An Automated Complement Fixation Procedure for Detecting Antibody to *N. gonorrhoeae*

WILLIAM L. PEACOCK, Jr., B.A.

FOR many years complement fixation (CF) tests have been used in the serologic detection of gonorrhea (*1–9*). Reports in 1965 (*4–8*) favored the CF test, particularly with regard to serums from patients with anamnestic and clinical evidence of infection. The use of Thayer-Martin medium (*10*) focused attention on infected women with minimal or no symptoms, but as yet there is little information on the serologic reactivity of serums from such patients. In the work described in this paper, a CF technique was used to determine the reactivity of two antigens with several hundred serums, including 101 serums from women whose specimens yielded cultures positive for *Neisseria gonorrhoeae*. The complement fixation tests were facilitated by an instrument which automatically made the serial dilutions and added the reagents for the test (fig. 1).

Mr. Peacock is a research microbiologist, Venereal Disease Research Laboratory, Venereal Disease Branch, State and Community Services Division, Center for Disease Control, Health Services and Mental Health Administration, Public Health Service. Tearsheet requests to William L. Peacock, 1600 Clifton Road, NE., Atlanta, Ga. 30333.

Materials and Methods

Sheep red blood cells. Sheep red blood cells (*A*) were collected in 3.8 percent sodium citrate (23 ml. of blood to 27 ml. sodium citrate), washed three times in veronal buffered diluent (VBD) pH 7.3, and centrifuged to determine the volume of packed red blood cells. A 2.8 percent red blood cell suspension was used in the test.

Complement. Guinea pig lyophilized complement (C′) (*B*) was stored at 5°C. Reconstituted complement was stored in 0.5 ml. and 1.0 ml. amounts at —50°C. Each day tests were performed, C′ was thawed at 5°C. and diluted to yield $5C'H_{50}$ units per 0.05 ml. in veronal buffered diluent ($1C'H_{50}$ equals the amount of C′ needed to lyse 50 percent of sensitized sheep red blood cells).

Hemolysin. This reagent (*C*) (*11*) was diluted 1:100 in veronal buffered diluent as follows: 94 ml. VBD, 4.0 ml. 5 percent phenol in 0.85 percent NaCL, and 2.0 ml. of 50 percent glycerinized hemolysin. This stock 1:100 hemolysin was stored at 5°C. Optimal titer was determined for each new lot of 1:100 hemolysin and for each new lot of red blood cells.

Antigen. N. gonorrhoeae F 62 type 1 protoplasm was prepared as described by Martin and co-workers (*12*). The protoplasm antigen was lyophilized (shell frozen) and stored at room temperature (22°–25°C.). The dried material was reconstituted with veronal buffered diluent, 1 mg. per ml., and titrated (checkerboard) with positive pooled serums from patients with gonorrhea (*13*). The tited of the protoplasm antigen was 1:32 to 1:64 dilution.

The Wellcome antigen (*D*)

was stored at 5°C. and was titrated as described. The titer of the Wellcome antigen was 1:32.

Serums from four groups of persons were tested. The two groups with uncomplicated gonorrhea were 101 women and 87 men. All of these patients yielded specimens which were cultured and found positive for *N. gonorrhoeae.*

Those in the other two groups, all women, were presumed not to have gonorrhea. Specimens taken from the cervix of 61 of these women were cultured and found negative for *N. gonorrhoeae.* The other serums were taken from 66 celibate members of a religious order. All serums were stored at —20°C. and heated at 56°C. for 30 minutes before use.

Complement fixation technique. The laboratory branch complement fixation (LBCF) method (*14*) was utilized and augmented with the Autotiter (fig. 1) (*E*), an instrument which has been described elsewhere (*15, 16*).

For automation of the LBCF procedure, 0.05 ml. of heated (56°C. for 30 minutes) serum was manually added to the first U-shaped cup of each row of the clear autotray plates (Canalco 8 by 15 cups), using 0.05 ml. capillary tubes (*F*). Veronal buffered diluent, 0.025 ml., was automatically added to cups 2–10. Each serum was automatically diluted in twofold dilutions from 1:2 to 1:512 in veronal buffered diluent and 0.025 ml. of antigen added to each dilution by the Autotiter. Complement (0.05 ml.) was automatically added to each cup, and the reactants were incubated overnight at 5°C.

The next morning the Autotiter was used to add 0.025 ml. sensitized sheep red blood cells to all cups. The trays were shaken and incubated at 37°C. for 30 minutes. After the plates were centrifuged at 300 g's for 3 to 5 minutes, the reactions were ready by comparing each cup with standards of 0 percent to 100 percent hemolysis in 10 percent increments.

A reading of 50 percent hemolysis or less was considered reactive if the control cup (no antigen) showed 75–100 percent hemolysis. Serum was considered anticomplementary if the control cup showed 75 percent or less hemolysis at the starting dilution of 1:2.

Results

Results are presented in figure 2. With the protoplasm antigen, serums from celibate women and women whose specimens were negative when cultured showed 4 percent reactivity. Serums from women whose specimens were positive showed 80 percent reactivity at 1:2 dilution. Serums from men yielding specimens which were positive were 50 percent reactive at 1:2.

With the Wellcome antigen (*D*), the serums from women whose specimens were positive when cultured and from infected men were slightly less reactive than with protoplasm antigen; whereas the serums from celibate women and from women whose specimens were negative when cultured were slightly more reactive.

With both antigens, three se-

Figure 1. Automatic serial dilution instrument used in an LBCF micromethod application for detecting antibody to *N. gonorrhoeae*

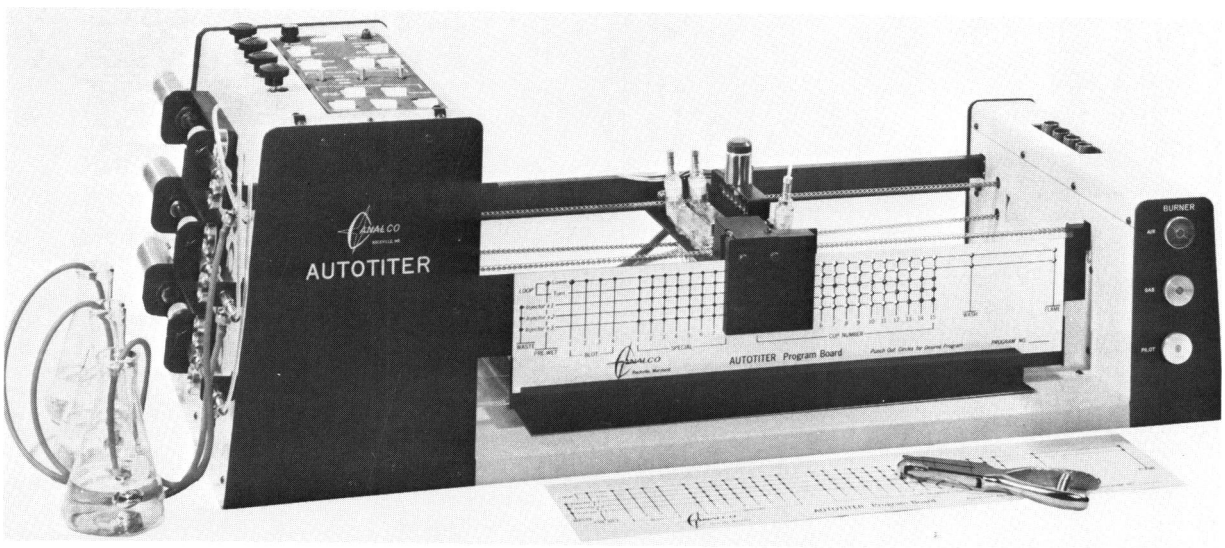
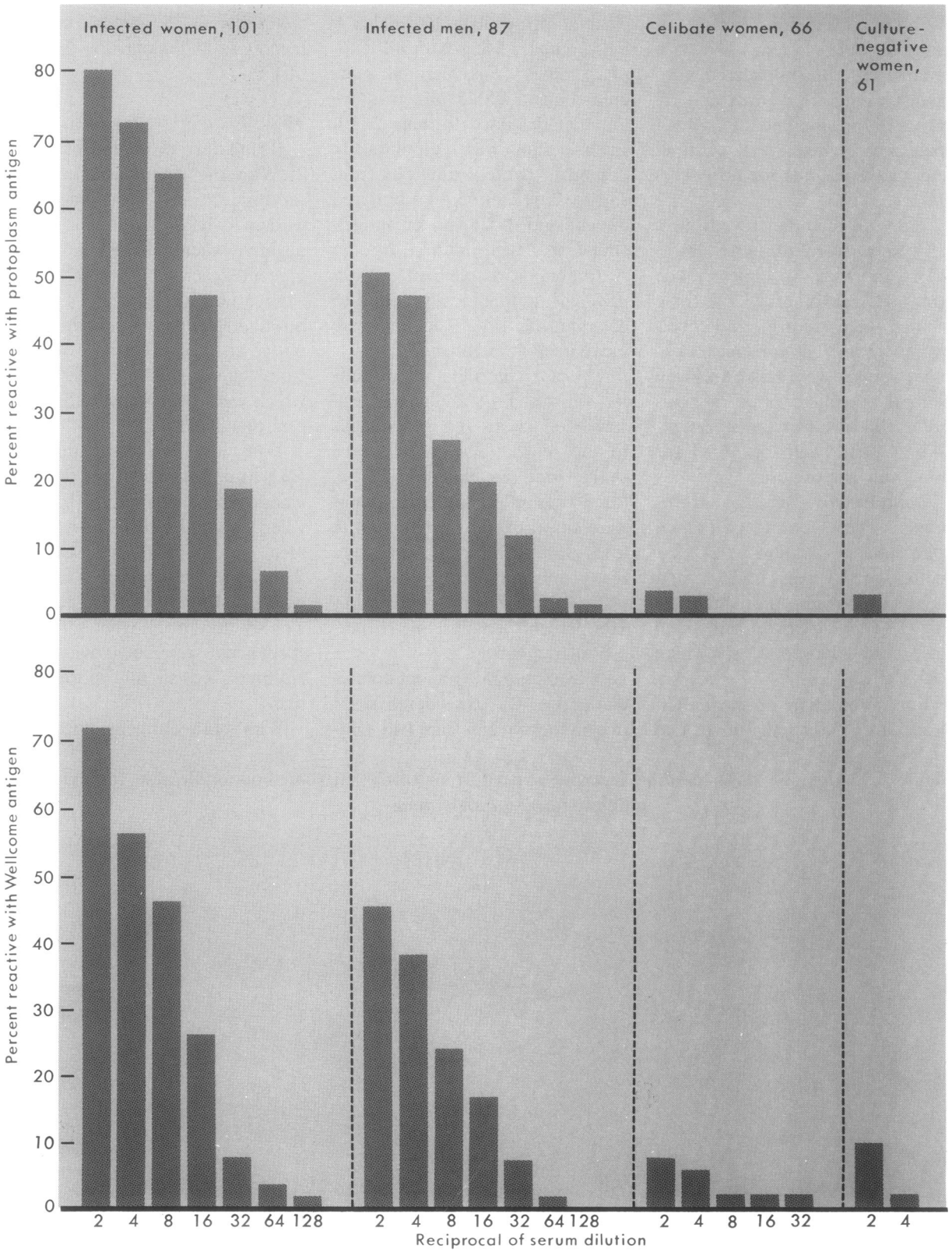


Figure 2. Percentage of reactions of four groups of serums with protoplasm and Wellcome antigens



rums from patients with gonococcal arthritis were positive at the 1:2 dilution and remained reactive when diluted 1:32, while three serums from patients with nongonococcal arthritis were negative at 1:2.

Discussion

Magnusson and Kjellander asserted in 1965 that the gonococcal complement fixation reaction "has unjustly fallen into far too deep obscurity" (*5*). The data in this study support that position.

Prior to the work of Magnusson and Kjellander, the important female carrier group, in which the patients are relatively asymptomatic, had received scant attention from investigators interested in the serology of gonorrhea. These authors found 50 percent of the asymptomatic female carriers yielded serum samples that reacted positively, which is less than the 80 percent reactivity found in this study using a 1:2 serum dilution and protoplasm antigen. The Wellcome antigen (*D*), which is commercially available, yielded 72 percent positive reactions under the same conditions.

In serums from infected men, 50 percent were positive at a 1:2 dilution using protoplasm antigen and 45 percent with the Wellcome antigen which is greater than the 21 percent detected by Magnusson and Kjellander. However, the percentages of positive reactivity on the presumedly noninfected group of serums was 1.3 percent in their study in contrast to 4 percent in this study.

As can be seen in figure 2, the apparent specificity can be increased by raising the minimum titer which is considered significant, but this increase is accomplished at the price of sensitivity. For example, at an 1:8 serum dilution the protoplasm antigen did not react with any serum in the presumedly noninfected group, but still reacted with 65 percent of serums from infected women.

The nature of the gonococcal antigen(s) reactive in the laboratory branch complement fixation method is not known beyond the initial description of protoplasm (*12, 13*). Although a single lot of protoplasm from the laboratory F 62 type 1 strain was used throughout this study, a few preliminary experiments indicated that similar titers of reactivity were obtained using equal weights of (*a*) protoplasm prepared from each of four recently isolated gonococcal strains or (*b*) a sonicate of F 62 type 1 organism.

This second observation suggests that simple sonicates might substitute for the more difficult to prepare protoplasm. No difference in reactivity per unit weight was noted when protoplasm from type 1 cells was compared with protoplasm from F 62 type 4 cells. Whole F 62 type 1 and F 62 type 4 gonococci, in suspension, gave a lower titer of reactivity than did an identical weight of F 62 type 1 protoplasm.

Magnusson and Kjellander used a heat-killed antigen (60°C. for 30 minutes) suspension of intact gonococci from 20 strains. According to information furnished by the manufacturer, the Wellcome antigen is prepared by a modification of the method of LeMinor (*3*). In that method, whole cells disrupted by alkali digestion or by ultrasonic treatment were acid precipitated.

To the best of our knowledge at the Venereal Disease Research Laboratory, this is the first report of the use of the Autotiter for the performance of a complement fixation reaction. The instrument functioned without mechanical difficulty and enabled me to increase greatly the output per unit time compared with manual methods. This assay could also be performed by manual methods using standard equipment in most serology laboratories.

REFERENCES

(*1*) Price, I. N. O.: The complement fixation test for gonorrhea. A new technique and its clinical application. London County Council (1933).

(*2*) Torrey, J. C.: A comparative study of antigens for the gonococcal complement fixation test. J Immunol 38: 413–430 (1940).

(*3*) LeMinor, L.: Preparation d'un antigene pour la practique des gonoreactions. Ann Inst Pasteur 75: 383–386 (1948).

(*4*) Labzoffsky, N. A., and Kelen, A. E.: New complement fixing antigen for serodiagnosis of gonorrhea. Can J Microbiol 7: 715–723 (1961).

(*5*) Magnusson, B., and Kjellander, J.: Gonococcal complement-fixation test in complicated and uncomplicated gonorrhea. Br J Vener Dis 41: 127–131 (1965).

(*6*) Anderson, K., and Brendish, G.: The specificity of the gonococcal complement-fixation test. Br J Vener Dis 2: 491–492 (1965).

(*7*) Lange, P. K., Reyn, A., Bentzon, M. W., and Lind, I.: Gonococcal complement fixation reaction (GR) in pulmonary patients in Greenland. Proc Fenno-Scand Assoc Derm 128: 101–106 (1965).

(*8*) Schmidt, J., Niordson, A.M., Reyn, A., and Bentzon, M. W.: Spiramycin in the treatment of acute gonorrhea. Br J Vener Dis 41: 120–126 (1965).

(*9*) Reising, G., Schmale, J. D., Danielsson, D. G., and Thayer, J. D.: Reactivity of two selected antigens of *Neisseria gonorrhoeae*. Appl Microbiol 18: 337–339 (1969).

(*10*) Martin, J. E., Billings, T. E., Hackney, J. F., and Thayer, J. D.: Primary isolation of *N. gonorrhoeae* with a new commercial medium. Public Health Rep 82: 361–363, April 1967.

(*11*) Darter, L. A.: Procedure for production of antisheep hemolysin. J Lab Clin Med 41: 653–654, April 1953.

(*12*) Martin, J. E., et al.: Preparation of cell walls and protoplasm of Neisseria with the Ribi cell fractionator. J Bacteriol 97: 1009–1011 (1969).

(*13*) Danielsson, D. G., Schmale, J. D., Peacock, W. L., Jr., and Thayer, J. D.: Antigens of *Neisseria gonorrhoeae*. Characterization by gel filtration complement fixation and agar-gel diffusion of antigens of gonococcal protoplasm. J Bacteriol 97: 1012–1017 (1969).

(*14*) Standardized diagnostic complement fixation method and adaptation to micro test.

1. Laboratory Branch Task Force: Laboratory Branch complement fixation method.

2. Casey, H. L.: Adaptation of LBCF method to micro technique. PHS Publication No. 1228 (Public Health Monogr No. 74). U.S. Government Printing Office, Washington, D.C., 1965.

(*15*) Goss, W. A., and Cimijotti, N. B.: Evaluation of an automatic diluting device for microbiological applications. Appl Microbiol 16:1414–1416 (1968).

(*16*) Cox, P. M., Logan, L. D., and Norins, L. C.: Automated quantitative micro hemagglutination assay for *Treponema pallidum* antibodies. Appl Microbiol 18: 485–489 (1969).

EQUIPMENT REFERENCES

(*A*) Sheep red blood cells, Scientific Resources Branch, Laboratory Division, Center for Disease Control, Atlanta, Ga. 30333.

(*B*) Guinea pig lyophilized complement, Texas Biological Laboratories, Inc., P.O. Box 722, Fort Worth, Tex.

(*C*) Hemolysin, Reagents Testing and Evaluation Sub-Unit, Venereal Disease Research Laboratory, Center for Disease Control, Atlanta, Ga. 30333.

(*D*) Wellcome gonococcal antigen, Wellcome Research Laboratories, Beckenham, England.

(*E*) Autotiter, Canalco, Inc., Rockville, Md. 20852.

(*F*) Capillary tubes, Hynson, Westcott and Dunning, Inc., Baltimore, Md. 21201.

PEACOCK, WILLIAM L, Jr. (Center for disease Control): *An automated complement fixation procedure for detecting antibody to* N. gonorrhoeae. *HSMHA Health Reports, Vol. 86, August 1971 pp. 706–710*

A micro complement fixation technique was automated using the Autotiter. This instrument dilutes serum specimens and adds reagents automatically. In this study, the reactivity of two antigens were compared, a gonococcal protoplasm antigen prepared in the Venereal Disease Research Laboratory and a gonococcal complement fixation antigen prepared at the Wellcome Research Laboratory.

Serums from men and women with gonorrhea and serums from women presumed not to have gonorrhea were tested using both antigens. Serums were automatically diluted from 1:2 to 1:512.

At a 1:2 serum dilution the gonococcal protoplasm antigen reacted with 80 percent of the serums from infected women, 50 percent of the serums from infected men, and 4 percent of the serums from presumedly noninfected persons. Wellcome gonococcal antigen tested under similar conditions using the same serums reacted with 72 percent of the serums from infected women, 45 percent of the serums from infected men, and 10 percent of the serums from presumedly noninfected persons.

Serums from three patients with gonococcal arthritis were strongly positive with both antigens tested, while serums from three patients with nongonococcal arthritis were negative with both antigens. It is concluded that complement fixation assays, especially when automated, merit further attention in the development of serologic tests for gonorrhea.