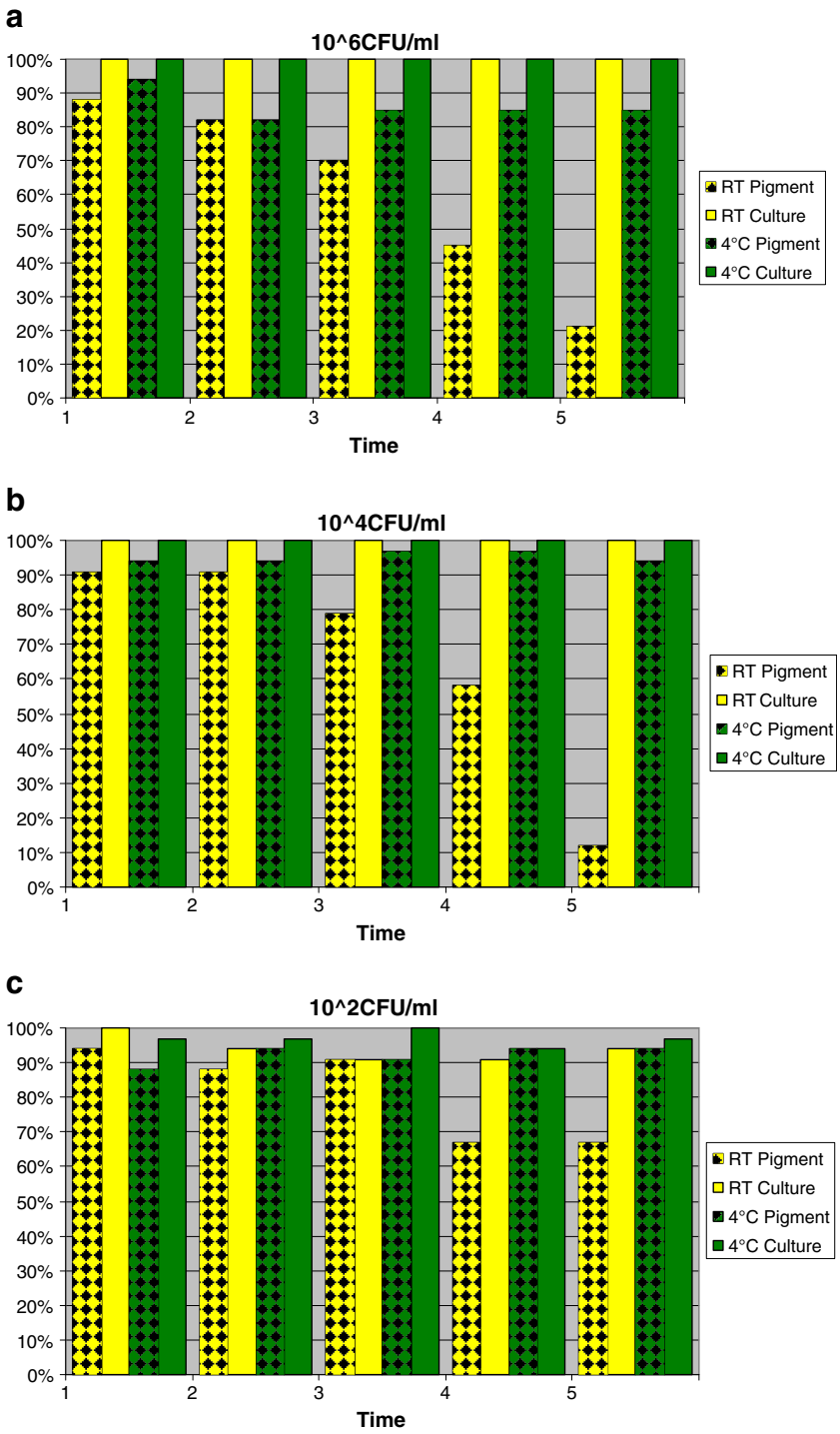

Fig. 3 Detection of orange pigment and GBS-positive cultures in GT after subsequent incubation at 37°C for 24 h after an initial incubation for 1, 2, 3, 4, or 7 days at RT or 4°C using three different inocula (study phase II)

extended transport times of swab specimens at RT or higher could reduce the culture sensitivity for AT but possibly not for GT.

Acknowledgments The Granada tubes, Amies transport media, and Columbia III agar with 5% sheep blood used in this study were provided by bioMérieux free of charge.

Conflicts of interest Alex van Belkum is an employee of bioMérieux. There are no conflicts of interest to disclose.

References

1. Muller AE, Oostvogel PM, Steegers EA, Dörr PJ (2006) Morbidity related to maternal group B streptococcal infections. Acta Obstet Gynecol Scand 85(9):1027–1037
2. Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC) (2010) Prevention of perinatal group B streptococcal disease—revised guidelines from CDC, 2010. MMWR Recomm Rep 59(RR-10):1–36
3. Bergseng H, Bevanger L, Rygg M, Bergh K (2007) Real-time PCR targeting the sip gene for detection of group B *Streptococcus* colonization in pregnant women at delivery. J Med Microbiol 56 (Pt 2):223–228
4. Campbell JR, Hillier SL, Krohn MA, Ferrieri P, Zaleznik DF, Baker CJ (2000) Group B streptococcal colonization and serotype-specific immunity in pregnant women at delivery. Obstet Gynecol 96(4):498–503
5. Valkenburg-van den Berg AW, Sprij AJ, Oostvogel PM, Mutsaers JA, Renes WB, Rosendaal FR, Joep Dörr P (2006) Prevalence of colonisation with group B Streptococci in pregnant women of a multi-ethnic population in The Netherlands. Eur J Obstet Gynecol Reprod Biol 124(2):178–183
6. Schrag SJ, Zell ER, Lynfield R, Roome A, Arnold KE, Craig AS, Harrison LH, Reingold A, Stefonek K, Smith G, Gamble M, Schuchat A; Active Bacterial Core Surveillance Team (2002) A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. N Engl J Med 347(4):233–239
7. Money DM, Dobson S; Canadian Paediatric Society, Infectious Diseases Committee (2004) The prevention of early-onset neonatal group B streptococcal disease. J Obstet Gynaecol Can 26(9):826–840
8. Håkansson S, Axemo P, Bremme K, Bryngelsson AL, Wallin MC, Ekström CM, Granlund M, Jacobsson B, Källén K, Spetz E, Tessin I; Swedish Working Group For The Prevention of Perinatal Group B Streptococcal Infections (2008) Group B streptococcal carriage in Sweden: a national study on risk factors for mother and infant colonisation. Acta Obstet Gynecol Scand 87(1):50–58
9. Ostroff RM, Steaffens JW (1995) Effect of specimen storage, antibiotics, and feminine hygiene products on the detection of group B *Streptococcus* by culture and the STREP B OIA test. Diagn Microbiol Infect Dis 22(3):253–259
10. Rosa-Fraile M, Camacho-Muñoz E, Rodríguez-Granger J, Liébana-Martos C (2005) Specimen storage in transport medium and detection of group B streptococci by culture. J Clin Microbiol 43(2):928–930
11. Stoner KA, Rabe LK, Hillier SL (2004) Effect of transport time, temperature, and concentration on the survival of group B streptococci in amies transport medium. J Clin Microbiol 42 (11):5385–5387
12. Martinho F, Prieto E, Pinto D, Castro RM, Morais AM, Salgado L, Exposto Fda L (2008) Evaluation of liquid biphasic Granada medium and instant liquid biphasic Granada medium for group B streptococcus detection. Enferm Infecc Microbiol Clin 26(2):69–71
13. Heelan JS, Struminsky J, Lauro P, Sung CJ (2005) Evaluation of a new selective enrichment broth for detection of group B streptococci in pregnant women. J Clin Microbiol 43(2):896–897
14. van Elzakker E, Yahiaoui R, Visser C, Oostvogel P, Muller A, Ho YR, Wu JJ, van Belkum A (2009) Epidemiology of and prenatal molecular distinction between invasive and colonizing group B streptococci in The Netherlands and Taiwan. Eur J Clin Microbiol Infect Dis 28(8):921–928
15. Gil EG, Rodríguez MC, Bartolomé R, Berjano B, Cabero L, Andreu A (1999) Evaluation of the Granada agar plate for detection of vaginal and rectal group B streptococci in pregnant women. J Clin Microbiol 37(8):2648–2651
16. Overman SB, Eley DD, Jacobs BE, Ribes JA (2002) Evaluation of methods to increase the sensitivity and timeliness of detection of *Streptococcus agalactiae* in pregnant women. J Clin Microbiol 40 (11):4329–4331
17. Perry JD, Oliver M, Nicholson A, Wright J, Gould FK (2006) Evaluation of a new chromogenic agar medium for isolation and identification of Group B streptococci. Lett Appl Microbiol 43 (6):615–618
18. Rosa-Fraile M, Rodriguez-Granger J, Cueto-Lopez M, Sampedro A, Gaye EB, Haro JM, Andreu A (1999) Use of Granada medium to detect group B streptococcal colonization in pregnant women. J Clin Microbiol 37(8):2674–2677
19. Tazi A, Réglier-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 Methods 73(3):263–265