Improved Dry-Swab Transportation for Streptococcal Specimens

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LARGE-SCALE CULTURING of throat swabbings for group A streptococci is currently advocated as essential to primary prevention of the rheumatic sequelae of streptococcal infections (*1*). This proposal is based upon a conclusion that streptococcal infections cannot be diagnosed accurately without supporting laboratory evidence (*2*).

Physicians can obtain this evidence in many States and in some municipalities simply by mailing or delivering specimens of throat swabbings to a public health laboratory (*3*, *4*). Since delays varying from 24 to 72 hours may occur between collection and culture of the specimens, a transport outfit or kit which assures viability of the streptococci after transit is mandatory.

Outfits which keep the specimen moist in either a nutritional or nonnutritional base until cultivation on blood agar have been proposed (*5–9*). This principle was applied in the nutritive-free, 1 percent agar gel transport medium which the laboratory of the Connecticut State Department of Health used for many years in its collection outfit for throat swabbings. The swab specimen was inserted into the agar gel in a cotton-stoppered tube for transmission to the laboratory. The mailing outfit included a metal screw-capped inner cylinder and a fiberboard screw-capped outer container. The screw-capped containers and test tubes were expensive but were reusable. The costs for washing, sterilizing, making the agar gel, and reassembling the outfits were absorbed with other overhead expenses; these labor costs were not at the time prohibitive because the volume of specimens did not exceed 5,000 annually.

Heightened interest in group A streptococci has led, however, to an unprecedented increase in requests for examinations of throat swabbings. Hence, cost and convenience have become very important and have compelled the development of a mailing outfit that will be at once less expensive, more convenient for large-volume processing, commercially available, disposable, and effective in preserving streptococcal viability.

The Hollinger filter strip method (*10–12*) appeared to fulfill these requirements, but it has several disadvantages. After collection, proper usage requires physicians to air-dry the strip before sealing and mailing, and busy physicians may not at all times perform this task judiciously. Compliance with the recommendation to remove the strip 4 to 6 hours after its application to the blood plate necessitates staffing a laboratory beyond normal closing hours for specimens received in the late afternoon. Moreover, there is no swab to incubate in broth for later use in preparing smears for fluorescent antibody staining, a step our laboratory has found convenient and successful (*4*).

A dry-swab outfit for mailing (*13*, *14*) ap-

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peared more suited to our purpose, but conflicting evidence regarding the efficacy of such a procedure (*7*, *15*, *16*) fostered some reservations and prompted our studies.

This report pinpoints conditions which adversely affect the reliability of dry-swab mailing procedures and provides evidence favoring the use of a silica gel desiccant to counteract these adverse conditions. The supporting staff of the streptococcus unit of our diagnostic microbiology laboratory supplied the technical assistance needed to obtain the data. Based on these studies, a practical and effective dry-swab collection outfit for streptococcal specimens has been developed.

Materials and Methods

Sterile, individually packaged, cotton swabs were used for all studies.

Streptococcus pyogenes group A (National Communicable Disease Center, Public Health Service, strain SS–132) was used to measure survival on dry swabs under a variety of experimental conditions.

Sterile, diluted rabbit serum (one part normal rabbit serum and nine parts phosphate-buffered saline, 0.01 M, pH 7.2) was used to prepare streptococcal cell suspensions and to simulate throat exudate. Pilot studies had shown that the cells remained viable but failed either to multiply or to die off appreciably in this menstruum during 3 days at room temperature. However, cells in saline, buffered saline, or buffered distilled water died off rapidly under similar circumstances.

To seed the swabs, a small portion of the growth from an 18–24-hour blood agar plate was suspended in the sterile diluted rabbit serum to a density measuring 90 percent transmission on a Bausch and Lomb spectronic 20 spectrophotometer and then further diluted 10 times. Approximately 0.5 ml. of the cell suspension was spread on the surface of a firm, nonnutritive agar plate (1.5 percent Ionagar, Colab, in phosphate-buffered saline), and excess fluid was removed with a sterile pipet. Partially covered plates were placed in a 30° C. incubator for 5 to 10 minutes to dry. This dry surface was used as the inoculum, and the swabs were rolled over the dried inoculum three or four times. Seeding the swabs by this method produced colony counts of 100 to 300 on streak tryptose blood agar plates after overnight incubation at 35° C. in an atmosphere of 90 percent N_2 and 10 percent CO_2.

The tryptose blood agar was made according to the following formula: Bacto agar 15 gm. (1.5 percent), tryptose 20 gm. (2 percent), NaCl 5 gm. (0.5 percent), distilled H_2O 1,000 ml.; final pH 7.2; defibrinated sheep blood (6–7 percent), added when plates are poured. To permit critical detection of loss in viability of the streptococci, the test swabs were semiquantitatively seeded with small, rather than large, numbers of cells. Seeding the swabs by dipping or by direct application of the cell suspension was avoided to eliminate any effect that the fluid menstruum itself might have on survival of streptococci under these circumstances.

Seeded swabs were placed immediately in sterile 15 by 100 mm. screw-capped vials, some of which contained silica gel. Certain of the vials without silica gel were allowed to remain within the experimental environments with their caps removed, while others were tightly closed. All the vials containing silica gel crystals were tightly closed.

Test environments were improvised by using covered 3-gallon-capacity screw-capped glass jars containing either thoroughly moistened paper towels or approximately 200 gm. of silica gel. Relative humidities achieved in the jars under these conditions were approximately 95 percent for the jars with the towels and 25 percent for those with the silica gel, as measured by the "wet-dry" bulb method. To achieve the test temperatures, we placed the jars in incubators regulated at 10° C. (50° F.), 20° C. (68° F.), and 30° C. (86° F.). Although the temperatures selected do not include the extremes of heat and cold to which specimens in transit may be subjected, they are reasonably representative of conditions in Connecticut at different times of the year.

A throat-swab collection and mailing outfit which incorporated the convenient use of silica gel as a desiccant during transport was then evaluated under actual conditions of use in comparison with a similar outfit without silica gel. A packaged sterile cotton swab, a pouch or packet containing silica gel crystals, a heavy self-sealing envelope, and a manila pre-

Table 1. Effect of simulated exudate on the recovery of streptococci from swabs held over a 72-hour period at different temperatures

Amount of exudate added	Held at 50° F. for—				Held at 68° F. for—				Held at 86° F. for—			
	0 hour	24 hours	48 hours	72 hours	0 hour	24 hours	48 hours	72 hours	0 hour	24 hours	48 hours	72 hours
None	+	+	+	+	+	+	+	+	+	+	±	±
0.04	+	+	+	+	+	+	0	0	+	0	0	0
0.08	+	+	+	+	+	+	0	0	+	±	0	0
0.16	+	+	+	+	+	+	±	0	+	±	0	0

NOTE: Estimated colony counts on blood agar plates after overnight incubation at 35° C. under atmosphere of CO_2 and N_2. 0=no colonies, ±=fewer than 100, +=100 or more.

addressed return envelope comprised the kit mailed to physicians (*A–D*). The outfits were assembled in the central services area of the laboratory division.

Results

Effect of exudate on streptococcal survival. That swabs become moistened with exudate to varying degrees during collection is a reasonable assumption. The possibility that this moist condition might, under certain circumstances, influence recovery of streptococci from dry swabs was investigated.

Seeding of the swabs was immediately followed by application of the simulated exudate drop by drop from a calibrated Pasteur pipet. Next, the swabs were placed and held in tightly closed, screw-capped vials at the three temperature levels mentioned. At intervals, some vials were removed and the swabs streaked onto sheep blood agar plates. Colonies of beta hemolytic streptococci were counted after overnight incubation at 35° C. in an atmosphere of 90 percent N_2 and 10 percent CO_2.

The data in table 1 indicate that the amount of fluid (simulated exudate) present on the swab may affect the recovery of streptococci from originally dry swabs and suggest that this effect depends upon the temperature at which the swab has been held. Recovery of organisms when swabs were held through a 72-hour period at 50° F. (refrigerator temperature) was excellent regardless of the amount of exudate. After exposure of swabs to 68° and 86° F., recovery was satisfactory after the 72-hour holding period only from swabs to which no exudate had been added. Recovery of organisms from the exudated swabs held at 68° F. was poor, and no colonies were observed on plates streaked with swabs held longer than 48 hours. Recovery from exudated swabs exposed to a temperature of 86° F. was even less productive. This experiment was repeated numerous times with similar results.

Effect of ambient temperature and humidity. Dry-swab specimens transported through the mails normally undergo varying conditions of temperature and humidity and are affected by other factors such as type of mailing container and speed of mail service. Using swabs seeded with cells to which 0.04 ml. of simulated exudate had been added, we tested the effect of several controlled environmental conditions on the survival of streptococci. Swabs so prepared were immediately placed in uncapped vials and held in the test environments for 3 days. At intervals, the swabs were removed and streaked on blood plates.

Table 2 shows that neither the low nor high

Table 2. Effect of temperature and relative humidity on the recovery of streptococci from dry swabs held in uncapped vials

Holding period (hours)	At 50° F.		At 86° F.	
	25 percent humidity	95 percent humidity	25 percent humidity	95 percent humidity
0	+	+	+	+
24	+	+	+	0
48	+	+	+	0
72	+	+	±	0

NOTE: See note, table 1.

levels of humidity adversely affected the recovery of streptococci from swabs held at the relatively cold temperature of 50° F. When similar swabs were held at 86° F. in a highly humid environment, however, the streptococci died off rapidly. Swabs held at the same warm temperature, but in a dry environment, yielded streptococci for the 3-day test period.

Effect of silica gel desiccant on recovery. Improved recovery of streptococci from dry swabs in contact with silica gel was reported by Hollinger (*10*) and Hosty (*11*), but the reason for the enhancement was not explained fully. A question arose as to whether the effectiveness of the desiccant might be due to its potential to nullify certain adverse effects of the exudate and environmental conditions upon the streptococci.

An experiment was planned to test the desiccant with seeded and "exudated" swabs under varying conditions. Swabs seeded with cells and 0.04 ml. of the simulated exudate were prepared for each set of test conditions and held in either uncapped vials, closed vials, or closed vials containing approximately 1.5 gm. of silica gel. Glass jars were used to produce the environmental test conditions.

Table 3 shows that recovery of streptococci was effective from swabs held at 50° F. at both low and high humidity. At 50° F., silica gel offered no particular advantage to survival of the streptococci. At 86° F. with low humidity, swabs held in the uncapped vials yielded streptococci for the entire test period. It is important to note, however, that swabs held in closed vials under similar environmental conditions failed to yield streptococci after a 24-hour holding period. Once again it is apparent that the moisture from the exudate seriously affected the viability of the organisms when they were retained within a small sealed-off space. At the higher temperature with high ambient humidity (in jars), streptococci on swabs in both closed and uncapped vials died off rapidly, whereas swabs in contact with silica gel in sealed vials yielded streptococci for the entire test period, even though a temperature of 86° F. and high humidity were maintained in the atmosphere surrounding the vials.

Silica gel outfit in routine use. Studies with field specimens clearly show how essential desication (silica gel) is in dry-swab transportation. In one study, 728 pairs of throat swabbings were collected, and each pair was forwarded simultaneously by the physician to the laboratory. One of each pair was transported in a sealed, foil-lined envelope without silica gel and the other, in a similar envelope containing the desiccant. The results were as follows:

Streptococci found	*No silica gel*		*In silica gel*	
	Number	*Percent*	*Number*	*Percent*
Beta hemolytic	112	15.3	148	20.3
Group A	87	12.0	111	15.2

The study was carried out during June and July, a period when the incidence of streptococcal infections is low in Connecticut. The yield was greater from swabs submitted in silica gel (5.0 percent more beta hemolytic streptococci and 3.2 percent more group A streptococci). A simple statistical comparison of these results shows a significant difference

Table 3. Effect of silica gel on the recovery of streptococci from seeded and exudated swabs held at different temperatures and relative humidities

Holding period (hours)	Swabs held at 50° F. in—				Swabs held at 86° F. in—			
	Uncapped vials		Closed vials[1]		Uncapped vials		Closed vials[1]	
	25 percent humidity	95 percent humidity	Without silica gel	With silica gel	25 percent humidity	95 percent humidity	Without silica gel	With silica gel
0	+	+	+	+	+	+	+	+
24	+	+	+	+	+	0	0	+
48	+	+	+	+	+	0	0	+
72	+	+	+	+	+	0	0	+

[1] Closed vials were not affected by varying humidity within the jars in which they were held.

NOTE: See note, table 1.

Table 4. Comparative recovery of streptococci from routinely mailed specimens randomly selected each day over a 6-month period of 1966, transported with and without silica gel

Sampling period	Number of specimens of each type	Swab not in silica gel				Swab in silica gel			
		Positive beta hemolytic streptococci		Positive group A streptococci		Positive beta hemolytic streptococci		Positive group A streptococci	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
June 15–July 31	437	75	17.1	59	13.5	105	23.4	70	16.0
Aug. 1–Sept. 15	552	79	14.3	54	9.8	115	20.8	63	11.3
Sept. 16–Oct. 31	500	117	23.4	84	16.8	142	28.4	101	20.2
Nov. 1–Dec. 15	645	125	19.4	106	16.5	195	30.0	173	27.0
Total	2,134	396	18.7	303	14.2	557	26.0	407	19.2

NOTE: A random selection of equal numbers of each type was made daily.

(chi-square=6.1) in favor of silica gel for the transport of beta hemolytic streptococci, but its superiority for the transport of group A streptococci is not clearly demonstrated (chi-square=3.4). Further work in which a somewhat different type of comparison was made suggests that the less favorable result for group A streptococci may have been influenced by the low incidence of group A streptococcal infections in the material studied.

Another opportunity to evaluate the silica gel outfit occurred during a time when both the newly developed outfits and similar dry-swab outfits without silica gel were in use. A comparison of results with both types of specimen outfits received during the 6 months of the year when Connecticut experiences widely variable climatic conditions is shown in table 4. Determinations of chi-square values for the various sets of data shown are as follows:

	Chi-square	
Time of year	*Beta hemolytic streptococci*	*Group A strepto-cocci*
June 15–July 31	6.3	1.1
Aug. 1–Sept. 15	8.1	.8
Sept. 16–Oct. 31	3.3	1.9
Nov. 1–Dec. 15	20.4	20.5
June 15–Dec. 15	35.0	18.3

The highly significant chi-square values for the entire 6-month period offer conclusive evidence that the use of silica gel as described is an essential for survival of streptococci transported on swabs which are to be examined either for beta hemolytic streptococci, or more specifically, for group A streptococci. It is interesting to note that this conclusion could not have been reached without inclusion of data from a portion of the season (November 1–December 15) when group A streptococcal infections begin to reach significant proportions in Connecticut. The untested inference is that the numbers of group A streptococci originally present on the swabs examined affected comparisons made from June 15 through October 31. Their presence probably accounts for the fact that the data on recovery of group A streptococci from paired swabs approached but failed to achieve statistical significance.

Discussion

Both laboratory controlled studies and data from clinical specimens presented strong evidence favoring the use of silica gel for dry-swab transportation of routine specimens.

The mailing outfit we tested was designed to provide durability sufficient to confine the specimen during unusually rough intransit handling, as well as to afford impermeability to moisture, convenience for physicians and laboratory personnel, disposability, an indefinite shelf life, and compactness, so that it could be easily stored in the physician's office or his bag.

The swab specimen is inserted immediately after collection into a packet containing silica gel crystals. Doublebacked adhesive tape on the packet provides for resealing. The sealed specimen is inserted into the self-sealing Kraft-foil

envelope, which is submitted to the laboratory, along with the specimen invoice, in a Columbia-clasp, self-addressed manila envelope. Thus, three substantial layers are provided to protect and confine the specimen during transit.

On arrival in the laboratory, such specimens can be processed with ease. Accession numbers are stamped on the foil envelopes and on the corresponding specimen invoices. These envelopes are opened along the top with heavy shears or an electric opener. Each packet containing the swabbing and silica gel is removed from its opened foil envelope for culture. The packet is opened by unfolding the sealed corners. Since contaminated gel crystals may adhere to the swab, it is removed gently over a towel soaked with bactericidal agent, on which adherent crystals may fall. Crystals of the proper mesh size and quality have not been found to interfere with cultural or FA procedures, whereas a powdery form of silica gel is troublesome, as well as more hazardous.

The swabbing is removed, dipped in a prenumbered tube of Todd-Hewitt broth, streaked on a tryptose blood plate, and then placed into the same tube for overnight incubation at 35°C. The plate is also incubated overnight at the same temperature in an atmosphere of 90 percent N_2 and 10 percent CO_2. Appearance of beta hemolytic colonies on an incubated plate is the criterion for applying fluorescent antibody staining for group A streptococci to the corresponding broth culture; other broth cultures are considered negative (*4*).

Whether or not organisms other than streptococci will consistently survive this method of transportation has not been determined. Until this question is resolved, the method cannot be recommended for transportation of other organisms.

While the knowledge that streptococci survive well on dried surfaces is not new, application of this principle to the transportation of streptococcal throat cultures is relatively recent (*13*, *14*) and as yet not widely accepted (*3*, *17*, *18*). Poor experiences with dry-swab methods for streptococcal specimens may have been due to (*a*) use of saline, which we found to affect adversely the viability of streptococci held in suspension, (*b*) failure to consider the amount of attendant fluid on experimental swabs, (*c*) failure to consider ambient temperature and humidity during the holding period, and (*d*) failure to desiccate the swabs rapidly.

If swabs could be held in a mechanical refrigerator or some other dry environment or could be collected with little or no moistening with exudate, use of a drying agent such as silica gel would be unnecessary. Since these two conditions are uncontrollable in the culture program of a central laboratory, silica gel in conjunction with dry-swab transportation is recommended.

During transportation of throat swabs kept dry by exposure to silica gel, the numbers of beta hemolytic streptococci may be altered or overgrowth may occur because of concomitant flora. The significance in respect to the clinical condition of the patient of the relative numbers of colonies appearing on the blood agar plates would be debatable under such conditions. Our results suggest that rapid drying of swabs immediately after collection and transporting them in contact with silica gel could contribute to an evaluation of the numbers found by preserving the viability of the streptococci present on the swab and at the same time prevent overgrowth by other flora.

Summary

Exposure under controlled conditions of group A streptococci to relative humidities of 25 and 95 percent at holding temperatures of 50°, 68°, and 86° F. led to the development of a disposable and inexpensive dry-swab outfit for collecting and transporting throat swabbings. The outfit provides for immediate and continuous contact of the material collected on dry swabs from suspected throats with a silica gel desiccant in a sealed, Kraft laminated-to-foil pouch. Recoveries in a central laboratory of group A streptococci from delayed cultures (1 to 3 days after collection) were consistently and significantly greater with such outfits than when similar outfits without silica gel were used. Assembly of the outfit is convenient for laboratory personnel, it stores compactly in a physician's office or in his bag, and the components are available commercially.

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EQUIPMENT REFERENCES

(*A*) Cotton swabs, sterile, packaged singly, #3066, Acme Cotton Products Co., Inc., Valley Stream, N.Y., $6 per 1,000.

(*B*) Silica gel packets, to be delivered in airtight cans. Pouch: heat-sealed polyfoil bag, outside dimensions 2 by 4½ in., 25#, bleached; S.K., 0.00035 foil, coated with 0.001 polyethylene, printed one color with instructions for use, containing approximately 1.5 gm. of silica gel, mesh size 12–28, grade #08 blended with approximately 3–5 percent indicator blue (grade #46); contents of pouch must pass usual sterility tests in laboratory. Doublecoated paper tape adhesive patch with release liners to be placed on silica gel packets (used to seal packet after specimens have been inserted), overall dimensions 2 by ¾ in.; adhesive area 2 by ½ in., adhesive to be 3M–#410 doublecoated or equivalent; unprinted. Carter Rice Storrs & Bement, Inc., 179 Park Ave., East Hartford, Conn. 06108, $4,145 per 100,000; $9,875 per 250,000.

(*C*) Kraft laminated-to-foil envelopes (to hold silica gel packet with collected specimen), 3 by 7½ in. outside dimensions (2¼ by 7⅛ in. inside dimensions), open end, ⅜ in. seal on two sides and one end, plus 1 in. lip with ¾ in. adhesive. Material to be used to be equal to MIL–B–131C class 2 amend. 2; instructions for use to be printed in red on one side. Carter Rice Storrs & Bement, Inc., $1,990 per 100,000, or Chatfield Paper Co., 233–239 State St., P.O. Box 1850, New Haven, Conn. 06508, $3,075 per 100,000.

(*D*) Envelopes, manila, Columbia clasp (standard sizes stocked by almost all envelope companies).

1. Outside, return, for submitting specimens to laboratory, 5 by 7½ in., 32 lb., No. 35, printed with address of laboratory and space for sender's name and address in upper left corner, $1,030 per 100,000.
2. Outside, for mailing outfits to physicians (holds 1–4 outfits)—5½ by 8¼ in., 32 lb., No. 50, flap on left side (to facilitate use in mailing machine), printed with laboratory return address in upper left corner, $288.75 per 25,000.
3. Outside, for mailing outfits to physicians (holds 12 outfits), 9 by 12 in., 32 lb., No 90, printed with laboratory return address in upper left corner, $426 per 25,000.

NOTE: Prices on all supplies are those quoted by suppliers to the Connecticut State Department of Health for the quantities indicated; other sources for comparable materials are probably available elsewhere.