DELAYED CULTURE OF GROUP-A STREPTOCOCCI: AN EVALUATION OF VARIABLES IN METHODS OF EXAMINING THROAT SWABS

DIANA R. MARTIN, J. M. STANHOPE* AND LESLEY A. FINCH
*National Health Institute, Wellington 2, and * Epidemiology Unit,
Wellington Hospital, Wellington 2, New Zealand*

THIS laboratory is assisting in an epidemiological study of a population in which the incidence of rheumatic fever and rheumatic heart disease is five times greater than the New Zealand national average (Stanhope, 1975 and 1977). Because it was not possible to examine large numbers of throat swabs locally, it was necessary to find a transport medium in which the survival of group-A streptococci could be assured.

Group-A streptococci survive well on dried swabs (Rubbo and Benjamin, 1951). Methods advocated for the transport of dried throat secretions include inserting the swab into a desiccant of silica gel contained either in a screw-capped tube (Hosty *et al.*, 1964) or in a foil pack (Redys, Hibbard and Borman, 1968), or scrubbing the swab on to the surface of a sterile piece of filter paper and posting this to the laboratory (Hollinger and Lindberg, 1958); but other workers have posted the dry swab in its tube to the laboratory (Lattimer, Siegel and de Celles, 1963). For our purposes, the use of either plain tubes or tubes containing silica gel offered the best method of transport.

It has been claimed that serum-coated swabs (Cooper, 1957) or Dacron swabs (Hollinger and Lindberg, 1958; Hosty *et al.*, 1964) have an advantage over plain cotton swabs for the isolation of group-A streptococci. Because general practitioners were to participate in our study, it was necessary to establish whether a variation in the type of swab would influence the rate of isolation of group-A streptococci.

The aim of the present study was to establish a suitable method for the isolation of group-A streptococci from throat swabs by comparing the rate of isolation if the swab type only was varied and by determining whether the presence or absence of silica gel in the transporting tube affected the results obtained. These variables could not be assessed critically without also comparing the effect of delayed plating as opposed to immediate plating of swabs. A statistical approach was used to examine each variable independently and in combination with others.

MATERIALS AND METHODS

Key variables. The following key variables were tested: (1) the use of silica gel for the transport of the swab to the laboratory in comparison with transport in a similar tube without silica gel; (2) the use of serum-coated swabs for sampling in comparison with the use of plain cotton swabs; (3) the use of an immediate isolation technique (processing of the swab within 4 h of collection) in comparison with delayed isolation (storage for 4 days at room temperature in the dark to simulate a delay of 4 days whilst in postal transit).

Swabs. Serum-coated and plain cotton swabs (Exogen Ltd, 1967 Dumbarton Road, Glasgow G14).

Silica gel (Ajax Chemicals Ltd, Sydney, Australia, grade 123, mesh size 18–35); c. 2 g per tube were used.

Tubes. Pyrex with metal caps; all tubes, whether containing silica gel or not, were sterilised in the hot-air oven (160°C, 1 h).

Collection of swabs. Primary-school children were used as subjects for this study; duplicate throat swabs were obtained simultaneously from each child. The subject's head

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was held erect, the tongue depressed with a disposable spatula and the two swabs held firmly together were rubbed in a circular movement over both tonsils and tonsillar fossae. To achieve consistency all swabbing was undertaken by two of the authors (D. R. M. and L. A. F.). Swabs were immediately placed in the appropriate pre-labelled tubes.

Design of experiment. Each child swabbed was allotted to one of the three "variable groups"—(1) varying gel, (2) varying swab and (3) varying plating—in which the other two of the three key variables (see above) were held constant and assumed not to influence the variable being tested. Thus for each major group there were four possible minor subdivisions that would be made (table I), and these were labelled *a, b, c, d*, etc.

To ensure randomisation, the first child sampled was allotted to group *a* with swabs and treatment of the swabs appropriate to that group, the second child to group *b* and so on, with repetition of these 12 groups until the necessary number of children had been sampled. The sample size was determined by statistical methods based on an expected percentage carriage rate.

Isolation technique. To avoid the consequences of spillage of contaminated silica-gel crystals, swabs were carefully removed from the tubes over a Savlon-soaked paper towel. The swab was then plunged into a pre-labelled 5-ml screw-capped bottle containing 2 ml of Todd-Hewitt broth, moved around to moisten and to remove adherent silica-gel particles,

TABLE I
Effect of three key variables in technique on the isolation of group-A streptococci from paired throat swabs

First alternative	Second alternative	Variables held constant	Number of persons sampled	Number of isolations of group-A streptococci by		
				first method only	second method only	both methods
Key variable no. 1						
Transport in silica gel	Transport in plain tube	<i>a</i> Immediate plating, serum swab	66	2	0	3
		<i>b</i> Delayed plating, serum swab	65	0	1	9
		<i>c</i> Immediate plating, plain swab	66	1	1	5
		<i>d</i> Delayed plating, plain swab	66	3	1	5
Key variable no. 2						
Serum swab	Plain swab	<i>e</i> Immediate plating, silica gel	66	1	1	2
		<i>f</i> Delayed plating, silica gel	66	1	0	6
		<i>g</i> Immediate plating, no gel	65	2	0	3
		<i>h</i> Delayed plating, no gel	65	0	1	2
Key variable no. 3						
Delayed plating*	Immediate plating†	<i>i</i> Serum swab, silica gel	66	3	0	3
		<i>j</i> Serum swab, no gel	66	2	1	2
		<i>k</i> Plain swab, silica gel	66	2	2	1
		<i>l</i> Plain swab, no gel	66	6	1	1

* After 4 days in dark at room temperature.

† Within 4 h of swabbing.

drained against the inner surface of the bottle, and then rubbed over the surface of a plate of each of the two isolation media before being broken off into the Todd Hewitt broth for incubation. The first plate seeded in each case was one of plain blood agar (5% v/v of defibrinated sheep blood in Trypticase Soy Agar, Baltimore Biological Laboratories) and the second plate was crystal-violet blood agar (blood agar with the addition of 1 in 10⁶ crystal violet). Blood-agar (BA) plates were incubated anaerobically and crystal-violet blood agar (CVBA) in 95% air + 5% CO₂ for 18–24 h before examination. After incubation for 18–24 h, Todd-Hewitt broths were seeded on to CVBA.

Bacitracin sensitivity. All β -haemolytic colonies suspected of being streptococci were tested for bacitracin sensitivity on BA plates with commercially prepared paper disks containing 0.04 units of antibiotic. Any organism that gave a zone of inhibition was retained for serological grouping.

Serological grouping. Extracts of cultures (37°C, 18–24 h) in Todd-Hewitt broth were made with 0.2M HCl (100°C, 10 min.), and were tested against the group-A antiserum by the precipitation test in capillary tubes (Swift, Wilson and Lancefield, 1943).

RESULTS

A total of 809 primary school children between the ages of 5 and 13 years were sampled. The results for 20 children could not be included because in each case one of the swabs had adhered to the wall of the transporting tube and could not be removed without damage. Of the strains presumptively identified as a group-A streptococci by bacitracin sensitivity, only one failed to be confirmed on extraction of the group antigen; it was a member of group C.

The total number of isolations of group-A streptococci obtained in the three "variable groups" was as follows (see table I): group 1: with silica gel 28, without silica gel 25; group 2: on serum swab 17; on plain swab 15; group 3: delayed plating 20; immediate plating 11. The assumption was made that the two swabs collected simultaneously from the same child were comparable samples of the throat flora. Analysis of the effects of the three key variables was therefore made within swab pairs by McNemar's test for the significance of paired differences (Siegel, 1956). This yielded an χ^2 value of 3.7647 ($P = 0.0523$) for delayed versus immediate plating; this suggested a superiority for delayed plating over immediate plating of only marginal statistical significance. For serum versus plain swabs, the χ^2 value

TABLE II
Reorganisation of data to show rates of isolation and proportions of failures of isolation of group-A streptococci

Variables in technique			Isolation rate*	Proportion of failures†
Time before plating	Type of swab	Silica gel		
4 days	Serum	Present	22/197	1/23‡
4 days	Serum	Absent	16/196	2/18
4 days	Plain	Present	17/198	4/21
4 days	Plain	Absent	16/197	4/20
<4 h	Serum	Present	11/198	4/15
<4 h	Serum	Absent	11/197	4/15
<4 h	Plain	Present	12/198	4/16
<4 h	Plain	Absent	11/197	9/20§

* Number of isolations/number of swabs examined.

† See *Results* for method of calculation.

‡ Binomial test: $P < 0.005$.

§ Binomial test: $P < 0.001$.

was 0.1667; for silica gel versus no gel, it was 0.444; neither of these results were of statistical significance.

If the pairing of swabs was disregarded, the data could be reorganised as shown in table II. The four groups in which plating was delayed yielded higher recovery rates than the four in which it was performed immediately ($\chi^2 = 5.8840$, $P = 0.0153$). A proportion "failed" was determined for each of the eight possible combinations of key-variable alternatives. This was the proportion of positive isolations the combination failed to yield that had been obtained by any other combination differing from it by no more than one key variable. The proportion was tested for divergence from a pooled failure rate by means of the binomial test (Remington and Schork, 1970). These results are also shown in table II, and indicate that the delayed-plating, serum-swab, silica-gel method was superior to the alternatives, whilst the immediate-plating, plain-swab, no-gel method was unreliable.

DISCUSSION

Our results indicated that methods in which there was a considerable delay in plating throat swabs tended to give higher isolation rates than did methods in which the swabs were plated within 4 h of collection: other workers have made similar observations (Hollinger and Lindberg, 1958; Taplin and Landsell, 1973). We noted that growth was less often confluent in the cultures of throat swabs that had been plated after the longer delay and that β -haemolytic colonies were more easily observed, particularly when present in small numbers. Drying of the swab during transit to the laboratory thus appeared to be responsible for eliminating some throat organisms that would otherwise have competed for growth on the primary plate.

Because we proposed in future studies to enlist the help of general practitioners, we wished to know whether it would be necessary to supply swabs of a particular type. Our results showed that no significant difference in isolation rates occurred when serum and plain swabs were compared. However, when other factors were taken into consideration, there was evidence that immediate plating of plain swabs that had been transported in tubes without silica gel gave unreliable results. It is possible that the continued presence of moisture in the tubes without desiccant may have been responsible for the inferior results obtained by this method. Rubbo and Benjamin (1951) demonstrated that group-A streptococci remained viable on serum swabs and were apparently uninfluenced by the presence of moisture, whereas on plain swabs these organisms died off rapidly in a moist environment. Redys *et al.* (1968) found that high humidities reduced the survival time of group-A streptococci on swabs, but that silica gel prevented the deleterious effect of humidity on the streptococci. Isolation of streptococci from calcium-alginate swabs desiccated in silica gel was possible for up to 1 month after sampling (Taplin and Landsell, 1973). The observations of Hosty *et al.* (1964) also suggested that silica gel affected the survival of group-A streptococci. In its absence, there was a 29% reduction in the isolation rate on Dacron swabs and a 50% reduction on cotton swabs over a period of 3 days; in the presence of silica gel there was no such difference.

Under the conditions prevailing during our study we were not able to show that the presence or the absence of silica gel significantly influenced the number of group-A streptococci isolated, either when considered as a separate factor or in combination with other factors. It was noted, however, that in the absence of silica gel swabs tended to stick to the base of the transporting tubes and were difficult to remove. The presence of silica gel thus appears to offer a practical advantage in the transporting of swabs to the laboratory; and when cultures were made from plain swabs within 4 h of collection, the use of silica gel increased the number of isolations.

An alternative to the use of silica gel is to rub the swab on to a strip of filter paper, dry this in air, and mail it to the laboratory (Hollinger and Lindberg, 1958). This technique has been successfully applied in field studies (Hollinger *et al.*, 1960; Smith *et al.*, 1965). Major disadvantages of this system appear to be (1) the necessity to smear and air dry the filter-paper strips, which presents difficulties when large numbers of throat swabs are being

collected at a time, (2) the lack of control over variations in humidity, and (3) the necessity to remove the filter-paper strip from the culture plates 6 h after inoculation.

In most of the studies cited, a statistical approach was not used to demonstrate the significance of the findings. The statistical evidence obtained in our study supported our decision to post swabs to a central laboratory, and to use serum-coated swabs in tubes containing silica gel.

SUMMARY

We studied the effects on the frequency of isolation of group-A streptococci from throat swabs of school children of three variables: (1) plating the cultures within 4 h of collecting the swabs or after storage for 4 days at room temperature; (2) using a plain or a serum-coated cotton swab; and (3) including silica gel in the swab tube or omitting this. Under the conditions of our experiment, only delayed plating of the swab gave a statistically significant advantage. When the delayed-isolation technique was used, neither the swab type nor the presence or absence of silica gel significantly influenced the result. The least advantageous combination of variables was: plating within 4 h, a plain swab, and the absence of silica gel.

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