multilocus sequence typing of the isolates would be required.

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RESEARCH NOTE

Evaluation of Group B *Streptococcus* Differential Agar for detection and isolation of *Streptococcus agalactiae*

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ABSTRACT

In total, 320 vaginal or rectal swabs were cultured on Granada medium (GM) or Group B *Streptococcus* Differential Agar (GBSDA), and were also inoculated into LIM broth (Todd–Hewitt broth supplemented with selective antibiotics), for detection of group B *Streptococcus* (GBS). Overall, GBS isolates were detected on 53 of the 320 swabs; 47 of these isolates grew on both GM and GBSDA, five only on GBSDA, and one only following

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subculture from LIM broth. GBSDA appears to be a valid alternative to GM for the growth of GBS isolates from pregnant women.

Keywords Detection, GBS Differential Agar, group B streptococcus, isolation, *Streptococcus agalactiae*

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Streptococcus agalactiae (group B *Streptococcus*; GBS) is a significant cause of perinatal and neonatal infections worldwide [1]. To detect GBS colonisation in pregnant women, the CDC recommends isolation of the bacterium from vaginal and anorectal swab samples by growth in a selective enrichment medium, such as Todd–Hewitt broth or LIM broth (Todd–Hewitt broth supplemented with selective antibiotics), followed by subculture on sheep blood agar [2]; however, this procedure may require 48 h to complete.

In recent years, modifications of Islam medium, first described in 1977, have been tested for their suitability for detecting and isolating GBS [3,4]. Such modified media include Granada medium (GM) [3,5], on which β-haemolytic strains of *S. agalactiae* produce orange-to-salmon colonies [3], thereby allowing identification of GBS in a single step. GM shows a sensitivity of >90% for the detection of GBS [6–9], although there have been reports of lower sensitivity [10] that may be explained by poor storage conditions in the laboratory or during transportation [11]. However, the occurrence (1–3%) of non-pigmented GBS strains, as well as possible masking of the orange colour in GM by the saprophytic flora that normally colonises the vagina, make alternative procedures desirable. The Group B *Streptococcus* Differential Agar (GBSDA; BD Biosciences, San Jose, CA, USA) is a modification of New GM with improved stability and selectivity. The present study aimed to assess the performance of GBSDA in the isolation and identification of GBS, using GM as the standard for comparison.

Between October and December 2003, 320 swab samples (68 rectal, 196 vaginal, 56 vagino-anorectal) were collected from pregnant women and inoculated, in an antenatal clinic in random order, on to GM (Biomedics, Madrid, Spain) and GBSDA, and finally into LIM broth (BD Biosciences). Plates were incubated under anaerobic conditions at 37°C and examined after 24 and 48 h for red–orange colonies (indicating the presence of GBS). A pigment-producing *S. agalactiae* strain, ATCC 12386, was used as a positive control for detection of red–orange pigmentation. After incubation for 24 h under anaerobic conditions, the LIM broth cultures were subcultured on to Columbia agar containing sheep blood 5% v/v (BD Stacker Plates; BBL, Franklin Lakes, NJ, USA) for detection of colonies showing β-haemolysis.

Colonies with a red–orange appearance on GM and GBSDA plates, or showing β-haemolysis on Columbia agar with sheep blood, were agglutinated with Lancefield group-specific antisera (Streptocard Enzyme Latex Test; BBL). Red–orange colonies detected on one medium only were agglutinated, and were also identified using the MicroScan system with the Positive Combo Panel 1S A (Dade International, West Sacramento, CA, USA). β-Haemolytic colonies, recovered on Columbia agar containing sheep blood from samples that failed to yield red–orange colonies on GM or GBSDA, were also agglutinated with specific antisera and identified with the MicroScan system. The association of qualitative variables was evaluated by chi-square test and Fisher's exact test.

Overall, GBS isolates were obtained from 53 of 320 swabs, including 52 swabs that yielded red–orange colonies, 47 of them on both GM and GBSDA, and a further five only on GBSDA. One swab was not identified as GBS-positive on either GBSDA or GM, but only following subculture in LIM broth. Of the five swabs that yielded a positive result with GBSDA but not with GM, two were positive after 24 h, and three were positive after 48 h. Of the remaining positive swabs, most yielded red–orange colonies on both GM and GBS after incubation for 24 h; longer periods of incubation did not improve the detection of GBS colonies. No non-haemolytic GBS strains were detected, but control (non-clinical) GBS strains that were weakly β-haemolytic on sheep blood agar were found to be weakly pigmented on GM and GBSDA, the latter with lighter pigmentation, Thus, it seems that a blood agar plate should also be inoculated in order to detect all strains of GBS, including non-haemolytic strains.

Table 1 summarises the main microbiological properties of GBS isolates growing on GM and

Table 1. Microbiological properties of swabs yielding positive results on Granada medium (GM) and Group B *Streptococcus* Differential Agar (GBSDA)

	% swabs positive on		
	GM	**GBSDA**	**p**[a]
Growth score[b]			
NG	11.3	1.8	0.294
+	45.3	60.4	
++	18.9	15.1	
+++	17	17.0	
++++	7.5	5.7	
Pigmentation (%)			
Yellow	4.2	1.9	0.084
Pale orange	15.0	5.7	
Orange	48.9	38.4	
Deep orange	31.9	54.0	
Colony diameter			
< 1 mm	44.7	80.8	< **0.001**
1–2 mm	55.3	19.2	
> 2 mm	0	0	
Growth of saprophytes[c]			
VR	35.9	47.2	0.460
+	33.9	35.9	
++	18.9	9.4	
+++	9.4	7.5	
++++	1.9	0	

[a]p values in bold are statistically significant.
[b]+, weak growth, usually < 10 CFU; ++, moderate growth, usually 10–100 CFU; +++, high growth, usually between 100 and 1000 CFU; ++++, very high growth, innumerable colonies; NG, no growth.
[c]The presence of contaminant saprophytic flora: < 10 CFU, very rare (VR); +, ≥ 10 CFU but only in the first quadrant of the plate; ++, colonies reaching the second quadrant of the plate; ++, colonies reaching the third quadrant of the plate; +++, colonies reaching the fourth quadrant of the plate.

GBSDA. Only the difference in colony size was statistically significant, although GBSDA recovered *c*. 10% more GBS strains than did GM, and *c*. 20% of the isolates had a stronger pigmentation on GBSDA.

The usefulness of GM for the detection of GBS has been demonstrated previously [6,9,11]. Moreover, the use of selective broth for the isolation and detection of GBS has been reported to have good sensitivity, although the technique is time-consuming [9]. In the present study, one GBS isolate was recovered that had failed to be identified on GM or GBSDA, providing further evidence that, although time-consuming, this is a sensitive method for recovering *S. agalactiae*. Since swabs were inoculated into LIM broth only after the GM and GBSDA plates were inoculated, the sensitivity may have been underestimated. However, the cost of screening is higher if the enrichment procedure is used.

There are advantages in the use of GBSDA rather than GM, in that the intensity of colony pigmentation on GBSDA is stronger, the saprophytic flora is more suppressed, and GBSDA may be more sensitive, although the differences were not statistically significant in the present study. The time and costs involved are similar to those required for GM, and less than those required for the LIM broth enrichment method. It was therefore concluded that GBSDA is a valid alternative to GM for the isolation and detection of *S. agalactiae* from pregnant women.

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