Evaluation of the Gonosticon Dri Dot Test in Females with a Low Incidence of Gonorrhea

JOHN D. DYCKMAN, REUBEN D. WENDE, AND ROBERT P. WILLIAMS

Department of Microbiology and Immunology, Baylor College of Medicine, and Houston City Health
Department Laboratory, Houston, Texas 77025

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A group of 765 females attending a Planned Parenthood Clinic was screened for gonorrhea by inoculating Thayer-Martin plates and Transgrow bottles with specimens from the cervix. Blood was obtained at the same time and tested for anti-gonococcal antibody by using the Gonosticon Dri Dot test. In this low-incidence group, 18 positive cultures were detected by culture on Thayer-Martin plates, whereas Transgrow detected only 15 positive cultures. Of the 18 patients with gonorrhea, 11 exhibited reactive serum (agglutination of the latex particles). In the total population, 64% of the patients had nonreactive serum (no agglutination) and negative cultures; 25% had reactive serum and negative cultures. When this latter group was subdivided on the basis of race, blacks and Latin Americans were found to have a higher incidence of reactive serum with a corresponding negative culture than was found in whites. Patients who were originally culture positive and nonreactive in the Gonosticon Test were retested; three out of four patients retested within 6 to 11 days after the initial screening had converted to a positive Gonosticon test.

Gonorrhea is epidemic and constitutes one of the major health problems throughout the world. In 1971 there were an estimated 2.5 million cases in the United States, with a 15% increase expected in 1972 (4). At this rate of increase, there should have been over 3 million cases of gonorrhea in the United States in 1973, although only 823,380 cases were reported to the Center for Disease Control (5). The task of screening and treatment of patients is complicated by the fact that approximately 90% of the females who are infected with Neisseria gonorrhoeae remain asymptomatic and thus will not be prompted to seek medical attention (6).

Diagnosis of gonorrhea was aided greatly by the development of the selective Thayer-Martin medium (8, 9). However, screening of large populations by culture necessitates a pelvic examination in females and urethral swab in males. Also, screening by culture methods alone has not reduced the incidence or rate of increase of gonorrhea.

Many authorities have emphasized the need for a rapid serological test to simplify and speed the screening process. Recently, a new serological test, the Gonostatic Dri Dot (Organon Inc., West Orange, N.J.), was developed to aid in screening large population groups. Our investigation has evaluated the specificity and sensitivity of this test in a group of females in whom the incidence of positive cultures for N. gonorrhoeae was relatively low.

MATERIAL AND METHODS

Media. Modified Thayer-Martin medium contained GC medium base (BBL), 1% hemoglobin (Difco), 1% defined supplement (Iso-VitaleX; BBL), vancomycin (3.0 μg/ml), colistin (7.5 μg/ml), nystatin (12.5 U/ml), and trimethoprim (5.0 μg/ml). Bottles of Transgrow medium (3) were obtained from the Texas State Health Department in Austin. The medium in these bottles was of the same composition as the modified Thayer-Martin medium except that the amount of agar was doubled to 2%. The Transgrow bottles were filled with CO₂ to a final concentration of 20%. Chocolate agar contained the same constituents as modified Thayer-Martin medium, but the antimicrobial inhibitors were omitted. For sugar fermentations, CTA medium (BBL) containing 1% dextrose, sucrose, maltose, lactose, mannitol, or fructose was used.

Patients. A group of 765 women attending a Planned Parenthood Clinic was selected at random for this study. Data on the patient's age, race, and method of birth control were obtained from information furnished by the patients.

Culture and identification of bacteria. Specimens for culture were taken by a single, sterile cotton swab from the cervix and were inoculated immediately onto petri plates containing modified Thayer-Martin medium and into Transgrow bottles. The
sequence of inoculation was randomized. The bottles were processed at the Houston City Health Department Laboratory by incubation at 35 C in a CO₂ incubator providing an atmosphere of 8 to 10% CO₂. Specimens were examined after incubation for 24 and 48 h. At 48 h, all bottles were flooded with oxidase reagent (N,N-dimethyl-p-phenylenediamine mono-hydrochloride; Eastman Kodak), and oxidase-positive colonies were Gram stained. If colonies of oxidase-positive, gram-negative diplococci were found, the culture was recorded as presumptively positive for the presence of *N. gonorrhoeae*.

The Thayer-Martin plates were streaked to isolate colonies and then were incubated in candle jars at 35 C for 48 h. The plates were examined daily and suspicious colonies were isolated on chocolate agar. Isolated colonies of oxidase-positive, gram-negative diplococci were confirmed as *N. gonorrhoeae* by the pattern of sugar fermentations as well as by the inability to grow on nutrient agar slants at 37 C in candle jars.

### Performance of the Gonosticon Test

Blood samples were collected in stoppered Vacutainer tubes and allowed to clot. The unclotted portion was decanted and centrifuged, and the clear supernatant fluid was saved. Samples (0.05 ml) of serum were tested for the presence of antibodies against *N. gonorrhoeae* with the Gonosticon Dri Dot test (Organon, Inc., West Orange, N.J.) according to the method described by the manufacturer. Specimens were recorded as nonreactive if no visible agglutination of the latex particles was seen. Reactive sera, as evidenced by latex agglutination, were graded from 1+ to 4+ on the basis of the size of the agglutinated latex particles. If a slight coarseness of the latex suspension was seen at the end of the test, the result was recorded as a borderline reaction and was interpreted as an inconclusive test, being neither reactive nor nonreactive. In all cases the Gonosticon test was performed within 24 h after the blood was collected. To aid in the objectivity of this study, the Gonosticon Dri Dot test was performed only once on each specimen of serum and before the corresponding cultures were examined.

### RESULTS

A comparison of the results of the Gonosticon test with cultures grown on plates containing Thayer-Martin medium is presented in Table 1. A total of 18 positive cultures were obtained. Only 15 positive cultures were detected with the Transgrow bottles, consistent with the difference in sensitivity of these two media previously reported (10). Serum from 11 of these positive cases was reactive in the Gonosticon test. Negative cultures with corresponding nonreactive sera were obtained from 63.9% of the patients. Reactive serum with a corresponding negative culture was present in 23.5% of these females. *N. meningitidis* was cultured from the cervix of one patient who exhibited a nonreactive Gonosticon test.

Six subjects yielded a positive culture with a nonreactive Gonosticon test. All of these females were retested to determine whether antibodies would appear in the serum at a later date (Table 2). Of the four patients retested within 11 days of the initial screening, three had converted to reactive serum. Five had converted to reactive serum within 1 month, and three of the five exhibited a strongly positive test. One patient had failed to convert when she was retested 2 months after the initial screening.

During this study we noted that the females who had a reactive Gonosticon test with a corresponding negative culture were predominantly nonwhite Americans. When all patients were grouped by race to determine the percentage of each racial group exhibiting a reactive Gonosticon test with a negative culture (Table 3), over 41% of the black and 33% of the Latin American females were Gonosticon reactive and culture negative. Only 14.2% of the white females were in this category. The difference between white and black groups was significant at a value of *P* < 0.001 by use of a chi-square test (Yates correction). No significant differences were demonstrable between blacks and Latin Americans or between whites and Latin Americans. Also, no significant differences in reactivity to the Gonosticon test were evident when patients were grouped on the basis of age or method of birth control.

The percentage of each racial group yielding a positive culture regardless of the Gonosticon result was also determined (Table 3). In this case, no significant differences were seen between the number of positive cultures from these racial groups.

Table 4 lists the gradations of the Gonosticon reactions of all 765 females. A reactive Gonosticon test was present in 61% of the females with a

### Table 1. Comparison of the results of the Gonosticon Dri Dot test with results of cultures grown on Thayer-Martin medium

| Gonosticon result* | Culture results* | Total |
|--------------------|------------------|-------|
|                    | Positive | Negative |       |
| Reactive           | 11 (1.4)  | 180 (23.5) | 191   |
| Borderline         | 1 (0.1)   | 78 (10.2)  | 79    |
| Nonreactive        | 6 (0.8)   | 489 (63.9) | 495   |
| Total              | 18       | 747       | 765   |

* Procedures as described in reference 5.

* Cultures were incubated for 48 h in candle jars at 35 C. A positive culture indicates identification as *N. gonorrhoeae* of isolated colonies by subculture. Numbers in parentheses indicate percentages.
TABLE 2. Results of the Gonosticon Dri Dot test after retest of females with an initial positive culture and a nonreactive serum

| Patient's no. | Date of initial test | Date of second test | Gonosticon reaction, second test | Date of third test | Gonosticon reaction, third test |
|---------------|----------------------|---------------------|---------------------------------|-------------------|-------------------------------|
| 246           | 11/13                | 1/17                | Nonreactive                     | ND*               | ND                            |
| 566           | 12/4                 | 12/10               | Nonreactive                     | 12/27             | 1+                            |
| 587           | 12/7                 | 1/4                 | 2+                              | ND                | ND                            |
| 589           | 12/7                 | 12/14               | 3+                              | ND                | ND                            |
| 611           | 12/11                | 12/18               | 3+                              | ND                | ND                            |
| 741           | 12/20                | 12/31               | 4+                              | ND                | ND                            |

* ND, Not done.

TABLE 3. Incidence by racial group of females with a reactive Gonosticon test and a negative culture

| Racial group\(^a\) | No. of patients | Gonosticon reactive with negative culture\(^b\) | Gonosticon reactive, borderline, or negative with positive culture\(^c\) |
|---------------------|----------------|---------------------------------------------|-----------------------------------------------------------------|
| White               | 464            | 66 (14.2)                                   | 11 (2.4)                                                        |
| Black               | 188            | 78 (41.3)                                   | 7 (3.7)                                                        |
| Latin American      | 102            | 34 (53)                                     | 0                                                               |
| Other\(^d\)         | 8              | 0                                            | 0                                                               |
| Total               | 737\(^e\)      | 178\(^e\)                                   | 18                                                              |

* Racial group as designated by each patient.
* Numbers in parentheses indicate percentages.
* Of the white group, six were Gonosticon reactive and one reaction was borderline. Five of the black group were reactive, and two were negative.
* This group included four Orientals, two Asian Indians, one American Indian, and one Israeli.
* Two of the 765 patients designated no racial group. Both had a reactive Gonosticon test and a negative culture.

positive culture, whereas only 24% of the females with negative cultures were reactive. Of the females from whom N. gonorrhoeae was isolated, 33% were nonreactive by the Gonosticon test whereas 65% with negative cultures were nonreactive. The group with positive cultures had a higher percentage in each of the grades of reactivity, 1+ through 4+. This difference between culture-positive and culture-negative groups was found to be significant at \( P < 0.001 \).

Unfortunately, complete medical records for these females were unavailable. In only six of the 180 patients who exhibited a reactive Gonosticon test with a negative culture could a history of gonococcal infection be obtained (Table 5). Prior records indicated isolation of N. gonorrhoeae from four patients 6 months to 1 year before they were tested in this study. The remaining two patients had gonorrhea approximately 2 months before inclusion in this study. Five of these patients exhibited a 2+ Gonosticon reaction at the time of screening, and one exhibited at 3+ reaction.

**DISCUSSION**

Our data indicated that the Gonosticon Dri Dot test detected 61% of the cases of gonorrhea that were detectable by a single culture from the cervix. Five of the six females who had a nonreactive Gonosticon test at the time of positive culture converted to a reactive Gonosticon test within 1 month. We presumed that these patients were initially nonreactive because the disease was in too early a stage to promote antibody formation against the gonococci. The reason for lack of conversion in one patient is unknown.

Of the total population studied, 24% exhibited a reactive Gonosticon test with a corresponding negative culture. Several possibilities exist for such a combination of results. The first is that a certain percentage of these females had gonorrhea that was not detected by culture. Several studies indicate that between 13 and 54% of gonorrheal infections may be missed when a single culture is taken from the cervix (1, 2, 7). Repeated cultures from the cervix, as well as cultures from other sites such as the rectum and pharynx, provide a more thorough screening procedure. Unfortunately, such a protocol was not possible in this investigation. The second possibility is that some of these females had antibodies to N. gonorrhoeae due to a prior infection. The data obtained from the few patients with a known past history implied that such antibodies might persist in the peripheral blood for a long time. A third possibility is that some of these patients had antibodies against N. meningitidis that cross-reacted with the N. gonorrhoeae antigen used in the Gonosticon Dri Dot test. Finally, a certain percentage of these
reactive sera could be due to nonspecific cross-reactivity of unknown origin. The differences in the percentage of various racial groups yielding a positive Gonosticon test with a negative culture is of interest. Future studies may examine these differences in greater depth in an attempt to provide an explanation for them.

The Gonosticon Dri Dot test is not a diagnostic test for gonorrhea and should not be interpreted as such. It was found to be about 72% reliable when compared with the results of a single cervical culture. The Gonosticon test failed to detect 39% of the cases of gonorrhea that were evident by a positive culture, and 24% of the total population examined was reactive in the Gonosticon test although cultures were negative.

The Gonosticon Dri Dot test may have clinical value as an aid in screening groups with a low incidence of gonorrhea. In conjunction with culture, a nonreactive Gonosticon test would reinforce the finding of a negative culture. A reactive Gonosticon test with a negative culture might indicate that additional cultures from various sites are warranted. In support of this suggestion, the three females whose specimens showed no growth of N. gonorrhoeae in Transgrow medium but did have positive cultures on Thayer-Martin medium also had reactive Gonosticon tests. The Gonosticon test could also be used in patients who would not ordinarily be cultured to widen the screened population and to select those patients to be called back for a culture. Future research and developments should be directed towards investigation of these possible uses of the test as well as improvement of the sensitivity and specificity of this potentially valuable tool for screening patients for gonorrhea.

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