An Evaluation of Methods for Culturing Group A Streptococci From the Pharynx

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NEW METHODS which promise more accurate diagnosis of group A streptococcal infections of the pharynx are worthy of note for two reasons: First, prompt diagnosis permits the beginning of appropriate antimicrobial therapy, the effectiveness of which precludes suppurative complications (*1*). Second, as Rammelkamp and others (*1*, *2*) have clearly demonstrated, the eradication of streptococci within a period of 9 days or less after onset of infection prevents the development of rheumatic fever.

Diagnosis of group A streptococcal infections, based on clinical findings alone, may be accurate in 70 percent of all cases but frequently is less reliable (*3*). The proper use of current laboratory methods adds significantly to the validity of the diagnosis, although no method permits 100 percent accuracy (*4*). Physicians can be expected to improve their diagnostic skill by systematically correlating clinical observations and laboratory findings, but the achievement of highest possible accuracy in the identification of group A streptococcal infections rests on the continued improvement of bacteriological techniques. In a recent review, Stollerman (*5*) has summarized the culturing methods presently considered to be most effective.

Although certain modifications in bacteriological techniques may improve the accuracy in diagnosing streptococcal infections, they often are achieved at the cost of complexity and higher expense and consequently are of relatively limited value in clinical practice. Ideally, improvements should be directed toward greater reliability of these methods and at the same time be easily adaptable for use in clinical laboratories or in physicians' offices. This study was designed to evaluate various methods, several of them easily applicable in clinical practice, for the bacteriological diagnosis of group A streptococcal infections of the pharynx.

Our study was based on two previous investigations. The first, by Joe (*6*), showed that enteric pathogens survived for considerable periods of time in the dry state on filter paper, whereas nonpathogens of the same species did not survive. Hollinger and Lindberg (*7*, *8*) applied this approach, with dacron swabs and filter paper strips, to the identification of group A streptococcal infections and demonstrated that these organisms could be recovered with relative ease after drying on filter paper, whereas most other organisms found in the throat do not survive. As a result of the Hollinger and Lindberg report, a specially designed

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filter paper pack (*A*) was devised for use in transporting secretions obtained by pharnygeal swabbing to the laboratory.

Study 1

Throughout these studies, the culture medium used for isolation of streptococci was Difco blood agar base to which 4 percent defibrinated sheep's blood was added. The streptococci were classified in respect to group by the Lancefield method. The filter paper packets (*A*) were used when the filter paper technique was followed.

Between January 5 and May 8, 1961, multiple throat cultures were obtained from 420 persons, military personnel and their dependents, at Lowry Air Force Base, Denver, Colo., with the cooperation of Col. James Espy, chief of the AFB medical service. All these subjects were family contacts of patients with bacteriologically proved group A streptococcal infections. Pharyngeal cultures were obtained from each individual by four techniques:

1. Cotton: A cotton swab was rotated over the pharynx of the subject and then immediately used to streak a blood agar plate.
2. Dacron: The same procedure as for technique 1 except that a dacron swab was substituted.
3. Dacron hold: A dacron swab was used and then placed in a sterile, dry test tube for 1 week before it was used to streak a blood agar plate.
4. Dacron filter paper: A dacron swab was used and immediately rubbed on the surface of a sterile filter paper strip, which was dried in air. The strip was put into a sterile packet for 24 hours, after which it was removed aseptically and placed, exposed side down, on a blood agar plate. After incubation for 4 hours at 37° C., the paper was removed, and the plate streaked with a sterile loop.

It was found essential that the filter paper strip be dried before being returned to the packet. Similarly, removal of the strip and streaking of the blood agar plate after 4 hours of incubation was noted to be of equal importance for best results.

The four procedures were used in each subject; they were alternated systematically so as to negate any influence on the results of the order of culture. In both instances of direct plating, the growth was relatively heavy, and dilution streaking was used to simplify colony isolation. When streaking was delayed the plates were easier to read, as growth was less luxuriant. The plates were read after being incubated for 24 hours at 37° C.

All colonies which, on the basis of morphologic and microscopic examination, appeared to be beta-hemolytic streptococci were subjected to Lancefield grouping. When 100 individuals were found to have one or more cultures positive for group A organisms, the study was closed. Four hundred and twenty subjects were examined before this point was reached.

Results. The findings for the 100 subjects from whom group A streptococci were isolated, on pharyngeal swabs, by one or more of the four techniques used (table 1) showed the highest percentage of recovery (89 percent) was achieved with the dacron swab, filter paper strip method. When a dacron swab was plated immediately on a blood agar plate, the results were only slightly less favorable. Of the other two techniques, the cotton swab, plated immediately, gave better results than the dacron swab held in the dry state for a week, but the former was distinctly less satisfactory than the dacron swab, filter paper method. Further, it was noted repeatedly that when the dacron swab, filter paper strip was placed on the agar plate, the number of organisms, except for alpha- and beta-hemolytic streptococci, were greatly lessened, which simplified ultimate identification of group A streptococci.

Despite the relatively high yield of group A organisms with the dacron swab, filter paper

Table 1. Number and percent of 100 cultures positive for group A streptococci, by technique used

Technique used	Cultures positive for group A streptococci	
	Number	Percent
Cotton	75	75
Dacron	83	83
Dacron hold	49	49
Dacron filter paper	89	89

technique, it is nonetheless apparent that in 11 instances group A streptococci were not recovered by this method.

Study 2

When study 1 was concluded, we decided to compare the dacron swab, filter paper technique with a pour plate method. Accordingly, we made arrangements with Col. Roland Sigafoos, post hospital commandant, to obtain pharyngeal cultures from Army personnel and their dependents at Fort Carson, Colo. Between February 26 and May 7, 1962, we took pharyngeal swabs from 239 subjects, all family contacts of patients with bacteriologically proved group A streptococcal pharyngitis. Eight cultures were obtained from each subject by the following techniques. Three dacron swabs were held together and used to culture the pharynx of each individual. The swabs were placed in a sterile test tube and allowed to dry for 30 minutes.

1. Dacron filter paper: One swab was rubbed on filter paper, and the paper strip was handled as outlined in study 1.
2. Dacron delayed: A second swab was held in the sterile test tube for 4 hours and then streaked on a blood agar plate.
3. Dacron broth: The third swab was placed in a test tube containing 2.0 ml. of Todd-Hewitt broth; then the swab was discarded and the broth was incubated for 2 hours at 37° C., after which a loopful was streaked on a blood agar plate.
4. Dacron pour plate: One ml. of the same broth was transferred to a sterile petri dish, and 10 ml. of BBL trypticase soy agar with 5 percent sheep red blood cells were added; after appropriate mixing, the agar was allowed to harden and was then incubated at 37° C.

All plates were read after 18 hours.

A second set of cultures was made, identical in all respects to the first set, except that cotton rather than dacron swabs were used. The cultures in this series were identified respectively as cotton filter paper, cotton delayed, cotton broth, and cotton pour plate. We used the two types of swabs alternately to eliminate the order of swabbing as a factor in the results. When 72 subjects were found to have positive cultures for group A streptococci, the study was terminated.

Results. As in the first study, the highest percentage of recovery of group A streptococci was achieved when the dacron filter paper strip method was used (table 2). The results were almost as favorable when a cotton swab was used with filter paper. Pour plates from either dacron or cotton swabs had comparable percentages of group A streptococci. When streaking of the plates was delayed for 4 hours, the results were identical, and there was little difference in streptococcal recovery when the two kinds of swabs were incubated for 2 hours before streaking.

It should be noted that no attempt was made to determine the number of colonies of streptococci obtained by any of the procedures. When a single colony of group A organisms, proved by Lancefield grouping, was identified, the culture was considered to be positive. Despite the fact that the dacron filter paper method was superior to others, it did not result in as high a percentage of positives as were obtained by a combination of techniques. In this respect, the findings in studies 1 and 2 were essentially the same. In both studies, accuracy with the dacron filter paper strip was approximately 90 percent.

Discussion

On the basis of observations in this study as well as previous experience, we believe that the dacron swab, filter paper strip technique has dis-

Table 2. Number and percent of 72 cultures positive for group A streptococci, by technique used

Technique used	Cultures positive for group A streptococci	
	Number	Percent
Dacron:		
Filter paper	66	91.7
Delayed	51	70.8
Broth	54	75.0
Pour plate	62	86.1
Cotton:		
Filter paper	61	84.7
Delayed	51	70.8
Broth	50	69.4
Pour plate	61	84.7

tinct advantages in enhancing the recovery of group A streptococci from pharyngeal cultures. This method was introduced as a means of obviating or at least minimizing the deleterious effect of ensuing delays between the time a pharyngeal swab is made and the specimen streaked on a blood agar plate and incubated. Hollinger and Lindberg demonstrated that group A streptococci survived on a dried filter paper strip for a period of 2 to 10 days (*7*), and we have confirmed their observations. Further, our investigations showed that this method is identified with a higher recovery of group A streptococci than is obtained by a number of other commonly used methods.

The filter paper strip technique makes it possible to safely mail specimens for culture; therefore, the technique lends itself well to widespread application by State or regional public health agencies. For the physician in practice or for mass surveys in the field, the filter paper strip method is extremely useful (*9*). The additional expense entailed is limited to the cost of the packets, and this cost is probably compensated for as the relatively low yield of organisms other than streptococci usually obviates subcultures.

It is of interest that in study 2, the dacron swab, filter paper method resulted in a higher percentage of recovery than our pour plate method.

Since the drying inherent when the filter paper strip method is used inhibits nonstreptococcal organisms, it may be that the streptococci grow more favorably because of decreased competition for nutrients, but this interpretation is purely speculative. Despite several studies emphasizing the effectiveness of the pour plate method (*10*, *11*), it has never been widely adopted, probably because of the difficulties in "fishing" colonies for subculture. The filter paper strip method is as effective and certainly easier to use.

As stated, the cultures for our studies were obtained from healthy carriers who were family contacts of patients with proved group A streptococcal infections. Although it has been suggested that, in the presence of infection, positive cultures can be obtained with less effective methods (*3*), clear-cut evidence in support of this concept is lacking. However, the dacron swab, filter paper strip technique has the dual advantages of effectiveness and simplicity and is, therefore, broadly applicable whether cultures are being sought from patients or carriers.

It is important to reiterate the importance to the physician of systematically correlating clinical and laboratory findings in streptococcal pharyngitis, for in this way diagnostic skill can be improved. More important, however, is the fact that a throat culture, when properly done, represents the most effective means available for the diagnosis of group A streptococcal infections, which in turn provides a sound basis for antimicrobial therapy and the prevention of suppurative and nonsuppurative complications.

Summary

In two studies carried out on 659 family contacts of patients with proved group A streptococcal pharyngitis, it was demonstrated that the dacron swab, filter paper strip technique for throat cultures was identified with a higher percentage recovery of group A streptococci than other commonly used methods. The importance of confirming a diagnosis by means of appropriate cultures is emphasized.

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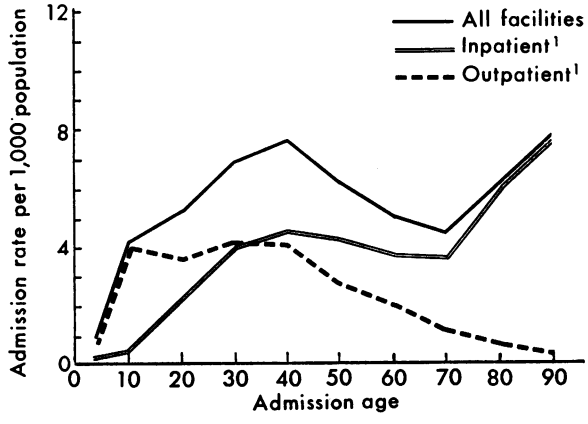
EQUIPMENT REFERENCE

(A) Culpak Kits (R), Diagnostic Associates, Walnut Creek, Calif.

Erratum

Figure 1 of the article, "Services Received by Maryland Residents in Facilities Directed by a Psychiatrist. First Year of a State Case Register," by Anita K. Bahn, Kurt Gorwitz, Gerald D. Klee, Morton Kramer, and Isadore Tuerk, published in PUBLIC HEALTH REPORTS, May 1965, page 408, contains an error. Labels for the lines representing outpatient and inpatient facilities were inadvertently reversed. The correct figure is reproduced here.

Figure 1. Age-specific admission rates to psychiatric facilities by type of facility, Maryland psychiatric case register, fiscal 1962



[1] Includes persons admitted to both inpatient and outpatient facilities.