FIELD APPLICATION OF FLUORESCENT ANTIBODY TECHNIQUE FOR IDENTIFICATION OF GROUP A STREPTOCOCCUS

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THE VIGOROUS treatment of streptococcal infections to prevent the sequelae of rheumatic fever and acute glomerulonephritis has received considerable emphasis in recent years. Early, accurate diagnosis of group A streptococcal infections associated with the complications, however, has not kept pace with therapeutic advances. Physicians often experience considerable delay in obtaining a laboratory diagnosis and must administer therapy to acute throat infections without knowing the etiological agent. The delay is due to the necessity of isolating a pure streptococcal culture, with subsequent preparation of extracts for use in precipitin grouping tests. Though beta hemolytic streptococcal organisms can be identified readily and quickly, the correct designation of the infecting streptococcal group requires several more days of testing.

Coons and his associates first described in 1942 a technique of bacterial identification which might offer rapid diagnosis (*1*). This was the fluorescent antibody technique, described more definitively in subsequent writings by Coons and Kaplan as an antigen-antibody reaction to identify mumps virus, pneumococcal polysaccarides, and rickettsia (*2*). Moody, Goldman, Thomason, and Cherry applied this same method to the identification of other organisms (*3–5*).

Moody, Ellis, and Updyke first developed an accurate method for the identification of streptococci using the fluorescent antibody technique (*6*). They showed that specific fluorescent antibody could be prepared and used for grouping streptococci in dried smears by staining only group A streptococci and also that a specific fluorescein-labeled antibody could be used to identify groups B, C, D, F, and G. Occasional cross reactions between organisms of groups A, C, and G were corrected by absorbing group A fluorescent antibody with group C organisms. This did not affect in any way the affinity of the antibody for group A cells.

As a result of this discovery, we proposed a study of the fluorescent antibody technique for streptococcal identification under field conditions using the facilities of a local health department. The study was conceived on the principle that the technique would give private physicians a diagnosis on suspected group A streptococcal disease within 4 to 5 hours after a specimen was submitted rather than the 3 to 5 days ordinarily required. The experimental design emphasized quality and correctness of technique rather than performance of a large number of tests.

Funds for the study were supplied by the Heart Disease Control Branch of the Public Health Service, and technical supervision, as well as the various reagents used in performing the tests, was provided by the Special Research Unit of the PHS Communicable Disease Center. The bureau of laboratories of the Maryland Department of Public Health, the Montgomery

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County Medical Society, and the Montgomery County Tuberculosis and Heart Association gave the plan their wholehearted support.

Methods

The field trial of the fluorescent antibody technique was divided into two parts by a change in the method of collecting and submitting specimens. From January through November 1959, specimens were collected by rotating two swabs together in the throat. The swabs were then placed in a sterile culture tube and submitted to the laboratory within 2 hours. Because of complaints from physicians about the rigid submission policies, a filter paper collection kit, which could be mailed to the laboratory, was used throughout the rest of the study.

Because of the large numbers of specimens sent in during the study, only the first six specimens received on Monday through Wednesday could be compared by complete laboratory identification. All specimens, however, received fluorescent antibody determination on both broth smear and culture. All labeled antibody, normal rabbit globulin, and precipitin grouping antiserums were supplied by the Communicable Disease Center, and reference cultures and fluorescent antibody smears were sent periodically to the Center.

Swab Collection

One swab was washed in 0.5 ml. Todd-Hewitt broth, and melted sheep blood neopeptone agar was inoculated, poured into petri dishes, and incubated 24 to 48 hours at 35° C. Beta hemolytic colonies of streptococci were selected, and acid-heat extracts were prepared from packed cells of the resulting growth in Todd-Hewitt broth in accordance with Lancefield's method (*7*). Extracts were grouped in precipitin tests set up with antiserums for groups A, B, C, D, F, and G streptococci.

The other swab was placed in 2.0 ml. of Todd-Hewitt broth and incubated 2 to 4 hours at 35° C. The swab was then squeezed against the side of the tube and discarded. The broth was cultured and beta hemolytic streptococci were grouped as described above.

The remaining broth was centrifuged and the sediment washed once in buffered physiological saline, pH 7.2. Duplicate smears were made from the final sediment and allowed to air dry. After fixation with 95 percent ethyl alcohol, the smears were rinsed in distilled water and blotted dry. One smear was stained 30 minutes with fluorescein isothiocyanate-labeled globulin prepared from group A streptococcus antiserum and the other with labeled normal rabbit globulin. Excess and unreacted conjugate was removed by rinsing 10 minutes in two changes of buffered saline. The smears were finally rinsed in distilled water, blotted dry, mounted with glycerol saline (9 parts glycerin plus 1 part saline), and examined under the microscope for fluorescence. Smears were examined for intensity of the fluorescence reaction and concentration of cocci in chains. If typically greenish-yellow cocci in chains were observed in smears stained with group A conjugate, but not with normal rabbit conjugate, a positive report was made to the physician by telephone. He was told the results of the fluorescent antibody reaction and that a definitive written report would follow after conventional tests were completed.

Filter Paper Collection

When a filter paper specimen was received, the paper was aseptically removed from the kit and placed in a tube of Todd-Hewitt broth. This was incubated 3 to 5 hours. An additional 1 to 2 hours of incubation was necessary to obtain the amount of growth equivalent to that obtained after 2 to 4 hours incubation of broth inoculated with swabs. After incubation the paper was aseptically removed from the broth and placed on a culture plate of blood neopeptone agar and cross streaked with a loop for colony isolation. This was incubated at 35° C. for 24 to 48 hours.

The broth was centrifuged 10 minutes at 3,500 to 3,600 rpm and treated identically as the broth cultures previously described. Smears from the sediment (control and test) were also made, stained, and examined in the same fashion.

Results

During the first phase of the study, 158 swab specimens were examined. Comparison of the results obtained with the two conventional cultural methods indicated that the 2- to 4-hour

broth procedure is the more sensitive. All 64 specimens found positive at all were found positive by this method, whereas only 57 (89 percent) were positive by the direct swab method. Results of the fluorescent antibody test were therefore compared only with the results of the 2- to 4-hour broth method. Results of these two techniques showed agreement on 150, or 95 percent, of the 158 specimens (see table).

During the second phase of the study 998 specimens were examined. Comparison of 392 specimens by conventional culture on 3- to 5-hour broth and fluorescent antibody tests resulted in 94.4 percent agreement of the two methods (see table). On an additional 603 specimens conventional cultures were not grouped by precipitin tests, but beta hemolytic colonies were confirmed by fluorescent antibody tests, and these results were in 98.8 percent agreement with the results of the fluorescent antibody test on 3- to 5-hour broth cultures.

Discussion

The fluorescent antibody technique for rapid identification of group A streptococci is a practical, economical laboratory method which can easily be made available to the practicing physician. Specimens taken from patients can be mailed using filter paper kits as described or conventional cotton swabs protected by a glass tube. Physicians can receive results in 4 to 5 hours if specimens are brought to the laboratory and often in 24 hours if specimens are mailed locally. The results of this study indicate that the fluorescent antibody technique is as specific as precipitin grouping. All group A organisms were stained by this method.

Group A streptococci represented 538 specimens (93.4 percent) of the total of 576 specimens identified as beta hemolytic streptococci. Other groups were B, 10 specimens; C, 3 specimens; D, 2 specimens; F, 3 specimens; G, 7 specimens. Thirteen other specimens were identified simply as not belonging to group A. For an additional 603 specimens, no group identification other than group A was made. One strain of G organisms gave a 1+ intensity reaction in fluorescent antibody smear, but only group G organisms were recovered in culture.

Occasionally a staphylococcal strain was encountered which gave a weak fluorescent reaction. Such strains could be distinguished as staphylococci by their morphology and presented little difficulty since only typically stained cocci in chains giving good fluorescent reactions were reported.

Comparison of fluorescent antibody method and conventional culture method of identifying group A beta hemolytic streptococcus

Conventional culture	Fluorescent antibody method		
	Positive	Negative	Total
	Swab collection of specimens [1]		
Positive	57	6	63
Negative	2	93	95
Total	59	99	158
	Filter paper collection of specimens [2]		
Positive	158	14	172
Negative	8	[3] 212	220
Total	166	226	392

[1] Broth incubated 2 to 4 hours.
[2] Broth incubated 3 to 5 hours.
[3] 3 specimens showed absolutely no growth by either method.

Summary and Conclusions

In a study of the field application of the fluorescent antibody technique for identification of group A beta hemolytic streptococcus, the technique was found to be 95 percent or better in agreement with the conventional 2- to 4-hour broth culture method. The fluorescent antibody technique is a rapid, accurate, practical laboratory procedure that can be used by any local or State public health agency or private laboratory.

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Conference on Gastroenteric Morbidity

"A gastroenteric episode is an acute illness manifested by one or more of the following symptoms: nausea, vomiting, diarrhea, abdominal cramps or abdominal pain, with or without fever, which may be associated with other symptoms."

This definition of acute gastroenteric illnesses appeared to have general agreement at the Conference on Methods of Improving Reporting of Gastroenteric Morbidity, held October 12–13, 1960, at the Robert A. Taft Sanitary Engineering Center of the Public Health Service in Cincinnati, Ohio, with joint sponsorship of the National Vital Statistics Division.

Conferees agreed that gastroenteric episodes should include both foodborne and waterborne illness, but exclude organic and allergenic gastroenteric disease.

It was also agreed that acute gastroenteritis could be classified according to: (1) means of spread: (*a*) ingestion (foodborne, waterborne, and other), (*b*) inhalation (airborne and other), and (*c*) contact, and (2) etiology (infectious or intoxication).

Acute gastroenteritis is the second leading cause of acute morbidity, after acute respiratory disease, and is a substantial cause of mortality. In 1959 in the United States, 4,899 deaths were ascribed to gastroenteritis, including diarrhea of the newborn: 492 for infants under 4 weeks old, 2,862 for children 4 weeks to 2 years, and 1,545 deaths at 2 years of age and over.

More gastroenteric episodes could be prevented if knowledge of their occurrence were more complete, it was pointed out. Adequate morbidity reporting should reveal the extent of gastroenteritis and stimulate increased activity by State and local health departments. Epidemiologic followup of reported cases should be provided. Since active participation of local health departments is necessary for substantial improvement in reporting gastroenteric morbidity, efforts to improve reporting should be more intensive in metropolitan areas where good health services exist.

The need for strong health activities was stressed, with epidemiologic investigation in all localities accomplished by a team of epidemiologists and sanitarians, supported by laboratory facilities and staff. These teams might operate on a local basis or on a cooperative local-State or local-State-national basis, using suitable existing facilities.

Subcommittees were appointed to develop a list of reference laboratories available to State and local health departments for examination of materials implicated as the cause of acute gastroenteritis and to promulgate information concerning its occurrence and prevention.

The committee, chaired by Dr. William D. Schrack, Jr., director of the division of communicable disease control, Pennsylvania State Department of Health, invites suggestions from anyone interested in the problem of acute gastroenteritis.