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Microflocculation Assay for Gonococcal Antibody

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An antigen suspension consisting of cholesterol-lecithin particles sensitized with an extract of gonococci was used in a flocculation assay for the detection of human antibodies to *Neisseria gonorrhoeae*. With culturally and clinically diagnosed cases of uncomplicated gonorrhea, sera from 70 (49.6%) of 141 males and 207 (79.0%) of 262 females were reactive in the assay. Sera from four (7.3%) of 55 males and 11 (5.4%) of 203 females, all presumed normal, were reactive. The assay utilized equipment available in most clinical and public health laboratories.

Vogel et al. (5) described a flocculation technique in which the extract of a specific etiological agent was carried on a cholesterol-lecithin particle. This report describes a flocculation assay for gonococcal antibodies by using an extract of the gonococcus carried on a similar particle. The results of testing several hundred sera indicate that a workable assay has been developed.

MATERIALS AND METHODS

Source of sera. Presumed normal sera were obtained from blood donors, nuns, and monks. Sera from gonococcal patients were collected at several venereal disease clinics in the United States. Patients were diagnosed as having a gonococcal infection if urethral cultures in the male and cervical cultures in the female revealed the presence of oxidase-positive colonies of gram-negative diplococci on Thayer-Martin medium (4).

Bacterial growth. F62 type I gonococci (1) were grown on GC Medium Base (Difco) containing a defined supplement (1). The cultured organisms were harvested into sterile, distilled water cooled in an ice bath.

Extract preparation. The gonococci, suspended in distilled water and cooled in an ice bath, were subjected for 1 hr to the action of a Raytheon 250 w, 10 kc magnetrostrictive oscillator cooled with water at approximately 2 C. The resulting sonically treated material was then centrifuged at 43,500 × *g* for 1 hr at 5 C. After centrifugation, the supernatant fluid was decanted and lyophilized.

Preparation of antigen suspension. (i) A 2-mg amount (set originally by trial) of the dehydrated supernatant fluid of F62-T1 sonically treated material was reconstituted with 1 ml of Sorensen's buffer (*p*H 7.0) in a plastic tube (12 by 75 mm). (ii) The reconstituted supernatant was transferred to a 30-ml glass-stoppered flat bottom bottle. (iii) The bottle was tilted so that the liquid lay to one side, and 0.25 ml of cholesterol-lecithin mixture (4.0 mg of lecithin per 10 ml of 0.9% cholesterol in absolute alcohol) was blown by pipette directly into the extract. The tip of the pipette rested just below the bottle neck. (iv) The bottle was stoppered, and the mixture was shaken from bottom to top approximately 30 times in 15 sec. The mixture was allowed to stand for 1 hr at room temperature. Acceptability of the antigen suspension was determined by check testing with control sera. Flocculation (described below) with sera from diagnosed cases of gonorrhea and no flocculation with sera from presumed normals was an acceptable pattern.

Preparation of cholesterol-lecithin mixture. The optimum amount of lecithin for the antigen suspension was determined by adding successive increments of lecithin (0.05 mg/ml) to 0.9% cholesterol with a final concentration of lecithin varying from 0.3 to 0.5 mg/ml of 0.9% cholesterol. Antigen suspensions were made with each of these cholesterol-lecithin mixtures by the procedure above. Sera obtained from cases of gonorrhea diagnosed clinically and in the laboratory and from individuals presumed never to have had gonorrhea were tested with each of the prepared antigen suspensions. The ratio of lecithin to 0.9% cholesterol which showed the greatest sensitivity with serum from diagnosed cases of gonorrhea and clearly negative results with serum from presumed normals was selected for the assay. By using the technique described in the assay procedure below, it was found that adding 4.0 mg of lecithin in absolute alcohol to 10 ml of 0.9% cholesterol in absolute alcohol gave optimal results.

Assay procedure. (i) A 0.025-ml amount (measured with a capillary pipette) of whole serum, unheated, was pipetted onto a glass slide (2 by 3 inches 5.08 by 7.52 cm) with paraffin rings approximately 14 mm in diameter. (ii) A 0.025-ml amount (measured with a calibrated pipette dropper) of antigen suspension was added to each serum. (iii) The antigen suspension and serum were stirred with a toothpick in a manner to spread the mixture over as much of the surface area within the ring as possible. (iv) The slide was rotated on a rotating apparatus [adjustable to 180 rev/min, circumscribing a circle 0.75 inch (1.9 cm) in diameter on a horizontal plane] at 140 rev/min for 10 min under a moisture chamber. (v) At 100× magnification, any clumping of antigen particles (flocculation)

TABLE 1. *Gonococcal (GC) antibody microflocculation assay of human sera*

Source of sera	No. tested	No. reactive	Amt reactive (%)
Presumed normals			
Male	55	4	7.3
Female	203	11	5.4
GC patients			
Male	141	70	49.6
Female	262	207	79.0

was interpreted as reactive; no clumping or slight roughness, nonreactive.

RESULTS

Preliminary work with heated and unheated sera indicated that there was no appreciable difference in reactivities. For this reason the sera in this study were assayed unheated.

As the results of two different groups of sera (one coded and the other uncoded) were not significantly different, they have been combined for presentation. Of the male and female sera 7.3 and 5.4%, respectively, in the presumed normal groups reacted with the antigen suspension (Table 1). The gonorrheal patient group results indicated 49.6% of the male sera and 79.0% of the female sera to be reactive.

To determine the reproducibility of the antigen preparation, two preparations (made from same ingredients) were tested by using duplicate samples of 50 sera. Twenty-five of the sera were from patients known to have gonococcal antibody and had been found reactive in a previous testing. The other 25 sera were from presumed normal individuals who had been nonreactive on previous testing. There was agreement 48 of 50 times when comparing the reactivities of the two preparations.

The antigen suspensions have been found stable up to 2 weeks stored at 5 C when not in use at room temperature.

DISCUSSION

It is not clear why a significant number of infected patients were sero-negative. At this time there is no definitive information as to when, during the course of a gonorrheal infection, detectable antibody levels appear. However, even if such information were known it is probably that there is great variation in individual levels.

The higher precentage reactivity in infected females compared to infected males was also of interest. Males usually develop troublesome symptoms of the disease within a short time after infection and seek medical attention. Infected females, because of the relatively asymptomatic nature of their infections in most cases, may carry the organisms for prolonged periods and thus have greater opportunity to develop detectable antibody levels.

Fewer than 8% of the presumed normal sera assayed in this study showed reactivity. These reactions could indicate cross-reactions, for the sonically treated supernatant fluid of the gonococcus is a mosaic of many antigens, some of which may be shared by other *Neisseria*, or organisms of other genera, or both (2, 3). Another possibility is that the antibodies detected were, in fact, due to a previously treated infection or an active infection, which was undetected at the time the sera were drawn.

Data presented in this report indicate the feasibility of using such an assay to detect gonococcal antibodies in human sera. Work is now in progress to refine the assay as a serological test for gonorrhea.

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