EVALUATION OF FA AND CONVENTIONAL TECHNIQUES FOR IDENTIFYING GROUP A BETA HEMOLYTIC STREPTOCOCCI

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PROMPT RECOGNITION of group A beta hemolytic streptococci by use of the fluorescent antibody technique (*1–4*) permits early and adequate treatment of streptococcal infections so that initial and recurrent attacks of rheumatic fever may be prevented. However, the degree of accuracy obtained with the fluorescent antibody (FA) technique depends on the specific methodology used in its application.

The study reported here was undertaken to determine the most rapid and accurate method to identify group A beta hemolytic streptococci among several modifications of the FA technique, and to compare these modifications with each other and with conventional bacteriological procedures.

Materials and Methods

A total of 1,115 throat cultures were collected from 6- to 12-year-old children attending three schools within a 1-mile radius of the National Children's Cardiac Hospital, Miami, Fla. Each weekday morning public health nurses assigned to the project contacted the schools to obtain that day's list of absentees. After ascertaining the cause of absence, by telephone or visit, the nurses took throat specimens, using a double-swab technique, from each child absent because of respiratory illness.

The swabs were placed in a tube containing 1 ml. of Todd-Hewitt broth and immediately taken to the laboratory where they were incubated in 2 ml. of Todd-Hewitt broth at 37° C. for 3 hours. Pour plates were made from this 3-hour suspension (see illustration), and the plates were incubated for 18 hours at 37° C. Each culture was studied by the conventional microprecipitin grouping technique (*5*), by FA techniques, and by a number of modified techniques, which are described below.

Three streak plate techniques, using commercially obtained blood agar plates (sterile blood agar plates containing 4 percent defibrinated sheep red blood cells) were evaluated. The first technique (S1) required preparation of a streak plate directly from one of the paired swabs on arrival at the laboratory. In the second streak plate technique (S2), the plate was prepared from a swab after initial 3-hour incubation in Todd-Hewitt broth. In the S3 technique, a streak plate was made from the broth suspension after 24 hours' incubation. Each streak plate was prepared by rolling a swab across the plate and then streaking by the standard technique. The agar was not routinely stabbed.

Five modifications of the fluorescent antibody technique were evaluated. Commercially prepared group A antiserum conjugated with fluorescein isothiocyanate (*A*) was used. The F1 technique employed a slide prepared from the 3-hour broth sediment. Technique F2 required a slide made from the 24-hour broth sus-

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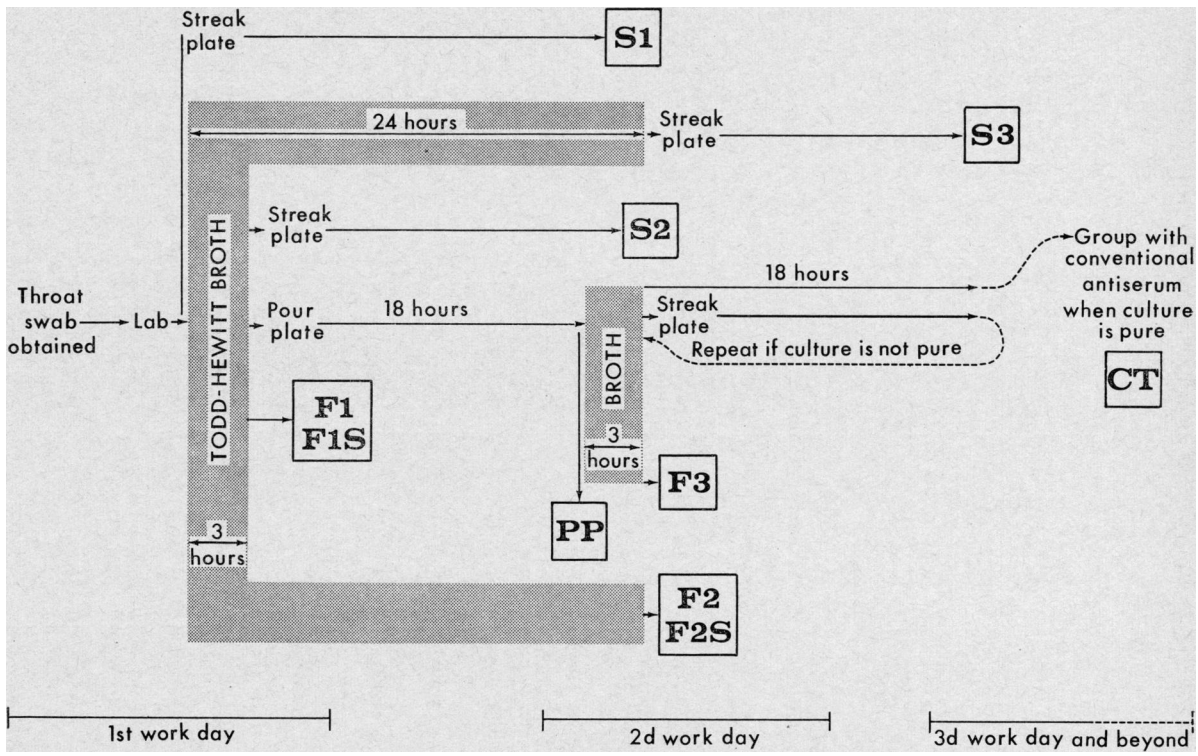
pension. In technique F3, a colony was picked from the 18-hour pour plate and incubated in broth for 3 hours. A fluorescent slide was prepared from this broth. F1S and F2S differed from F1 and F2 in that slides were prepared from F1 and F2 centrifuged sediment instead of broth suspension.

In the preparation of the fluorescent slides, one 4-mm. loopful of suspension or sediment was placed on a clean glass slide, the slide was dried in an incubator at 37° C., and fixed in 95 percent ethanol for 1 minute. After fixing, the slides were allowed to dry at room temperature and then stained with the fluorescent antiserum for 30 minutes. The slides were washed with 0.01 M sodium potassium phosphate buffered saline, and a coverslip was mounted over buffered glycerol-saline (9 parts glycerine : 1 part buffered saline). The slides were then read with the fluorescent microscope, a Leitz Ortholux equipped with an HBO 200 mercury vapor lamp light source and BG 12 and OG 1 filters. A slide, freshly prepared each day, of a known group A streptococcus was used as a control.

The control determinant for the presence or absence of group A beta hemolytic streptococci was Lancefield's conventional microprecipitin grouping technique. For this procedure, a representative colony was picked from each pour plate after the 18-hour incubation period. The picked colony was incubated in broth for 3 hours at 37° C. A streak plate was then prepared from this broth, and both streak plate and subinoculated broth were incubated for 18 hours at 37° C. If the culture on the streak plate proved to be a pure isolate, the broth was used to group the organism against rabbit anti-streptococcus grouping serum (*B*) by the



Schematic outline of 10 bacteriological and immunofluorescent procedures used for identification of group A beta hemolytic streptococci in throat cultures

NOTE: CT, conventional microprecipitin technique; S1, S2, and S3, streak plate techniques; F1, F1S, F2, F2S, and F3, fluorescent antibody techniques; PP, pour plate technique. F1 and F1S are the only techniques that can be completed on the first work day (day on which culture is obtained). S1, S2, F2, F2S, F3, and PP can be completed in the morning of the second day (day after culture is obtained). S3 cannot be completed until the third day. CT can be completed on the third day only if the subculture is pure; otherwise it cannot be completed until the fourth day or later.

microprecipitin technique. If the streak plate was not pure, another colony was picked and the procedure repeated until a pure culture was obtained.

Results

Of the total 1,115 cultures, 177 (15.9 percent) were positive for the presence of group A beta hemolytic streptococci, as confirmed by the conventional grouping procedure (table 1).

The pour plate technique alone identified 176 of the 177 group A organisms. However, the beta hemolytic rate was high with the technique (107 cultures were beta hemolytic on pour plate and ultimately proved not to be group A beta hemolytic streptococci). Only one culture was false negative and 831 were true negatives. All 1,115 cultures were studied by this technique.

The streak plate techniques proved to be less accurate. Of 320 cultures (table 1), the S1 technique identified 30 beta hemolytic organisms, of which 20 were group A beta hemolytic streptococci and 10 were not; it failed to identify 17 additional group A strains. Using the S2 technique (320 cultures), only 15 of the 37 were identified, with 23 non-group A strains. The S3 technique was used 98 times, identifying only 7 of 17 strains of group A streptococci, with one non-group A. The same 320 throat cultures were used for the S1 and S2 evaluations; the 98 S3 evaluations were derived from the same group. Only 10 of the 15 organisms identified by the S2 technique had also been identified by the S1 technique. All seven of the organisms identified by the S3 technique also had been identified by S1. There was S1–S2–S3 agreement in only four of the seven identifications.

The F3 fluorescent antibody technique showed excellent results. It identified all of the 162 group A streptococci among the 956 cultures studied; 3 strains (0.31 percent of the 956 throat swabs studied) were not corrobo-

Table 2. Streptococci isolated from 1,115 throat cultures, by serologic groups

Streptococcal group	Number recovered	Percentage of total
A	177	65.8
B	6	2.2
C	9	3.4
D	1	.4
F	8	3.0
G	41	15.2
Non-groupable streptococci	27	10.0
Total	269	100.0

Table 1. Results of various bacteriological techniques used for identification of group A beta hemolytic streptococci among 1,115 throat cultures, Miami, Fla., December 1961–May 1962

Technique	Number of throat cultures	Group A beta hemolytic streptococci			True negatives	Other beta hemolytic organisms or cross reactions	Percentage accuracy of method	
		Actual number	Number correctly identified	False negatives			Sensitivity	Specificity
Conventional	1,115	177	177	0	938	0	100.0	100.0
Pour plate	1,115	177	176	1	831	[1] 107	99.4	88.6
Streak plate:								
S1	320	37	20	17	273	[1] 10	54.1	96.5
S2	320	37	15	22	260	[1] 23	40.5	91.9
S3	98	17	7	10	80	[1] 1	41.2	98.8
Fluorescent antibody:								
F1	647	68	20	48	572	[2] 7	29.4	98.8
F2	831	161	44	117	665	[2] 5	27.3	99.3
F3	956	162	162	0	791	[2] 3	100.0	99.6
F1S	129	31	24	7	97	[2] 1	77.4	99.0
F2S	467	109	70	39	355	[2] 3	64.2	99.2

[1] Other beta hemolytic organisms.
[2] Cross-reacting organisms.

rated by the conventional technique. The F1 and F2 techniques identified 20 of 68 and 44 of 161 group A organisms respectively. Seven strains with the F1 technique and five with the F2 technique fluoresced, but were not identified as group A strains by the conventional technique. These represent cross reactions. The F1 studies were performed on 647 cultures and the F2 on 831 cultures. F1S and F2S identified 24 of 31 and 70 of 109 organisms. Additionally, one F1S and three F2S strains cross reacted.

Other streptococci observed by the conventional technique in addition to the 177 group A organisms (table 2) were 6 group B, 9 group C, 1 group D, 8 group F, 41 group G, and 27 non-groupable strains.

Discussion

The ultimate aim of this study was to determine the most satisfactory technique available to identify group A streptococci so that appropriate treatment might be instituted for the purpose of preventing the non-suppurative complications of streptococcal infections. The more specific goal of this phase of the study was to determine the bacteriological technique, or combination of techniques, best suited to rapid and accurate identification of the group A beta hemolytic streptococcus. Therefore, several techniques were studied concurrently. Whenever a particular technique was shown to have little or no value for purposes of identification of group A streptococci, the technique was modified or discarded and replaced by another procedure.

The pour plate technique (reading the pour plate alone as the means of identification) was excellent as a means of positive identification (176 of 177 strains) when read by an experienced technician. However, this technique yielded a high beta hemolytic, non-group A rate (107 of a total of 1,115 cultures, 9.6 percent). Thus the pour plate technique cannot be relied upon alone, since it would result in the unnecessary use of penicillin in all instances identified as beta hemolytic colonies—107 in this case.

None of the streak plate procedures was satisfactory, since these techniques failed to identify a large percentage of the group A organisms (table 1).

Of the FA techniques employed, F1 and F2 were extremely poor, showing false negative rates which were more than double the true positive rates (table 1). F1S and F2S were somewhat better, with lower false negative rates. However, the number of false negatives was still quite high.

By far the most satisfactory technique of those studied was F3. With this method, all 162 of the group A organisms studied were correctly identified; there were only 3 false positives (due to cross reactions) in a total of 956 cultures. The problem of cross reactions has been studied (*6*) and even this small source of error may be eliminated in the future.

The F3 technique is a combination of conventional bacteriological procedures and fluorescent antibody techniques. Is there, then, any particular advantage of the F3 technique over the conventional technique? A brief reiteration of the steps of the procedures (see illustration) will answer this question. In the F3 technique, the swab is incubated for 3 hours in Todd-Hewitt broth; a pour plate is then prepared and incubated for 18 hours; and finally a colony is picked from the pour plate, incubated for 3 hours in broth, and a fluorescent slide is prepared and read. The total time for this procedure including transportation of the swab to the laboratory is 25 hours. The time requirements for the first part of the conventional technique are identical. In addition, however, 18 hours' incubation time is required for each subculture and at least one subculture is mandatory.

In practical terms, therefore, the fluorescent antibody technique takes 1 day from throat swab to identification, while the conventional technique requires a minimum of 2 days, assuming that a pure culture is obtained on the first subculture. We feel that time is an extremely important factor in terms of patients' incentive in seeking proper medical care. When the patient is advised to seek treatment within 24 hours after the onset of illness, he complies in almost all instances. However, as time passes, especially if a child's symptoms become less severe or disappear, appropriate medical treatment tends to be bypassed.

Therefore, we believe that the fluorescent antibody technique, more specifically the F3

procedure described here, provides the most practical approach for the successful identification of group A beta hemolytic streptococci and the subsequent treatment thereof.

Summary

To determine the most rapid and accurate method of identifying group A beta hemolytic streptococci, several modifications of the fluorescent antibody technique were compared with each other and with various conventional techniques. A total of 1,115 throat cultures were studied. The conventional microprecipitin technique was used as the standard to determine the group identity of the beta hemolytic streptococci.

When pour plates were read directly by an experienced technician, beta hemolysis was observed in 283 strains of 1,115 cultures studied. Subsequently, 176 proved to be group A streptococci; 107 were other beta hemolytic organisms. One additional group A strain was not recognized by the pour plate technique.

Five fluorescent antibody technical modifications were evaluated. Best results were obtained when throat swabs were inoculated in Todd-Hewitt broth and incubated for 3 hours, followed by preparation of pour plates which were then incubated for an additional 18 hours at 37°C. Hemolytic colonies were picked from the pour plates after incubation and placed in broth for 3 hours' further incubation. Fluorescent slides were prepared from broth suspensions. Of 956 cultures surveyed (of the original 1,115 cultures) by this technique, all 162 strains of group A beta hemolytic streptococci were identified.

The other four fluorescent modifications met with varying degrees of success but none approached the accuracy of the above technique. Various streak plate techniques were considerably less successful.

The modified fluorescent antibody technique recommended provides a rapid and accurate method for the identification of group A beta hemolytic streptococci, so that prompt treatment may be instituted for the ultimate purpose of preventing the non-suppurative complications of streptococcal infections: rheumatic fever and glomerulonephritis.

REFERENCES

(*1*) Moody, M. D., Ellis, E. C., and Updyke, E. L.: Staining bacterial smears with fluorescent antibody. IV. Grouping streptococci with fluorescent antibody. J Bact 75: 553–560 (1958).

(*2*) Wolfe, M. D., and Cameron, A. M.: Fluorescent antibody technique versus cultural methods as a routine procedure for finding β-hemolytic streptococci in throat cultures. Public Health Lab 17: 76–82 (1959).

(*3*) Peeples, W. J., Spielman, D. W., and Moody, M. D.: Field application of fluorescent antibody technique for identification of group A streptococcus. Public Health Rep 76: 651–654 (1961).

(*4*) Shuey, H. E., and Estela, L. A.: Fluorescent antibody methods as a help in the clinical diagnosis of streptococcal sore throat. Armed Services Technical Information Agency, June 1961.

(*5*) Lancefield, R. C.: A micro-precipitin technique for classifying hemolytic streptococci and improved methods for producing antisera. Proc Soc Exp Biol Med 38: 473–478 (1938).

(*6*) Jablon, J. M., and Brust, B.: Suppression of cross-reactions in fluorescent antibody identification of group A streptococci. Bacteriological Proceedings, 1963, abstract M 137, p. 89.

EQUIPMENT REFERENCES

(*A*) Group A antiserum conjugated with fluorescein isothiocyanate: Sylvania Chemical Company, Bloomfield, N.J.

(*B*) Rabbit antistreptococcus grouping serum: Difco Laboratories, Detroit, Mich.