

Review

Microbiological diagnosis of gonorrhoea

A E Jephcott

Gonorrhoea is caused by *Neisseria gonorrhoeae*. This is a delicate and fastidious organism which dies rapidly if exposed to desiccating or oxidising conditions and requires a moist carbon dioxide enriched atmosphere and a nutrient medium if it is to be cultured successfully. For these reasons diagnosis by culture was regarded as difficult and uncertain, but many decades ago the problems were overcome, and culture became the method of first choice for diagnosis, and remains the "gold standard" against which others are measured.¹ However, culture is by no means the only method available for diagnosis of gonorrhoea, and others offer alternative advantages such as speed, robustness, or technical simplicity, and no single method is appropriate in all situations.

In the typical UK microbiology laboratory specimens to be examined for gonococci will usually have been taken from patients in whom there is a significant likelihood of infection, and, on the results obtained, therapy is likely to be administered. Prevalences will vary, but are likely to be highest in patients attending genitourinary medicine clinics and somewhat lower in other patient groups. However, specimens may also be received as part of continuous or intermittent monitoring of particular population groups such as antenatal clinic patients. Here the aim, while including the treatment of any infected patients identified, is primarily to establish background knowledge of the prevalence of infection in that community. Tests employed in these differing situations will need to meet different performance criteria.

Where a clinician is seeking to identify infection in an individual patient the sensitivity of the test (that is, the likelihood of a genuine infection being detected by the test) is of paramount importance, whereas the risk of encountering a false positive result—which relates to the specificity of the test, will be of lesser importance.² However, in a situation where infection rates are low the problems of encountering false positive results will become more significant, so that the specificity of the test system employed will take on increasing importance and some sensitivity may have to be sacrificed. Moreover, in any situation the likelihood of any test result, either positive or negative, being accurate (the so called positive and negative predictive values) will depend on the number of genuine cases present in the population tested, as well as on the sensitivity and specificity of the test.² Thus, when numbers are low specificity becomes increasingly important, whereas in a population with a high prevalence of infection, optimal sensitivity should be sought.

Elsewhere in the world the availability of

laboratory facilities, and the technical expertise available in these, can be very different from those normally found in the UK, and the prevalence of infection may well be far higher. All these variables will affect the choice of the optimal test to employ—as will financial considerations and the expectations of the population involved. Problems of maintaining viability of the organism until cultured are unlikely to present problems in the usual UK clinic situation, whereas in field exercises or in rural surveys elsewhere in the world these may be of cardinal importance.

Diagnostic strategies*1 Direct visualisation of N gonorrhoeae cells in biological samples.*

- (i) Methylene blue stained smear
- (ii) Gram stained smear
- (iii) Fluorescent antibody stained smear
 - (a) Polyclonal antibodies
 - (b) Monoclonal antibodies

2 Direct detection of N gonorrhoeae cell components in biological samples.

- (i) Solid phase immunoassay
- (ii) Nucleic acid hybridisation
 - (a) Nucleic acid probing
 - (b) Nucleic acid amplification

3 Cultural detection of N gonorrhoeae in biological samples.

- (i) Direct inoculation of culture medium
- (ii) Use of transport medium and delayed inoculation of culture medium
- (iii) Transport cum culture systems

Identification of cultured gonococci.

 - (a) Presumptive gonococcus identification
 - (b) Carbohydrate utilisation
 - (c) Fluorescent antibody staining
 - (d) Co-agglutination
 - (e) Lectin agglutination
 - (f) Chromogenic substrates of constitutive enzymes
 - (g) Genetic probing

4 Specimens and sites for examination

- (A) Genital secretions
 - (i) Urethral swab
 - (ii) Cervical swab
 - (iii) Rectal/anal swab
- (B) Distant infection sites
 - (i) Conjunctival swab
 - (ii) Pharyngeal swab
 - (iii) Salpinges, peritoneal cavity, etc
- (C) Metastatic infection sites
 - (i) Skin
 - (ii) Joint aspirate
 - (iii) Blood

- (iv) Cerebrospinal fluid
(D) First voided urine

Direct methods

ADVANTAGES

- 1 Rapid—diagnosis can be reached before patient leaves clinic
- 2 Often cheap
- 3 Independent of constraints necessary for maintaining viability of organisms

DISADVANTAGES

- 1 Technical skill usually required in clinic/survey centre
- 2 Varying levels of accuracy (specificity) or of sensitivity
- 3 Absence of antibiotic sensitivity information

Cultural methods

ADVANTAGES

- 1 Greater sensitivity
- 2 Almost 100% specificity
- 3 Antibiotic sensitivity information obtainable

DISADVANTAGES

- 1 Delay inevitable
- 2 Cost
- 3 Laboratory facilities required
- 4 Technical expertise required
- 5 Viability of organisms must be maintained until culture is made

Direct methods

DIRECT VISUALISATION OF GONOCOCCI IN CLINICAL MATERIALS BY MICROSCOPY OF STAINED SMEARS

This is the traditional method. Gonococci appear as bean-shaped diplococci, located in pairs with their long axes parallel, characteristically lying inside polymorphonuclear cells. They are Gram negative. Originally a methylene blue stain was considered to demonstrate these characteristic intracellular diplococci very clearly.³ The likelihood of intracellular bean-shaped diplococci being anything other than gonococci is low (staphylococci can occur intracellularly but do so with a low frequency and are not the same shape); nevertheless the Gram stain has now generally replaced methylene blue staining.³ This can demonstrate the Gram negative character of visualised microbes, but a lack of clarity and precise definition is the price paid. However, a Gram stained smear examined by an experienced worker will detect almost all gonococcal infections in symptomatic men with a very low false positive rate. Thus Goodhart *et al*⁴ reported the probability of gonorrhoea to be 94.8% in smear positive symptomatic men, with only a 7.4% likelihood of infection in smear negative men. In women cervical smear examination is less reliable. Thus, the same authors reported that whereas there was a 97.3% probability of infection in patients when a cervical smear showed intracellular Gram negative diplococci (GNDC) 48.8% of infections could not be detected by this means.

Moreover, sensitivity of the test for men dropped to 53.9% when asymptomatic sexually active men were examined, and also fell dramatically when a less experienced operator read the slides. These figures are similar to those of other reports.^{5,6}

DIRECT VISUALISATION OF GONOCOCCI IN CLINICAL MATERIALS BY FLUORESCENT ANTIBODY STAINED SMEARS

These are considered to be more specific, using antibody to detect gonococci and a bright green label bonded onto this to make them stand out in the visual field. The technique originally⁷ received favourable reports, but some found it to be little better than a Gram stained smear and discussed theoretical disadvantages.^{8,9} These include problems of non-specific absorption of fluorescein, autofluorescence, and difficulty of large antibody molecules penetrating through the macromolecular gel of genital secretions dried onto the slide. It also requires more technical skill than is usually available in a clinic situation, and availability of an expensive fluorescence microscope.

Older antisera were raised in rabbits and were so called "polyclonal". These had to be made specific by absorption with other species of bacteria and with tissue powders. They have been superseded by mixtures of monoclonal antibodies. Using these last Ison *et al*¹⁰ found (in a population with a high prevalence of infection) a sensitivity of 84.4% for men with a specificity of 100% (being some 10% less sensitive than the Gram stain). In women the sensitivity was 65% and specificity 98% for urethral samples with values of 72% and 94% respectively for cervical samples. These were better than the 40% sensitivity but 100% specificity achieved by examination of Gram stained films by the same team, but fall far short of those obtainable by culture.

In view of the technical difficulties, costs, and lack of significantly increased diagnostic gain over the Gram film, this rapid method is not used widely.

SOLID PHASE IMMUNOASSAY DETECTION OF N GONORRHOEAE IN CLINICAL MATERIALS

A solid phase enzyme linked immunoassay (ELISA) is commercially available and is sold as "Gonozyme" (Abbott Laboratories, Chicago, IL, USA). Its advantages are that it gives a rapid result, is relatively technically undemanding, and can be performed without expensive equipment. Moreover, it can be carried out on dead organisms. This can allow it to be employed after prolonged transportation of the specimen, but complicates matters if used as a test of cure. Danielsson *et al*¹¹ assessed it in a clinic population and found a sensitivity of 87% for men and 91% for women (specificity 94.3% and 100% respectively). Whereas the results in men were no better than could be obtained by Gram film examination, in women they were almost as good as those obtained by culture. They suggested that the test might have a place as a rapid alternative to culture for women, especially if transport delays were a problem. Others^{12,13} obtained similar findings

and showed that, whereas it could be used for test of cure in males, persisting gonococcal antigen in cervical secretions rendered it unsuitable as a test of cure in females. Moreover, this test is only recommended for genital sites; rectal and pharyngeal specimens cannot be examined owing to cross reactions with bacterial antigens found at these sites. This significantly reduces confidence in results obtained at genital sites also, and is reflected in inferior test results in populations with lower incidences of infections, and in these false positive rates become unacceptably high. Thus, in a population with a 6.9% prevalence the predictive value of a positive test fell to 37.2%, indicating that the test should not be used in place of culture in such clinical situations.¹⁴

NUCLEIC ACID HYBRIDISATION DETECTION OF *N GONORRHOEAE* IN CLINICAL MATERIALS

All nucleic acid detection methods have the advantage that they can be carried out on dead organisms and thus are suitable for testing in situations where culture is impracticable or inconvenient. They have proved highly sensitive and specific in many situations, to the extent that they now have replaced cultural methods in many clinical laboratories in the United States.¹⁵ However their theoretically highly sensitive and specific performance has not always been achieved in practice.¹⁶ Problems of non-specific inhibition by genital secretions have reduced sensitivity, and specificity problems have also been encountered (see below).

Commercial versions tend to be expensive. All these factors must make them less attractive for population screening in the field or in other situations where difficulties maintaining viability for culture would otherwise exploit their major virtue.

Ribosomal RNA probing

The Gen-Probe PACE 2 system (Gen-Probe Inc San Diego, CA, USA)¹⁷ uses a magnetic bead separation stage and a chemiluminescent labelled DNA probe to detect gonococcal rRNA. It is very quick but requires expensive kits and equipment. In a high prevalence population sensitivity rates between 100% and 94.2% with specificities between 99.8% and 99.9% for specimens from both sexes and including those from non-genital sites have been reported by authors who felt that the method was more sensitive and more reliable than culture.¹⁸ Test of cure appeared reliable but numbers were too small to be dogmatic.¹⁸ Others made similar observations but were less confident concerning probe positive culture negative results,¹⁹ and advised that whereas the technique is acceptable in high risk populations where culture is not available, borderline cases with a low relative light unit emissions should be confirmed to avoid false positive results. This problem has now been overcome with the introduction of a confirmatory Probe Competition Assay from the same manufacturers,²⁰ best employed in retesting specimens yielding low initial signals with the PACE 2 system.

Plasmid DNA probing

Probes made complementary to the 2.6 MDa cryptic plasmid and to the 4.4 MDa penicillinase coding plasmid of gonococci have been tested.²¹ The technique used radioactive labels and was designed for reference laboratory screening of populations on a different continent, and as such fulfilled its purpose admirably. It gave results comparable with those obtained by culture and permitted the detection of penicillinase production. It is not a practicable proposition in the UK situation, and will inevitably fail to detect the small but significant proportion of gonococci which do not carry the 2.6 MDa plasmid.²²

Polymerase/ligase chain reaction techniques

These techniques rely upon selective amplification of sections of the genome of gonococci.

The polymerase chain reaction (PCR) technique is theoretically very sensitive and specific. Favourable results were obtained with a system which used a portion of the 2.6 MDa plasmid that is also represented on the gonococcal chromosome,²³ thus circumventing the problem alluded to above. Roche Molecular Systems Inc (Branchburg, NJ, USA) have extended their Amplicor PCR system to include a second generation multiplex test which simultaneously seeks *N gonorrhoeae* and *Chlamydia trachomatis*. Moreover, this test kit includes an internal control to detect possible false negative results due to inhibition of the system. Pre-release tests of their original system showed 100% sensitivity and 99% specificity in clinical samples.²⁴ The PCR has also been used successfully in a nested PCR technique to demonstrate gonococcal DNA in sterile synovial fluids of patients with gonococcal arthritis.^{25, 26}

A ligase chain reaction technique detecting Opa and Pilin genes was developed by Birkenmeyer and Armstrong.²⁷ Positive reactions (where appropriate) were obtained in tests of a large number of cultured gonococci and other organisms. Testing of a limited number of clinical specimens detected 100% of known infections but reacted with two apparently negative specimens in addition. In a multicentre study²⁸ of over 2000 male urethral and cervical specimens sensitivities of 97.3 and 98.5% and specificities of 99.6% and 99.8% for male and female specimens respectively were obtained, and another study examining genital and extra-genital swab and first voided urine samples from both sexes found the overall performance superior to culture in all areas.¹⁵ These good comparative results depend, among other things, on the efficiency of the culture system employed and should be treated with caution. However, the test, taking only 4 hours, clearly should be considered where cost is not the limiting factor and where transportation of viable specimens presents difficulties.

Cultural diagnosis of gonorrhoea

CHOICE OF MEDIUM AND CULTURAL CONDITIONS

Gonococci require humid conditions, an atmosphere containing between 3% and 10%

carbon dioxide, and a temperature of incubation of 35–36°C.³ Modern carbon dioxide incubators will provide these conditions reliably but candle jars, sulphuric acid/sodium bicarbonate mixtures, or GasPak (Oxoid Ltd, Basingstoke, Hants) carbon dioxide sachets all produce adequate environments. Temperature control of modern incubators is reliable but good practice dictates that the temperature is monitored daily using a thermometer which has been calibrated against an instrument of known accuracy.

Media should be nutritious and poured to a generous depth. Choice of basal medium is not critical but it should not be overheated during production and should be enriched with 5–10% heated or freeze thaw lysed blood. Serum, yeast extract, or IsoVitaleX (Becton Dickinson, Cockeysville, MD, USA) alone or in combination may be added.

Antibiotics may be incorporated to make the medium selective for gonococci. Their usefulness depends on the degree of contamination likely to be encountered. This in turn will reflect the site sampled (thus rectal and pharyngeal swabs must be plated onto selective media), the degree of skill with which they are taken, and the degree of separation obtained during the plating out process. The usual antibiotics added are vancomycin 2–3 µg/ml, colistin 6–7.5 µg/ml, trimethoprim 1.5–8 µg/ml, and nystatin 12.5 µg/ml.²⁹ Lincomycin (1 µg/ml) may replace the vancomycin, and amphotericin B (1 µg/ml) may replace the nystatin.³⁰

Some gonococci will not grow on selective media,^{31 32} and in all cases a diminished efficiency of plating will require that larger numbers of gonococci are needed in the initial inoculum for successful culture on these media. The use of a non-selective plate in parallel has been recommended to overcome this problem³¹ and while using a highly selective medium in tandem with a non-selective medium gives the best chance of isolating gonococci, if a single medium is employed (as is almost invariably the case) this should be only moderately selective.³³ Media using the lower antibiotic concentrations or those omitting trimethoprim or employing lincomycin in place of vancomycin provide compromise choices.^{31 30}

TRANSPORT CONDITIONS

If plating of the specimen is to be delayed, gonococci in clinical specimens must be protected because of their susceptibility to desiccation and oxidation. Original transport media were nutritious semisolids in which the organisms survived well but subsequent isolation rates were very poor because of overgrowth by contaminants.³⁴ This difficulty was overcome by holding organisms in “suspended animation” in semisolid buffered non-nutrient systems.^{35 36} Survival is much improved at 4°C³ but should not be relied upon with complete certainty for more than 12 hours,³⁷ although performance up to 72 hours is acceptable. In the author’s hands the system has proved superior to a direct plating culture system.³³

Direct inoculation of culture plates in the clinic is advocated as the method of choice for avoiding premature death of gonococci on swabs. For this to be successful close liaison with the servicing laboratory is essential. An efficient system involving a supply of fresh plates, properly stored at 4°C, skilfully inoculated and either rapidly transported to the laboratory or incubated until transferred to the laboratory must be set up. For optimal isolation rates plates should be introduced into a carbon dioxide enriched atmosphere within 2 hours of inoculation.³⁸ This may pose problems as may difficulty in transporting bulky containers. However, when these requirements can be met the system has much to recommend it.

Transport cum culture systems have been designed to overcome the disadvantages of conventional direct plating systems while retaining their advantages.³⁸ These systems comprise a selective culture medium provided with an individual carbon dioxide enriched environment. The original system employed a pregassed bottle (Transgrow),³⁸ but developments using small plates which employ a citric acid/sodium bicarbonate tablet to produce carbon dioxide and which retain this in a gas impermeable plastic envelope are more popular, and allow better access to colonies for confirmation of identity and sensitivity testing.³⁹ These are very “user friendly” and have good shelf lives making them ideal for single use in the doctor’s office in the United States, but are expensive. Trials have shown them to be reliable and rapid.^{40 41}

In practice in the United Kingdom some clinics employ transport media while others arrange for a direct plating service, logistics often dictating the method chosen. Field surveys (when using culture) will usually employ a transport medium method. “Transgrow” type methods are best employed where high risk patients requiring rapid results are examined in situations without ready access to an adjacent laboratory. Private rooms and isolated STD clinics are most likely to fit this situation. They are also appropriate for field surveys, offering longer viability in transit than Stuart’s medium,³⁷ particularly so in conditions of high ambient temperatures.³⁷

Identification of cultured gonococci

Plates should be examined at 24–48 hours of incubation. Gonococci appear as small greyish-white oxidase positive colonies. Morphologically they show their diplococcal appearance to a far lesser extent than *in vivo*. Oxidase positive Gram negative cocci which derive from genital sites and have grown on a selective culture medium have been designated “presumptive gonococci”.⁴² These have a very high probability of being gonococci, and reliance on these criteria for identification suffices in many parts of the world, but is considered inadequate in most developed countries. Confirmation of identity of cultured gonococci may be obtained by a number of techniques.

ACIDIFICATION OF CARBOHYDRATE CONTAINING MEDIA

Gonococci will acidify media containing glucose but not those containing maltose, sucrose, or lactose. Heavy pure inocula are needed and the medium necessarily must be clear yet nutritious. Originally animal sera were used to enrich these, but some contain maltase. Flynn and Waitkins⁴³ described a successful serum free medium, but a cystine trypticase agar basal medium (Becton Dickinson, Cockeysville, MD, USA) finds much favour. Both media are acceptable but require heavy inocula of pure cultures and 18 hours of incubation for the most reliable results. Modifications of the technique using lightly buffered sugar substrate solutions^{44,45} to detect preformed enzymes allow reading in a matter of hours, and these are now available as "kit tests". Thus the RIM-N kit is a micro test using an enzyme enhancer giving results within 1 hour (RIM-N; American MicroScan Campbell, CA, USA). It showed 96% agreement with CTA sugar testing.⁴⁶

Other rapid carbohydrate utilisation test kits which give comparable results⁴⁷ include: Neisseria-Kwik (Micro BioLogics, St Cloud, MN, USA); Gonobio-Test (IAF Products Inc Lavall, Quebec, Canada); Minitex (Miniaturised Microorganism Differentiation System; BBL Microbiology Systems, Cockeysville, MD, USA).

FLUORESCENT ANTIBODY STAINING

By testing cultures rather than smears of secretions most of the problems discussed earlier are avoided. Moreover pure cultures are not necessary. Thus whereas it is usual to test identifiable colonies after 24–48 hours of incubation, the process can be speeded up and examining a blind sweep of growth at 18 hours has been shown to be very successful in detecting gonococci.⁴⁸

Sera originally used for this method were polyclonal. These were found to perform as well as carbohydrate "fermentation" tests by some but others were less successful.^{33,48,49} However, these sera have been replaced by a pool of monoclonal antibodies raised against epitopes on the major outer membrane proteins (MOMPs) of strains of *N gonorrhoeae*. This, as the MicroTrak test (Syva Co, Palo Alto, CA, USA) when evaluated in 1988⁵⁰ correctly identified all strains examined. Others at that time confirmed very high sensitivity and specificity.^{51,52} However as time has passed it has become apparent that certain strains circulating in some localities are not detected by these antibodies. Thus, in 1993 a survey revealed that 4.6% of strains of *N gonorrhoeae* which reacted with a polyclonal reagent failed to react with the monoclonal antibodies,⁵³ and in 1995 up to 20% of isolates were found to be unreactive.⁵⁴ It was concluded that this test was unsuitable for use as the sole means of identifying cultured gonococci in the localities examined.

CO-AGGLUTINATION

Identification of cultured gonococci by co-agglutination of antibody coated staphylococci with easily prepared extracts on a slide is quick,

cheap, and very sensitive and specific. It was originally described in 1974.⁵⁵ A system using polyclonal antibody (Phadebact Gonococcus Test: Pharmacia Diagnostics AB, Uppsala, Sweden) and two systems using different pools of monoclonal antibodies are available (Gono Gen, New Horizons Diagnostic Company, Columbia, MD, USA; and Phadebact GC Omni Test, Pharmacia Diagnostics AB, Uppsala, Sweden). The original monoclonal and the polyclonal systems both gave over 97.5% agreement with neisserial strains in an "in use" test. The monoclonal system retained specificity when challenged with non-gonococcal *Neisseria* and *Branhamella* species but the polyclonal antibodies system fared less well.⁵⁶ Comparison of the two monoclonal systems⁵⁷ showed that whereas the GC Omni test gave false positive reactions with *N lactamica* and *N meningitidis* which could be overcome by modifying the extraction process, the Gono Gen system gave no false positive reactions but failed to react with occasional gonococci. A recent reassessment⁵⁴ of the Swedish monoclonal system—as the Phadebact Monoclonal GC test (Boule Diagnostics AB, Huddinge, Sweden) indicated that it retains its ability to detect those gonococci no longer identified by the alternative monoclonal antibodies set when using either fluorescent antibody or coagglutination formats.

The problem of varying responses to serological reagents was addressed by Evins *et al.*⁵⁸

LECTIN AGGLUTINATION

Lectins are glycoproteins which bind specifically to individual carbohydrate moieties.⁵⁹ Thus wheatgerm lectin binds to β -D-1.4 N acetylglucosamine.⁶⁰ Capitalising on this specificity Schaefer *et al.*⁶¹ described a rapid test to differentiate cultured gonococci from other Neisseriae. Problems of lack of reproducibility and the agglutination of non-capsulated meningococci were encountered,^{62,63} but modifications to the technique, using DNase to overcome autoagglutinability and a second lectin, and supplementing the test with three chromogenic enzyme substrates (see above) were claimed to have overcome these difficulties and to yield a reliable 30 minute test system.^{64,65} However, current exploitation of lectin agglutination has been directed to the use of an array of lectins for differentiating between strains of gonococci to enhance subtyping methods rather than as diagnostic reagents.^{66–68}

CHROMOGENIC ENZYME SUBSTRATES

D'Amato *et al.*⁶⁹ in 1978 developed a system of chromogenic substrates which, by detecting 10 constitutive enzymes, would permit the identification of cultured gonococci within a few hours. This has now been refined as the basis of the Gonocheck II (E Y Laboratories Inc, San Mateo, CA, USA) which gave 100% agreement with eighty neisserial isolates when tested by Brown and Thomas⁷⁰ and finds widespread use in today's diagnostic laboratory. Reactions obtained are not specific only to gonococci, and for this reason the system

must be used only on specimens isolated on media selective for pathogenic *Neisseriae* as *N sicca* and *N mucosa* will give misleading results.⁷⁰ However, these will not cause confusion as they do not grow on gonococcal selective media. Enzymes detected are γ glutamyl aminopeptidase, prolyl aminopeptidase, and β galactosidase. The apiNH system (bioMerieux, Marcy-l'Etoile, France) employs chromogenic reagents detecting a range of enzymes and also seeks conventional sugar acidification. This battery of 13 tests permits identification of *N gonorrhoeae* and a range of other bacteria, but again requires application of preliminary selective criteria if results are to be relied upon.⁷¹

NUCLEIC ACID HYBRIDISATION (GENETIC PROBING)

A synthetic oligonucleotide probe specific for gonococcal rRNA has been developed by Rossau *et al.*⁷² This was used in a dot hybridisation test on cultured *Neisseriae* and proved 100% specific and 100% sensitive. Pure cultures were not necessary. Use of the gonococcal 2.6 MDa plasmid as a probe was less sensitive. These authors employed a radioactive label, but the AccuProbe *N gonorrhoeae* Culture Confirmation Test (Gen-Probe Inc, San Diego, CA, USA) has also shown 100% sensitivity and specificity, using a commercially available chemiluminescent rRNA probe and gives results in 30 minutes.⁷³ This rapid non-radioactive system must have much to recommend it, but its cost will prevent widespread adoption in most situations. A biotinylated DNA probe on dot paddles (Ortho Diagnostic Systems, Neckargemund, Germany) was rapid and sensitive when evaluated but was felt not to be sufficiently specific for routine use.⁷⁴

Specimen type—sites examined

URETHRA, CERVIX, AND RECTUM

In the male the urethra should be sampled. In the female the primary site of infection is the cervix, but the urethra is frequently infected also and should also be sampled, thus Schmale *et al.*⁷⁵ found over 70% of urethral cultures positive. The female rectum is also often culture positive in infected women. This is usually a result of spread of infection from infected vaginal secretions and does not necessarily imply anal intercourse. Nicol⁷⁶ demonstrated gonococci in the rectums of 35% of a group of women gonorrhoea contacts and recommended that rectal testing should be part of the routine examination, and should always be included in tests of cure. Others using selective media found 45% of infected women had positive anorectal cultures, and in 5.6% this site alone tested positive.⁷⁷

DISTANT INFECTION SITES

Throat swabs should sample the tonsillar crypts and the posterior pharynx. A selective medium will be required and caution with the use of Gonozyme has already been mentioned. Young and coworkers report a significant superiority for direct gene probing over other test systems.⁷⁸

CONJUNCTIVA

Conjunctival pus, and pus from other sites, should be treated as genital swabs.

JOINTS AND BLOOD

Blood and joint aspirates may be treated as are similar cultures from non-STD patients but a PCR test for gonococcal DNA in joint fluid has recently been shown to provide rapid accurate results.^{25 26}

SKIN

Skin lesions should be punctured with a sterile lancet and tissue fluid expressed. This may be cultured as a pus specimen, but a direct smear on a slide stained by Gram or fluorescent antibody method can yield a rapid answer, but the former will not differentiate between meningococcal or gonococcal infection.

FIRST VOIDED URINE

Whereas a proportion of gonococci are killed in acidic concentrated urine⁷⁹ testing of a "socially acceptable" specimen offers many advantages. Centrifuged urine was reported as an effective alternative to a urethral swab in Moore *et al.*⁸⁰ yielding a sensitivity of 94% in a high prevalence population. Similarly uncentrifuged male urine culture has yielded 94% of infections diagnosed by culture or Gram smear,⁸¹ and the Gonozyme test has given results comparable with culture. Sensitivities of 91.7% and 93% with specificities of 97.9% and 99% for uncentrifuged urine, have been reported.^{82 83} These results were so encouraging that use of these methods was advocated as a non-invasive technique for detecting gonorrhoea in men. Predilution of urine deposit seems to give best results,⁸⁴ results remain stable on urine sediment stored for up to 7 days before testing. This reinforces the advantage of transportability of this system.⁸⁵

Culture of urine in women has proved less successful. Thus, Chapel and Smeltzer⁸⁶ were only able to grow gonococci from the centrifuged deposit of 72.3% of female cases of gonorrhoea, but as the site of primary infection in women is the cervix (see above) this is unsurprising.

Molecular techniques have also been employed successfully on these specimens. Thus the Gen-Probe PACE 2 yielded 98.3% sensitivity and 96.4% specificity with symptomatic male urine.⁸⁷ By combining this with a similar test for *Chlamydia trachomatis* a non-invasive socially acceptable sensitive and transportable system is available for the diagnosis or screening for two sexually transmitted diseases of major importance. In women also the great sensitivity of these tests has proved of value. Thus the ligase chain reaction technique has been shown to be 94.7 and 94.6% sensitive and 100% specific when testing first voided urine samples from STD clinics.^{15 88}

Biological variability

In the animal kingdom the reproductive process is sexual and involves a reassortment of genes at each generation. Thus each indi-

vidual is genetically unique. However, in the bacterial kingdom reproduction is a vegetative process with no stage of recombination. Such genetic alteration as does occur takes place by mutation in individual cells or by transfer of genetic material between cells by conjugation or by transformation. These are usually uncommon events and many bacterial species are highly clonal, being stable in character for many years and showing little variation throughout the world.⁸⁹ Gonococci, in contrast, are uniquely competent to exchange DNA by transformation and in consequence are highly variable and non-clonal.⁹⁰ Thus their characteristics alter and it is to be expected that no test seeking a relatively small number of identifying enzymes or antigenic markers will ever detect all gonococci, and strains will be encountered that show features of gonococci and also of other *Neisseriae*. One such example is *N gonorrhoeae* sub sp *kochii*, which has certain characteristics of the meningococcus and is often encountered in Egypt.⁹¹ Others are only met rarely⁹² but all series of cultures will include biochemically aberrant strains. Thus final identification must be based on an Adansonian concept and, in practice, judgment will always be necessary as the final arbiter. Knapp⁹³ recommends that important or difficult decisions should be based on tests relying on more than one diagnostic principle, but opines that a very small minority of isolates will never be satisfactorily speciated.

Conclusion

A very wide range of test procedures are currently available for the diagnosis of gonorrhoea. Choice of a particular system will be based on the weighing of the relative advantages and disadvantages of all those available, and significantly different decisions may be reached, entirely correctly, in the myriad differing circumstances which will be encountered in the UK and elsewhere in the world.

- Ison CA. Laboratory methods in genitourinary medicine—methods of diagnosing gonorrhoea. *Gentourin Med* 1990; 66:453–9.
- Vecchio TJ. Predictive value of a single test in unselected populations. *N Engl J Med* 1966;274:1171–3.
- Reyn A. Laboratory diagnosis of gonococcal infections. *Bull World Health Organ* 1965;32:449–69.
- Goodhart ME, Ogden J, Zaidi AA, Kraus SJ. Factors affecting the performance of smear and culture tests for the detection of *Neisseria gonorrhoeae*. *Sex Transm Dis* 1982;9:63–9.
- Handsfield HH, Lipman TO, Harnisch JP, Tronca E, Holmes KK. Asymptomatic gonorrhoea in men. Diagnosis, natural course prevalence and significance. *N Engl J Med* 1974;290:117–23.
- Barlow D, Phillips I. Gonorrhoea in women. Diagnostic clinical and laboratory aspects. *Lancet* 1978;i:761–4.
- Deacon WE, Peacock WL, Freeman EM, Harris A, Bunch WL. Fluorescent antibody tests for the detection of the gonococcus in women. *Public Health Rep (Wash)* 1960; 75:125–9.
- Thin RNT. Immunofluorescent method for diagnosis of gonorrhoea in women. *Br J Vener Dis* 1970;46:27–30.
- Lind I. Identification of *Neisseria gonorrhoeae* by means of fluorescent antibody technique. *Acta Path Microbiol Scand* 1967;70:613–29.
- Ison CA, McLean K, Gedney J, Munday PE, Coghill D, Smith R, et al. Evaluation of a direct immunofluorescence test for diagnosing gonorrhoea. *J Clin Pathol* 1985; 38:1142–5.
- Danielsson D, Moi H, Forslin L. Diagnosis of urogenital gonorrhoea by detecting gonococcal antigen with a solid phase enzyme immunoassay (Gonozyme). *J Clin Pathol* 1983;36:674–7.
- Schachter J, McCormack WM, Smith RF, Parks RM, Bailey R, Ohlin AC. Enzyme immunoassay for diagnosis of gonorrhoea. *J Clin Microbiol* 1984;19:57–9.
- Granato PA, Roefaro M. Comparative evaluation of enzyme immunoassay and culture for the laboratory diagnosis of gonorrhoea. *Am J Clin Pathol* 1985;83: 613–8.
- Nachamkin I, Sondheimer SJ, Barbagallo S, Barth S. Detection of *Neisseria gonorrhoeae* in cervical swab using the Gonozyme enzyme immunoassay. Clinical evaluation in a university family planning clinic. *Am J Clin Pathol* 1984;82:461–5.
- Starey A, Ching S-F, Teodorowicz L, Lee H. Comparison of ligase chain reaction and culture for detection of *Neisseria gonorrhoeae* in genital and extragenital specimens. *J Clin Microbiol* 1997;35:239–42.
- Wright CJ, Jerse AE, Cohen MS, Cannon JG, Seiffert HS. Non-representative PCR amplification of variable gene sequences in clinical specimens containing dilute complex mixtures of microorganisms. *J Clin Microbiol* 1994; 32:464–8.
- Granato PA. PACE (Probe Assay—Chemiluminescence Enhanced). A magnetic bead assay for the noncultural diagnosis of gonorrhoea. *Methods Mol Biol* 1994;28: 209–16.
- Vlasopolder F, Mutsaers JA, Blog F, Notowicz A. Value of DNA probe assay (Gen-Probe) compared with that of culture for the diagnosis of gonococcal infection. *J Clin Microbiol* 1993;31:107–10.
- Stary A, Kopp W, Zahel B, Nerad S, Teodorowicz L, Hörting-Möller I. Comparison of DNA-probe test and culture for the detection of *Neisseria gonorrhoeae* in genital samples. *Sex Transm Dis* 1993;20:243–7.
- Woods GL, Garza DM. Use of Gen-Probe Probe Competition Assay as a supplement to probes for direct detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urogenital specimens. *J Clin Microbiol* 1996; 34:177–8.
- Perine PL, Totten PA, Holmes KK, Sng EH, Ratnam AV, Widy-Wersky R, et al. Evaluation of a DNA-hybridisation method for detection of African and Asian strains of *Neisseria gonorrhoeae* in men with urethritis. *J Infect Dis* 1985;152:59–63.
- Abeck D, Alexander F, Johnson AP, Zaba R, Korting HC, Taylor-Robinson D. Prevalence of gonococci lacking the 2.6 megadalton cryptic plasmid. *Lancet* 1987;i:1440.
- Ho BS, Feng WG, Wong BK, Egglestone SI. Polymerase chain reaction for the detection of *Neisseria gonorrhoeae* in clinical samples. *J Clin Pathol* 1992;45:439–42.
- Purohit A, Koslov E, Uhrin M, Rosenstrauss M, Wiesenfeld HC, Sweet RL. Extending Amplicor to detect both *Neisseria* and *Chlamydia*. Abstract presented at American Society of Microbiology Meeting, Las Vegas, Nevada, 24–27 May 1994.
- Liebling MR, Arkfeld DG, Michelini GA, Nishio MJ, Eng BJ, Jin T, et al. Identification of *Neisseria gonorrhoeae* in synovial fluid using the polymerase chain reaction. *Arthritis Rheum* 1994;37:707–9.
- Li F, Bulbul R, Schumacher HR, Kieber-Emmons T, Callegari PE, Von Feldt JM, et al. Molecular detection of bacterial DNA in venereal-associated arthritis. *Arthritis Rheum* 1996;39:950–8.
- Birkenmeyer L, Armstrong AS. Preliminary evaluation of the ligase chain reaction for specific detection of *Neisseria gonorrhoeae*. *J Clin Microbiol* 1992;30:3089–94.
- Ching S, Lee H, Hook EW, Jacobs MR, Zenilman J. Ligase chain reaction for detection of *Neisseria gonorrhoeae* in urogenital swab. *J Clin Microbiol* 1995;33:3111–4.
- Martin JE, Armstrong JH, Smith PB. New system for cultivation of *Neisseria gonorrhoeae*. *Appl Microbiol* 1974;27: 802–5.
- Young H. Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *Br J Vener Dis* 1978; 54:36–40.
- Reyn A, Bentzon MW. Comparison of a selective and a non selective medium for the diagnosis of gonorrhoea to ascertain the sensitivity of *Neisseria gonorrhoeae* to vancomycin. *Br J Vener Dis* 1972;48:363–8.
- Brorson JE, Holmberg I, Nygren B, Seeberg S. Vancomycin sensitive strains of *Neisseria gonorrhoeae*. A problem for the diagnostic laboratory. *Br J Vener Dis* 1973; 49:452–3.
- Jephcott AE. Laboratory diagnosis of gonorrhoea. MD Thesis, University of Sheffield, 1977.
- Reymann F. Cultivation of gonococci as diagnostic method in gonorrhoea in women; transport of material for bacteriological examination for gonococci. *Acta Dermat-venereol Stockh* 1944;25:9–28.
- Stuart RD. The diagnosis and control of gonorrhoea by bacteriological cultures, with preliminary report on new method of transporting clinical material. *Glasgow Med J* 1946;27:131–42.
- Amies CR. A modified formula for the preparation of Stuart's transport medium. *Can J Public Health* 1967; 58:296–300.
- Van Dyck E, Piot P, Meheus E. Bench-level laboratory manual for sexually transmitted diseases. WHO/VDT/89. Geneva: World Health Organisation, 1989:443.
- Martin JE, Lester A. Transgrow: a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *Health Serv Ment Health Admin Rep* 1971;86:30–3.
- Martin JE, Jackson RL. A biological environmental chamber for the culture of *Neisseria gonorrhoeae*. *J Am Vener Dis Assoc* 1975;2:28–30.

- 40 Jephcott AE, Morton RS, Turner EB. Use of transport & culture medium combined with immunofluorescence for the diagnosis of gonorrhoea. *Lancet* 1974;2:1311-3.
- 41 Jephcott AE, Bhattacharyya MN, Jackson DH. Improved transport and culture system for the rapid diagnosis of gonorrhoea in women. *Br J Vener Dis* 1976;52:250-2.
- 42 Schroeter AL, Pazin GJ. Gonorrhoea. *Ann Intern Med* 1970;72:553-9.
- 43 Flynn J, Waitkins SA. A serum-free medium for testing fermentation reactions in *Neisseria gonorrhoeae*. *J Clin Pathol* 1972;25:525-7.
- 44 Young H, Paterson IC, McDonald DR. Rapid carbohydrate utilization test for the identification of *Neisseria gonorrhoeae*. *Br J Vener Dis* 1976;52:172-5.
- 45 Kellogg DS, Turner EM. Rapid fermentation confirmation of *Neisseria gonorrhoeae*. *Appl Microbiol* 1973;25:550-2.
- 46 Laird RC, Kelly MT. Evaluation of a one-hour test for the identification of *Neisseria* species. *J Clin Microbiol* 1985;22:238-40.
- 47 Dillon JR, Carballo M, Pauze M. Evaluation of eight methods for identification of pathogenic *Neisseria* species: Neisseria-Kwik, RIM-N, Gonobio-Test, Minitex, Gonocheck II, Gonogen, Phadebact Monoclonal GC OMNI Test, and Syva MicroTrak Test. *J Clin Microbiol* 1988;26:493-7.
- 48 Lind I. Combined use of fluorescent antibody technique and culture on selective medium for the identification of *Neisseriae gonorrhoeae*. *Acta Path Microbiol Scand* 1969;76:279-87.
- 49 Freundlich LF, Rosenthal SL, Hochberg FP, Trogele MR. Comparison of methods for the immunological identification of *Neisseria gonorrhoeae* in clinical specimens using commercially obtained reagents. *Am J Clin Pathol* 1982;77:456-8.
- 50 Ison CA, Tanna A, Easmon CS. Evaluation of a fluorescent monoclonal antibody reagent for identification of cultured *Neisseria gonorrhoeae*. *J Med Microbiol* 1988;26:121-3.
- 51 Welch WD, Cartwright G. Fluorescent monoclonal antibody compared with carbohydrate utilisation for rapid identification of *Neisseria gonorrhoeae*. *J Clin Microbiol* 1988;26:293-6.
- 52 Moyes A, Young H. Fluorescent monoclonal antibody test for the confirmation of *Neisseria gonorrhoeae*. *Med Lab Sci* 1989;46:6-10.
- 53 Beebe JL, Rau MP, Flageolle S, Calhoun B, Knapp J. Incidence of *Neisseria gonorrhoeae* isolates negative by Syva direct fluorescent antibody test but positive by Gen-Probe Accuprobe test in sexually transmitted disease clinic population. *J Clin Microbiol* 1993;31:2535-7.
- 54 Turner A, Gough KR, Jephcott AE. A comparison of three methods for the culture confirmation of *Neisseria gonorrhoeae* strains currently circulating in the UK. *J Clin Pathol* 1995;48:919-23.
- 55 Danielsson D, Kronvall G. Slide agglutination method for the serological identification of *Neisseria gonorrhoeae* with anti-gonococcal antibodies absorbed to protein A-containing staphylococci. *Appl Microbiol* 1974;27:368-74.
- 56 Young H, Reid K. Immunological identification of *Neisseria gonorrhoeae* with monoclonal and polyclonal antibody coagglutination reagents. *J Clin Pathol* 1984;37:1276-81.
- 57 Andand CM, Gubash SM, Shaw H. Serologic confirmation of *Neisseria gonorrhoeae* by monoclonal antibody-based coagglutination procedures. *J Clin Microbiol* 1988;26:2283-6.
- 58 Evins GM, Pigott NE, Knapp JS, DeWitt W.E. Panel of reference strains for evaluating reagents used to identify gonococci. *J Clin Microbiol* 1988;26:354-7.
- 59 Goldstein J, Hughes RC, Monsigny M, Osawa T, Sharon N. What should be called a lectin? *Nature* 1980;285:66.
- 60 Pistole TG. Interaction of bacteria and fungi with lectins and lectin-like substances. *Ann Rev Microbiol* 1981;35:85-112.
- 61 Schaefer RL, Keller KF, Doyle RJ. Lectins in diagnostic microbiology: use of wheatgerm agglutinin for laboratory identification of *Neisseria gonorrhoeae*. *J Clin Microbiol* 1979;10:669-72.
- 62 Curtis GDW, Slack MPE. Wheat-germ agglutination of *Neisseria gonorrhoeae*. A laboratory investigation. *Br J Vener Dis* 1981;57:253-5.
- 63 Frasch CE. Role of lipid polysaccharide in wheat germ agglutinin-mediated agglutination of *Neisseria meningitidis* and *Neisseria gonorrhoeae*. *J Clin Microbiol* 1980;12:498-501.
- 64 Yajko DM, Chu A, Hadley WK. Rapid confirmatory identification of *Neisseria gonorrhoeae* with lectins and chromogenic substrates. *J Clin Microbiol* 1984;19:380-2.
- 65 Doyle RJ, Nedjat-Haiem F, Keller KF, Frasch CE. Diagnostic value of interactions between members of the family Neisseriaceae and lectins. *J Clin Microbiol* 1984;19:383-7.
- 66 Moyes A, Young H. Typing of *Neisseria gonorrhoeae* by auxotype, serovar and lectin agglutination. *Br J Biomed Sci* 1993;50:295-300.
- 67 Moyes A, Young H. An analysis of lectin agglutination as a means of sub-dividing gonococcal serovars. *J Med Microbiol* 1992;37:51-5.
- 68 Vasquez JA, Berron S. Lectin agglutination test as an epidemiological marker for *Neisseria gonorrhoeae*. *Genitourin Med* 1990;66:302-5.
- 69 D'Amato RF, Eriquez LA, Tomfohrde KM, Singerman E. Rapid identification of *Neisseria gonorrhoeae* and *Neisseria meningitidis* by using enzymatic profiles. *J Clin Microbiol* 1978;7:77-81.
- 70 Brown JD, Thomas KR. Rapid enzyme system for the identification of pathogenic *Neisseria* spp. *J Clin Microbiol* 1985;21:857-8.
- 71 Janda WM, Zigler KL, Bradna JJ. API QuadFERM+ with rapid DNAase for identification of *Neisseria* spp and *Branhamella catarrhalis*. *J Clin Microbiol* 1987;25:203-6.
- 72 Rossau R, Duhamel M, Dyck E van, Piot P, Heuverswyn H van. Evaluation of an rRNA-derived oligonucleotide probe for culture confirmation of *Neisseria gonorrhoeae*. *J Clin Microbiol* 1990;28:944-8.
- 73 Lewis JS, Kranig-Brown D, Trainor DA. DNA probe confirmatory test for *Neisseria gonorrhoeae*. *J Clin Microbiol* 1990;28:2349-50.
- 74 Näher H, Kohl P, Petzoldt D. Evaluation of non-radioactive DNA probe for confirmatory identification of *Neisseria gonorrhoeae*. *Int J Med Microbiol* 1989;272:181-5.
- 75 Schmale JD, Martin JE, Domesick G. Observations on the culture diagnosis of gonorrhoea in women. *J Am Med Assoc* 1969;210:312-4.
- 76 Nicol CS. Some aspects of gonorrhoea in the female—with special reference to infection of the rectum. *Br J Vener Dis* 1948;24:26-37.
- 77 Bhattacharyya MN, Jephcott AE. Diagnosis of gonorrhoea in women: role of rectal sample. *Br J Vener Dis* 1974;50:109-12.
- 78 Young H, Anderson J, Moyes A, McMillan A. Non-cultural detection of rectal and pharyngeal gonorrhoea by the Gen-Probe PACE 2 Assay. *Genitourin Med* 1997;73:59-62.
- 79 Noble RC, Parekh MC. Bactericidal properties of urine for *Neisseria gonorrhoeae*. *Sex Transm Dis* 1987;14:221-6.
- 80 Moore G, Pittard WB III, Mosca N, O'Brien WM. Gonorrhoea detection by urine sediment culture. *J Am Med Assoc* 1973;224:1499-501.
- 81 Feng WC, Medeiros AA, Murray ES. Diagnosis of gonorrhoea in male patients by culture of centrifuged first-voided urine. *J Am Med Assoc* 1977;237:896-7.
- 82 Rudrik JT, Waller JM, Britt EM. Efficiency of an enzyme immunoassay with centrifuged first-voided urine for detection of gonorrhoeae in males. *J Clin Microbiol* 1984;20:577-8.
- 83 Schachter J, Pang F, Parks RM, Smith RF, Armstrong AS. Use of Gonozyme on urine sediment for diagnosis of gonorrhoea in males. *J Clin Microbiol* 1986;23:124-5.
- 84 Roongpisuthipong A, Lewis JS, Kraus SJ, Morse SA. Gonococcal urethritis diagnosed from enzyme immunoassay of urine sediment. *Sex Transm Dis* 1988;15:1992-5.
- 85 Kuroki T, Watanabe Y, Asai Y, Yamai S. Application of urine sediment for diagnosis of gonococcal urethritis by enzyme immunoassay. *Kansenshogaku Zasshi—J Japan Ass Infect Dis* 1994;68:607-11.
- 86 Chapel TA, Smeltzer M. Culture of urinary sediment for the diagnosis of gonorrhoea in women. *Br J Vener Dis* 1975;51:25-7.
- 87 Jemal C, Lebar W, Schubiner H. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine specimens by nucleic acid hybridisation. (Abst P 242). Abstracts of International Society for Sexually Transmitted Diseases Research. Banff Canada. International Society for Sexually Transmitted Diseases Research 1991:120.
- 88 Smith KR, Ching S, Lee H, Ohhashi Y, Hu HY, Fisher HC, et al. Evaluation of ligase chain reaction for use with urine for the identification of *Neisseria gonorrhoeae* in females attending a sexually transmitted disease clinic. *J Clin Microbiol* 1995;33:455-7.
- 89 Smith JM, Smith NH, O'Rourke M, Spratt BG. How clonal are bacteria? *Proc Natl Acad Sci USA* 1993;90:4384-8.
- 90 O'Rourke M, Stevens E. Genetic structure of *Neisseria gonorrhoeae* populations: a non-clonal pathogen. *J Gen Microbiol* 1993;139:2603-11.
- 91 Mazloum H, Totten PA, Brooks GF, Dawson CR, Faklow S, James JF, et al. An unusual *Neisseria* isolated from conjunctival cultures in rural Egypt. *J Infect Dis* 1986;154:212-4.
- 92 Hodge DS, Aston FE, Terro R, Ali AS. Organism resembling *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *J Clin Microbiol* 1987;25:1546-7.
- 93 Knapp JS. Historical perspectives and identification of *Neisseria* and related species. *Clin Microbiol Rev* 1988;1:415-31.