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Indirect fluorescence test for the detection of anti-gonococcal antibodies

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The use of an indirect fluorescence test for the detection of anti-gonococcal antibodies was described by Hess, Hunter, and Ziff (1965). They reported positive reactions at a dilution of 1 in 100 or more with sera from nineteen of 22 patients with definite gonococcal arthritis, in seventeen of 35 in whom the diagnosis was probable, and in six of a series of 105 patients with miscellaneous diseases, other forms of arthritis, and normal individuals. Ojajärvi and Aho (1968) reported positive results at a serum dilution of 1 in 10 in 35 of 91 sera from patients with bacteriologically proven gonorrhoea, eleven of 201 seamen attending for pre-employment tests, and two of sixty healthy controls. These workers absorbed sera with autoclaved gonococci before testing to remove normal antibody. Welch and O'Reilly (1973) obtained positive results with 52 per cent. of sera from fifty infected males, in 79 per cent. of 120 infected females, and in 4 per cent. of a control series of 170 patients. The selection of a suitable strain of gonococcus for use as antigen was discussed by O'Reilly, Welch, and Kellogg (1973). The present report describes preliminary results with a similar technique.

Material and methods

SERA

These were selected from routine specimens sent for serological tests for syphilis from patients from whom gonococci had been isolated. For presumed normal sera, routine specimens from antenatal patients and blood donors were used. Sera were inactivated for 30 min. at 56°C. before testing.

ANTIGEN

This was prepared from a subculture from the primary isolate of a strain of gonococcus subsequently confirmed by fermentation tests. Growth was suspended in distilled water by means of a vortex mixer and diluted further to give only barely visible turbidity. Aliquots of 0.25 ml. were stored at —20°C. and were found to maintain their reactivity for at least 3 months under these conditions. For use, an aliquot was thawed rapidly and re-suspended on a vortex mixer. A 2 mm. loopful was spread within four 0·4 cm. circles inscribed on slides separated by grease-pencil marks to prevent mixing of sera. Slides were dried in an incubator for 20 min. and not fixed. Unused antigen suspension was discarded.

TEST PROCEDURE

Two-fold dilutions of serum from 1 in 4 to 1 in 32 were made in phosphate buffered saline (PBS), pH 7·2 and added to the dried films of gonococcal antigen. After incubation at 36°C. in a moist chamber for 30 min., excess serum was washed off in running tap water and the slides washed in two changes of PBS for 5 min. each. Anti-human immunoglobulin conjugate (Wellcome Reagents) at its optimal titre (1 in 240) was added and the slides incubated for 30 min. at 36°C., washed in PBS as before, rinsed in distilled water, and allowed to dry. They were mounted in buffered glycerin, pH 9·0.

READING

Slides were examined by darkground illumination on a Leitz Ortholux microscope with a halogen quartz illuminator, a Balzer FITC 3 interference filter, and 520 barrier filter. A $\times 54$ immersion objective N.A. 0·95 and $\times 10$ oculars were used. Gonococci showed a rim of peripheral fluorescence which was graded 0 to 4+. Assessment was made on isolated organisms in several fields and definite bright ++ fluorescence of the majority of isolated cocci taken as a positive result. Clumps of cocci often showed some fluorescence when isolated organisms showed no or only faint fluorescence; this was disregarded when assessing results.

Results

(1) *Tests on known sera*

Sera were selected from patients from whose secretions gonococci had been grown; these were almost all sera taken at the patient's first attendance at the clinic. As a control series, sera from blood donors and antenatal patients sent for routine serological tests for syphilis were examined. The results are shown in Table I (opposite).

If a titre of 1 in 16 is taken as significant, there was a well-marked differentiation between the results with sera from infected patients and the control

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TABLE I *GcIFA test results on sera from 204 patients with gonorrhoea and 182 controls*

Category	*No. of sera*	*Serum titre*					
		⩽4	8	16	⩾32	*Reactive ⩾16*	
						No.	*Per cent.*
Males, culture positive	119	78	17	12	12	24	20
Females, culture positive	85	24	9	22	30	52	61
Blood donors	34	28	6	—	—	0	
Antenatal patients	148	111	30	3	4	7	4·7

group. In the latter only seven of 182 sera (3·8 per cent.) were reactive, a figure comparable to that found by Welch and O'Reilly (1973). The proportion of positive results in sera from infected patients was considerably lower than that found by the American authors, especially in sera from infected males. Titres tended to be higher among the sera from infected females than in those from infected males; the highest titre found was 1 in 256.

(2) *Tests on unknown sera*

Tests were carried out on 310 unselected sera from patients attending the Whitechapel Clinic which had been sent to the laboratory for routine serological tests for syphilis. 25 sera were found to be reactive at a dilution of 1 in 16 or 1 in 32, none gave titres above 1 in 32. A clinical assessment of the status of these patients is shown in Table II.

TABLE II *Clinical assessment of 25 patients with reactive GcIFA screening tests*

Sera tested: 310. Positive: 25 (males 19, females 6)

Smear and/or culture	*Other evidence*	*Patients*
Positive		8
Negative	P.H. gonorrhoea	8+1 doubtful
Negative	Gc contact	2
Negative	No evidence	6 { 4 males NSU; 1 female, *T. vaginalis*; 1 female, warts

In this group there were six sera from patients with no supporting evidence of present or past gonorrhoea, an incidence of presumed false positive results of 2 per cent.

(3) *Tests for early (IgM) antibody*

The results in the previous group of sera suggested that the test might be found positive in patients with past as well as active infection. It seemed possible that early antibody response might be IgM in nature, so a further group of sera was tested with a monospecific anti-human IgM conjugate; the results are summarized in Table III.

These results showed a greater degree of reactivity with sera from infected males (32 per cent.) than in tests with a broad spectrum conjugate which detected mainly IgG antibody (20 per cent., Table I). The reverse was true of sera from infected females. This could be explained by a shorter duration of infection in males than in females but the two series of tests were done on different groups of sera. Cohen, Norins, and Julian (1967) have shown that a high concentration of IgG antibody may inhibit the reactivity of IgM antibody against gonococci, presumably by competition for antigen sites. The incidence of presumed false positive results (5 per cent.) was similar to that found with the broad spectrum conjugate.

Discussion

Many of the difficulties with serological tests for gonorrhoea stem from the sharing of antigens between the gonococcus and other *Neisseria*, particularly the meningococcus, as well as other organisms. Also, uncomplicated gonorrhoea is a surface infection of mucous membranes which does not favour a strong antibody response to infection. Many sera contain low titres of naturally occurring antibody which reacts with gonococci. The presence of varying proportions of meningococcal carriers in the population and the prevalence of patients with serum antibodies to meningococci complicates the problem. Jones and Tobin (1972), in a survey in the Bolton and Manchester area, found haemagglutinating antibodies to meningococci of groups A, B, or C in 343 of 990 sera from adults; titres ranged up to 1 in 128 but most were positive at 1 in 8 to 1 in 16. The meningococcus and gonococcus have antigenic affinities and some cross-reactions between antibody to

TABLE III *GcIFA test results with monospecific anti-human IgM conjugate*

Category	*No. of sera*	*Serum titre*					
		⩽4	8	16	⩾32	*Reactive ⩾16*	
						No.	*Per cent.*
Males, culture positive	63	33	10	8	12	20	32
Females, culture positive	55	25	6	18	6	24	43
Antenatal sera	42	37	3	2	—	2	5

the two organisms may well occur. Cohen (1967) has shown that naturally occurring anti-gonococcal antibody may be found in the immunoglobulin classes IgG, IgM, and IgA. The 'normal' IgG antibody was thought to react mainly with heat stable antigens on the gonococcus, whereas immune IgG reacted with heat labile surface components. Ojajärvi and Aho (1968) exploited this distinction by absorbing sera with heated gonococci before testing and obtained 38 per cent positive results in tests on 91 patients with bacteriologically proven gonorrhoea (sex not stated); two out of sixty sera from control patients also gave positive results at a screening dilution of 1 in 10.

O'Reilly and others (1973) showed that strains of gonococci differed in their reactivity with a panel of sera from infected patients; they emphasized the need for careful selection of the strain to be used as antigen in the indirect FA test so as to achieve the widest spread of reactivity with sera from infected patients. Their observations have been confirmed during the present study; they presumably reflect the presence of variations between the antigenic composition of gonococcal strains.

These preliminary studies confirm the suggestion of earlier workers that an indirect fluorescence technique is a practicable method of detecting anti-gonococcal antibody. Although the results showed a reasonable specificity, the finding of only 61 per cent. of positive results in females with uncomplicated infections indicates that it is not sufficiently sensitive as a screening method for detecting such infections. It may be possible to improve these results by selecting a more reactive strain for use as antigen or by removal of natural antibody by absorption of sera with heated gonococci before testing. Attempts to detect IgM antibody as a means of detecting early active infection in the female were not encouraging.

Summary

(1) An indirect fluorescence test for the detection of anti-gonococcal antibody is described.

(2) Positive results at a serum dilution of 1 in 16 or above were obtained with sera from 20 per cent. of males and 61 per cent. of females with bacteriologically proven gonorrhoea. 3·8 per cent. of presumed false positive results were given by sera from patients presumed not to have gonorrhoea.

(3) In different groups of sera, tests for IgM anti-gonococcal antibody were positive in 32 per cent. of those from males but in only 43 per cent. of those from females with positive cultures.

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Le test de fluorescence indirecte dans la détection des anticorps gonococciques

SOMMAIRE

(1) On décrit un test de fluorescence indirecte pour la détection des anticorps anti-gonococciques.

(2) On a obtenu des résultats positifs dans le sérum dilué à 1 pour 16 et au dessus dans le sérum de 20 pour cent des hommes et de 61 pour cent des femmes ayant une gonococcie prouvée bactériologiquement. 3,8 pour cent de résultats présumés faussement positifs furent fournis par les sérums de malades présumés n'ayant pas de gonococcie.

(3) Dans différents groupes de sérums, chez les sujets à cultures positives, la recherche de l'anticorps anti-gonococcique IgM fut positive chez 32 pour cent des hommes mais seulement chez 43 pour cent des femmes.