

CULTURE DIAGNOSIS OF GONORRHOEA

A Comparison of the Yield with Selective and Non-selective Gonococcal Culture Media Inoculated in the Clinic and after Transport of Specimens

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Abstract. Seventy men and 75 females attending the out-patient clinic for venereal diseases in Örebro, Sweden, were examined for gonococcal (GC) infections by direct microscopy, culture on selective and non-selective GC media in the clinic and after transport of specimens for 18-20 hours in modified Stuart transport medium (STM). The highest yield of a diagnosis of gonorrhoea was obtained by the combined use of selective and non-selective GC culture media and by inoculating these in the clinic. Compared with culture, direct microscopy gave a good reproducibility in the male group while it was quite inadequate in the female group. With regard to individual patients, a good reproducibility of positive results was obtained by culture in the clinic as compared with those obtained in the laboratory after transport of the specimens; in the male group a diagnosis was reproduced in 95%, and in the female group in 97%. However, if only one GC medium had been used in the clinic, selective or non-selective, 6% of the females would have escaped a diagnosis of gonorrhoea. After transport of the specimens to the laboratory 9% of the females would have escaped a positive diagnosis if only selective GC medium had been used, and as many as 12% if only non-selective GC medium had been used. The epidemiological significance of these findings is discussed.

A diagnosis of gonorrhoea cannot be made on clinical grounds in either women or men but must be based on the demonstration of gonococcal organisms. Direct microscopy of a methylene-blue or a Gram-stained smear still remains a rapid and accurate diagnostic method in men when performed by an experienced investigator, but it has proved inadequate in women (1, 2, 3, 7, 10). Immunofluorescent techniques have proven to be rapid and sensitive tools for the diagnosis of gonorrhoea in both men and women (1, 2, 4, 10) but their use is still confined to well equipped laboratories.

Neisseria gonorrhoeae is a fastidious micro-organism, a fact which for a long time made culture diagnosis of gonorrhoea unrewarding. However, the introduction of a new transport medium by Stuart (13, 14) and selective culture medium by Thayer & Martin (15) have achieved improved results in the bacteriological isolation of gonococci from clinical specimens.

Since 1963 medical authorities in Sweden have recommended their physicians to carry out culture routinely in both men and women with suspected gonorrhoea. Physicians in Sweden, as in other countries in Scandinavia, usually have to send the specimens to the laboratory by post, the transport time usually being 16-24 hours, sometimes longer. For this purpose the specimens are transported to the laboratory in Stuart transport medium (STM) as modified by Ringertz in 1960 (12). This modified STM has been in general use in Scandinavian countries for the last 10 years. The efficiency of the modified STM has been evaluated in laboratory experimental studies (5, 6, 12) but there are very few field studies. In a study from 1965 Danielsson reported a loss of 14% of the positive yield when the specimens were transported to the laboratory as compared with inoculation in the clinic (2). Since then a new transport kit for the modified STM and selective culture medium have come into general use in Sweden (6, 8). The purpose of the present work was to compare the yield of culture carried out in the clinic using selective and non-selective culture medium, with that of culture performed in the corresponding manner in the laboratory after transport of specimens in the new kit for

Table 1. Comparison of diagnostic results obtained in the group of male patients by culture after transport of specimens, culture at the clinic, and direct microscopy

Inoculation on plates at the clinic	Culture ^{STM} Pos.	Culture ^{STM} Pos.	Culture ^{STM} Neg.	Culture ^{STM} Neg.	Total
	Dir. Microsc. Pos.	Dir. Microsc. Neg.	Dir. Microsc. Pos.	Dir. Microsc. Neg.	
Positive	19	1	0	1	21
Negative	0	0	2	47	49
Total	20		50		70

the modified STM. The yield of direct microscopy was also compared with that of culture.

MATERIALS AND METHODS

Scope of investigation

The study comprises 70 men and 75 women who were examined and treated at the out-patient clinic for venereal diseases in Örebro, a town in central Sweden with 85 000 inhabitants. The patients attended the clinic for consultation on suspicion of gonorrhoeal infection or because they were reported as sources of infection.

Collection and handling of specimens, bacteriological examinations

Direct microscopy. Specimens were taken from the urethra of males and females and from the rectal mucosa of females with a blunt curette. Specimens were taken from the cervix with a wooden applicator covered with cotton wool. Smears were made, gently fixed by heat, stained with methylene blue and viewed at a magnification of 1 000 $\times$. A specimen was considered positive when groups of coffee-bean shaped diplococci typical of gonococci could be observed intracellularly in polymorphonuclear leucocytes. Positive smears were saved for subsequent IFL examination in the event of a discrepancy between direct microscopy and culture.

Culture of specimens after transportation in STM. The routine procedure of the VD out-patient clinic in Örebro was followed for the collection of specimens for transport to the bacteriological laboratory of the Central County Hospital in Örebro. Briefly, this was as follows: specimens were taken from the urethra in males and from the urethra, cervix and rectum from the females with the use of cotton-tipped wooden sticks impregnated with charcoal. The swabs were transported to the laboratory using the new transport kit with modified Stuart transport medium as described by Kallings (8) and Gästrin & Kallings (6). The transport kit was handled by the local postal service and taken care of by the bacteriological laboratory after 18–20 hours' transportation. The specimens were cultured on a Thayer-Martin plate (selective GC medium) prepared from Difco GC-medium base plus haemoglobin and Isovitalex and with Vancomycin, Colistin and Nystatin (11, 15). Each specimen was also cultured on a corresponding plate without antibiotics (non-selective GC medium). The plates

were incubated for 2 days at 35°C in 5–10% CO₂, and then tested for oxidase-positive colonies, from which smears were made and tested with fluorescein isothiocyanate (FITC)-labelled antigenococcus globulin as described elsewhere (1, 3).

Direct inoculation of plates in the clinic. Specimens were collected with a platinum loop from the urethra of males and from the urethra, cervix and rectal mucosa of the females. The specimens were immediately inoculated onto selective and non-selective GC medium (the rectal specimen on selective GC medium only) and the plates were incubated without delay in a candle jar. This was kept in room temperature for 1 to 2 hours and then transferred to the bacteriological laboratory by car which did not take more than 5–10 minutes. The candle jars with inoculated plates were incubated at 35°C for 2 days and then inspected and tested for gonococcal colonies as described above.

RESULTS

The male group

The results of the bacteriological tests carried out on the male group are presented in Table 1.

It will be seen that direct microscopy, inoculation of plates in the clinic, and culture after transport in modified STM, gave corresponding positive results in 19 patients. The diagnosis of gonorrhoea was arrived at in 2 additional patients by culture alone, one of which was diagnosed only with the aid of a plate inoculated at the clinic. This patient had no symptoms at all and only a few colonies grew on the plate. He attended the clinic for examination because he was the partner of a woman with septic gonococcal dermatitis. Table 1 also shows that 2 patients were considered positive by direct microscopy but were negative on culture. Reexamination of the smears with FITC-labelled antigenococcus globulin, however, gave negative results. It can be seen from the table that negative results were obtained in 47 patients with all three methods.

Table II. Comparison of diagnostic results obtained in the group of women by culture after transport of specimens, culture at the clinic, and direct microscopy

Number of positive cases by culture based on the combined results with non-selective and selective GC media

Inoculation on plates at the clinic	Culture ^{STM} Pos.	Culture ^{STM} Pos.	Culture ^{STM} Neg.	Culture ^{STM} Neg.	Total
	Dir. Microsc. Pos.	Dir. Microsc. Neg.	Dir. Microsc. Pos.	Dir. Microsc. Neg.	
Positive	5	26	0	1	32
Negative	0	0	1	42	43
Total	31		44		75

The female group

The results of the bacteriological tests carried out on the female group are presented in Table II.

It will be seen that direct microscopy, inoculation of plates in the clinic and culture after transport in STM gave corresponding positive results in only 5 women while a diagnosis of gonorrhoea was arrived at in an additional 27 patients by culture, one of which was diagnosed only with the aid of plates inoculated in the clinic. One patient was considered positive by direct microscopy but was negative in culture. All three methods gave negative results in 42 patients.

Tables III and IV show a comparison of the number of positive female cases diagnosed by non-selective and selective GC media inoculated in the clinic, or in the laboratory after transport in STM.

It will be seen from Table III that a diagnosis of gonorrhoea was arrived at in 30 women by inoculating specimens on non-selective or selective GC media in the clinic. However, the combined use of these two media gave 32 positive cases, i.e. an increase of 6.2%.

Table IV shows that after transport of specimens in STM, a diagnosis of gonorrhoea was arrived at in 28 women by inoculating specimens on non-selective GC medium and in 29 women with selective GC medium, i.e. an increase of 3.4%. However, the combined use of these two media for specimens transported to the laboratory in STM gave 31 positive cases, i.e. an increase of 9.7% compared with non-selective medium alone, and an increase of 6.4% as compared with selective medium alone. The combined use of the two media for inoculating specimens in the clinic or after transport in STM gave cor-

responding results in 31 out of the total of 32 positive females.

A comparison was made on the total yield of positive sites obtained in women by culture in the clinic and by culture after transport in STM with the use of selective and non-selective GC media. The results are summarized in Table V.

It will be seen that the combined use of the two media gave 64 positive sites when the specimens were inoculated in the clinic as compared with 58 positive sites when culture was performed after transport in STM, i.e. a difference of 9.3%. It should be mentioned that the rectal specimen for practical reasons was only inoculated on selec-

Table III. Comparison of the results of 32 female cases diagnosed by culture on selective and non-selective GC media at the clinic

Selective media	Non-selective media		
	Positive	Negative	Total
Positive	28	2	30
Negative	2	43	45
Total	30	45	75

Table IV. Comparison of the results of 31 female cases diagnosed by culture on selective and non-selective GC media after transport of specimens in modified STM

Selective media	Non-selective media		
	Positive	Negative	Total
Positive	26	3	29
Negative	2	44	46
Total	28	47	75

Table V. Comparison of positive sites in the female group with regard to specimens cultured on selective and non selective GC media in the clinic and after transport in STM

	Culture in the clinic			Culture after transport in STM		
	Select. medium	Non-select. medium	Sel. Non. sel.	Select. medium	Non-select. medium	Sel. Non. sel.
Urethra	17	20	23	19	17	22
Cervix	27	29	31	28	27	28
Rectum	10	N.D.	10	8	2	8
Total	54	49	64	55	46	58

N.D. = not done.

tive GC media in the clinic. This did not, however, influence the number of positive sites (see below). Table V also shows that inoculating specimens on selective GC medium in the clinic or after transport in STM to the laboratory gave nearly the same results: there was actually one more positive specimen after transport. A loss of positive sites of 16.3 % is noted when specimens after transport in STM are cultured on non-selective GC medium as compared with selective medium. Two-thirds of this loss is found to be due to rectal specimens for which the selective GC medium is superior to the non-selective one. Table V also shows that the combined use of selective and non-selective GC medium in the clinic gave 15.6% more positive sites than the use of selective medium alone. After transport in STM the corresponding figure will be 5.2%.

DISCUSSION

In patients attending a venereal disease clinic, the highest yield of a culture diagnosis of gonorrhoea was obtained by the combined use of selective and non-selective GC culture media and by inoculating these media in the clinic. In the female group, in which specimens were cultured from urethra, cervix and rectum, the total number of positive sites was nearly 10% greater when culturing the specimens in selective and non-selective media in the clinic than with culture on corresponding media after 18–20 hours' transport in modified STM. However, with regard to the individual patients with a culture diagnosis of gonorrhoea, there was a good reproducibility of the results obtained by culture in the clinic as compared with those obtained in the laboratory

after transport of the specimens. Thus a diagnosis of gonorrhoea was reproduced in 95% of the males and in as many as 97% of the females after transport of the specimens, which indicates a high efficiency of the modified STM.

Women with gonorrhoea are generally considered to be a reservoir of gonococci because more than 50% of them have no symptoms of an infection (9). It is therefore of special interest to look at the individual results obtained in the female group with the use of selective versus non-selective culture media inoculated in the clinic versus the laboratory. Compared with the combined results obtained with both selective and non-selective culture media in the clinic, it will be seen that 6% of the females would have escaped the diagnosis of gonorrhoea if only one type of culture medium, selective or non-selective, had been inoculated at the clinic. A corresponding comparison with the results obtained in the laboratory shows that 9% of the females would have escaped the diagnosis of gonorrhoea if only selective culture media had been used, and as many as 12% if only non-selective culture media had been used. These interesting results may be interpreted in various ways.

It is a well known bacteriological fact that the number of positive isolates will increase by increasing the number of investigated specimens in one and the same patient. This might explain the discrepancy between the use of a single culture medium and the combined use of two since there may be differences in how representative each specimen is. However, even though the number of positive patients is too small for a statistical evaluation, the present investigation shows that the selective culture medium is supe-

rior to the non-selective one with regard to rectal specimens. These findings are in agreement with other reports (3, 6). In this connection it should be emphasized that in large series, some 3–5% of females with gonorrhoea are diagnosed only by the isolation of gonococci from rectal specimen cultured on selective medium (3, 6). It is of interest to note, however, that in the present investigation there are no obvious differences between selective and non-selective media with regard to urethral and cervical specimens.

In a previous investigation in 1965 Danielsson noted a loss of 14% of positive women after transport of their specimens in STM to the laboratory (2). Non-selective culture media were used in that work. A comparison of corresponding results with non-selective media in the present investigation shows a loss of 7% of positive women. This difference between the two studies may be explained by the use of a new kit for the modified STM (6, 8). This kit prevents oxidation of the STM, thus increasing its efficiency as has been shown in laboratory experimental studies (5, 6).

The present investigation confirmed that direct microscopy of smears is too insensitive to be used alone as a diagnostic method in women, though it still is an adequate method in men. There are reports, however, of an increasing number of males with asymptomatic gonorrhoea. Lidén in 1969 reported a figure of 20% (9) and most of these males were diagnosed only by culture. It is therefore recommended that culture be made routinely in all male patients suspected of having gonorrhoea.

Some bacteriological laboratories in Scandinavia, and also in other countries, nowadays carry out culture of gonococcal specimens only on one medium and exclusively on selective GC medium, mostly because of the heavy load of gonococcal specimens sent in to the laboratories. The present investigation shows, however, that in this way at least 6 to 9% of women with gonorrhoea may escape specific diagnosis. Many publications about treatment of gonorrhoea show similar figures with regard to treatment failures. This means that despite our improved bacteriological techniques for culture diagnosis there is a reservoir of women with undetected gonorrhoea at least as large as 12 to 18%. We would therefore like

to question what this may mean from an epidemiological point of view? It is stated in "Today's VD Control Problem 1970" (16) that much sustained effort is needed to bring gonorrhoea under control. It is obvious from the figures presented in this work that, beside socio-medical factors, the diagnostic methods for detecting gonorrhoea, i.e. both bacteriological and serological techniques, must be further improved and that further work is needed to elucidate the epidemiological significance of the "undetected" female gonorrhoeal reservoir.

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