DETECTING TREPONEMA PALLIDUM IN PRIMARY LESIONS BY THE FLUORESCENT ANTIBODY TECHNIQUE

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A FLUORESCENT TECHNIQUE (*1*) using intact *Treponema pallidum* (Nichols strain) as known antigen and fluorescein-labeled antiglobulin as an indicator of a treponemal antibody complex has been widely tested as a serologic test for syphilis and may some day replace the *T. pallidum* immobilization (TPI) test. The fluorescent technique has also been employed in the identification of unknown antigens; for example, recent reports (*2,3*) have shown the feasibility of rapid and specific identification of micro-organisms in smears stained with specific labeled fluorescent antibody. Until recently these techniques had not been applied to the field of venereal disease (*4*). This paper reports experimental studies and a field trial study in which the same principles have been applied to the identification of *T. pallidum* from primary lesions.

Materials and Methods

Rabbit propagated *T. pallidum* (Nichols strain) was used in the experimental studies. Rabbit testicular tissue was extracted as outlined for the TPI test (*5*). The extracted treponemes were frozen in aliquots of 0.5 ml. at −20° C. until used. Treponemes were also lyophilized and stored over $CaCl_4$ at 6° C. until used. In the experimental studies, slides were made from treponemes suspended in different diluents, air dried, and then held at varied temperatures to test the effects of diluents and temperatures on the ease of their identification by fluorescent antibody techniques.

One-half of the slides were fixed with acetone immediately; the other half were not fixed until they were to be stained for testing. The delay in acetone fixation was 3–5 days, and was designed to simulate what would be expected when freshly prepared slides were transported through the mails to a central laboratory for testing.

In the field trials, exudate from lesions suspected of containing spirochetes was placed on a slide, air dried, and held until tested. Material for the field trial studies was supplied by Dr. Andelman and his staff at the Chicago Clinic, where we were permitted to make our slides and dark-field studies. Serum from a known syphilitic patient (fluorescent treponemal antibody (FTA) reactive) was heated at 56° C. for 30 minutes, diluted 1:5 in Bactohemagglutination buffer and employed as the known or identifying antibody. Rabbit anti-human globulin labeled with fluorescein isothiocyanate was used as the counterstain to visualize the treponemes. Stained slides were examined for fluorescence using a Leitz UAM-UV assembly with a dark-field condenser and a 4 mm. dry objective. Fluorescence was graded (the average reading of three slides) from a brilliant fluorescence (4+) to minimal fluorescence (1+) to provide an evaluation of the possible effect of temperature and diluents on fluorescence. The number of spirochetes was also graded to evaluate the retention of the spirochetes on the slide during the drying, fixing, and staining process. The range of tem-

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peratures was selected to simulate what might be expected if slides from primary lesions were transported great distances over any part of the world.

Test Procedure

A diamond-point stylus was used to cut circles of approximately 1 cm. diameter on slides 1 by 3 inches, 0.95 ± 0.05 mm. thick so that the specimen area could be located at later observation.

Slides for the experimental tests were made by pipetting one drop (0.01 ml.) of thawed *T. pallidum* or reconstituted lyophilized *T. pallidum* containing 10–15 spirochetes per high dry field onto the circled areas of the slides (*6*). Field trial slides from primary lesions were made by touching the circled area of the slide to the serous exudate of the lesion.

In both methods, the smears were allowed to air dry, fixed in acetone for 10 minutes, and again air dried. Approximately 0.05 ml. of a 1:5 dilution of a known reactive syphilitic serum was heated at 56° C. for 30 minutes and overlaid on each test area. The slides were then left in a moist chamber for 30 minutes at room temperature, washed in two changes of phosphate buffered saline pH 7.2 for a period of 10 minutes, rinsed in distilled water, and air dried. The same procedure was then carried out with an overlay of fluorescein-labeled anti-human globulin.

After air drying, the slides were mounted in buffered glycerine. In the field trial studies, a duplicate slide was made for examination for treponemes by the conventional dark-field method as a basis for determining the sensitivity of the fluorescent antibody method compared with the dark-field method.

Results

Laboratory Studies

Using a laboratory propagated strain of *T. pallidum* (Nichols strains), spirochetes were easily identified by the indirect fluorescent antibody technique.

Table 1 shows the effect of delayed fixation of slides. In this experiment, the slides were held for 3–5 days at different temperatures prior to fixation, employing different diluents. Physiological saline and Bacto-hemagglutination buffer gave the best results. With these there was no decrease in the intensity of fluorescence nor in the number of spirochetes in a variety of storage temperatures. Bovine albumin was unsuitable as a diluent both because of background fluorescence and because the number of organisms decreased considerably compared with those treponemes suspended in either physiological saline or Bacto-hemagglutination buffer. Human serum, although pretested to be nonreactive by the VDRL and FTA tests, was not as good a diluent as physiological saline or Bacto-hemagglutination buffer.

Table 2 shows the results of immediate

Table 1. Effect of delayed fixation [1] of slides on results of fluorescent antibody technique for detecting Treponema pallidum

Diluent	Degree of fluorescence [2] and relative numbers [3] observed at—							
	−20° C.		6° C.		24° C.		37° C.	
	Fluorescence	Relative number	Fluorescence	Relative number	Fluorescence	Relative number	Fluorescence	Relative number
Normal human serum	4+	1+	4+	1+	2+	1+	2+	1+
Physiological saline	4+	4+	4+	4+	4+	4+	4+	4+
Bacto-hemagglutination buffer	4+	4+	4+	4+	4+	4+	4+	4+

[1] Slides stored 3–5 days at indicated temperatures before fixation and staining.
[2] Graded as 4+ (brilliant) to 1+ (minimal).
[3] Graded as 4+ (10–15 organisms/field), 3+ (5–10 organisms/field), 2+ (2–5 organisms/field), and 1+ (0–2 organisms/field).

fixation, using different diluents and storage temperatures prior to testing. Under these conditions human serum, physiological saline, and Bacto-hemagglutination buffer proved equally effective in preserving the degree of fluorescence and the number of organisms at the various storage temperatures.

Field Trial Studies

Very satisfactory results were obtained by the indirect fluorescent antibody technique with material from 13 patients suspected of having either primary or secondary lesions of syphilis, compared with results of dark-field examination, even when some of the slides were mailed to the laboratory for fluorescent antibody studies, a delay in fixation and staining for at least 3–5 days from the time the slides were made until their arrival at the laboratory.

Four smears were made from patient No. 13. Two were fixed immediately, and two, 7 days later. No difference could be detected in fluorescence or number of organisms observed in the two sets of smears. In general the results of fluorescent identification were superior to those using the dark-field method because of the ease with which the spirochetes were discernible and the rapidity with which they could be identified

Table 2. Effect of immediate fixation [1] of slides on results of fluorescent antibody technique for detecting Treponema pallidum

Diluent	Degree of fluorescence and relative numbers [2] observed at—							
	−20° C.		6° C.		24° C.		37° C.	
	Fluorescence	Relative number	Fluorescence	Relative number	Fluorescence	Relative number	Fluorescence	Relative number
Normal human serum	4+	1+	4+	4+	4+	4+	4+	4+
Physiological saline	4+	4+	4+	4+	4+	4+	4+	4+
Bacto-hemagglutination buffer	4+	4+	4+	4+	4+	4+	4+	4+

[1] Slides fixed immediately upon drying, then stored for 3–5 days at indicated temperatures before staining.
[2] Graded same as in table 1.

Table 3. Comparative results with darkfield and fluorescent antibody techniques for detecting Treponema pallidum on slides made from primary and secondary lesions of syphilis

Case No.	Type of lesion	Results		Number of organisms [1]
		Dark-field method	Fluorescent antibody method	
1	Secondary	Negative	Negative	0
2	Suspected primary	do	do	0
3	do	Positive	Positive	1+
4	do	do	do	4+
5	do	do	do	1+
6	do	do	do	3+
7	do	do	do	3+
8	do	do	do	2+
9	do	do	do	4+
10	do	do	do	1+
11	do	do	do	4+
12	do	do	do	2+
13 [2]	do	do	do	3+

[1] Numbers graded as 4+ (3 or more organisms/field), 3+ (1–3 organisms/field), 2+ (1 organism in 2–5 fields), and 1+ (1 organism in 6 or more fields).
[2] 4 slides made for fluorescent examination.

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amidst cellular debris. Table 3 shows that there was 100 percent agreement between results with fluorescent antibody and results with the conventional dark-field techniques in both the negative and positive slides.

Discussion

From early work on the FTA test (*1*) it appeared obvious that treponemes could be transported on a slide and retain their antigenic properties, as indicated by their ability to form an antigen-antibody complex with serum from syphilitic individuals. This paper has demonstrated that *T. pallidum* can be identified under a variety of conditions of storage and temperature by the fluorescent antibody technique. The method proved equal to the conventional dark-field examination method, even when slides were not fixed and stained for as long as a week after being made. This makes the method very useful in areas removed from trained personnel and microscopes for dark-field studies. By using the technique outlined, prepared slides could be mailed from any part of the world to a central laboratory for handling by trained personnel, thereby eliminating transferring the person to a base laboratory for diagnosis. The technique may also be applicable to the diagnosis of other treponemal diseases, such as yaws and bejel, which often occur in areas of the world with poorly developed local laboratory facilities.

Summary

A method is described for the indentification of *Treponema pallidum* on slides from primary syphilitic lesions, using the fluorescent antibody technique. Such slides may be mailed to a base laboratory for examination, thus permitting diagnosis in areas removed from laboratory facilities. Preliminary observations indicate that the method is comparable to the dark-field method of demonstrating *T. pallidum.*

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Film Archives Transferred to CDC

On January 1, 1962, responsibility for the Public Health Service's medical motion picture archives was transferred from the National Library of Medicine in Washington, D.C., to the National Medical Audiovisual Facility, Medical Audiovisual Branch, Communicable Disease Center, Atlanta, Ga. The collection of films on medicine and health-related sciences is organized and indexed to serve the medical community.

Extensive film cataloging, formerly done by the National Library, has also become a function of the National Medical Audiovisual Facility. The facility is intended to become a national resource for development, production, and distribution of audiovisual materials.