Enzymatic detection of *Neisseria gonorrhoeae*

MILTON M TAKEGUCHI,* HOWARD H WEETALL, DAVID K SMITH,
HUGH C McDONALD, KIM A LIVSEY, CLARENCE C DETAR,
AND THOMAS A CHAPEL†

From the *Research and Development Laboratories, Sullivan Science Park, Corning, New York, and the
†Department of Syphilology and Dermatology, Wayne State University School of Medicine, Detroit,
Michigan, USA

SUMMARY In a study using a non-serological enzymatic approach for the detection of *Neisseria gonorrhoeae* in cervical and urethral swabs, the technique was shown to be technically feasible. The enzyme, 1, 2-propanediol oxidoreductase, was used as a presumptive diagnostic marker for *N. gonorrhoeae*. Enzymatic activity was measured with a fluorometer. Two assay procedures were performed: (a) enzyme detection (two-tube and three-tube assays) requiring 60 minutes; and (b) enzyme inhibition (EI) (90-minute and modified 20-minute assays). Sensitivities of the two-tube, three-tube, and the 90-minute EI assays with male urethral specimens from a high-prevalence population were 80%, 84%, and 81% respectively. The specificities of these assays in a low-prevalence male population were not determined. Sensitivity of the 90-minute EI assay in a high-prevalence female group was 77% and specificity in a low-prevalence female group was 75%. The modified EI assay was tested only in a low-prevalence female group and had 87% specificity. Although the specificity of the assays needs improvement, several advantages—including early case detection, rapid availability of results, detection of current active infections, and the possibility of automation—are intrinsic in this enzymatic approach.

Introduction

Cultural isolation and identification are the most definitive diagnostic means presently available for detecting gonorrhoea in women and will very likely continue to be the principal diagnostic procedures in the foreseeable future.¹ Any test which can rapidly determine the presence of an active gonococcal infection would be an invaluable adjunct.

Recently, we reported the isolation and characterisation of a previously undescribed enzyme, 1, 2-propanediol oxidoreductase, from *Neisseria gonorrhoeae*.² Examination of various micro-organisms in the female genital tract showed that appreciable concentrations of this particular enzyme were present in only *Neisseria* and *Acinetobacter* species.² Of these organisms, *N. gonorrhoeae* showed higher levels of activity than most of the other organisms. We decided therefore to use the enzymatic approach for the presumptive diagnosis of gonorrhoea in a clinical context. This report

describes four variations in the use of enzymatic assays for detecting gonorrhoea in a small number of patients in three separate clinical trials.

BACTERIA

Neisseria gonorrhoeae strain F62 (obtained from Dr D S Kellogg Jr, Center for Disease Control, Atlanta, Georgia), was the strain used in lysis and sensitivity experiments.

GONOCOCCAL LYSIS

A calcium alginate-tipped swab (Inolex Corporation, Glenwood, Illinois) was used to collect gonococci growing on GC Medium (GCM, Difco Laboratories, Detroit, Michigan) supplemented with 1% IsoVitalex enrichment (Baltimore Biological Laboratories, Cockeysville, Maryland). The swab containing gonococci was then placed in 10 ml of 0.1 mol/l Tris buffer, pH 9.0, containing 0.001 mol/l cysteine for one hour at room temperature (24°C). The degree of cell lysis in this buffer was determined at various intervals by phase microscopy, scanning electron microscopy, and indirectly by standard bacteriological plate counts. Propanediol oxidoreductase activity was measured after storage for 20, 45, and 115 hours in the buffer at -20°C.

Address for reprints: Dr M M Takeguchi, Research and Development Laboratories, Sullivan Science Park, Corning Glass Works, Corning, New York 14830, USA

Received for publication 24 October 1979

DETECTION OF PROPANEDIOL
OXIDOREDUCTASE

In a two-tube assay, 0.5 ml of patient sample (described below) was mixed with 2.5 ml of 0.1 mol/l Tris buffer, pH 9.0, containing 0.25 mol/l NH_4Cl , 5.02 mmol/l nicotinamide adenine dinucleotide (NAD), and 0.438 mmol/l 1, 2-propanediol. A control tube contained the same constituents with the exception of the 1, 2-propanediol. After incubation for one hour at 50°C, a fluorometer (Turner Model 111, Turner Associates, Palo Alto, California) was calibrated to zero with the control tube. The propanediol oxidoreductase activity in the test tube was then measured and expressed as fluorescence units (fu) per ml of patient sample.

A three-tube assay was also used, in which the two-tube assay was expanded to include a third tube with patient sample and Tris buffer containing NH_4Cl , NAD, and 0.362 mmol/l glycerol. The assay was conducted as above with the enzymatic activities in the propanediol and in the glycerol tubes being measured. The activity with the glycerol was then subtracted from the activity with propanediol to yield the net fu/ml with this three-tube assay.

INHIBITION OF PROPANEDIOL
OXIDOREDUCTASE

Initially a 0.5-ml volume of patient sample was added to each of two tubes: one tube contained 0.1 ml normal goat serum and one tube, 0.1 ml of goat anti-propanediol oxidoreductase serum. Preparation and characterisation of this goat antiserum is described elsewhere.³ After incubation for 30 minutes at room temperature (24°C), 2.4 ml of 0.1 mol/l Tris buffer containing 0.25 mol/l NH_4Cl , 5.02 mmol/l NAD, and 0.438 mmol/l 1, 2-propanediol were added to both tubes. These were then incubated for one hour at 50°C. A fluorometer was calibrated to zero with the immune serum tube. The activity in the normal serum tube was then measured. This assay was referred to as the 90-minute enzyme inhibition (EI) assay.

Eventually, the procedure was modified as follows to reduce the total assay time: 1.0 ml of patient sample and 0.2 ml of normal or immune goat serum were used. Both tubes were incubated for five minutes at 24°C. Then, 0.5 ml of Tris buffer containing the same concentrations of NH_4Cl , NAD, and propanediol as before were added to each tube. The tubes were incubated for 15 minutes at 50°C and read in the fluorometer as before.

PATIENT SAMPLES

Urethral swabs from 121 male patients from a population in which the prevalence of gonorrhoea

was at least 32% were tested initially. These samples were collected with cotton-tipped swabs, which were first used for cultural diagnosis then for enzyme assays. Subsequent studies used separate calcium alginate swabs for culture and for enzyme assays.

Cervical swabs from 117 women in a high-prevalence (32%) group and from 100 women in a low-prevalence (2%) group were tested. All swabs were placed immediately in 2.5 ml Tris-cysteine buffer and were refrigerated until tested. Time lapse between collection of samples and testing ranged from 1.5 to five hours. Swabs were mixed in the buffer, squeezed against the inside of the tube to remove as much buffer as possible, and discarded. This buffer (or patient sample) was then subjected to the enzyme assays.

INTERPRETATION OF FLUORESCENCE UNITS

To maximise assay efficiency interpretative criteria in fluorescence units (fu) were established statistically and defined as the agreement between the enzyme assay and cultural results in a hypothetical 50/50 mixture of positive and negative samples. The criteria for each assay were: in the two-tube assay, $\text{fu} \geq 5$ was positive and $\text{fu} < 4$ was negative; in the three-tube assay, $\text{fu} \geq 2$ was positive and $\text{fu} < 1$ was negative; and in the 90-minute enzyme inhibition assay, $\text{fu} \geq 1$ (≥ 2 in the modified 20-minute procedure) was positive and $\text{fu} = 0$ (≤ 1 in the modified procedure) was negative.

SENSITIVITY AND SPECIFICITY

The sensitivity and specificity of our assay were computed assuming the cultural method had 100% sensitivity and specificity.

CULTURE

A separate swab was used to sample simultaneously the cervix for cultural diagnosis by recommended techniques.⁴

Results

Gonococci were lysed rapidly in the Tris-cysteine buffer (table I). More than 99% of the cells were lysed in 15 minutes. Propanediol oxidoreductase activity in these lysates was detected even after prolonged storage at -20°C (table II). The effect of this Tris-cysteine buffer on the cellular integrity of other microbial species was not specifically examined in these studies. The results of testing different gonococcal concentrations with the 90-minute EI assay are shown in table III. Detectable activity ranged from 720 fu with 4.5×10^5 colony forming units per test (cfu/test) to 2 fu with 450 cfu/test. Activity was not detected at concentrations equal to

TABLE I *Lysis of N gonorrhoeae in 0.1 mol/l Tris/1.0 mmol/l cysteine buffer*

Time (mins)	Viable gonococci (cfu/ml)*	% Lysis
0	4.7×10^{11}	
5	2.1×10^{11}	55.32
10	3.2×10^{10}	93.19
15	1.8×10^8	99.96
30	8.4×10^6	>99.99
45	7.2×10^5	>99.99
60	7.8×10^4	>99.99

*Determined by standard bacteriological plate counts

TABLE II *Detection of propanediol oxidoreductase activity in gonococcal lysate*

Storage* time (hours)	% of initial enzymatic activity
20	40
45	40
115	27

*Storage at -20°C TABLE III *Enzymatic activity of different gonococcal concentrations*

Gonococcal concentration (cfu)	Enzymatic activity (fu)
4.5×10^5	720
4.5×10^4	52
4.5×10^3	10
4.5×10^2	2
4.5×10^1	0
4.5×10^{-1}	0

cfu = colony forming units/test

fu = fluorescence unit determined by 90-minute enzyme inhibition assay

or less than 45 cfu/test. Similar tests with the modified enzyme inhibition assay indicated that gonococcal concentrations equal to or greater than 90 cfu could be detected.

Initial results with male urethral samples from a group with a high prevalence of gonorrhoea are shown in table IV. The three assays performed well; they correctly identified 80-84% of all culture-positive specimens and 92-97% of all culture-negative specimens.

TABLE IV *Sensitivity and specificity of three enzymatic assays for gonorrhoea in a high-prevalence male population*

Enzymatic assay	No of samples	% Sensitivity	% Specificity
Two-tube	121	80 (35/44)	97 (75/77)
Three-tube	121	84 (37/44)	92 (71/77)
Inhibition	89	81 (30/37)	96 (50/52)

TABLE V *Sensitivity and specificity of the 90-minute enzyme inhibition assay in a female group with a high prevalence of gonorrhoea*

Culture results	No of samples	% Sensitivity	% Specificity
Positive	43	77 (33/43)	
Negative	74		59 (44/74)

In subsequent trials only the EI assays and female samples were used. The 90-minute EI test results with female cervical swabs from the same high-prevalence group are summarised in table V. Thirty-three (77%) of the 43 culture-positive samples also gave positive results by the enzyme assay. In this test group, the specificity of the assay was 59%. The modified inhibition assay was not tested in this group.

However, the modified, as well as the 90-minute EI, assays were used in a female population in which the prevalence of gonorrhoea was low. The 90-minute EI assay detected 1/2 (50%) of the culture-positive samples and 74/98 (75%) of the culture-negative samples. The modified assay detected both culture-positive samples and 85/98 (87%) of the culture-negative samples.

Discussion

A definitive diagnosis of gonorrhoea is most often achieved today by bacteriological culture.⁴ Screening programmes aimed at detecting asymptomatic carriers⁵⁻⁷ also depend primarily on this method. Although the culture method is highly specific, it has many defects. Sensitivity is questionable, ranging anywhere from 40 to 90%. The process for obtaining specimens for culture is cumbersome and impractical for large-scale screening, and the 24 to 48 hours' incubation time required makes the method ineffective as a screening technique.⁸ Furthermore, cultural screening programmes are expensive.⁹

For some time now, many investigators have been attempting to develop a serological test for gonorrhoea.¹⁰ A good serological test may lead to more widespread screening since collection of specimens is simple, and blood samples routinely drawn for serological tests for syphilis are already available. However, many reported serological tests produce a high rate of false-positive results and also fail to detect recently infected individuals.¹⁰ For these reasons, even serological tests are not yet ideal for screening for gonorrhoea.

In this paper, we have described a new non-serological enzymatic assay for the detection of *N gonorrhoeae*. This approach could overcome some of the major disadvantages of both the cultural method and serological tests.

Like most laboratory tests which analyse a clinical specimen, a result with this enzymatic approach depended to a large extent on the condition of the specimen examined. Initial efforts in this study were therefore concerned with the development of a reliable transport system for genital secretions. Our results indicated that the Tris-cysteine buffer was an excellent means of transporting specimens, during which time gonococci were lysed and propanediol oxidoreductase activity was maintained.

Experiments with pure gonococcal lysates suggested that this enzymatic approach was capable of detecting low concentrations of gonococci. These concentrations were within the range suggested as the required sensitivity of a test for non-viable gonococcal antigens in clinical specimens.¹¹

Results of the initial trial with male specimens indicated that the sensitivity of all three assays compared favourably with many of the documented serological tests.¹⁰ Improved sensitivity may have been observed if swabs that had not been used previously for culture had been tested.

Since a low-prevalence male group was not tested, the exact specificities of these assays were not known and accurate comparison with serological tests was not possible. However, it is reasonable to assume that male swabs will not contain the diverse types and numbers of organisms which may crossreact in the assays and which may be present in female swabs. Thus, the 92-97% specificity observed in the high-prevalence group may be indicative of the specificity in a low-prevalence male group.

In the second and third trials, we concentrated on female specimens, since this is where a rapid, non-cultural test would be most advantageous. We used the inhibition approach because we considered our goat antiserum would promote specificity and not affect sensitivity. In the high-prevalence group, the sensitivity of the 90-minute assay was 77%, which is within the range of sensitivities reported for serological tests.¹⁰ This sensitivity is also approximately equivalent to the 80% sensitivity generally assumed for the cultural method.⁸ Unfortunately, the modified assay was not tested in this population. The observed sensitivity however coupled with other advantages, such as the rapid availability of test results and the non-dependency on antibody-response time should lead to early detection and treatment of cases of gonorrhoea. Furthermore, only current active infections should be detected as persistent antigenococcal antibodies present after treatment should not interfere.

Both the 90-minute and the modified EI assays were tested in a low-prevalence female group. Although only two culture-positive samples were tested, the modified assay appeared to be more sensitive than the 90-minute assay. The specificities of the modified assay and of the 90-minute assay in this low-prevalence group were 87% and 75% respectively. Therefore, many false-positive results were observed. Possibly, many of these may not have been caused by microbial crossreactivity but were due to non-microbial factors such as cervical tissues or enzymes or by unresolved technical factors in the methodology. Alternatively, false-positive results by our assay in these comparative studies with culture results may not necessarily have all been incorrect. The sensitivity of our cultural procedure was not determined. Also, cultural results may have been falsely negative since sensitivity on culture may be as low as 40%.⁸ This would have severely affected the estimate of our specificity. Nonetheless, a specificity of nearly 100% must be attained for the assay to be truly effective in screening programmes.

In summary, these studies have shown the promise of a novel and rapid approach for the detection of *N. gonorrhoeae* in urethral and cervical specimens. Further studies and comprehensive clinical evaluation are needed to perfect this approach.

References

1. Gaafar HA, D'Arcangelis DC. Fluorescent antibody test for the serological diagnosis of gonorrhea. *J Clin Microbiol* 1976; **3**:438-42.
2. Takeguchi MM, Livsey KA, Detar CC, *et al.* The occurrence of a 1, 2-propanediol oxidoreductase in micro-organisms and its use as a possible diagnostic marker for *Neisseria gonorrhoeae*. *J Gen Microbiol* 1980 (in press).
3. McDonald HC, Takeguchi MM, Detar CC, *et al.* The isolation and characterization of a 1, 2-propanediol oxidoreductase from *Neisseria gonorrhoeae*. *J Gen Microbiol* 1980 (in press).
4. Kellogg DS, Holmes KK, Hill GA. Laboratory diagnosis of gonorrhea. In: Marcus S, Sherris JC eds. *Cumitech 4* Washington, DC: American Society for Microbiology. 1980.
5. Crawford G, Knapp JS, Hale J, Holmes KK. Asymptomatic gonorrhea in man: caused by gonococci with unique nutritional requirements. *Science* 1977; **196**:1352-3.
6. Pariser H. Asymptomatic gonorrhoea. *Med Clin North Am* 1972; **56**:1127-32.
7. Pariser H, Marino AF. Gonorrhea—frequently unrecognized reservoirs. *South Med J* 1970; **63**:198-201.
8. Norins LC. The case for gonococcal serology. *J Infect Dis* 1974; **130**:677-9.
9. Gaafar HA. Evaluation of the fluorescent gonococcal test—heated. *J Clin Microbiol* 1976; **4**:423-8.
10. Koransky JR, Jacobs NF. Serologic testing for gonorrhea. *Sex Transm Dis* 1977; **4**:27-31.
11. Lowe TL, Kraus SJ. Quantitation of *Neisseria gonorrhoeae* from women with gonorrhea. *J Infect Dis* 1976; **133**:621-6.