Evaluation of bacteriological swabs and transport media in the recovery of group B streptococci on laboratory media

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SUMMARY The survival of group B streptococci on a variety of swabs, held either in their containers or in transport media for periods of up to 48 hours, at room temperature and at 4°C, has been assessed. Results indicated that holding swabs in transport media did not favour prolonged survival of the streptococci and that the yield of organisms was much greater from swabs held in their ordinary plastic tubes. A holding temperature of 4°C rather than room temperature is recommended if any delay in plating out swabs is anticipated.

In the early 1930s, group B streptococci were commonly isolated from the vagina of healthy post-partum women but were seldom implicated in infective obstetric complications (Lancefield and Hate, 1935). Only sporadic cases of infections such as endocarditis, puerperal sepsis, and septic abortion were reported (Congdon, 1935; Fry, 1938; Hill and Butler, 1940).

During the last two decades, however, group B streptococci have painted quite a different clinical picture, both in types of infections caused and in their severity. They have been increasingly associated with infections in adults in whom they appear to have a bimodal age distribution: a young female population in which group B streptococcal infection occurs as a complication of pregnancy on the post-partum period, and an older age group in which infection is often associated with the 'compromised host'. Urinary tract and respiratory tract infections, meningitis and arthritis, and many other infections may occur in such persons (Bayer et al., 1976). The most dramatic change in the pathogenic potential of group B streptococci has been in the newborn and in infants, in whom they can cause severe septicaemia and meningitis. Whereas in the early 1960s coliform organisms were by far the major neonatal pathogens, the incidence of group B streptococci in early neonatal bacteraemia has shown a marked increase in the last few years (Jeffrey et al., 1977).

In epidemiological studies of the prevalence of group B streptococci and their role in disease, several variables must be borne in mind as these can seriously affect findings. They include time of culture, site of culture, and the swab.

Ferrieri et al. (1977) reported that, in their studies on pregnant women, 20 were negative for group B streptococci during the third trimester of pregnancy but were positive during labour. They also reported that 19 of the babies born who were negative for group B streptococci at birth were positive by the time of discharge, whereas 14 originally positive became negative. The site of swabbing is also important. Anthony et al. (1975) stated that urethral swabs from females were positive more often than vaginal swabs, and Ross (unpublished) found that urethral swabs were more frequently positive than cervical swabs taken from the same patient in women attending antenatal clinics and in patients attending clinics for sexually transmitted diseases, although in the latter group rectal swabs were most commonly positive.

Regarding the swab itself, there are those who prefer to use a tip made from a fibre such as Dacron (Hollinger and Rantz, 1959) or calcium alginate (Ellner and Ellner, 1966) rather than the more usual cotton-wool variety. Other variables include whether plain or coated swabs are used (Bartlett and Hughes, 1969; Ross, 1977) and whether or not moisture is present on the swab (Rubbo and Benjamin, 1951). Various workers have suggested that swabs should be placed in transport media to minimise the effect of environmental factors during transit (Pike, 1945; Stuart, 1956), and the use of silica gel to counteract possible deleterious effects of moisture has also been recommended (Taplin and Landsell, 1973).
The purpose of this paper is to compare and contrast the performance of various swabs and transport media in maintaining the viability of group B streptococci, in an attempt to ascertain optimal methods that can be applied to epidemiological studies and to the diagnosis of infections caused by these increasingly common organisms.

### Material and methods

Twenty strains of group B streptococci were used, as follows. Five reference strains, representing the different serotypes (1a, 1b, 1c, II, III), were supplied by the Streptococcal Reference Laboratory, Colindale, London; seven strains were freshly isolated from the upper respiratory tract of patients in the Royal Infirmary, Edinburgh; and a further eight strains were vaginal isolates from pregnant women attending the same hospital.

#### SWABS

The varieties of swabs and transport media used are shown in Table 1. Swabs obtained commercially, with and without transport media, were used in addition to plain swabs in Pike's and Stuart's media, and in silica gel prepared in the laboratory. Swabs were loaded with one drop (0.02 ml) of a dilution of a group B streptococcal culture grown overnight in Todd-Hewitt broth, and four drops of 0.08 ml saline (0.85% w/v sodium chloride); the streptococcal suspension and saline were mixed together on a small area of the inner surface of a plastic petri dish; the swab was rotated during the loading process to ensure an equal distribution of organisms on the swab tip. The swabs were then held either in their plastic containers or in transport media for prescribed periods up to 96 hours. The holding temperatures were 4°C and room temperature (16-22°C). Pairs of swabs of each type were streaked out twice on to each half of 5-10% human blood agar plates at 0, 8, 24, and 48 hours, and, after aerobic incubation overnight at 37°C, surface colony counts were performed.

The initial number of organisms taken up by each swab was determined by surface viable counts from 10^4 to 10^8 dilutions of the original culture. At every time interval the average chain length of each colony-forming unit was calculated for the different swab types by rotating the swab head on a glass microscope slide and performing Gram stains.

#### Statistical methods

To evaluate the effects of time, temperature, and type of swab on the recovery rate of each group of organisms, data were combined and analysed as a randomised block experiment, the treatments being time, temperature, and plating. Experimental blocks were the various groups of organisms. 2 × standard error (SE) = significant difference.

### Results

The mean percentage recovery of group B streptococci from swabs plated at 0 hours is shown in Table 2. Table 3 shows that over a period of 48 hours there was no significant loss of recovery from albumen and charcoal swabs at room temperature. A surprisingly high recovery of group B streptococci was obtained from Dacron swabs at 8 hours; at 24 and 48 hours growth on plates was confluent. Plain cotton swabs showed a slight loss of recoverable organisms with

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**Table 1** Swabs and transport media

<table>
<thead>
<tr>
<th>Swabs in transport media</th>
<th>Prepared in laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial</td>
<td></td>
</tr>
<tr>
<td>Cotton-wool in Amies'</td>
<td>Cotton-wool in Pike's</td>
</tr>
<tr>
<td>Cotton-wool in charcoal Amies'</td>
<td>Cotton-wool in Stuart's</td>
</tr>
<tr>
<td>Alginate in Amies'</td>
<td>Cotton-wool in silica gel</td>
</tr>
<tr>
<td>Dacron in Amies'</td>
<td></td>
</tr>
<tr>
<td>Dacron in charcoal Amies'</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 2** Recovery of group B streptococci on solid media from swabs not held in transport media

<table>
<thead>
<tr>
<th></th>
<th>Mean recovery % at 0 hours</th>
</tr>
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<tbody>
<tr>
<td>Plain</td>
<td>8.2</td>
</tr>
<tr>
<td>Albumen</td>
<td>7.5</td>
</tr>
<tr>
<td>Alginate</td>
<td>6.0</td>
</tr>
<tr>
<td>Charcoal</td>
<td>8.5</td>
</tr>
<tr>
<td>Dacron</td>
<td>10.8</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.0</td>
</tr>
</tbody>
</table>

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**Table 3** Effect of time and temperature on recovery of group B streptococci from swabs not held in transport media

<table>
<thead>
<tr>
<th>Mean recovery % at time (hours)</th>
<th>Room 4°C</th>
<th>Room 4°C</th>
<th>Room 4°C</th>
<th>Room 4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>24</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Plain</td>
<td>12.9</td>
<td>9.4</td>
<td>7.6</td>
<td>9.7</td>
</tr>
<tr>
<td>Albumen</td>
<td>8.6</td>
<td>9.9</td>
<td>9.3</td>
<td>6.2</td>
</tr>
<tr>
<td>Alginate</td>
<td>5.3</td>
<td>9.7</td>
<td>11.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Charcoal</td>
<td>21.8</td>
<td>14.0</td>
<td>TNC</td>
<td>24.8</td>
</tr>
<tr>
<td>Dacron</td>
<td>21.8</td>
<td>14.0</td>
<td>TNC</td>
<td>20.3</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.3</td>
<td></td>
<td></td>
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</table>

TNC = too numerous to count
time, while the number of colony-forming units produced by plating alginate swabs dropped significantly after 24 hours.

The effect of temperature on recovery rate of group B streptococci over 48 hours is also shown in Table 3. There was little difference between swabs held at room temperature and at 4°C, although the type of statistical test used could not include any result recorded as ‘too numerous to count’ or confluent growth. Nearly all strains held on Dacron swabs at room temperature produced confluent growth after 24 hours, whereas at 4°C, although counts were high, it was still possible to count the colonies.

Table 4 shows the recovery rate from swabs held in commercial and laboratory-prepared transport media at room temperature and at 4°C for 8, 24, and 48 hours, and it can be seen that, of all the various combinations of swabs and media, only the recovery from Dacron in Amies’ and in charcoal Amies’ at 4°C is comparable to that obtained from swabs not immersed in transport media. Holding swabs in transport media at room temperature beyond 8 hours meant that in the majority of experiments colonies were too numerous to count.

In these studies, the bacterial concentration of the initial inoculum placed on to the swab varied, although it was standardised within each set of experiments. In the statistical analysis it was shown that the percentage recovery rates were similar, regardless of whether large or small inocula were used. Copies of the statistical analyses, which, because of the amount of data involved, are not reproduced here, may be obtained from the authors.

For each swab at each time and temperature interval, chain lengths were calculated, and these were not found to vary throughout the experiment.

Discussion

The overall conclusion from this study is that, with the exception of Dacron swabs held in Amies’ or in charcoal-Amies’ media, the recovery of group B streptococci from a variety of swabs not held in transport medium was much superior to that obtained from swabs held in transport media, whether commercially produced or prepared in the departmental laboratory. Also, Dacron swabs held in plastic containers produced higher recovery rates than those held in the Amies’ media, although at 24 and 48 hours colonies were too numerous to count (TNC) at room temperature. Such a TNC result can be unhelpful because, far from the organisms dying off on the swab, they multiply on it, and this multiplication makes it impossible to predict what the size of the original inoculum was, and therefore invalidates the possibility of even semi-quantitative analyses. In addition, overgrowth by other commensal organisms cannot be prevented.

Satisfactory results were obtained from all plain swabs, with the exception of the alginate variety, although the results from alginate swabs held in their containers were infinitely better than those obtained from transport media; whereas the plain type produced fewer colonies as time progressed, although at 48 hours a recovery rate of 2-3% was still found, when immersed in transport media the swab never produced a recovery rate of more than 1% at any period of time, either at room temperature or at 4°C.

Swabs held in Pike’s and Stuart’s media did not give a good yield of group B streptococci at 4°C, and at room temperature the colonies were TNC after 8 hours. Holding swabs in silica gel produced uniformly low counts at all times, at both temperatures, and this procedure cannot therefore be recommended for the culture of group B streptococci, although Martin et al. (1977) advocated the use of silica gel in the transport of Streptococcus pyogenes.

As with many other organisms, particularly pathogens of the upper respiratory tract (Ross and Lough, 1978), holding swabs at 4°C not only maintained a satisfactory recovery rate of group B streptococci for 48 hours but also prevented a vast
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multiplication of these and any other accompanying contaminant organisms on the swab. This was particularly the case with swabs held in transport media.

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References

Lancefield, R. C., and Hare, R. (1935). The serological differentiation of pathogenic and non-pathogenic strains of hemolytic streptococci from parturient women. Journal of Experimental Medicine, 61, 335-349.

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