Fluorescent Antibody Tests for Detection of the Gonococcus in Women

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THE successful application of the fluorescent antibody (FA) method for the identification of *Neisseria gonorrhoeae* in men prompted an investigation of the use of this method for the detection of gonococcus in women. The preparation and use of fluorescein-labeled antiserums for the detection of the gonococcus in males was described in a previous publication (*1*).

Conventional culture procedures for *N. gonorrhoeae* identification in females, though recognized as superior to any other methods presently available, are slow, cumbersome, and costly in performance. Because of this, the culture method has been largely abandoned in many laboratories. The development of a more rapid, and a less complicated gonococcal detection method would therefore appear to have much to offer in future venereal disease programs aimed at the control of gonococcal infections.

In a recent study of gonorrhea in female contacts, Goldstein (*2*) found 16 percent positive by culture. Mahoney and associates (*3*) reported 21 percent positive in an examination of 2,429 women of the prostitute class. Stuart and Crookes (*4*) identified the gonococcus in 20.3 percent of 2,288 women examined at the main venereal disease clinic of the Provincial Division of Social Hygiene, Edmonton. H. R. Morton found 47.4 percent of the female contacts in his study group to be positive (personal communication). From these and other reports (*5*), it may be concluded that the culture method, when performed under the most favorable circumstances, is capable of detecting the gonococcus in women in from 16 to 47.4 percent of the cases.

The aim of this study has been the development of a rapid fluorescent antibody procedure for *N. gonorrhoeae* detection, capable of obtaining equal or superior results to those reported for the culture method.

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Materials and Methods

Female subjects, constituting the study group, were named contacts of men with gonorrhea seen at the clinic of the division of venereal disease control, Fulton County Health Department, Atlanta, Ga. The usual methods were used in performing pelvic examinations, and no special or unusual techniques were employed in obtaining specimens.

Direct Fluorescent Antibody Method

Specimens were obtained from three sites; the urethra, vagina, and cervix, by means of sterile, cotton-tipped applicator sticks. Slides were prepared in duplicate for smears from each site. Smears were fixed and stained for 1 hour at 37° C., and conjugates were prepared as described previously (*1*), except in the present study a 24-hour, heat-killed (100° C. for

1 hour) *Aerobacter cloacae* culture (Jordan strain) was used for control and removal of free fluorescein. Conjugates which stained heat-killed *A. cloacae* smears were absorbed with an equal volume of saline-washed, packed cells. Absorption with an equal volume of saline-washed, dried beef bone marrow (Difco) was also used for the same purpose and with similar results. Leitz and Reichert ultraviolet light microscope assemblies were used for determining fluorescents. A desirable contrast between background and specific *N. gonorrhoeae* fluorescents was obtained by the proper selection of filters. A blue background was used to define the gonococcus in an intracellular position.

The recognition of *N. gonorrhoeae* by direct FA constituted a complete test or identification (fig. 1). Photomicrographs were recorded on Super Anscochrome daylight film using a basic exposure time of 5 minutes.

Delayed Fluorescent Antibody Method

Slants (30 mm. butt and 30 mm. slant) were prepared from Difco GC medium base plus hemoglobin and supplement B. This medium was placed in 15- by 125-mm. tubes sealed with culture tube closures B16 (*7*). Specimens were collected by means of sterile, cotton-tipped applicator sticks as described for the direct FA procedure. Slants were inoculated immediately after specimen collection by rotating and rubbing the swab over the surface of the medium. The stick was then broken so that the cotton swab remained in the tube, supported by the butt. After inoculation, slants were immediately placed in a candle-jar and held at room temperature until subsequent inoculations were performed from another patient, at which time the jar was again opened. After completion of specimen collections (4-6 hours), candle-jars were incubated for 16–20 hours at 35° C. Slant growth was mixed by the original swab left in the tube. This swab was also used to prepare heavy smears. These were allowed to air-dry. All delayed FA smears were fixed for 10 minutes in 3 percent formalin in phosphate buffered saline pH 7.2. This was followed by a distilled water rinse. Slides were finally blotted and allowed to air-dry. Subsequent staining with fluorescent antibody and microscope observations were the same as for direct FA. The demonstration of *N. gonorrhoeae* constituted the complete test (fig. 2).

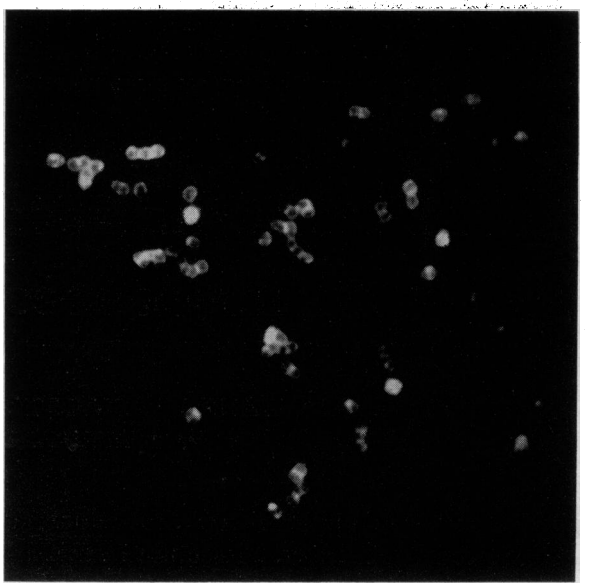

Figure 1. Identification of Neisseria gonorrhoeae in vaginal smear by the direct fluorescent antibody technique

Culture Method

The medium used for petri plates, the method of obtaining the specimen, inoculation and candle-jar procedure were as described under delayed FA. After plates were incubated for 24 hours at 35° C., they were examined for oxidase-positive colonies, and purification by replatings was instigated. CTA medium (BBL) plus 0.5 percent carbohydrate and 0.1 percent cornstarch was used for fermentation studies.

Results

Table 1 compares the direct and delayed fluorescent antibody methods with the conventional culture identification of the gonococcus. In the detection of individuals harboring *N. gonorrhoeae*, culture and the delayed FA procedures appear to be in agreement, each demonstrating 58 percent positive results. The delayed FA method, however, shows a higher degree of sensitivity in relation to total sites tested, 71 compared with 67 for the culture

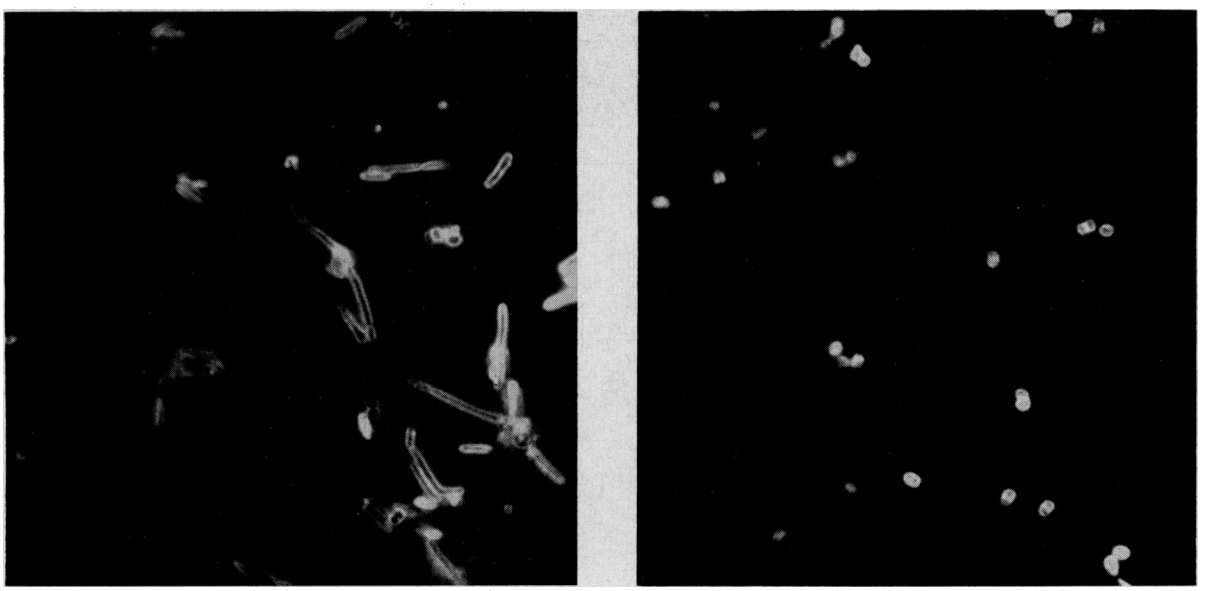

Figure 2. Delayed fluorescent antibody method: Note on left heavy contamination as seen by tungsten illumination. On right-hand side is the same field under ultraviolet illumination showing well-defined gonococci.

technique. It will be noted in this regard that if culture alone had been used on cervical examinations, two individuals harboring *N. gonorrhoeae* would have gone undetected. Similar findings were also obtained in urethral examinations, 22 positives being detected by the delayed FA, and 20 by culture.

In contrast to the delayed FA technique, the direct FA procedure obtained positive results in 26 percent of all patients, or 41.4 percent of those proved by the delayed FA technique. In no case did direct FA demonstrate positive results without also obtaining similar findings by the delayed FA method. Invariably, when one or more sites (vagina, urethra, or cervix) were found positive by direct FA, two or more sites in the same individual were detected by the delayed FA method.

A further comparison of the direct and the delayed FA procedures is shown in table 2. As in the first series of patients, 58 percent of this group also demonstrated positive findings by the delayed FA method. Direct FA detected 24 percent of the individuals harboring *N. gonorrhoeae*. The delayed technique produced positive results in urethral smears in 38 percent of the patients, 44 percent positive were demonstrated from the vagina, and 46 percent from the cervix smears. If positive sites are combined, vagina and urethra examinations account for 50 percent of the gonococcus detections, vagina and cervix for 55 percent, and a combination of all three sites for the highest result, or 58 percent.

Discussion

Direct FA identification of the gonococcus in females as demonstrated in this study may be accomplished in approximately 1 hour. It is obvious, however, that *N. gonorrhoeae* detection by this method is limited and dependent upon quantity of pathogens at the site at the particular time of examination. This effect is minimal when the delayed FA procedure is used. If one considers fluorescent antibody identification from the practical application standpoint, a saving of 3 to 9 days over the conventional culture procedures is effected. Other savings, of course, include the technician's time, culture media, and equipment.

One of the unexpected results was the high percentage of positive findings in vaginal examinations. Although culture findings (table 1) appear to be nearly equal to those demonstrated by the delayed FA technique, one must consider that cultures were performed under nearly ideal conditions. It should be empha-

Table 1. Comparison of culture and direct and delayed fluorescent antibody methods for identifying gonococcus, 50 female contacts

Method	Positive vagina		Positive cervix		Positive urethra		Total sites positive		Total patients positive	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Direct FA	13	26	4	8	5	10	22	14.6	13	26
Delayed FA	24	48	25	50	22	44	71	47.3	29	58
Culture	24	48	23	46	20	40	67	44.6	29	58

sized, particularly in relation to vaginal examinations, that culture results were obtained only through laborious platings and multiple isolation attempts prior to fermentation studies. Vaginal examinations (presumptive and confirmation by fermentations) frequently required 10 days or more for completion. This was also true for other examination sites where heavy contamination complicated *N. gonorrhoeae* identification.

The following are offered as well-defined procedures for the rapid identification of *N. gonorrhoeae* in females by the direct and delayed FA methods.

1. The greatest yield of positive findings may be expected if specimens from the vagina, cervix, and urethra are included in the examination. The vagina and urethra, as a combination will result in satisfactory findings, and may be used where complete clinic facilities are not available, such as examination table and speculum.

2. Duplicate smears from each site should be prepared. These are used for direct FA identification. At least one slant may also be prepared from each site and examined by the delayed FA procedure.

3. Direct FA slides demonstrating positive results constitute a complete examination. Delayed FA needs completion only if the direct procedure fails to demonstrate gonococci. If desired, delayed FA may be used to confirm direct FA findings.

4. Fixation of air-dried smears (direct or delayed) is best accomplished by 10 minutes in 3 percent formalin in phosphate buffered saline pH 7.2. This is followed by a thorough washing in distilled water, and finally blotting before application of fluorescent antibody. Positive findings from any site constitute a completed examination.

Summary

Fluorescent antibody methods have been developed for the rapid identification of *Neisseria gonorrhoeae* in women. A combination of the direct and delayed fluorescent antibody methods was clearly demonstrated as superior to the conventional culture method. The delayed FA method gave a slightly higher yield of positive results in less time than the conventional culture method. The delayed FA method was superior to the direct method alone. The value of vaginal examinations in addition to the customary urethral and cervical tests is indicated.

Table 2. Comparison of the direct and delayed fluorescent antibody methods for identifying gonococcus, 100 female contacts

Method	Positive vagina		Positive cervix		Positive urethra		Total sites positive		Total patients positive	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Direct FA	9	9	8	8	7	7	24	8.0	24	24
Delayed FA	44	44	46	46	38	38	128	42.6	58	58

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