

Evaluation of liquid biphasic Granada medium and instant liquid biphasic Granada medium for group B streptococcus detection

Filipa Martinho^a, Emília Prieto^a, Dulce Pinto^a, Rita Maria Castro^a, Ana Maria Morais^b, Luísa Salgado^c and Filomena da Luz Exposto^a

^aSexually Transmitted Diseases Unit, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa. ^bCentro de Saúde de Oeiras. ^cHospital Fernando da Fonseca, Amadora, Lisbon, Portugal.

INTRODUCTION. Group B streptococci (GBS) are transmitted from the mother to the newborn. Prevention of neonatal infection is achieved by intrapartum prophylaxis given to mothers colonized with GBS at 35 to 37 weeks of pregnancy.

MATERIALS AND METHODS. Liquid biphasic Granada medium (LB) and instant liquid biphasic Granada medium (ILB) were evaluated for GBS detection. Vaginal swabs obtained from 300 women were inoculated onto LB or ILB, or onto Todd-Hewitt broth and analyzed with the ATB system (comparison method).

RESULTS. Prevalence of GBS was 20% (61/300). LB and Todd-Hewitt with ATB detected GBS in 20% of women, and ILB in 19% of women. No growth was observed at four hours in any of the media studied. At 10 h and 14 h, identification of GBS was possible in 43/300 (14%) and 53/300 (18%) of ILB cultures, respectively, and in 32/300 (11%) and 46/300 (15%) of LB cultures.

CONCLUSION. All the media used are suitable for GBS detection. The majority of GBS were identified in ILB and LB cultures at 10 h and 14 h.

Key words: Group B streptococci. Granada medium. Instant Granada medium. Liquid biphasic medium. Group B streptococcus identification.

Evaluación del medio Granada líquido bifásico y del medio Granada líquido bifásico instantáneo para la detección de estreptococo del grupo B

INTRODUCCIÓN. Los estreptococos del grupo B (SGB) se transmiten de la madre al recién nacido. La prevención de la infección neonatal se logra mediante la profilaxis intraparto de las madres colonizadas por SGB en las semanas 35 a 37 de gestación.

MATERIALES Y MÉTODOS. Se ha evaluado el uso del medio Granada líquido bifásico (LB) y del medio Granada líquido

bifásico instantáneo (LBI) para la detección de SGB.

Se tomaron muestras vaginales con torunda de 300 mujeres, se inocularon las torundas en LB, LBI o en caldo Todd-Hewitt, y se analizaron los resultados con el sistema ATB (método de comparación).

RESULTADOS. La prevalencia de SGB fue del 20% (61/300). Los medios LB y Todd-Hewitt con ATB detectaron SGB en el 20% de mujeres, y el medio LBI en el 19%. No se observó crecimiento a las 4 h en ninguno de los medios estudiados. A las 10 y 14 h se pudo identificar SGB en 43/300 (14%) y en 53/300 (18%) de los cultivos en LBI, respectivamente, y en 32/300 (11%) y en 46/300 (15%) de los cultivos en LB.

CONCLUSIÓN. Todos los medios usados son adecuados para la detección de SGB. La mayoría de SGB se identificaron en LBI y en LB a las 10 y a las 14 h.

Palabras clave: Estreptococo del grupo B. Medio Granada. Medio Granada instantáneo. Medio líquido bifásico. Identificación de estreptococo del grupo B.

Introduction

Group B streptococcus (GBS) remains a leading cause of neonatal mortality and morbidity in many countries, despite major efforts to detect colonized pregnant women^{1,2}. GBS disease is caused by transmission of the microorganism from the mother to the newborn after membrane rupture^{1,3}. The most effective strategy to prevent early-onset GBS disease is detection of GBS in the vagina and rectum of pregnant women at 35 to 37 weeks of gestation, and administration of intrapartum antibiotic prophylaxis in those who are colonized^{3,4}. In Portugal, the prevalence of GBS in pregnant woman ranges from 11.7% to 31.2% (unpublished data of the authors).

In 1996, the Centers for Disease Control and Prevention (CDC) published guidelines designed to decrease the risk of neonatal GBS disease, in which the above-mentioned strategy was advocated. It was also recommended that swabs should be inoculated onto a selective broth medium supplemented with nalidixic acid and gentamicin or colistin^{4,5}. Specimens should then be incubated for 18h, subcultured onto blood agar plates and suspected colonies of GBS identified through antigen detection, genetic probes or by CAMP test.

Correspondence: Dr. F. Nogueira.
Rua da Junqueira, 96. 1349-008 Lisbon, Portugal.
E-mail: filipan@ihmt.unl.pt

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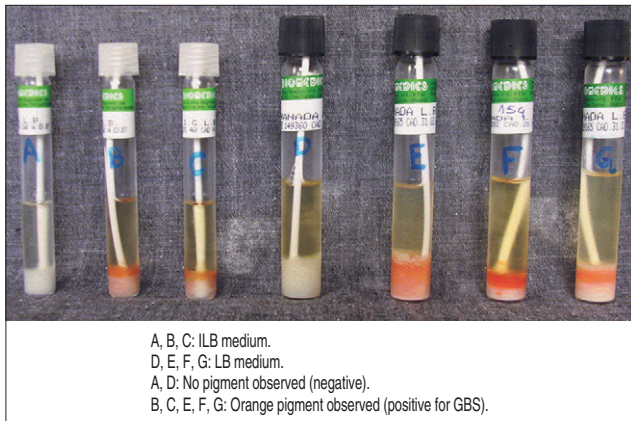


Figure 1. A presence of orange pigment in Granada media.

Granada agar is a selective and differential medium for rapid detection of beta-hemolytic GBS and has been recommended by several authors for GBS detection⁶⁻⁸. Other Granada media have been recently commercialized, such as liquid biphasic Granada (LB) and instant liquid biphasic Granada (ILB), which seem to have some advantages, such as shorter time requirements (4 h, 10 h) for identifying GBS (both LB and ILB), easy reading, and a longer shelf life (ILB)⁹.

The objectives of the present study were to evaluate liquid biphasic Granada medium and instant liquid biphasic Granada medium as compared to the reference method (Todd-Hewitt broth and subculture into blood agar) for the detection of GBS.

Materials and methods

From January to April 2006, triplicate swabs were used to collect vaginal specimens from 300 women consulting at Fernando da Fonseca Hospital, Amadora, and Oeiras Primary Health Center, Oeiras (Portugal). Swabs were transported to our laboratory at the Instituto de Higiene Medicina Tropical in Stuart transport medium and each swab was inoculated onto one of the media used in this study:

- Todd-Hewitt broth with nalidixic acid (15 µg/mL) and gentamicin (8 µg/mL) (Biomedics, Spain).
- LB (Biomedics, Spain).
- ILB (Biomedics, Spain).

The culture media were incubated in an anaerobic atmosphere, at 36 °C for 48 h, with the exception of Todd-Hewitt broth, which was subcultured onto Columbia blood agar (Biomedics, Spain) after 18 h. Blood agar plates were inspected at 24 h and 48 h. All other media were examined at 4 h, 10 h, 14 h, 18 h, 24 h and 48 h.

Beta-hemolytic colonies growing in blood agar were identified as GBS by CAMP. The appearance of an orange pigment in any of the Granada media was indicative of the presence of GBS.

Results

Group B streptococci were identified in 61 of 300 women (20%) on Granada LB medium, in 60 women (20%) on Todd-Hewitt broth with the ATB system, and in 56 (19%) women on ILB medium.

There was no GBS growth in Todd-Hewitt with antibiotics after 24 h incubation or in any of the other media after 18h. No GBS growth was observed at 4 h in any media. However, at 10 h and 14h, the presence of the orange pigment was observed (fig. 1) in 43/300 (14%) and 53/300 (18%) of the ILB tubes and in 32/300 (11%) and 46/300 (15%) of the LB tubes, respectively (table 1).

Conclusions

As previously stated, the screening approach strategy recommend by the CDC³ is based on GBS detection in the vagina and rectum of women at 35 to 37 weeks of pregnancy. Therefore, optimum prevention of neonatal GBS infection will only be achieved if all colonized women are detected during pregnancy and treated intrapartum. Therefore, sensitive and rapid laboratory techniques are necessary to identify GBS-carrying women to facilitate implementation of intrapartum antibiotic prophylaxis.

In this study, we assessed different culture media for GBS growth, comparing sensitivities and time of incubation needed for identification of the microorganism. The sensitivity of the Todd-Hewitt broth technique found in our study was high, 98% (60/61), which is in keeping with reported values^{10,11}. Granada LB presented the highest sensitivity (100%) and Granada ILB a sensitivity of 92% (56/61).

The ILB medium proved to be less sensitive than the detection rate described by De la Rosa et al⁶. The fact that some of the ILB tubes were less concentrated (less amount of powder for the same 3 mL of distilled H₂O) and/or errors in the lyophilization process could explain the lower detection rate found. Improved quality control is likely to resolve these problems.

The shortest incubation period needed for identification of GBS was 10h. At that time, ILB presented the highest sensitivity, while at 14h, 87% of GBS had grown on this medium and 75% on LB. It is also worth noting that no growth was observed after 18 h in either of the Granada media evaluated in the present study and after 24 h in the blood agar plates. A shorter incubation period, as is obtained with ILB, is important since fast results are useful, particularly in women starting premature labor.

In conclusion, it seems that all the media used in our study are suitable for GBS growth. Nonetheless, ILB and LB offer some advantages, since the majority of GBS grown in these media can be identified at 10 h and 14 h.

TABLE 1. Group B streptococci culture results in different media

| Medium | Hours | 4 hours N° positive/total (%) | 10 hours N° positive/total (%) | 14 hours N° positive/total (%) | 18 hours N° positive/total (%) |
|----------------------|-------|----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Todd-Hewitt with ATB | | * | * | * | 60/300 (20) |
| Granada LB | | 0 | 32/300 (11) | 46/300 (15) | 61/300 (20) |
| Granada ILB | | 0 | 43/300 (14) | 53/300 (18) | 56/300 (19) |

*No GBS growth was observed.

Since ILB presented results similar to those obtained with LB and it can be kept at room temperature for up to 2 years, it may be a good option when refrigeration is not available or when long storage periods are needed. Further studies should be performed to determine whether the incubation period needs to be increased to more than 18 hours, since no growth of GBS was observed after this time.

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