Throat cultures from patients with clinical respiratory infections were examined after having been mailed to the laboratory. The authors conclude that the mail-in methods studied are effective for the recovery of beta hemolytic streptococci.

EVALUATION OF THE RECOVERY OF BETA HEMOLYTIC STREPTOCOCCI FROM TWO MAIL-IN METHODS

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STUDIES aimed at the primary prevention of acute rheumatic fever have been predicated on the principle and practice of prompt and adequate treatment of respiratory infections due to Group A beta hemolytic streptococci.[1,2] In spite of the realization that a positive throat culture has proved to be the most effective method for the detection and most reliable indication for treatment of streptococcal disease, this procedure has not been widely used by practicing physicians. The reasons for this appear to be threefold: first, many physicians feel that they can diagnose streptococcal disease clinically with reasonable accuracy; second, the cost of the usual throat culture is frequently prohibitive; and third, the presently acceptable technics require elaborate adjacent laboratory facilities.

The difficulty, experienced by clinicians, in accurately diagnosing streptococcal infections, without throat cultures, has been documented.[3-6] Investigations, aimed at simplifying the laboratory diagnosis of beta hemolytic streptococcal disease and at the same time, making the facility more accessible to physicians, have been numerous.[7-10]

A pilot study was begun four years ago to encourage physicians to take throat cultures for accurate diagnosis as a guide to adequate therapy of beta hemolytic streptococcal infections. The response to the study was very encouraging. However, the method in use at the time, which was supplying sterile sheep's blood agar plates by messenger, proved to be too expensive and cumbersome for application to a city-wide service. Hollinger and Lindberg[9] had reported on the use of a filter paper kit, which appeared to be as accurate for the recovery of streptococci as the plate and could be sent through the mail. Catanzaro, et al.,[11] demonstrated in a controlled study that adequate penicillin therapy aimed at eradicating beta hemolytic streptococci from the throat would prevent primary attacks of acute rheumatic fever, even if delayed as long as nine days after the onset of pharyngitis. The present study was undertaken in order to find a method which was as accurate as the sheep's blood agar plate, immediately streaked for the diagnosis of beta hemolytic streptococci, and had the additional advantage of requiring no messenger service.

Materials and Methods

Three separate throat culture studies were done. Study No. 1 compared the

accuracy of recovery of beta hemolytic streptococci from a mailed serum impregnated dacron swab and filter paper strip (culpak-kit) * to the recovery from a paired swab inoculated immediately onto a sheep's blood agar plate. Dacron swabs had been recommended for use with the filter paper strip because they were less absorbent.[9] The swabs originally were impregnated with serum because of the work of Rubbo and Benjamin,[12] who demonstrated that serum swabs (cotton and wool, absorbent and nonabsorbent) significantly increased survival time and recovery rate of beta hemolytic streptococci, even from carriers.

Throat cultures were obtained from the private patients of four cooperating physicians and the pediatric patients from two clinics in the city. Paired dacron swabs were used to swab the patient's throats. One swab was then inoculated onto a blood agar plate. The other swab was streaked onto a filter paper strip and then placed into a glass tube. The filter paper strip was allowed to dry, rewrapped in its container, and both the tubed swab and the filter paper strip were mailed to the laboratory. The blood agar plate was taken to the laboratory within two hours, streaked and incubated.

When the swab and filter paper arrived at the laboratory, usually within 24 hours, occasionally as long as 72 hours, the swab was streaked onto a blood agar plate and incubated. The filter paper strip was placed aseptically onto one-half of a blood agar plate and incubated for six hours. Then the strip was removed to the other half of the plate and incubated again. The results of the cultures were read after 18 to 24 hours' incubation. The positive cultures were graded 1 to 4 plus as follows: cultures showing less than 10 colonies of hemolytic streptococci were graded 1 plus, 10 to 50 colonies 2 plus, over 50 to 200 colonies 3 plus, and 4 plus if there was confluent growth of hemolytic streptococci. Subcultures were done when necessary, and all positive cultures were grouped using the Bacitracin disc technic.[13] The majority of the cultures were processed at the Bacteriology Laboratory of the Chicago Board of Health.

Study No. 2 was done to compare the accuracy of recovery of beta hemolytic streptococci from four types of swabs. Dacron swabs are more expensive than cotton swabs, and serum impregnation of swabs is time-consuming and expensive. It was desirable, therefore, to determine whether accuracy was enhanced by serum impregnation of swabs for the mail-in method. Six hundred and fifty throat cultures were taken, using one plain cotton swab, one serum impregnated swab, one plain dacron swab and one serum impregnated swab. All four swabs were swabbed over the throat simultaneously, rotating them carefully so that each swab contacted the pharyngeal area. The patients were very cooperative, being accustomed to this procedure, since they were involved in another study requiring their constant contact with the personnel who consequently had good rapport with them. A fifth swab was also used to swab the throat, and it was inoculated onto a sheep's blood agar plate immediately, streaked and incubated. The four swabs were placed in aluminum foil and mailed to the laboratory. When received in the laboratory, the swabs were streaked individually onto sheep's blood agar plates and incubated. The cultures were read as previously described. The blood agar plate inoculated by the fifth swab had been prepared from a dehydrated agar base and recovered only 63 per cent of the positive cultures; therefore, it was not possible to compare the four swabs to the plate,

* Supplied: Diagnostic Associates, Inc., Walnut Creek, Calif.

Table 1

Study No. 1—Evaluation of Two Mail-in Methods for the Recovery of Streptococci in 1,008 Triple Cultures

Methods	Positive Cultures	Per cent Recovery of Streptococci	Cultures Showing No Growth	
			Total	Positive by One Other Method
Immediate plating	336	86.5	1	0
Dacron swab (mail-in)	345	88.9	16	0
Filter paper strip (mail-in)	334	86.0	73	20

and the swabs were compared to each other in recovery of streptococci.

Study No. 3 was done in order to further test the interlaboratory reproducibility and accuracy of the mail-in method for throat cultures. Paired plain dacron swabs were used to swab the throat as previously described. All sheep's blood agar plates were prepared from fresh beef extract, tryptose agar, and sheep's blood. One swab was inoculated onto a plate immediately and the other swab was mailed to the laboratory. Two sources for the throat cultures were used. One source was a hospital clinic, and the other was the private office of a practicing physician. The plates from the first source were inoculated at the laboratory of the participating hospital clinic, and the paired swabs were mailed to another laboratory. The plates from the second source were inoculated, streaked, and incubated in the office, and the swabs were mailed to the laboratory. A messenger delivered the inoculated blood agar plates, after incubation, between the laboratories. The plates were read by the respective technicians at each laboratory as positive or negative and graded 1 to 4 plus. The plates were then returned to the respective laboratories, subcultured as necessary and grouped either by the Lancefield[14] or Bacitracin disc method[13] or both.

Results

Triple Cultures—Study No. 1

There were 1,008 triple cultures obtained during Study No. 1 from February, 1959, to July, 1959; 38 per cent of the cultures were positive. Comparison of the three methods revealed each to be accurate in recovering beta hemolytic streptococci (Table 1). Actually, the mailed swab appeared to be slightly superior but not statistically so ($p=0.99$). The least accurate method was the filter paper. This diminished accuracy was due to the high percentage, 7.2 per cent, of filter papers showing no growth. A small number of swabs, 1.5 per cent, showed no growth on culture. Of the 15 swabs showing no growth, none were positive by either of the other methods. However, 20 of the 76 filter paper strips showing no growth were positive by one or both of the other methods. Because of the large number of the filter paper strips showing no growth, it was desirable to know if this result was due to a difference in technic. All

Table 2

Study No. I—Evaluation of Dacron Swab Mail-in versus Immediate Plating in Relation to Degree of Positivity of the Culture, Total Positive Cultures—386, No Growth Cultures Counted as Negative

Dacron Swab	Immediate Plating			
	Negative	1-2+	3-4+	Total
Negative	621	31	11	663
1-2+	39	56	25	120
3-4+	12	56	157	225
Total	672	143	193	1,008

sources, but one, had some filter paper strips showing no growth, but more than half of the total number were from two sources. The disadvantage of the filter paper strip appears to be the necessity to transfer moisture to the filter paper and the required air drying. These factors may make the filter paper, in inexperienced hands, somewhat less reliable than the mailed dacron swab. In addition, comparison of the efficiency of the three methods revealed that the original culture plate prepared from the filter paper strip rarely showed colony isolation. As a result, the filter paper strip required many more subcultures for positive diagnosis.

Data were also collected which related to the degree of positivity of cultures. Many previous studies have demonstrated that a single culture for streptococci during the carrier state is likely to be negative, and that multiple cultures enhance the chance of recovering the organism, irrespective of the culture method used.[15,16] An evaluation was therefore made of the reliability of the three culture methods for recovery of small inocula of streptococci. The results indicated that, although there was disagreement in the recovery of streptococci in many instances, this disagreement was distributed in a random manner among the three methods studied, with some superiority of the swab being noted (Tables 2 and 3). The latter manifested itself in more luxuriant growth from the swab associated with less positive cultures from the plate and filter paper (Table 2). This difference between the plate and swab is statistically significant, p being less than 0.001.

When the cultures showing disagreement were divided according to positivity of the cultures, it was noted that when the culture was only 1 to 2 plus, the disagreement approached 50 per cent. However, when the culture was 3 to 4 plus, presumably representing active clinical infection, the disagreement averaged less than 10 per cent.

The relationship of the positive cultures to specific clinical symptoms was somewhat difficult to assess because individual symptoms and signs were not considered as single entities. It was noted, however, that the presence of significant cervical adenopathy definitely increased the probability of a positive culture. Patients with coryzal symptoms and pharyngitis without exudate had 35 per cent positive cultures. However, when the above symptoms were associated with cervical adenopathy, 54 per cent of patients had positive cultures; pharyngitis with exu-

Table 3

Study No. I—Evaluation of Filter Paper Strip Mail-in versus Immediate Plating in Relation to Degree of Positivity of the Culture

Filter Paper Strip	Immediate Plating			
	Negative	1-2+	3-4+	Total
Negative	618	35	21	674
1-2+	41	68	56	165
3-4+	13	44	112	169
Total	672	147	189	1,008

Table 4

Study No. 2—Evaluation of Four Types of Mail-in Swabs for the Recovery of Streptococci. Total Cultures=650, Total Positive Cultures=263.

Type of Swab	Number Positive	Per cent Recovery
Plain cotton swab	223	84.7
Serum cotton swab	233	88.6
Plain dacron swab	234	88.7
Serum dacron swab	238	90.5

date was associated with 46 per cent positive cultures; with associated adenopathy, 62 per cent were positive. Other investigators[17,18] have noted similar figures for culture results in exudative pharyngitis.

Analysis of the recovery of streptococci from the mailed swabs and the filter paper strips during both cold and hot weather revealed no difference between the plate and mailed cultures. The cultures showing no growth were equally prevalent throughout the study. At the completion of the above study, it was evident that the mail-in method was as reliable for the recovery of beta hemolytic streptococci from throat swabs as the sheep's blood agar plate inoculated immediately. Further studies were aimed at determining the type of swab to be used.

Quadruple Cultures—Study No. 2

Table 4 shows the relative accuracy of the four swabs. There were 263 positive cultures. The least accurate method was the plain cotton swab. All three other swabs were about 90 per cent accurate. Since it was observed that the plain dacron swab compared favorably to both types of serum impregnated swab, the plain dacron swab was selected. The differences between the plain cotton swab and the other three swabs are not statistically significant (p is greater than 0.90). However, the data indicate that both of the dacron swabs and the serum impregnated cotton swabs recovered more organisms than the plain cotton swab. For a city-wide program it seems advantageous to select the method which is most accurate and economical. Table 5 shows the comparison between the plain cotton swab and the plain dacron swab in relation to positivity of the cultures. As can be noted, most of the discrepancy between the two swabs was found in the cultures showing small inocula.

Double Cultures—Study No. 3

There were 376 paired cultures obtained during Study No. 3, of which 179 were positive or 47 per cent. Group A organisms were found in 87 per cent of the positive cultures. The results of the initial readings made by the two laboratories agreed very closely.

Of the 179 cultures positive by one or both methods, 154 were positive on the sheep's blood agar plate and 149 were positive by the mailed dacron swabs; an accuracy of 86 per cent and 83 per cent respectively. Comparing the results of the cultures by source, it was noted that a preponderance of plate-positive, swab-negative cultures, 80 per cent of which were non-Group A organisms, were from one laboratory.

Table 5

Study No. 2—Comparison of Recovery of Streptococci from Two Types of Mail-in Swabs

Plain Cotton Swab	Plain Dacron Swab			
	Negative	1-2+	3-4+	Total
Negative	395	28	4	427
1-2+	20	66	24	110
3-4+	1	19	93	113
Total	416	113	121	650

Table 6

Study No. 3—Evaluation of Mail-in Method for the Recovery of Streptococci in 376 Double Cultures. Total Positive Cultures = 179.

Dacron Swab	Immediate Plating			
	Negative	1-2+	3-4+	Total
Negative	198	29	—	227
1-2+	22	51	8	81
3-4+	2	32	34	68
Total	222	112	42	376

Table 6 shows the comparison between the two methods. Again noted is the larger number of 3-4 plus cultures from the dacron swab, and the fact that the major discrepancies exist in the 1-2 plus cultures.

Table 7 summarizes the data from Study No. 1 and No. 3. There was a total of 1,367 cultures, of which 564 (41 per cent) were positive. There were 490 positive cultures detected by the plate, inoculated immediately, compared to 493 positive cultures from the dacron swab, an accuracy of 86.8 per cent and 87.2 per cent respectively. Again noted is the preponderance of 3-4 plus cultures from the dacron swab as compared to the plate.

During the Quadruple Culture Study No. 2, it had been noted that the plate prepared from dehydrated media recovered only 63 per cent of the positive cultures. One source for the throat cultures in Study No. 1 also used these plates, and when these cultures are subtracted from the total of 1,367 paired cultures, there remain 928 paired throat cultures. All of these cultures were inoculated onto sheep's blood agar plates, either immediately or after being mailed to the laboratory. The plates were prepared from fresh beef extract and tryptose agar. From this revised total, of which 409 were positive cultures, the plate detected 359 or 87.7 per cent, and the dacron swab detected 347 or 84.8 per cent.

The relative accuracy of the Bacitracin disc for grouping of beta hemolytic streptococci was also compared; 210 cultures were grouped, using both the Lancefield M precipitin reaction[14] and the Bacitracin disc method.[13] The Bacitracin disc is accurate in detecting Group A organisms, but 13 per cent of non-Group A organisms were also grouped as A by this method. No Group A organisms were grouped as non-A by this method. The discrepancy between the two methods, as shown in Table 8, is always in the direction of detecting excess Group A organisms. For routine laboratory throat culturing services, therefore, the Bacitracin disc method for grouping appears to be adequate.

Discussion

The results of these studies indicate that there is a feasible and accurate mail-in method for identifying beta hemolytic streptococci. This method is applicable to large urban areas, and is inexpensive enough to merit budgeting expenditures by most state and local health agencies. The dry dacron swab apparently does not trap the organisms or injure them and thus allows easy and intact passage onto the surface of solid

Table 7

Summary Chart—(Data from Study No. 1 and Study No. 3) Comparison of Mail-in Method versus Immediate Plating for Recovery of Streptococci. Total Positive Cultures—564.

Dacron Swab	Immediate Plating			
	Negative	1-2+	3-4+	Total
Negative	803	60	11	874
1-2+	60	107	33	200
3-4+	14	88	191	293
Total	877	255	235	1,367

Table 8

Study No. 3—Comparison of Grouping of Hemolytic Streptococci by Lancefield Precipitin and Bacitracin Disc Methods

Lancefield Grouping	Bacitracin Disc Grouping		
	Group A	Non-Group A	Total
Group A	168	—	168
Nongroup A	22	20	42
Total	190	20	210

media as long as 24-48 hours after acquisition of the throat culture.

The American Heart Association Committee on Prevention of Rheumatic Fever has indicated that community efforts toward primary prevention of rheumatic fever should be directed toward the proper recognition by the physician of the scope and nature of streptococcal infections. This implies that the physician should realize that clinical impression alone will not encompass the total disease spectrum that can be ascertained through the use of the throat culture. He should therefore employ this valuable tool in the proper management of all streptococcal respiratory disease. A mail-in service would offer this opportunity to the majority of physicians who wanted it. This service would increase in popularity as the physician realized that his diagnostic acumen alone cannot be relied upon for the diagnosis of all streptococcal disease.

It is anticipated that city-wide throat culturing with the proper use of antibiotics for bacterial upper respiratory infections would decrease the needless use of antibiotics as well as insure proper duration and dosage of specific therapy for streptococcal infections.

The effects of a throat culture program such as this method offers are threefold: first, to insure proper diagnosis and treatment of virtually all streptococcal infections; second, to eliminate many instances of the carrier state which could lead to continuation of infection and nonsuppurative complications; third, to explore the possibility of changing the present so-called irreducible minimum of new cases of acute rheumatic fever and acute nephritis seen yearly. This minimum has remained constant since the onset of the antibiotic era.[19]

The discrepancy encountered between the direct inoculated plate and the mail-in swab occurred primarily in the weekly positive cultures. The general feeling today is that these represent nonacute infections or so-called carrier state cultures or mild nonvirulent infections. These explanations are at best theoretical. Certainly there is need to evaluate and elucidate the risk of rheumatic fever as well as criteria of virulence as applied to individuals harboring 1 plus and 2 plus cultures of Group A streptococci.

Finally, the epidemiological value of city-wide throat culturing, made possible by a mail-in method, is limitless. With maximum use of this facility by both private physicians and clinics, public health departments can begin to tackle the problem of obtaining a more complete picture of the prevalence of streptococcal disease locally. Areas where high incidence of Group A streptococci appear might be ascertained. Should outbreaks of rheumatic fever appear concomitantly, the possibility of early mass prophylaxis would arise. Data concerning family contacts and index cases of streptococcal infection, as well as repeat infections per individual, would also fall within the realm of city-wide throat culturing programs.

The dacron throat culture kit is available commercially, and consists of a sterile kit of material including one dacron tipped swab and one wooden

tongue depressor, enclosed within a glassine paper envelope, hermetically sealed and covered with aluminum foil.* A standard precoded throat culture form is also to be used with this kit by all laboratories in our urban area. This will facilitate the reporting of streptococcal disease and provide a central area where all pertinent clinical information will be coded and easily accessible.

Summary

A total of 1,367 throat cultures from patients with clinical respiratory infections were examined; 41 per cent of the cultures were positive by one or more of the methods used. The dry dacron swab, mailed to the laboratory, was found to be as effective in recovering beta hemolytic streptococci as the sheep's blood agar plate inoculated immediately; the percentages were 87.0 per cent and 86.8 per cent respectively. One other mail-in method, a filter paper strip, was studied initially and found to be fairly effective in the recovery of beta hemolytic streptococci, but this method was associated with an unexpectedly high percentage of throat cultures showing no growth. From the above data, it can be concluded that the mail-in methods studied are effective for the recovery of beta hemolytic streptococci; and that, if a clinical infection is due to beta hemolytic streptococci, the probability of accurate diagnosis from a single throat culture is equally good whether it is plated immediately or mailed to the laboratory. Further, it can be stated that the discrepancies noted among the methods are primarily in the area of small inocula or non-Group A organisms.

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New Manual on TB

A "Guide for the Follow-up of Tuberculosis Cases, Contacts, Suspects" is just off the press. It was prepared by a special subcommittee of the APHA's Program Area Committee on Public Health Administration and has the endorsement of the American Thoracic Society, the Medical Section of the National Tuberculosis Association.

Since the last revision of the Guide in 1953, more has been learned about the epidemiology of tuberculosis; there are now sufficient beds available for all TB patients needing hospital care and the principal antituberculosis drugs have proved highly effective and their use combined with adequate public health technics can eventually virtually eradicate tuberculosis as a public health problem. Futhermore, as the length of hospital stay declines, the outpatient clinic assumes greater importance in the tuberculosis control program.

Of particular interest to public health physicians and nurses and to students is the chapter which covers the details of supervision of patients, contacts, and suspects. Terms such as household contacts, casual contacts, break in contact are defined. The details of medical and nursing supervision of patients, depending upon the activity of their disease, are fully outlined, as are those required in following contacts and suspects. A ready-reference chart summarizing the details of supervision is included in the book.