

Value of Desiccated Swabs for Streptococcal Epidemiology in the Field

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Streptococcal surveys in foreign countries or remote areas may be greatly enhanced by the use of calcium alginate swabs desiccated in sterile silica gel. Delays of up to 4 weeks before return to a base laboratory are feasible, and the need for fresh media or laboratory facilities in the field may be eliminated. Comparison of direct plating on crystal violet blood agar versus delayed silica gel preservation during surveys in Uganda, Haiti, Colombia, and Miami, Fla., showed no significant loss of positive cultures from skin lesions and suggests that desiccated swabs increase the recovery of bacitracin-sensitive *Streptococcus pyogenes* (presumptive group A) from throats.

Several methods have been proposed for the isolation of *Streptococcus pyogenes* under field conditions, including the use of selective media (8, 10), special preparation of swabs (3, 11), non-nutrient transport media, filter paper strips (5, 12), and desiccation of swabs in sterile silica gel (6, 9).

Most of the published data concern the isolation of *S. pyogenes* from the upper respiratory tract. Relatively little attention has been paid to methodology related to streptococcal infections of the skin.

Although we have by comparative testing arrived at a suitable medium for the primary isolation of *S. pyogenes* from both skin and throats, such luxuries were not always available in the field, and it became essential to evaluate methods of delayed culture or transport media to conduct field epidemiology in tropical and often remote areas.

We describe here our experiences in several countries in which we compared direct plating on fresh media in the field with cultures made from identical swabs which were kept in silica gel for up to 1 month before culture.

MATERIALS AND METHODS

Throat cultures were made from the tonsillar fossae. Skin lesions were cultured without prior cleansing except to remove crusts, debris, gross dirt, or medications with sterile gauze. Two calcium alginate swabs (Calgiswab Colab) were taken from the same skin lesion or throat. One swab was rolled over one-third of a freshly prepared double-layer plate of TSAB-CV made with Trypticase soy agar

(BBL) containing 1 μ g of crystal violet (Difco) per ml overlaid with a layer of Trypticase soy agar (BBL) containing 1 μ g of crystal violet (Difco) per ml and 6% sterile defibrinated sheep blood (Brown Laboratory, Topeka, Kan.). The plates were streaked with a platinum loop in the field to obtain isolated colonies and incubated aerobically at 37 C as soon as possible, usually within 4 hr.

The other swab was immediately placed in an aluminum foil packet of sterile silica gel (Carter Rice Storrs & Bement, East Hartford, Conn.), which was sealed and placed in an aluminum foil-lined Kraft envelope (same supplier) and sealed. On return to our laboratories in Miami, from 3 to 4 weeks later, the swabs in silica gel were plated directly on TSAB-CV in the same manner as in the field and on double-layered Trypticase soy agar sheep blood (TSAB). The same individuals took the cultures in all studies, and the evaluation of the delayed culture plates was made without knowledge of the results of the first culture made in the field, utilizing code numbers instead of names.

RESULTS

Surveys of infants and schoolchildren were made in Miami, Fla., Haiti, Uganda, and Colombia, South America.

A total of 223 skin lesions and 48 throats were cultured. Skin lesions yielded group A *S. pyogenes* from 78% of attempts by the direct plating method, compared with 76% of attempts by the delayed culture method. There was no statistically significant difference in the recovery of *S. pyogenes* between the two methods for skin cultures. Throat swabs yielded 4% by direct culture compared with 21% by the delayed technique (Tables 1 and 2). Although

the denominator is small, this represents a significant difference ($P < 0.05$) in favor of the delayed swab method for throat cultures.

In Uganda and Colombia an attempt was made to roughly quantitate the growth, and cultures were scored as scanty (1 to 10 colonies per plate), moderate (10 to 50), or profuse (50 or more) (Table 2).

Although 15 cultures yielded fewer colonies following desiccation, the overall scoring of positive or negative cultures yielded two additional positive cultures by the desiccation method compared with plating in the field. There was good correlation between the two methods as a means of identifying patients with infected skin lesions. There was a poor correlation from throats and none of the 10 positive cases picked up by the delayed swab would have been identified by direct plating (Tables 2 and 3).

Bacitracin-resistant (presumably not group A) strains of beta-hemolytic streptococci were isolated 16 times (7.2%) from skin lesions by direct plating and on 11 occasions (5.0%) by delayed culture.

Throat cultures yielded bacitracin-resistant strains three times by direct culture (6.4%) and on two occasions (4.3%) by delayed culture (Table 4).

Five skin lesions in Colombia yielded posi-

tive cultures for bacitracin-sensitive *S. pyogenes* on initial plating in the field, but not from the delayed culture technique. In every case, however, the initial culture was recorded as scanty (less than 10 colonies per plate).

DISCUSSION

During our surveys in tropical areas of the world, we have consistently recovered group A streptococci from over 90% of purulent skin infections. We attribute this high recovery rate to improved methodology. By comparative testing we have eliminated neomycin, naladixic acid, phenyl ethyl alcohol, and sodium azide in favor of crystal violet (Difco) as the selective agent in our medium. Rejection of the other agents was based on lower recovery rates, difficulty in reading hemolysis, or requirement for increased incubation time. Crystal violet blood agar (TSAB-CV) is preferred over plain blood agar in view of the lower contamination in the field and increased recovery of beta-hemolytic streptococci.

Double-layered plates are considered worth the additional time of preparation because of the unequivocal zones of hemolysis which this method permits, and our observation that the sheep cells are less likely to hemolyze on storage of plates. Sheep blood is chosen because of the excellent zones of hemolysis produced

TABLE 1. Recovery of group A *S. pyogenes* in four field surveys

Culture	No. of cultures taken	Positive direct plating	% Recovery	Positive delayed culture	% Recovery
Miami skin	27	21	78	24	89
Haiti skin	17	17	100	16	94
Uganda skin	86	64	74	63	73
Colombia skin	93	71	76	66	71
Total	223	173 ^a	78	169 ^a	76
Uganda throat	47	2 ^b	4	10 ^b	21

^a No significant difference between methods.

^b Significant difference in favor of delayed culture $P < 0.05$.

TABLE 2. Semi-quantitative results of group A *S. pyogenes* cultures

Culture	Direct plating TSAB-CV			Culture delayed 3 to 4 weeks		
	Scanty ^a	Moderate ^b	Profuse ^c	Scanty	Moderate	Profuse
Uganda skin (86) ^d	1	0	63	1	3	59
Uganda throat (48)	2	0	0	6	4	0
Colombia skin (93)	2	0	69	2	4	60
Total	5	0	132	9	11	119

^a Zero to 10 colonies.

^b Ten to 50 colonies.

^c Fifty or more colonies.

^d No. of cultures taken.

TABLE 3. Recovery of group A *S. pyogenes* from skin lesions

Delayed culture	Direct plating		Total
	Positive	Negative	
Positive	166	3	169 ^a
Negative	7	47	54
Total	173 ^a	50	223

^a No significant difference in recovery of *S. pyogenes* between methods.

and because it inhibits the growth of *Haemophilus hemolyticus*, which could be mistaken for beta-hemolytic streptococci (13).

Calcium alginate swabs are chosen on the basis of literature reports of increased recovery over cotton swabs (2, 4) and the fact that the fibers are partially soluble and probably release more bacteria on to the agar surface during the plating procedure.

When in one study (7) cutaneous lesions diagnosed clinically as streptococcal ecthyma were selected, our recovery rate of group A streptococci was 100%, so that we must conclude that further improvement in our basic methods are unlikely.

Streptococcal pyoderma, however, is a worldwide problem which is more prevalent among populations in the tropics with low standards of living, poor hygiene, and overcrowding, and carries with it the additional danger of acute glomerulonephritis (14).

There is a need for epidemiological methods which do not require media or sophisticated laboratory facilities in the country under study. The areas in which the streptococcal problems are most significant in many cases have few laboratory services, and teams operating in remote areas may be cut off from their base laboratory even when such a facility exists.

As early as 1965 we abandoned attempts to use Stuart transport medium because of the poor recovery of beta-hemolytic streptococci from clinical lesions and overgrowth by extraneous gram-negative bacteria and molds during transportation by airmail from Vietnam to Miami, Fla. Similar findings were reported by Amies and Douglas (1).

Desiccation of swabs for the subsequent recovery of beta-hemolytic streptococci has long been known. The Hollinger method utilizing filter paper strips was introduced in 1960 (5), and the silica gel method described here was advocated for throat swabs by Redys et al. in 1968 (9). Many of the published studies deal with survival of pure cultures in the laboratory. All have been related to recovery of hemolytic

streptococci from throats, and most have been based on survival over the few days required to mail a specimen to a laboratory.

Our studies indicate that calcium alginate swabs desiccated in sterile silica gel in the field provide a method which should enable accurate epidemiology of streptococcal infections of throat and skin to be conducted in any area of the world without the need for media, and with a 3- to 4-week delay before returning to a base laboratory. We observed that the swabs which had been desiccated yielded cleaner cultures than swabs plated in the field, and we achieved equal recovery rates from the delayed swabs regardless of whether they were plated on TSAB or TSAB-CV. Other studies we have conducted showed a significant advantage in using TSAB-CV in the field particularly in wet conditions, where contaminating gram-negative bacteria rendered TSAB almost useless. The desiccated swab, therefore, has an additional advantage of eliminating many other bacteria which do not survive drying. It is well recognized that the recovery of group A streptococci from the throat is capricious and that multiple swabs or use of two or more methods of recovery will yield additional positive cultures (12). Our study tends to confirm this and further suggests that, if only one method is practical, the silica gel swab may well be the best choice.

We experienced some difficulties with perforation of the aluminum foil packets caused by the sharp crystals of silica gel. These pinholes resulted in a 20% loss of packages prior to use under rough field conditions, due to absorption of water vapor as determined by change in color of the indicator incorporated in the silica gel. This aspect requires modification before the system can be completely acceptable.

Some questions have been raised concerning the cost of the system for large surveys, but our experience suggests that the elimination of in-country laboratory support, the difficulties of supplying fresh media in the field, and the potential for microbiological disasters brought

TABLE 4. Recovery of group A *S. pyogenes* from throats

Delayed culture	Direct plating		Total
	Positive	Negative	
Positive	0	10	10 ^a
Negative	2	36	38
Total	2 ^a	46	48

^a Significant difference in favor of desiccated swab $P < 0.05$.

about by supply breakdowns, power failures, transportation difficulties, and environmental contamination make the silica gel method well worth any additional costs in materials. Although the method, in our opinion, leaves no country inaccessible to streptococcologists, it offers similar advantages to the conduct of large-scale screening of, for example, school children or military recruits in the United States.

The method described here would, therefore, allow investigators to carry their specimens or ship them on passenger aircraft at the completion of their field work, and would also allow physicians to mail swabs to a receiving laboratory.

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