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Immunofluorescent method for diagnosis of gonorrhoea in women

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DEACON, Peacock, Freeman, and Harris (1959) showed that the fluorescent antibody (FA) staining technique can be used to demonstrate *Neisseria gonorrhoeae* in specimens of secretion taken from patients suffering from gonorrhoea. Since then this technique has been widely employed for research but has been less popular for routine use as it is expensive and time-consuming. Gallwey, Nicol, and Ridley (1967), for instance, reporting a sensitive direct FA staining technique, observed that it was tedious for routine use in a busy clinic or laboratory; the total time taken to stain smears by their method was one hour and many other reported methods take a similar time.

The purpose of this paper is to describe a rapid direct FA staining method using readily available reagents, and to report the results obtained in the first 9 months of its routine use in a busy clinic.

Material and methods

Optical equipment

The microscope used for this work was a Gillet and Sibert Conference Research instrument, with a 100 watt iodine quartz lamp, a dark ground condenser, and a monocular head. The primary filter was a combination of Wratten filters numbers 32 and 38A (Tomlinson, 1967) and the secondary filter was an Ilford number 108. Smears were scanned using a low power objective and suspicious areas examined using a × 100 objective with an iris diaphragm.

Staining materials

Rabbit anti-gonococcal serum conjugated with fluorescein isothiocyanate was obtained from Difco Laboratories. Each bottle was tested against a range of organisms recently isolated from patients.

Pooled human serum was supplied by the hospital microbiology department; before use it was tested by staining positive and negative control smears.

Naphthalene black, obtained from Messrs. Gurr, was made up in a concentration of 10 mg./ml. and diluted in the human serum to a concentration of 3 mg./ml. immediately before use.

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Staining technique

Specimens of secretions having been collected from 325 female patients with a wire loop, the material was thinly spread within a circle $\frac{1}{2}$ in. diameter engraved on a clean glass slide. Smears were dried in air and heat-fixed in a flame.

Equal parts of antigonococcal fluorescein conjugate and naphthalene black in human serum were mixed, and one drop of the mixture was spread over the smear. Slides were then incubated at 36°C. for 10 minutes, washed for 10 minutes in phosphate buffered saline of pH 7·2, and rinsed in distilled water. They were dried in air and mounted under buffered glycerin.

In addition to the specimens for the FA-stained smears, specimens for Gram-stained smears and for cultures were obtained from the urethra and cervix in all 325 cases, and from the rectum in 62.

Initially urethral and cervical cultures were made on hydrocoele agar, but later agar enriched with lysed horse blood was employed. Scott and Stone's selective culture medium (Scott and Stone, 1966) was used for the rectal cultures.

In all cases the vaginal secretions were also examined for *Candida albicans* and *Trichomonas vaginalis*. In 162 cases vaginal smears were prepared for FA staining.

Results

Preliminary assessment

In the preliminary assessment of this FA staining method, all strains of *N. gonorrhoeae* fluoresced brightly. The only other organism to fluoresce significantly was *Neisseria meningitidis*, but this was much less bright than *N. gonorrhoeae*. Nine strains of *N. meningitidis* were examined; the National Collection of Type Cultures kindly supplied one strain each from Groups A, B, C, and D, and the remainder were ungrouped strains freshly isolated from patients. Seven out of twelve strains of *Neisseria pharyngis* and *Neisseria catarrhalis* did not fluoresce at all and the remainder showed only minimal fluorescence. Thirteen strains of *Staphylococcus albus*, eighteen strains of *Escherichia coli*, and several strains of haemolytic streptococci, *Streptococcus faecalis*, *Bacillus proteus*, *Pseudomonas pyocyaneus*, *Diplococcus pneumoniae*, and *veillonella* species showed

no fluorescence. Nineteen strains of *Staphylococcus aureus* were also tested, including three (Numbers 6134, 6136, and 8530) from the National Collection of Type Cultures, but none showed any fluorescence. At least two smears of each strain were examined.

A collection of slides was prepared in the hospital microbiology department and read without prior knowledge of the organisms present; *N. gonorrhoeae* was correctly distinguished from *N. meningitidis* and other organisms.

No fluorescent organisms were identified in smears from 34 men subsequently diagnosed as suffering from non-gonococcal urethritis, and smears from 82 female contacts of such men were also negative. Finally, specimens from male patients whose Gram-stained smears had proved difficult to interpret were examined and in all cases the FA smear and culture result agreed.

Results in clinical material

The findings in 325 female patients all of whom had had recent contact with a male suffering from gonorrhoea are reported. At their first visit to the clinic gonorrhoea was diagnosed by one or more methods in 250 (76·9 per cent.) of the women (Table I): 197 were diagnosed by both the FA method and the conventional methods of Gram-stained smear and culture, 23 by conventional methods alone, and thirty (9·2 per cent.) by FA smears alone (Table II). Thus, a rapid FA diagnosis was made in 227 patients, 70 per cent. of the total.

TABLE I *Overall results in 325 female patients*

Result	*No.*	*Per cent.*
Cases positive by one or more methods	250	76·9
Cases negative by all methods	75	23·1
Total	325	100·0

TABLE II *Methods by which gonorrhoea was diagnosed*

Positive by FA and conventional methods	197
Positive by conventional methods alone	23
Positive by FA smears alone	30
Total	250

At each site FA smears gave a greater number of positive results than Gram-stained smears or cultures (Table III). The highest yield was from the cervix, where there were 61·8 per cent. positive FA smears compared with 47·1 per cent. positive Gram-stained smears and 52 per cent. positive cultures. The rectum gave fewer positive results, with 9·7 per cent. Gram-stained smears, 6·4 per cent. cultures, and 14·5 per cent. FA smears from 62 patients.

TABLE III *Numbers of cases positive at each site by each method*

Site	*No. examined*	*Method*					
		Gram stain		*Culture*		*FA smear*	
		No.	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*
Urethra	325	111	34·2	117	36·0	152	47·1
Cervix	325	153	47·1	169	52·0	201	61·8
Rectum	62	6	9·7	4	6·4	9	14·5

At the cervix one method was positive in 55 cases, two in 72 cases, and three in 108 cases. Three methods were negative in ninety cases, so that total agreement between all three methods was obtained in 61 per cent.

At the urethra one method was positive in 62 cases, two in 69 cases, and three in sixty cases. Three methods were negative in 134 cases, giving a total agreement of 60 per cent.

Although few positive specimens were obtained from the rectum, three methods agreed in 83 per cent. of the 62 patients studied.

Among the 162 patients from whom vaginal FA smears were collected, the number of positive vaginal findings was intermediate between those for the urethral and cervical FA smears (Table IV). Vaginal, urethral, and cervical FA smears were all positive in 64 of these cases and negative in 58, giving an agreement of 75·4 per cent. The vaginal FA smear and either the urethral or cervical FA smear were positive in a further 22 cases. Overall disagreement between the vaginal FA smear and both the cervical and urethral FA smears was found in 7·4 per cent of cases.

TABLE IV *Comparison of positive vaginal, cervical, and urethral FA smears in 162 cases*

Site	*Positive results*	
	No.	*Per cent.*
Vagina	88	54·3
Cervix	99	61·2
Urethra	78	48·1

All positive FA smears contained at least one group of brightly fluorescent organisms. Usually these groups were seen to be within a pus cell but sometimes the outline of the cell was not visible.

Discussion

This staining method was developed from the technique described by Gallwey and others (1967). Pooled human serum was used to inhibit fluorescence of staphylococci as demonstrated by Danielsson

(1965a). Naphthalene black was tried as a counterstain following the suggestion of Sommerville (1968) and was found to give less background fluorescence than lissamine rhodamine.

Smears took about 25 minutes to prepare. With shorter staining times gonococci rapidly faded while slides were being read, and with shorter washing times excess fluorescein remained on the slide making reading difficult. The rapid FA staining techniques described by Kellogg and Deacon (1964), Danielsson (1965b), and Sommerville (1968) were found to be unsatisfactory using these materials, since the gonococci fluoresced poorly and faded rapidly. In routine examinations, all female contacts of males known or suspected to have gonorrhoea are studied, and the technique fits smoothly into the clinic routine. Patients whose Gram-stained smears are negative are invited to wait while their FA smears are stained and read.

Although exhaustive tests have not been carried out, the preliminary results indicate that this method is specific for gonococci. Meningococci were the only other organisms which fluoresced significantly, but all the strains examined were readily distinguishable from gonococci which were much brighter.

Contrary to some workers' experience, no cross-reactions with other organisms were observed. The strains of *Staph. aureus* examined included three from the National Collection of Type Cultures which Danielsson (1965a) found to fluoresce brightly. Lind (1967) also observed cross-reactions with certain strains of staphylococci.

There was no difficulty with non-specific staining of leuococytes such as that described by Lind (1967). Routine collection of rectal smears was abandoned because so few positive smears were found, but background fluorescence was not a problem, unlike the experience of Gallwey and others (1967).

At the cervix and urethra, FA smears gave 10 per cent. more positive results than cultures. This is similar to the observations of Fry and Wilkinson (1964), Danielsson (1965b), and Gallwey and others (1967). However, many workers, such as Lind (1967), report that cultures give more positive results than FA stained smears of patients' secretions. Danielsson (1965a) and Fry and Wilkinson (1964) suggested that their higher proportion of positive FA smears was related to the use of counterstain. They used lissamine rhodamine, but naphthalene black appears to be equally effective.

Three patients diagnosed by FA smears alone are of interest. One had received penicillin one week before, one had received Septrin 2 weeks before, and one had been douching with antiseptics. Lucas and his colleagues (1967) observed that FA smears were positive in 34 per cent. of cases 2 to 4 days after treatment and that this percentage gradually fell over the next 3 weeks. It seems reasonable, therefore, to accept these positive findings. Most patients diagnosed by FA smears alone were treated at once, but five had investigations repeated and four had positive cultures at the second examination.

In the cases of the 162 patients from whom vaginal FA smears were collected, the number of positive findings was intermediate between those in the urethral and cervical FA smears. Lucas, Price, Thayer, and Schroeter (1967) reported similar observations in their large series of patients studied in the U.S.A. When the cervix can be observed, specimens should be taken from it, but when it cannot be seen a vaginal smear appears to be a useful alternative.

Diagnosis in cases of female patients suspected to be suffering from gonorrhoea may present many difficulties. Any reasonably simple diagnostic technique is valuable, especially when it is more sensitive than conventional methods and provides an answer quickly. This FA staining technique has been most useful in the management of women who attended the clinic only with reluctance and who appeared unlikely to attend again.

Summary

A rapid specific direct fluorescent antibody (FA) method for staining gonococci is described and the results of applying this method in the cases of 325 female contacts of men with gonorrhoea are presented and compared with findings by conventional smears and cultures.

250 cases (76·9 per cent.) were diagnosed by one or more methods at the first examination in a busy Venereal Diseases clinic in Central London; FA tests alone were positive in thirty (9·2 per cent.) and a rapid FA diagnosis was made in a total of 227 cases (70·0 per cent.) FA smears gave more positive results than cultures or Gram-stained smears, and smears from the cervix gave more positive results than those from other sites. Vaginal FA smears yielded fewer positive results than those from the cervix, but more than those from the urethra, and it is concluded that vaginal FA smears are useful in those cases in which the cervix cannot be seen.

This FA method is now used for all female contacts of men known or suspected to have gonorrhoea and it fits smoothly into the clinic routine. It has proved a valuable additional diagnostic technique.

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Utilisation de la méthode d'immunofluorescence pour le diagnostic de la gonococcie chez les femmes

SOMMAIRE

On décrit une méthode spécifique rapide, directe, de marquage des gonocoques par l'anticorps fluorescent (FA); les résultats de l'application de cette méthode à 325 femmes ayant été en contact avec des hommes atteints de gonococcie sont présentés et comparés avec les résultats des méthodes conventionnelles de coloration et de culture.

250 cas (76,9 pour cent) furent diagnostiqués par une ou plusieurs méthodes lors d'un premier examen dans une clinique vénéréologique active du centre de Londres; les tests FA furent seuls positifs dans trente cas (9,2 pour cent) et un diagnostic par la méthode FA rapide fut obtenu au total pour 227 cas (70,0 pour cent). Le marquage FA donna plus de résultats positifs que les cultures ou que la coloration des prélèvements au Gram, et les colorations cervicales donnèrent plus de résultats positifs que pour les autres localisations. La méthode FA pour les prélèvements vaginaux donna moins de rèsultats positifs que pour le col, mais plus que pour l'urètre et l'on conclut que le marquage FA pour le prélèvement vaginal est utile dans le cas où l'on ne peut pas examiner le col.