Evaluation of the Fluorescent Gonococcal Test-Heated

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In a 1-year evaluation of the indirect fluorescent antibody test for gonorrhea, 16,127 specimens from low- and high-risk groups were examined, and the results were compared with culture, history of exposure to a known patient, and clinical signs and symptoms. The results confirmed the usefulness of the indirect fluorescent antibody test for gonorrhea as a screening procedure, especially for women in the low-risk group. The test is more economic and probably more sensitive than culture for mass screening of asymptomatic women.

A major obstacle in controlling the gonorrhea epidemic has been the lack of a blood test suitable for mass screening (13). A nationwide control program based on bacteriological examination has been attempted for the past several years, but the incidence of gonorrhea has continued to rise in spite of dedicated efforts by public health workers and the expenditure of millions of dollars (31). The failure of that control program could be due to an inability to reach the asymptomatic patients in the target population, or to the inherent inadequacy of the culture method (31), or both.

We have recently (15) reported the development of an indirect fluorescent gonococcal test-heated (FGT-H) which we believed could be used to screen the population for asymptomatic infected women. In a selected population of infected and uninfected women, the method proved to have a sensitivity of 94.6% and a specificity of 96.7% (15). The present report summarizes a field evaluation of this test in different populations, with special emphasis on its usefulness for screening asymptomatic women.

MATERIALS AND METHODS

Blood specimens were accepted from any physician or institution in New York State. With each specimen a companion cervical or urethral specimen was requested for culture; this was to be mailed to our laboratory on the Gonogrow transport culture system (6). A history form (Fig. 1) was completed by the physician and sent with the specimens.

Each specimen was assigned to a low- or high-risk group. Men and women who had no past history of gonorrhea and who were judged clinically free of signs and symptoms which might indicate current gonorrhea infection were assigned to the low-risk group.

Upon arrival at our laboratory, the culture specimen was processed in the bacteriology laboratory as previously described (28). The blood specimen was

examined by the special serology group, and the data were collated by use of the State Health Department computer. The serological test was then evaluated for its sensitivity and specificity by sex in each group.

FGT-H test. The FGT-H test as published (15) has been adapted for mass screening. Briefly, antigen slides were prepared from *Neisseria gonorrhoeae* isolate B-370. All sera were diluted 1:10 in physiological saline and heat inactivated at 59°C. Each was then used to stain one spot on a 10-spot slide, which was read on a Leitz SM microscope fitted with a Ploem reflected-light system.

Sera that gave no, shadow, or 1+ fluorescence were reported as negative. Sera which gave 3 to 4+ fluorescence were reported as positive. Sera which gave intermediate readings were reexamined by comparing the fluorescence of 1:10 diluted serum portions before and after heat inactivation. Sera which gave 1 to 2+ fluorescence or higher with no appreciable reduction after heating were reported as borderline (1 to 2+) or positive (2+ or higher).

RESULTS

Between 1 November 1974 and 31 October 1975, we received 26,705 serum specimens for examination. These included 5,635 specimens received without matching specimens for culture and another 4,943 received with culture specimens but not enough information for proper evaluation. The remaining 16,127 (60%) were accompanied by both culture specimens and completed history forms. Of these, 2,526 (15.7%) were from men, of which 80% were submitted by venereal disease clinics; 13,601 (84.3%) were from women, mostly through family planning clinics.

Cultural findings. The cultural positivity rates were: low-risk group, men 1.6%, women 0.75%; high-risk group, men 21.4%, women 11.5%.

Sensitivity. The sensitivity of the FGT-H was measured in culture-positive cases and is

VENEREAL DISEASE				PATIENT'S NAME AND ADDRESS		
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PLEASE COMPLETE ITEMS BELOW WITH NO. 2 PENCIL				PHYSICIAN'S NAME AND ADDRESS		
TYPE OF INSTITUTION	DATE SPECIMEN TAKEN	TAKEN FOR	SOURCE OF CULTURE SPECIMEN	PHYSICIAN'S CODE		
FAMILY PLANNING ☐		GC ☐ SYPH ☐ SYPH ONLY ☐	CERVIX ☐			
PRIVATE PRACTICE ☐	MALE ☐ FEMALE ☐	SCREENING ☐	VAGINA ☐	FOR LABORATORY USE ONLY		
VD CLINIC ☐	SINGLE ☐ MARRIED ☐	DIAGNOSIS ☐	URETHRA ☐			
HOSPITAL (NON VD CLINIC) ☐	PREVIOUS SPECIMENS SUBMITTED FOR THIS PATIENT:	PREMARITAL ☐	RECTUM ☐	LAG PERIOD DAYS		
HEALTH DEPT. (NON VD CLINIC) ☐	NONE ☐ 1 ☐ 2 ☐ >2 ☐	PRENATAL ☐	THROAT ☐			
NEIGHBORHOOD HEALTH CENTER ☐	CULTURE SPECIMEN SENDING:	TREPONEMAL TEST ☐	OTHER (NOT BLOOD) ☐	NO IDENTITY		
FREE CLINIC ☐	NO ☐ YES ☐	TEST OF CURE ☐				
STUDENT HEALTH SERVICE ☐	MEDICAL HISTORY (THIS VISIT)			EXTRA SPECIMENS		
CORRECTIONAL INSTITUTION ☐	EXPOSURE	SYMPTOMS	PRIOR HISTORY			TREATMENT
MENTAL HYGIENE ☐	GC ☐ NO ☐ YES ☐	GC ☐ NO ☐ YES ☐	GC ☐ NO ☐ YES ☐	GC ☐ NO ☐ YES ☐	SUBMITTED:	
OTHER ☐	SYPH ☐	SYPH ☐	SYPH ☐	SYPH ☐		
	"LIKELY" DATE	ONSET DATE	WHEN?		UNSATISFACTORY:	
					INSUFFICIENT ☐ VISCOUS ☐	
					LEAKAGE ☐ CONTAMINATED ☐	
					HEMOLYZED ☐ DISCOLORED ☐	
					SEPARATION ☐ BROKEN ☐	
					MEDIA EXPIRED ☐	
					NEW YORK STATE HEALTH DEPARTMENT DIVISION OF LABORATORIES & RESEARCH ALBANY, NEW YORK 12241	

FIG. 1. History form completed by the physician to accompany each pair of blood and cervical/urethral specimens submitted for the FGT-H field evaluation.

reported as the percentage of culturally proven cases of gonorrhea which were read as borderline or positive. The sensitivity for women was 81% in the low-risk and 75% in the high-risk group (Table 1). The test was less sensitive for men in both groups.

To determine the effect of past infection on the sensitivity of the test, the results for patients with and without past gonorrheal infection were compared (Table 2). The sensitivity for women was independent of past history, but a statistically significant increase in sensitivity for men with a previous history of gonorrhea was observed ($P < 0.01$, chi-square test).

Specificity. The specificity of the FGT-H was calculated from specimens from women in the low-risk group who were bacteriologically negative. There were 11,771 specimens in this category, 91% from patients examined at family planning clinics. Only 876 sera (7.4%) gave a 2+ fluorescence or higher; these were considered false positives. An additional 9.1% gave a borderline reading of 1 to 2+ (Table 3). A higher percentage of sera gave positive fluorescence in specimens from women who were symptomatic and/or who had been exposed to infection but were bacteriologically negative. This increase was not observed for men or for patients with past history of infection.

Reproducibility. Fluorescent microscopy has

TABLE 1. Sensitivity of FGT-H in field evaluation

Group	Total no. examined	No. of culture positives (%)	No. of culture positives detected serologically (%)
Women			
Low risk	11,860	89 (0.75)	72 (80.9)
High risk	1,741	201 (11.50)	151 (75.1)
Men			
Low risk	700	11 (1.60)	5 (45.5)
High risk	1,826	394 (21.60)	259 (65.7)

several inherent problems with reproducibility, including the effect of antigen density and background fluorescence on the reading, the subjective interpretation of the intensity of the fluorescence, and the variability of the equipment used.

To evaluate the reproducibility of the FGT-H test, two experiments were run.

(i) **Antigen slides.** During a 12-month period more than 125 lots of antigen slides (each lot is 25 slides) prepared on different dates and from different subcultures were used. Both the sensitivity (79.0 ± 5.0) and the specificity (92 ± 2.5) of the test were reproducible from month to month (Table 4). There were two exceptions, however. Sensitivity dropped to 20% from 10 May to 20 May (antigen lot no. 82) but was bracketed by sensitivities of 75% and 88% in the

TABLE 2. *Effect of past infection on sensitivity of FGT-H*

Group	No.	No past infection		Past infection	
		FGT-H/culture ^a	(%)	FGT-H/culture	(%)
Men					
Asymptomatic	40	17/31	(55)	8/9	(89)
Symptomatic	365	120/198	(61)	119/167	(71)
Total	405	137/229	(60)	127/176	(72)
Women					
Asymptomatic	168	117/150	(78)	13/18	(72)
Symptomatic	122	56/75	(75)	37/47	(79)
Total	290	173/225	(77)	50/65	(77)

^a Ratio of number of FGT-H positives to cultured positives.

TABLE 3. *Effect of exposure and symptoms on rates of positive and borderline FGT-H reactions in culture-negative cases*

Criteria ^a		Men			Women		
Exposure	Signs and symptoms	No.	% Positive	% Borderline	No.	% Positive	% Borderline
—	—	689	12.3	14.8	11,771	7.4	9.1
—	+	538	11.2	11.9	238	10.9	10.5
+	—	200	25.5	11.5	328	20.1	13.7
+	+	251	18.7	12.4	263	27.8	9.5

^a These individuals had no history of past infection.

10-day periods immediately before and after, and similar results were observed from 27 September to 17 October: sensitivity dropped to 25% but was bracketed by sensitivities of around 90%.

(ii) **Technicians and instruments.** Differences were investigated in the subjective reading by two technicians on different days, by the same technicians on different days, and by two technicians reading the same slides with the same equipment. For this study, 71 specimens were first examined by two technicians on different days and on two different microscopes. Later, all the negatives and most of the positives were reexamined by one of the two technicians. There was 100% reproducibility on all specimens from patients with culture-proven gonorrhea and 85 to 87% reproducibility on the culture-negative sera (Table 5). The same results were obtained when two technicians using the same microscope examined 588 consecutive specimens.

Review of the individual readings suggested that the subjectivity might be limited to borderline sera. In this group the same technician might read the same serum as 1 to 2+, 2+, or 1+, which would be reported as borderline, positive, and negative, respectively. When the results for the 272 sera which were read as borderline on the first or second readings were tabulated (Table 6), the reproducibility was shown to be only 56.0%. However, this low

reproducibility tends to reduce specificity rather than sensitivity. None of the specimens from patients with positive cultures were read as negative, but a full 37.5% of those from patients with negative cultures were read as negative on retesting ($P < 0.01$), and only 6.5% were read as positive.

Effect of FGT-H screening on culture rates. The FGT-H appeared to be sensitive, specific, and reproducible within the limitations of fluorescent microscopy, but it was important to determine its value as an adjunct screening test, not only for diagnosis but also for excluding infection in asymptomatic patients. Table 7 shows the value of the FGT-H for rapid confirmation of the physician's clinical diagnosis of clinically positive patients and for excluding infection in asymptomatic women. The culture positivity rate increased from 0.75% to 8.00% for low-risk women and up to 51% for high-risk men when only FGT-H-positive or borderline cases were cultured. For asymptomatic women who were FGT-H negative, the culture positivity rate was 0.15%.

DISCUSSION

Definitive diagnosis of gonorrhea requires the isolation of *N. gonorrhoeae* from a patient's specimen. The fastidious nature of the organism has stimulated a wide acceptance of less-definitive methods such as the Gram stain for men with acute urethritis (5) and fluorescent

TABLE 4. Reproducibility of FGT-H for women during 12-month field evaluation

Mo	No. of specimens examined ^a	No. of cultures positive	Sensitivity (%) ^b	Specificity (%) ^c
1974				
November	1,130	45	80	95
December	765	32	81	94
1975				
January	1,014	40	78	94
February	1,104	38	84	89
March	831	29	79	93
April	1,704	44	70	91
May	1,086	26	58 ^d	94
June	1,160	26	77	93
July	665	24	79	92
August	900	31	90	86
September	1,075	33	67 ^d	92
October	1,209	23	74 ^d	93

^a All specimens from women in low-risk group plus culture-positive specimens from women in high-risk group.

^b Sensitivity = (FGT-H positives + borderlines/cultural positives) × 100.

^c Specificity = (clinical and cultured negatives, FGT-H negatives + borderlines/clinical and cultural negatives) × 100.

^d Decline in sensitivity associated with use of apparently unsatisfactory antigen lots used over 1 to 2 weeks in the corresponding months.

TABLE 5. Agreement of FGT-H readings by different technicians

Experimental condition	Specimens		Agreement ^a	
	Cultural result	No.	No.	%
Two technicians, each with different microscope; different days	+	25	25	100
	−	46	40	87
Rereading by original technician same microscope, later day	+	22	22	100
	−	46	39	85
Two technicians using same microscope	+	5	5	100
	−	583	500	86

^a Calculated as number reported positive (positive or borderline reading) by both technicians or reported as negative by both.

microscopy for women (41) and for men with prostatitis (9, 30). The introduction of the selective culture medium by Thayer and Martin (40) and the later development of the culture-transport Transgrow (29) improved the sensitivity of culture for detecting gonorrhea infection, especially in women, but it has never been ade-

TABLE 6. Reproducibility of FGT-H borderline readings

Specimens		FGT-H readings ^a			Change to negative (%)	
Cultural result	No.	B → B	B → +	B → −		
+	16	10	6	0	0	} P < 0.01
−	256	143	17	96	37.5	

^a B, Borderline.

quately evaluated as a screening procedure in the low-risk population (31).

Recently, the increased significance of primary pharyngeal and rectal infection (14, 24, 32, 33) has increased the number of sites to be examined. Laboratories have been inundated with specimens that require more work to differentiate *N. gonorrhoeae* from the other oxidase-positive, gram-negative cocci or coccobacilli commonly encountered at these sites. This unavoidable increase in the cost, combined with evidence that culture, even under optimal conditions, may be an insensitive (11, 22, 23, 25) as well as expensive (43; G. H. Reynolds and P. J. Wiesner, personal communication) screening tool, mandates that a reasonably sensitive and specific blood test be used as the primary screening tool to identify the population with probable gonorrhea infection. Such screening would allow for the best utilization of the available funds and manpower.

We have earlier reported (15) FGT-H results obtained with a selected population, mostly from venereal disease clinics. Previous disappointments with several reported serological methods when they were evaluated as a screening tool (10, 35, 36) prompted us to study the test with primary emphasis on the asymptomatic women who are the target of screening programs. Nearly 12,000 asymptomatic women were examined. They had a low positivity rate (0.75%) by culture but contributed 31% of all culturally positive cases in the 13,601 women studied.

The test detected 80.9% of the infected asymptomatic women. This sensitivity is similar to that with the preselected group from Albany County Venereal Disease Clinic (15) but is significantly lower than with the other two preselected groups ($P < 0.01$). This difference may reflect the difference between the populations. Unlike the field evaluation and Albany County Venereal Disease Clinic groups, groups 2 and 3 of the preselected population were patients diagnosed 1 to 6 weeks earlier, a delay that might have allowed for the appearance of detectable antibodies (4).

TABLE 7. Effect of FGT-H screening on culture yield

Specimens		Positivity rate (%)				Frequency distribution of cultural positive (%)	
History	Clinical ^a	Men (n = 2,526)		Women (n = 13,601)		Men	Women
		Un-screened	Screened (only FGT-H + or borderline)	Unscreened	Screened (only FGT-H + or borderline)		
+	+	32.0	51.2	19.6	28.9	42.7	21.0
-	+	18.0	45.5	14.1	38.0	53.8	46.8
+	-	3.0	8.1	2.4	3.8	0.7	1.4
-	-	1.6	5.6	0.75	8.2 ^b	2.7	30.7

^a Signs and symptoms and/or contacts.

^b For women in the low-risk group only who were FGT-H-negative, the culture positivity rate was 0.15%.

The sensitivity of the FGT-H in women was not altered significantly by prior history of infection with gonorrhea. This may be due to the asymptomatic or minimally symptomatic course usually observed in women. This subacute or chronic course might have an effect on the antibody level similar to that of previous infection.

The probable role of duration of infection is more clearly seen in men. Asymptomatic men with no history of past infection have demonstrable antibodies in only 55% of the cases. These men were most probably in the incubation period, which has been estimated as 2 to 8 days (2). Sensitivity increased significantly if the patient had a history of past infection with gonorrhea.

Only 7.4% of the sera from culture-negative, asymptomatic women were positive in the test. In some of these cases there may have been inaccurate information regarding history of past infection or contact with a case of gonorrhea, false-negative culture results, and/or cross-reacting antibodies (H. A. Gaafar and D. Schoonmaker, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C147, p. 50). Several suggested or proven factors that may result in false-negative culture results include susceptibility of *N. gonorrhoeae* to vancomycin (3, 8, 38), hemoglobin (20, 34), and other microorganisms (19); the phase of the menstrual cycle (25); the culture site (1, 28); the culture medium (12, 16, 26, 39); and delay in transit (17, 27). Actually, the sensitivity of the culture method in a screening program could be as low as 40% (31). Further confirmation that the low sensitivity of the culture is a major contributor to the "false positives" is the better correlation of serological test and clinical findings in women with no history of previous infection. In our population, only 18% of the women who were exposed to gonorrhea were culture positive, whereas 35.4% were FGT-H reactive (Table 3), which is more in

agreement with expected infection rate of 37.5% (42).

An additional 9.1% of the women in the low-risk group had a borderline reaction. This percentage is very similar to the 8.6% borderline reaction observed with the fluorescent treponemal antibody-absorption test in nonsyphilitic patients (7); and as with that test, a borderline reaction in the FGT-H should be repeated (21).

This 1-year field evaluation confirms the usefulness of the FGT-H test as a screening procedure for the detection of asymptomatic patients, especially those who have had the infection for more than 1 week. These asymptomatic patients may be responsible for the continuation of the epidemic (18), and they themselves may suffer from serious complications (37).

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