

Specimen Storage in Transport Medium and Detection of Group B Streptococci by Culture

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Recovery of group B streptococci (GBS) was assessed in 1,204 vaginorectal swabs stored in Amies transport medium at 4 or 21°C for 1 to 4 days either by direct inoculation onto Granada agar (GA) or by culture in blood agar (BA) and GA after a selective broth enrichment (SBE) step. Following storage at 4°C, GBS detection in GA was not affected after 72 h by either direct inoculation or SBE; however, GBS were not detected after SBE in the BA subculture in some samples after 48 h of storage and in GA after 96 h. After storage at 21°C, loss of GBS-positive results was significant after 48 h by direct inoculation in GA and after 96 h by SBE and BA subculture; some GBS-positive samples were not detected after 24 h of storage followed by SBE and BA subculture or after 48 h of storage followed by SBE and GA subculture. Storage of swabs in transport medium, even at 4°C, produced after 24 h an underestimation of the intensity of GBS colonization in most specimens. These data indicate that viability of GBS is not fully preserved by storage of vaginorectal swabs in Amies transport medium, mainly if they are not stored under refrigeration.

The recommendations of the Centers for Disease Control and Prevention (10) for prevention of infections with neonatal group B streptococci (GBS) include cultures from anogenital swabs on all pregnant women, collected at 35 to 37 weeks of gestation. The recommendations state that swabs could be placed into a nonnutritive transport medium (such as Amies or Stuart without charcoal) and that transport media will maintain GBS viability for up to 4 days at room temperature or under refrigeration. Nevertheless, the accepted practice (3) of placing vaginal and rectal swabs in a transport medium and transporting them (even after several days) to the clinical laboratory has been questioned, because it has been reported that the yield of GBS-positive specimens increases when swabs are inoculated directly into selective media, thereby avoiding transport media (11). Moreover, some reports suggest a rapidly declining number of GBS for GBS-positive swabs that are stored in transport media (8).

This study examines the effect of time and temperature on GBS viability and GBS recovery rates for vaginorectal swabs stored in Amies transport medium either when the swabs are inoculated directly in Granada agar (GA) plates (9) or a selective broth enrichment (SBE) step (1, 3) is used before inoculating GA and blood agar (BA) plates.

Swab collection and processing. Two vaginorectal swabs were collected from each of 1,204 pregnant women (at 35 to 37 weeks of gestation); after collection, the swabs were placed in Amies transport medium (Biomedics, Madrid, Spain) and sent to the laboratory. All specimens were processed within 4 h of collection.

In the first phase of the study, we studied 300 samples, using one vaginal swab from each woman. Each swab was placed in

a tube containing 0.8 ml of 0.85% NaCl and swirled vigorously. Nine additional swabs were immersed in this tube and then placed in tubes of Amies transport medium. One of the nine swabs was immediately used to inoculate a plate of GA. Of the remaining eight swabs, four were stored at 4°C and four were stored at 21°C. After 24, 48, 72, and 96 h, one swab stored at 4°C and one stored at 21°C were inoculated onto GA plates using the four-quadrant technique to allow semiquantization of GBS colonies (5). The GA plates were incubated anaerobically for 48 h, and GBS growth (red colonies) was graded as 0 (no growth), 1+, 2+, 3+, and 4+, defined as growth in the first, second, third, and fourth quadrants, respectively. In the second phase we studied 904 samples, using two vaginorectal swabs from each woman. One swab was processed as received in the laboratory, and the remaining swab was stored (before processing) at either 4 or 21°C for 24, 48, 72, or 96 h (113 swabs for each condition). All swabs were processed in the same way, i.e., placed them in tubes with 5 ml of selective broth (SB) (Todd-Hewitt with 8 µg of gentamicin per ml plus 15 µg of nalidixic acid per ml) (1). Tubes of SB were incubated for 18 h at 36°C and then were subcultured onto BA and GA plates, which were incubated for 48 h (GA anaerobically). Hemolytic GBS colonies were identified by typical beta-hemolysis, Gram staining, and antigen detection.

Phase 1 results. The results of semiquantitative growth from GBS-positive vaginorectal swabs stored in Amies medium and directly inoculated in GA (without previous SBE) are shown in Table 1. GBS were recovered from 41 of the 300 (13.7%) swabs inoculated immediately after being received by the laboratory, and GBS growth from these swabs was scored 4+ in 24 swabs, 3+ in 10 swabs, and 2+ in 7 swabs.

From swabs kept at 4°C, GBS colonies were recovered from the same 41 samples after 24, 48, and 72 h (100%) and from 39 samples after 96 h (95%) ($P > 0.05$; Cochran's test). Nevertheless, among these 41 initially positive swabs stored at 4°C, the number of GBS colonies declined during storage in 10

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TABLE 1. GBS growth from 41 GBS-positive vaginorectal swabs after storage in Amies transport medium and direct inoculation onto Granada agar

GBS culture result ^a	No. of swabs with indicated culture result after being stored for indicated time at indicated temp								
	Initial no.	4°C				22°C			
		24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
4+	24	20	12	8	4	15	10	5	1
3+	10	10	14	10	8	8	7	5	1
2+	7	8	7	14	17	10	12	17	18
1+	0	3	8	9	10	6	7	8	9
0	0	0	0	0	2	2	5	6	12
Total positive	41	41	41	41	39	39	36	35	29

^a For an explanation of culture results, see the text.

(24%) of the swabs stored for 24 h, in 26 (63%) of the swabs stored for 48 h, in 33 (80%) of the swabs stored for 72 h, and in 36 (88%) of the swabs stored for 96 h ($P < 0.01$; Friedman's test).

Among the 41 initially positive swabs kept at 21°C, GBS were recovered only from 39 (95%) after 24 h, from 36 (88%) after 48 h, from 35 (85%) after 72 h, and from 29 (71%) after 96 h ($P < 0.05$; Cochran's test). GBS were detected after 96 h of storage at 21°C in all swabs ($n = 24$) that initially scored 4+ and in 50% of swabs that initially scored 3+, and they were not detected when the initial score was 2+. Among the GBS-positive swabs stored at 21°C, a decline (or loss) in the number of GBS colonies was observed in 22 (54%), 30 (73%), 36 (88%), and 40 samples (98%), respectively, after 24, 48, 72, and 96 h of storage ($P < 0.01$; Friedman's test).

Phase 2 results. GBS recovery, after SBE, from the 904 vaginorectal swabs preserved in Amies transport medium is shown in Table 2. In the samples processed as received in the laboratory, GBS were recovered from 135 swabs (14.9%) in the GA subculture and from 132 (14.6%) of the swabs in the BA subculture ($P > 0.05$; binomial test).

Among the 69 initially positive samples stored at 4°C, and when the subculture was carried out on BA, GBS were not detected in one sample after 48 h, in two after 72 h, and in two after 96 h ($P > 0.05$; binomial test). From these five negative samples (in the BA subculture), GBS were lost only in one (after 96 h) when GA was used to subculture.

Among the 66 initially positive samples stored at 21°C in transport medium, and when the subculture was carried out on BA, GBS were not detected in one sample after 24 h, in two samples after 48 h, in three samples after 72 h, and in five

samples after 96 h ($P < 0.05$; binomial test). From these 11 negative samples (in the BA subculture), GBS were lost only in 6 samples when GA was used to subculture.

GBS colonies were not observed in any BA plate subculture when they were not present in the GA plate subculture. In all (Table 2), there were 15 cases in which GBS were recovered after the subculture onto GA but not when BA was used ($P < 0.001$ binomial test).

Conclusions. The ideal transport system should maintain the viability of bacteria in specimens without promoting multiplication. However, some bacteria, such as *Pseudomonas* spp., *Escherichia coli*, and *Enterococcus* spp., which can be present in vaginorectal samples, may proliferate during transport, and this proliferation can impair the recovery of GBS from vaginorectal swabs preserved in transport media (D. M. Warshauer, L. J. Bartell, and J. T. Paloucek, Abstr. 98th Gen. Meet. Am. Soc. Microbiol., abstr. C-444, 1998). It has been shown that storage of vaginorectal swabs in transport medium for a few hours does not result in a significant decrease of GBS detection after SBE (4), but longer storage of vaginorectal samples in transport media has not been studied. Our data show that 4 days of storage of vaginorectal swabs in Amies transport medium at 4°C does not result in a significant decrease in the ability of the culture in GA to detect GBS by either direct inoculation or following SBE. However, when the subculture from SBE is carried out in BA, storage of swabs in transport medium impairs the detection of some GBS-positive samples, even after 24 h at 4°C. On the other hand, storage of vaginorectal swabs in transport medium at 21°C compromises the ability of the culture to detect GBS colonization, even after SBE, and some positive samples are lost after 24 h, when BA plates are used to detect GBS colonies, or after 48 h, when subculture onto GA plates is used.

Results of this study confirm those of previous reports that GBS can be detected in the GA subculture after SBE from samples stored in transport medium for 24 h (12). Our data also confirm that subculture onto GA is more sensitive than subculture onto BA to detect GBS in vaginorectal swabs after SBE (9). This finding could be explained by the fact that in all the samples in which GBS were detected in GA but not in BA, there was a large growth of fecal bacteria in BA (after SBE) that did not allow the observation of GBS beta-hemolytic colonies while, in contrast, red GBS colonies were easily spotted in GA. Our results also show that storage of vaginorectal swabs in Amies medium, either at 4 or at 21°C, decreases GBS viability after 24 h. The drop in GBS viability during storage at 21°C can cause the loss of some GBS-positive samples in BA

TABLE 2. Number of GBS carriers detected (after selective enrichment) by direct and delayed culture (after being stored in Amies medium during the indicated time at the indicated temperature) in 8 groups of 113 vaginorectal samples

Subculture	No. at temp:							
	4°C				21°C			
	Direct/delayed 24 h	Direct/delayed 48 h	Direct/delayed 72 h	Direct/delayed 96 h	Direct/delayed 24 h	Direct/delayed 48 h	Direct/delayed 72 h	Direct/delayed 96 h
Blood agar	20/20	13/12	19/17	16/14	12/11	18/16	13/10	21/16 ^a
Granada agar	20/20	13/13	20/20	16/15	13/13	19/18	13/11	21/18

^a $P < 0.05$, binomial test.

subculture after 24 h. In light of these findings, the claim that vaginorectal swabs for GBS detection can be safely stored in transport medium for 4 days at room temperature perhaps should be reevaluated.

The density of GBS colonization has been correlated with the risk of neonatal infection, with a lower risk of infection if there is light maternal colonization at delivery (2, 6, 7). Because of this, if loss of GBS viability occurs as a result of specimen storage and a direct sampling technique is used to evaluate the intensity of colonization, the culture results can lead to an underestimation of the intensity of maternal GBS colonization and of the actual risk of GBS neonatal infection.

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