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Comparative assessment of microbiological methods for the diagnosis of gonorrhoea in women

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Gonorrhoea is generally accepted as being more difficult to diagnose in women than in men. In the case of women symptoms and signs are often absent or minimal, and most authorities will agree with Catterall (1970) that conventional tests (smears stained by Gram's method and culture for the organism on selective media) will lead to a diagnosis in only about 70 per cent. of infected cases at the first visit. Repeated examinations are needed in many cases, and problems will arise due to patients defaulting and re-infecting their sexual partners.

In recent years preparations of antigonococcal serum conjugated with fluorescein isothiocyanate (FITC) have become commercially available, and it has been claimed that their use increases diagnostic accuracy. This present study compares the results obtained by use of this conjugate with those obtained with conventional tests in the investigation of potentially infected women.

Patients

These were 232 women who attended the Whitechapel Clinic of The London Hospital between May, 1968, and April, 1970. 188 attended because a male sexual partner had been treated for gonorrhoea. Of these 83 were considered to be the source of infection for the male and were classified as 'source' or 'reservoir' contacts, and 105 were considered to have been exposed to infection by the male after he had contracted the disease and were classified as 'subsequent' or 'secondary' contacts (Dunlop, 1963; Hare, Lamb, and King, 1970; Dunlop, Lamb, and King, 1971). Nine other women attended as contacts of men treated for non-gonococcal urethritis, and 35 more for various other reasons.

Material

Antigonococcal FITC conjugate is made by Difco Laboratories, Detroit, Michigan, U.S.A., using the method of Deacon (Deacon, Peacock, Freeman, and Harris, 1959; Deacon, 1961). Supplied in a dehydrated form, it was reconstituted according to the makers' instructions and stored at —40°C. until used.

The conjugate was initially tested against members of the genus *Neisseria* and a range of other organisms found in the female genital tract. Fluorescence was assessed on a five-point scale and recorded as 0 to ++++. All strains of *Neisseria gonorrhoeae* examined gave good brilliance (+++ or ++++) when stained with the conjugate. Organisms from Groups A, B, C, D, and E of *Neisseria meningitidis* also gave +++ fluorescence and it was concluded that diagnostic confusion between meningococci and gonococci would be inevitable. Serial dilution of the conjugate in water or serum resulted in a progressive loss of brilliance on staining with both species. These findings were expected in view of the shared antigenicity between *N. gonorrhoeae* and *N. meningitidis* (Wilson, 1956); similar results have been reported by Danielsson (1965c) and Lind (1967). Absorption of the conjugate with Group A meningococci removed this cross-reaction, but left the conjugate too weak to be of diagnostic use for gonococci. It was considered that this cross-reaction is of only slight importance in the clinical management of patients for, although the meningococcus has been isolated from the genital tract (Carpenter and Charles, 1942; Armytage, 1944; Wax, 1950; Perez-Miravete, Bustos, and Avitia, 1967; Volk and Kraus, 1973), its presence there is most uncommon. *Neisseria sicca*, *N. flava*, *N. flavescens*, and *N. catarrhalis* gave only very weak reactions when stained with the conjugate.

A moderate reaction (++ brilliance) was observed with one strain of *Staphylococcus aureus* (NCTC 6136). Reactions with this and with other strains of staphylococci, and with some streptococci, have been reported by Danielsson (1965a) and Lind (1967, 1968). These were considered to be 'non-specific' reactions as defined by Pittman, Hebert, Cherry, and Taylor (1967), and Lind (1967) found that they could be removed without affecting the specific reaction by diluting the conjugate with normal rabbit serum. This was confirmed in the present study. No other staphylococcus or streptococcus tested reacted with the conjugate. Other micro-organisms tested were species of *Proteus* and *Pseudomonas*, many strains of *Escherichia coli*, diphtheroids, one strain of *Mima polymorpha* var. *oxidans*, *Trichomonas vaginalis*, and species

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of *Candida* including *C. albicans*. In no instance was any fluorescence observed.

Preparation of specimens

Material to be examined for the presence of gonococci was taken from the patient's vagina, cervical canal, urethra, and ano-rectal canal. Tests were prepared in the following order:

(*a*) *Smear for microscopy after Gram-staining*
Material was smeared on to a microscope slide using a platinum loop, and allowed to dry. It was then heat-fixed and stained by Gram's method.

(*b*) *Smear for microscopy after immunofluorescence staining*
Material was smeared on to a microscope slide using a platinum loop, and allowed to dry. The slide was then fixed in acetone, and stained with conjugate as described below. This is known as the direct fluorescent antibody test (direct FAT).

(*c*) *Culture for immunofluorescence staining*
Material was inoculated on to warmed selective culture medium (brain-heart infusion with defibrinated horse blood to which colistimethate, vancomycin, and nystatin had been added; Thayer and Martin 1966). This was then incubated at 37°C. for 14 to 22 hours in a moist carbon-dioxide enhanced atmosphere. The surface of the medium was scraped with a platinum loop and the deposit so obtained emulsified in saline on a microscope slide, allowed to dry, and fixed in acetone. The slide was then stained with conjugate as described below. This is known as the delayed fluorescent antibody test (delayed FAT).

(*d*) *Routine culture for gonococci*
A culture swab impregnated with charcoal was used to remove secretion and then immersed in Stuart's transport medium (Moffett, Young, and Stuart, 1948). Within the next 24 hrs selective culture medium was inoculated with the swab, and incubated as previously described for 48 hrs. If after this time typical colonies were found which reacted with oxidase reagent these were subcultured for confirmatory fermentation tests.

Slides for immunofluorescence staining were stored at 4°C. The procedure found to give the best display of gonococci was as follows:

(1) Slides were incubated for 30 min. at 37°C. with a mixture of ten parts of conjugate, nine parts of normal rabbit serum, and one part of napthalene black solution.

(2) Slides were then rinsed in tapwater, and washed for two 5-min. periods in phosphate-buffered saline (pH 7·2). They were then rinsed in distilled water.

(3) Slides were then dried in air, and mounted in buffered glycerine for examination.

Slides were examined using a Gillett and Siebert 'Conference' microscope fitted with a darkfield condenser and a quartz iodine light source. The primary filters were Wratten 32 and 38A, and the barrier filter was a Wratten 12. Organisms giving a +++ or ++++ brilliance were accepted as gonococci. In the direct FAT extracellular as well as intracellular organisms were accepted, but in most cases intracellular organisms were visible.

Results

Microbiological confirmation of the diagnosis of gonorrhoea was obtained by tests taken at the first examination in 186 of the 232 patients studied. The diagnosis was made on the appearance of the Gram-stained smears in 118 cases; on the results of routine cultures in 130 cases; on the results of the direct FAT in 157 cases; and on the results of the delayed FAT in 180 cases. Agreement and disagreement between the results of the various tests used is shown in Table I. There was complete agreement between the results of all methods in 106 cases, and in a further 23 cases routine culture confirmed the results of at least one other test. In one case routine culture gave a positive result when all other tests were negative. In no case was the positive result of the Gram-stained smear unconfirmed by another test.

TABLE I *Conventional and immunofluorescence tests for gonorrhoea: results in 232 cases*

Direct FAT	*Positive*	*Positive*	*Negative*	*Negative*
Delayed FAT	*Positive*	*Negative*	*Positive*	*Negative*
Smear positive Culture positive	106	1	4	0
Smear positive Culture negative	5	0	2	0
Smear negative Culture positive	12	0	6	1
Smear negative Culture negative	29	4	16	46

56 women were diagnosed as infected on the results of immunofluorescence tests (sometimes with Gram-stained smears) despite negative cultures. In order to discover any false positive results which might have arisen from the immunofluorescence methods, further evidence as to the likelihood of infection was sought in the case records of each woman in this group, and also in the records of her sexual partner or partners. This evidence is summarized in Table II. In Groups (1) and (2*a*) the confirmatory evidence of infection is very strong. In Groups (2*b*) and (2*c*) the evidence is somewhat less definite, but most clinicians would find it acceptable. The patients in Groups (3), (4), and (5) would also seem to be at a high risk of infection. Only in the case of the one patient in Group (6) is the diagnosis at variance with the history, and in this

TABLE II *Patients diagnosed as infected despite negative cultures: other evidence of infection*

Other evidence of infection	*No. of patients*
(1) Patient: culture positive on subsequent test	4
(2) Consort: known to have gonorrhoea	
(*a*) Culture positive	33
(*b*) Smear positive, culture negative	7
(*c*) Attended elsewhere (contact slip)	7
(3) Patient: history of recent gonorrhoea	2
(4) Consort: history of recent gonorrhoea	1
(5) Patient: known prostitute	1
(6) Consort: diagnosed as non-gonococcal urethritis	1
Total	56

case it is possible that the diagnosis of gonorrhoea in the male might have been missed.

A false positive result could have been obtained for another reason. Meningococci and gonococci have been reported to retain their affinity for fluorochrome conjugates up to 24 days after exposure to lethal doses of penicillin (Mitchell and Biegeleisen, 1965; Lucas, Price, Thayer, and Schroeter, 1967), and experiments in the present study confirmed these findings. Gonococci growing on a culture plate were incubated in a strong solution of penicillin (10 μg./ml.) for 24 hrs, washed, and then incubated again at 37°C. in a moist carbon-dioxide enhanced atmosphere. Organisms taken from the plate up to 8 days after exposure to penicillin gave good reactions when stained with the conjugate, although no growth was observed on the plate or in daily subcultures taken from it. It would seem possible that a similar situation could arise after treatment of gonorrhoea, with occasional non-viable organisms giving a false-positive reaction. This could lead to errors when using the direct FAT, but not when using the delayed FAT which depends for a positive result on some growth on the culture medium. Eleven patients were receiving antibiotics at the time of testing or had just finished a course; these drugs had been prescribed for conditions other than gonorrhoea. In five of the eleven patients gonococci were identified; in two cases by the direct and delayed FATs together and in three by the direct FAT alone. All of these women were the sexual partners of men treated for proven gonorrhoea, and it was impossible to decide in the last three cases whether or not the incidental treatment had been adequate.

The results for the complete study give no indication of the absolute efficiency of the various diagnostic methods, as the total number of infected cases cannot be known. Such an estimate can be made if the only patients considered are the alleged infective source contacts of men treated for gonorrhoea. Using the information obtained by interviewing male patients and all their female contacts, it was possible to find a group of 83 women, each of whom had almost certainly infected at least one man with gonorrhoea. Three of these were taking antibiotics at the time of attendance, and so cannot be included in this calculation. Of the remaining eighty, 76 were diagnosed as being infected with gonorrhoea at their first attendance; two were subsequently proven to be infected by conventional cultures (the FA tests were not repeated); and in two the diagnosis of gonorrhoea was never proven. The diagnostic efficiency of each method, and of combinations of methods, is shown in Table III. A combination of conventional tests gave a diagnostic sensitivity of 75 per cent., while the two immunofluorescence methods in combination gave a 95 per cent. sensitivity. The delayed FAT with a 90 per cent. sensitivity was the most efficient single test.

TABLE III *Results of diagnostic tests for gonorrhoea in 80 source contacts*

Method or methods used	*Positive*	
	Number	*Per cent.*
Gram-stained smear	55	69
Culture on selective medium	57	71
Direct FAT	66	82
Delayed FAT	72	90
Smear and/or culture	60	75
Direct and/or delayed FAT	76	95

In Table IV, the yield obtained by each method is shown for each of the four sites tested. The most useful site for testing by conventional methods is the cervix; for material from this site the diagnostic sensitivity is improved only marginally by the use of immunofluorescence tests. The yield of positives obtained by testing urethral material is considerably increased by the use of the delayed FAT. This is probably due to the fact that the size of inoculum needed for a positive delayed FAT is much lower

TABLE IV *Results of diagnostic tests at different sites: 232 patients*

Site	*Any method positive*		*Smear positive*		*Culture positive*		*Direct FAT positive*		*Delayed FAT positive*	
	No.	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*
Urethra	154	100	76	49	95	62	101	66	142	92
Cervix	153	100	86	56	114	75	114	75	127	83
Vagina	141	100	Not tested		85	60	83	59	119	84
Rectum	124	100	41	33	44	35	64	52	107	86

than that needed for a positive conventional culture; the chances of obtaining a positive conventional culture would also be lowered by the fact that this test was always taken last. At other sites, where material for testing was more plentiful, this latter factor would not be relevant. The greatest difference between the results of immunofluorescence and conventional tests seems to occur when they are used on rectal material; on this material the delayed FAT proved well over twice as sensitive as either conventional test, and the direct FAT also gave considerably improved results. The significance of these rectal findings will be discussed in a subsequent article (Hare, 1975).

Discussion

Since the introduction in 1960 of immunofluorescence methods for the diagnosis of gonorrhoea the results of many studies have been published. The findings of the principal trials which concerned female patients have been summarized in Table V. Because the types of patients studied (and hence the proportion of each group potentially infected) varied from trial to trial, the results have been standardized by expressing the most sensitive method in each trial as 100, and the others as percentages of this. In all studies but one (McGill, Moffett, Masterton, and Schofield, 1969), the delayed FAT proved to be of high sensitivity; in nine of twelve reports it was the most sensitive method used. In contrast, reports on the direct FAT are much less consistent; although in four of eleven studies it was found to be the most sensitive method, in three it was less than 60 per cent. as sensitive as other methods. In six studies the direct FAT was found to be more sensitive than conventional culture and in five the reverse applied.

It is therefore concluded that the delayed FAT is a reliable and sensitive test for gonorrhoea in women. The direct FAT is less sensitive and less reliable; it would seem not to have a place in routine work, but could be useful in the investigation of special cases and in research.

Summary

The results are reported of a comparison between conventional and immunofluorescence tests for the diagnosis of gonorrhoea in women. The delayed FAT was found to be reliable and more sensitive than conventional tests; it was positive in 90 per cent. of cases of gonorrhoea compared with 75 per cent. for a combination of conventional smears and cultures. The direct FAT was found to be less sensitive than the delayed FAT, and is not recommended for routine use.

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TABLE V *Results obtained in previous studies (best method expressed as* 100: *others as percentages of this)*

Study	*Test (percentage positive)*			
	Smear	*Culture*	*Direct FAT*	*Delayed FAT*
Deacon, Peacock, Freeman, Harris, and Bunch (1960)	n.d.	100	46	100
Danielsson (1963)	20	64	n.d.	100
Peacock and Thayer (1964)	n.d.	100	96	n.d.
Holman, Koornhof, and Hayden-Smith (1964)	64	84	80	100
Price (1964)	n.d.	64	42	100
Fry and Wilkinson (1964)	79	84	100	88
Danielsson (1965b)	58	92	100	97
Mouton (1966)	42	52	100	n.d.
Gallwey, Nicol, and Ridley (1967)	76	82	100	n.d.
Lind (1967)	65	94	58	100
Pariser and Farmer (1968)	53	n.d.	n.d.	100
Lucas, Price, Thayer, and Schroeter (1967)	n.d.	98	n.d.	100
McGill, Moffett, Masterton, and Schofield (1969)	100	85	n.d.	46
Thin, Williams, and Nicol (1971)	67	68	95	100
Present study	66	72	87	100

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Valeur relative des méthodes microbiologiques appliquées au diagnostic de la gonococcie féminine

SOMMAIRE

On rapporte les résultats d'une comparaison entre les tests conventionnels et l'immuno-fluorescence dans le diagnostic de la gonococcie féminine. Le FAT retardé a été trouvé plus sûr et plus sensible que les tests conventionnels ; il fut positif dans 90 pour cent des cas de gonococcie contre 75 pour cent pour la combinaison de la coloration courante des étalements avec les cultures. Le FAT direct fut trouvé moins sensible que FAT retardé et n'est pas recommandé pour l'utilisation en routine.