THE LABORATORY DIAGNOSIS OF GONORRHOEA IN THE FEMALE

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The inadequacy of the direct microscopic examination of Gram-stained preparations in the diagnosis of gonococcal infections in the female is generally accepted by bacteriologists and has recently been emphasized by Simpson and Deacon[1] in the USA, by the Swedish worker, Danielsson[2] and by Wilkinson[3] in the UK. Thus Danielsson[2] reports 18·5% and 55·6% positive direct and cultural results respectively in females suspected of gonorrhoea, compared with the somewhat better results of 59·6% and 71·2% respectively in male patients. The main difficulty in interpreting Gram-stained smears is the presence of a mixed bacterial flora many of which may mimic gonococci, and the absence or very scanty presence of intracellular diplococci. Effete staphylococci and enterococci, often found in couples, may stain as Gram-negative diplococci, bipolar-staining Gram-negative bacilli, and also coccobacilli, e.g. Mimeae (so called because they mimic Neisseria), as well as Veillonella and non-pathogenic Neisseria may all resemble gonococci and thus induce the unwary to make false-positive laboratory diagnoses. The overcautious, and often experienced, bacteriologist realizing the pitfalls will tend to miss positives.

In view of this it was decided to conduct a survey at the Alexandra Health Centre comparing the results of the various bacteriological methods available for establishing a diagnosis of gonococcal infection. These included conventional cultural methods, the use of Stuart's transport medium,[4] and the fluorescent antibody (FA) techniques as described by Deacon *et al.*[5]

PROCEDURE AND METHODS

Bantu female patients were used in this comparative survey; they all had much the same symptomatology, namely lower abdominal pain, burning on micturition, and a vaginal discharge. The majority of the patients were young adults with an average age of about 24 years. Of these 15% were pregnant and 4% were menstruating.

Specimens were taken from the urethra, cervix, and posterior fornix. The laboratory procedures were divided into 5 stages with various modifications.

1. *Procedure in the First 57 Cases*

Duplicate smears, heat fixed at the clinic, were prepared from the posterior fornix for Gram-staining and were examined both by the clinic technician and at the laboratory of the South African Institute for Medical Research.

Specimens from the 3 sites were inoculated with a wire loop onto blood-agar plates and immediately incubated at 37°C in a CO_2 atmosphere, using the candle-jar method.

Swabs previously treated in phosphate buffer and coated with inactivated charcoal were taken from the cervix and posterior fornix and inoculated into Stuart's medium.

The incubated blood-agar plates, the planted Stuart's media, as well as the smears, were sent to the Institute laboratory, reaching it usually within 36 hours after being taken, and were then examined bacteriologically for gonococci.

2. *Introduction of the Direct FA Technique* (60 patients)

This procedure was the same as that described under (1), but additional air-dried, alcohol-fixed smears for direct fluorescent antibody testing were taken and sent to the laboratory.

Difco fluorescein isothiocyanate conjugated serum, prepared from the 'K' gonococcal antigen and suitably absorbed out to give the desired specificity,[5] was used after being passed through a Sephadex chromatography column and absorbed by dried bovine bone marrow. The smears were examined by means of a fluorescent microscope with excitation filters to give blue light and a OG1 protective filter, or alternatively with 47 and 50 Zeiss filters. Dark-field illumination was used. Although this serum was not absorbed out with *Staphylococcus aureus,* our results were correlated with the presence or absence of staphylococci on culture in an attempt to avoid possible false-positive results.[6]

3. *The Use of the 'Delayed' FA Technique* (20 patients)

This procedure was the same as that described under (2), but swabs were also taken from the cervix and posterior fornix, planted on a Difco gonococcal medium, incubated at 37°C in a CO_2 atmosphere at the clinic, and

TABLE I. ANALYSIS OF RESULTS

	Direct						*Culture*						*Delayed FA Techniques*								
	Gram 'Presumptive'			*Gram 'Definite'*			*FA test*			*Blood agar*			*Stuart*			*Delayed FA test*			*Difco medium for transport (36 hours)*			*Stuart + delayed FA*		
	No.	+	%	No.	+	%	No.	+	%	No.	+	%	No.	+	%	No.	+	%	No.	+	%	No.	+	%
Urethra	—	—	—	—	—	—	—	—	—	57	23	40	—	—	—	—	—	—	—	—	—	—	—	—
Cervix	—	—	—	—	—	—	—	—	—	57	20	35	57	17	30	—	—	—	—	—	—	—	—	—
P. fornix	57	21	37	57	5	9	—	—	—	57	21	37	57	15	26	—	—	—	—	—	—	—	—	—
Patients	57	21	37	57	5	9	—	—	—	57	24	42	57	19	33	—	—	—	—	—	—	—	—	—
Urethra	—	—	—	—	—	—	—	—	—	60	19	32	—	—	—	—	—	—	—	—	—	—	—	—
Cervix	—	—	—	—	—	—	60	22	27	60	23	39	60	21	35	—	—	—	—	—	—	—	—	—
P. fornix	60	15	25	60	3	5	60	18	30	60	25	42	60	19	32	—	—	—	—	—	—	—	—	—
Patients	60	15	25	60	3	5	60	24	40	60	26	43	60	23	38	—	—	—	—	—	—	—	—	—
Urethra	—	—	—	—	—	—	—	—	—	20	8	40	—	—	—	—	—	—	—	—	—	—	—	—
Cervix	—	—	—	—	—	—	20	7	35	20	5	25	20	4	20	20	8	40	—	—	—	—	—	—
P. fornix	20	2	10	20	0	0	20	8	40	20	5	25	20	4	20	20	8	40	—	—	—	—	—	—
Patients	20	2	10	20	0	0	20	8	40	20	5	25	20	5	25	20	10	50	—	—	—	—	—	—
Urethra	—	—	—	—	—	—	—	—	—	66	17	24	—	—	—	—	—	—	—	—	—	—	—	—
Cervix	—	—	—	—	—	—	66	27	38	66	17	24	66	10	15	66	33	50	—	—	—	—	—	—
P. fornix	66	19	29	66	0	0	66	23	38	66	17	24	66	9	13	66	37	56	66	34	51	—	—	—
Patients	66	19	29	66	0	0	66	29	44	66	18	27	66	11	17	66	37	56	66	34	51	—	—	—
Urethra	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cervix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
P. fornix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	60	27	45	60	13	22	60	20	33
Total	203	57	28	203	8	4	292	105	36	609	200	33	406	99	24	232	113	49	126	47	29	60	20	33

then sent to the laboratory. Smears were then made, stained with the FA staining technique, and examined with a fluorescent microscope.

4. *Testing of Difco Gonococcal Medium as a Transport Medium with Delays of Less than 36 Hours* (66 patients)

This procedure was the same as that described under (3), but an additional swab was taken from the posterior fornix and planted on the Difco gonococcal medium. This was not incubated immediately, but was sent to the laboratory within 36 hours for incubation under CO_2 followed by FA testing.

5. *The Use of Stuart's Transport Medium combined with the Delayed FA Technique* (60 patients)

Swabs from the posterior fornix were planted onto two Difco gonococcal slopes and into one Stuart medium. One of the Difco gonococcal slopes was incubated immediately at the clinic and all three media were dispatched to the laboratory the next day. Smears were prepared from the incubated Difco slopes while the other Difco slopes were incubated at the laboratory. Smears were then prepared for FA testing. The Stuart's-medium swabs were planted onto Difco gonococcal slopes, incubated overnight under CO_2, and then examined with the FA technique.

RESULTS AND DISCUSSION

A detailed analysis of the results is given in Table I.

To facilitate and simplify a discussion of these methods, it is proposed to deal with the results and their implications under the following headings:

A. *Comparison of the Conventional Direct and the Cultural Investigations*

Microscopic examinations of Gram-stained preparations from the posterior fornices of 203 patients were done at the clinic and the laboratory; the combined findings are compared in Table II with the culture results from the same site in these patients.

In order to avoid confusion about the criteria constituting a positive direct microscopic result, it was decided to classify the results into 2 categories. The 'presumptive' positive results comprise those cases in which Gram-negative intracellular diplococci, on occasions extremely scanty, were observed which morphologically resembled gonococci. In this group, however, factors such as the possibility of effete Gram-positive cocci assuming Gram-negative properties, and morphological considerations such as minor differences in size and shape of the cocci, were ignored. The 'definite' cases are those that were frankly positive, with typical clusters of bean-shaped intracellular diplococci and in which, after due consideration of the nature of the mixed bacterial flora, no reasonable doubt could be entertained about the identity of the organisms.

TABLE II. DIRECT AND CULTURE RESULTS

Gram stain		*Culture*	*Totals*	
'Definite'	*'Presumptive'*		*Examined*	*Confirmed positive (all methods)*
8	57	68	203	91

In the 'presumptive' positive group 7 out of 57 positive results (12%) could not be confirmed by the sensitive fluorescent antibody tests.

Table II shows that the cultural results on blood agar are by far superior to the direct results. These results have the same trend as those reported by Danielsson,[2] who found 18·5% of females suspected of gonorrhoea positive on direct examination and 55·6% positive on culture. They also illustrate the three main problems of the direct microscopic examinations in females, viz. (*a*) when the criteria for identification are very strict many positive cases are missed, (*b*) the large number of cases where at the best only a presumptive diagnosis can be given and where the odd false-positive result may be reported, and (*c*) even when doubtful positive results are included, it remains an insensitive test compared with the cultural and fluorescent antibody methods.

Cultural examinations are therefore preferable to direct microscopy of Gram-stained smears from both the point of view of sensitivity and because a definite identification can be obtained.

B. *Direct Gram and FA Techniques*

These two methods were employed on swabs from the posterior fornices of 146 patients; the results are given in Table III, from which it can be seen that the direct FA

TABLE III. DIRECT GRAM AND FA RESULTS

Microscopic examinations		*Totals*	
Gram stain ('presumptive')	*FA direct*	*Examined*	*Confirmed positive (all methods)*
36	49	146	70

method gave an appreciably higher number of positive results compared with the direct Gram-stain technique, and that approximately 70% of the confirmed positive cases were diagnosed by the direct FA method.

Both tests entail only the making of a smear and simple fixation by the medical officer examining the patient, while the problems of immediate incubation or transport media do not apply. There can be no doubt about the value of the direct FA technique under these conditions and its superiority over the conventional direct examination of Gram-stained smears. The extra expense and work involved in the direct FA test seem to be well warranted. Furthermore, it is specific and a definite laboratory diagnosis can be given.

C. *Cultural Methods*

Table IV shows that in this series the delayed FA technique gave considerably better results than culture on blood agar. It is also apparent that no positive result was obtained by any of the other tests which was not also revealed by the delayed FA technique. Although it is admitted that better results might have been obtained with selective media containing antibiotics such as 'ristocetin' and 'polymyxin B'[7] or more specialized media for gonococci, our results are similar to those reported by Danielsson[2] in Sweden and Brown *et al.*[8] in the USA, and show clearly that the delayed FA test is the method of choice for the detection of gonococci in gonorrhoea in the female, especially in subacute or chronic cases. In both Danielsson's and Brown's series it was also shown to be the best method for the detection of gonococci in partially treated patients.

TABLE IV. CULTURAL RESULTS

		Total	
Culture on blood agar	*Delayed FA*	*Examine*	*Confirmed positive (all methods)*
44	86	172	86

In an attempt to determine the usefulness of blood agar as the culture medium for the delayed FA technique, both blood agar and the Difco gonococcal medium were used in parallel on 43 posterior fornix swabs. Of these cases 10 were positive on culture and 12 positive results were obtained from both the blood agar and Difco medium delayed FA tests. We found, however, that the Difco medium was superior from a technical point of view, the organisms often being present in greater numbers and showing brighter fluorescence when this medium was used.

The effect of delaying incubation of the Difco gonococcal medium after inoculation was studied, and it was found that with delays of less than 36 hours before incubation this medium gave 47 positive results, compared with 64 when incubation was started immediately after inoculation (see Table I). This medium was found to be a reasonably good transport medium when only short delays were anticipated, but with delays of more than 1 day before incubation there was marked reduction in the number of positive results.

D. *Stuart's Transport Medium*

Cervical and posterior fornix swabs from 203 patients were inoculated into Stuart's transport medium and examined in the laboratory after delays of 1 day or longer by subinoculating onto blood agar followed by a full cultural identification. The Stuart's transport medium results are compared in Table V with a parallel series of

TABLE V. STUART'S TRANSPORT MEDIUM AND CULTURE BLOOD-ON-AGAR

		Total	
Culture on blood agar	*Stuart's medium*	*Examined*	*Confirmed positive (all methods)*
133	99	406	175

culture results on blood agar from the same sites in the same patients. It can be seen that in this study Stuart's medium shows a sensitivity of about 74% compared with direct culture on blood agar.

Subinoculation from Stuart's medium onto Difco gonococcal medium, followed by the delayed FA techniques, gave superior results to Stuart's medium followed by ordinary culture. The results of Stuart's medium used as a transport medium for the delayed FA techniques compared with the FA technique after immediate incubation are given in Table VI. These results show that Stuart's transport medium is a very useful adjunct to the conventional culture methods in laboratories where delays in

transit are anticipated. It should also prove useful in laboratories where fluorescent antibody techniques are used and where delays of more than 1 day are likely to occur.

The obvious alternative method of Stuart's medium in instances where a delay is likely is the use of fixed smears for the direct FA method. Of 292 investigations when these 2 methods were used concurrently, the direct FA

TABLE VI. STUART'S MEDIUM COMBINED WITH DELAYED FA TECHNIQUE

Stuart's medium + delayed FA	*Delayed FA*	*Total*	
		Examined	*Confirmed positive (all methods)*
20	27	60	27

was positive in 105 out of 134 confirmed positive cases and culture from Stuart's medium in 67. The combined use of the delayed FA technique and Stuart's medium would undoubtedly have given an appreciably higher yield of positive results. An advantage of Stuart's medium over the direct FA test is that with the former method the culture is available for antibiotic sensitivity tests and in addition other pathogens, including *Trichomonas vaginalis* may be recovered.

E. *Sites*

From Table I it can be seen that more positive results were obtained when specimens were taken from 2 or 3 sites than from any one single site.

CONCLUSIONS AND SUMMARY

This survey deals with the bacteriological diagnosis of 263 Bantu females suspected of gonorrhoea on account of lower abdominal pain, burning on micturition, and a vaginal discharge.

It is shown that the recently described fluorescent antibody techniques have very definite advantages over the conventional methods.

A summary and an evaluation of the various methods are given below together with recommendations of procedures.

A. *Conventional Methods*

(i) *Microscopic examination of Gram-stained smears.* The best figures that could be obtained in this survey gave a diagnostic rate of about 28% of the total or 63% of the cases found positive by the other methods. Some of these microscopic results could not be confirmed by more sensitive methods and only 4% of the patients gave results that could confidently be reported as positive. These figures are too low and too unreliable to be of real value.

(ii) *Immediate incubation on blood agar.* This was found to be a better diagnostic method but requires an incubator and a candle jar within early reach. In this survey cultivation on blood agar achieved a positive incidence of 33% of all patients and 83% of positive cases obtained by other methods. A definite identification can be given and antibiotic sensitivity tests can be performed.

(iii) *Stuart's transport medium* gave positive results in 74% of cases that were positive on ordinary culture.

B. *Fluorescent Antibody Techniques*

These methods present a considerable advance in the diagnostic procedures in females. The yield of positive results in suspected non-acute infections in this study was as follows:

(i) Direct smears for FA testing: 36% from all sites (79% of positive results by all methods).

(ii) Delayed FA technique with incubation on Difco gonococcal medium: 49% (100% of positive results by all methods).

(iii) Stuart's transport medium combined with the delayed FA technique: 33% (74% of all positive results).

Both Stuart's and Difco gonococcal media can be used for culture and sensitivity tests as well as for the isolation of Candida, and Stuart's medium is in addition a good Trichomonas transport medium.

Procedures for Specimen Taking

(a) *Incubator immediately available.* Inoculate the specimen taken with a plain swab onto a Difco medium slope and leave the broken-off swab in the screw-capped bottle with the lid loosely closed to allow the entry of air. Incubate for about 12 hours at 37°C in a 10% CO_2 atmosphere, using the candle-jar method. Close the lid of the bottle and dispatch the incubated medium to the laboratory where it can be examined by the FA technique without further delay.

If a sing'e swab is taken the posterior fornix is preferred because of convenience and the better chance of finding other pathogens.

(b) *Incubator not available.* Fixed smears and inoculated Stuart's media should be submitted to the laboratory. (*i*) Make *smears,* preferably from cervix, urethra, and posterior fornix, and after air drying, fix them in absolute alcohol or rectified spirits for a few minutes. (*ii*) For *Stuart's transport medium* a specially prepared swab obtained from the laboratory is used. After the specimen has been taken, the swab is pushed into the medium, the swab handle is broken off, and the lid is securely closed. The bottle is then submitted to the laboratory without undue delay.

Stuart's media should be stored in a cool dark place and media that have turned blue down to the bottom of the bottle should not be used. If a single site is sampled the posterior fornix is preferred. Specimens from the posterior fornix, cervix and urethra gave comparable results in this survey. The highest incidence of positive results was obtained when all 3 sites were examined.

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