The Laboratory diagnosis of veneral diseases — II — The Laboratory diagnosis of gonorrhoea

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Gonorrhoea is caused by *Neisseria* Gonorrhoeae, a Gram negative diplococcus which was first described by Neisser in 1879.

The prevalence and incidence of gonorrhoea in Malaysia is difficult to ascertain since most patients would seek medical attention from private practitioners rather than from government institutions. Furthermore many cases may be treated by quacks or are self treated. Therefore statistics collected from government hospitals and clinics in this country do not present the true picture of venereal disease prevalence (Tow, 1964).

There is also the problem of undiagnosed or "hidden" cases. This is particularly so in the case of females where 75% of those affected may be asymptomatic (Fiumara, 1972) and therefore would seek neither diagnosis nor treatment. It is these "hidden" cases who form the main reservoir of infection, as they remain infective for long periods. An attack of gonorrhoea confers no immunity to subsequent re-infections and a person can often have repeated episodes caused by the same untreated partner ("ping-pong" gonorrhoea). Often even in cases where a correct diagnosis is made treatment may be inadequate and the patient may continue to be an asymptomatic carrier. The only sure way of ensuring that treatment has been adequate is to perform a laboratory test of cure.

The above emphasises the need for good laboratory support if the incidence of gonorrhoea is to be reduced.

It is the purpose of this paper to review recent trends in the laboratory diagnosis of gonorrhoea.

1. Direct smear examination.

Diagnosis of gonorrhoea in the male is relatively simple as perhaps 99% of those affected are symptomatic (Fiumara, 1972) and Gram staining of a direct smear from urethral discharge will show characteristic Gram negative intracellular diplococci. This constitutes sufficient basis for a diagnosis of gonorrhoea in the male (U.S. Dept. of Health, Education and Welfare 1970).

In the male there is normally no commensal gram negative diplococci in the genito-urinary tract.

Direct smear examination the male in 99% sensitive and specific in cases of acute gonorrhoea.

Direct smear examination however has a different significance in the female. 75% of those affected may be asymptomatic. While direct smear examination may have some value in the diagnosis of acute cases in the female it is about 30 – 40% less sensitive than culture (Caldwell et al 1971). Furthermore, as the female genital tract may have commensal *Neisseria,* the possibility of false positives cannot be ruled out.

It can thus be seen that while gram stained direct smears are useful in the diagnosis of gonorrhoea in most male patients it is of questionable value in females. For them diagnosis depends on the demonstration of *Neisseria gonorrhoeae* by culture methods.

2. Culture of Neisseria gonorrhoeae

Culture of *N. gonorrhoeae* is essential for diagnosis of gonorrhoea in females, in males where direct smear examination has been negative and as a test of cure for both males and females.

N. gonorrhoeae is a fastidious organism needing special growth requirements for in-vitro culture. Difficulties experienced in culturing may be due to sensitivity to inhibition rather than complexity of nutritional requirements (Reyn 1965). Another difficulty encountered is the inability of the gonococcus to withstand the delay of transporting specimens from the doctor to the laboratory. A suitable transport medium has therefore to be used in situations where direct culture is not possible.

A culture medium suitable for the isolation of gonococci from clinical specimens should not only be enriching to the gonococcus but selective as well. Unless their growths is checked, the commensal organisms normally found in the female genital tract will overgrow the gonococcus. This is also true for sites such as the rectum and the pharynx in cases of suspected infections of these regions.

In 1964 Thayer and Martin introduced a selective medium (Thayer and Martin, 1964) which they modified in 1966 (Thayer and Martin, 1966). This medium consists essentially of a conventional chocolate agar base to which is added enrichment such as "Supplement B" or "isovitalex" and three antibiotics, Vancomycin, Colistin and Nystatin. This combination makes the medium highly useful for the primary isolation of gonococci from conspicuously contaminated sites. The Vancomycin inhibits the Gram positive contaminants, Colistin the Gram negative contaminants and Nystatin the yeasts. Thayer – Martin medium therefore achieves the objective of allowing the growth of N. gonorrhoeae and N. *meningitidis* while suppressing the growth of contaminants including commensal *Neisseria.*

For subculturing and for isolation of gonococcus from sites which are normally sterile conventional chocolate agar medium would suffice.

Method of collecting specimens and inoculation of culture media:–

In men specimens of urethral discharge are obtained using a sterile bacteriological loop. In females cervical cultures are obtained using an ordinary cotton tipped swab which is introduced into the cervix after the cervical plug is removed. In females an additional specimen taken from the anal canal will be useful as some of them may only have rectal involvement (Schmale et al, 1969).

To inoculate the culture plate, the swab or loop is rolled directly to the prewarmed medium in a large "Z" pattern as soon as it is taken. The plate is then cross streaked immediately with a sterile wire loop. If this is not possible in the clinic, cross-streaking may be done on receipt of the specimen at the laboratory. Specimens after collection and inoculation are placed as soon as possible into a candle jar to provide a carbon dioxide atmosphere.

At improvised candle jar would simply consist of a tin with a tight fitting lid. After plates are placed in the tin, a candle is lit and the cover replaced. Consumption of oxygen within the tin by the lighted candle will provide the necessary carbon dioxide.

Incubation of the plates at 35.0 to 36.0°C is begun as soon as they reach the laboratory which should be on the same day.

In situations where specimens cannot reach the laboratory on the same day the above method is not suitable and transport media will have to be used.

The most widely used transport medium in Malaysia at the moment is Stuart's medium (Stuart et al, 1954) which is a non-nutrient, non toxic, holding medium which is designed to eleminate oxidation as a cause of death of the gonococcus. The usefulness of this medium is somewhat restricted for transport periods longer than 24 hours.

In 1971 the "Transgrow" medium was intro-

duced (Martin & Lester, 1971) and has been found to satisfy all the requirements of a good transport medium. Transgrow medium represents a further evolution of the Thayer – Martin medium differing from it in that it is contained in a flat screw capped bottle in which a controlled atmosphere of 10 per cent carbon dioxide is obtained by gassing, an increased amount of agar to lend rigidity to the medium and an increased percentage of dextrose. In transgrow, the transport and culture medium are one and the same and survival of gonococci occur even after 48 – 96 hours at ambient temperatures.

The transgrow medium is inoculated directly from the patient, incubated at 34-37°C at the clinic for 16-18 hours after which it is mailed to the laboratory for further processing.

Identification of N. gonorrhoeae

An oxidase test is carried out on colonies on both Thayer-Martin and Transgrow media. Oxidase positive colonies are gram stained and a preliminary report can be given based on oxidase reaction and characteristic gram staining.

For confirmation, fermentation tests are carried out on glucose, maltose and sucrose. Gonococci ferment only glucose and can thus be differentiated from the meningococcus and from commensal *Neisseria.*

The direct fluorescent antibody technique may also be used to confirm strains isolated in the laboratory as N. gonorrhoeae. This technique however is not recommended for the identification of *N. gonorrhoeae* on smears from clinical specimens although it has been used by some workers for this purpose because it lacks the necessary sensitivity (Shroeter and Lucas, 1972).

3. Test of cure

A test of cure is essential to ensure that treatment has been successful. This is particularly important in the case of females because remission of clinical symptoms is not synonymous with total eradication of the gonococcus.

The only test of cure is to culture specimens from infected sites. To preclude the possibilities of re-infection this is usually done at 7 and 14 days after treatment is completed (Shroeter & Lucas, 1972).

In males a urethral specimen would suffice. However in females both cervical and rectal cultures should be taken because 60% of all infected females have gonococcal proctitis and 30% of all treatment failures occur at the rectal site (Shroeter & Reynolds 1972).

4. Antibiotic Sensitivity Testing

With the current increase in strains of gonococci resistant to many antibiotics, antibiotic sensitivity testing may be of use in the treatment and control of gonorrhoea.

This will also assist in elucidating the cause of treatment failure. A suitable laboratory method would be to determine the minimal inhibitory concentration of the relevant antibiotics against the strain in question. This may be achieved using tubes of enriched chocolate agar medium containing several concentrations of the antibiotic to be tested (Djuanda & Warsa 1973).

5. Serological Tests

Tests to detect serum antibody levels against the gonococcus have not been very rewarding in the diagnosis of gonorrhoea. In acute cases, antibody is rarely present, and although it may be of some value in chronic infections, its value is diminished by the lack of specificity and the fact that antibodies tend to persist for many years in spite of clinical cure (Reyn, 1965).

SUMMARY

The laboratory has a useful role to play in the diagnosis and control of gonorrhoea.

In males majority of cases may be diagnosed on direct smear examination alone but in the case of females culture on Thayer-Martin medium and subsequent identification of colonies is essential.

A test of cure by culture should be done on both males and females after therapy. In the case of females both cervical and rectal cultures should be taken.

In situations where a laboratory is not at hand a suitable transport medium like Transgrow should be used. If this is not available Stuart's medium may have to be used although it is less efficient.

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REFERENCES

CALDWELL J.C. etl al, (1971) – Sensitivity and reproducibility of Thayer-Martin culture medium in diagnosing gonorrhoea in women. Am. J. Obstet. Gynaec. 109: 463-468.

DJUANDA S. & WARSA R. (1973) – M.I.C.'s for some semisynthetic penicillins and penicillin G against gonococcal strains. Asian J. of Med. 9 : 12-14.

FIUMARA N. J. (1972) – The diagnosis and treatment of gonorrhoea. Med. Clin. N. Amer. 56: No. 5, 1105-1113.

MARTIN J. E. & LESTER A. (1971) – Transgrow, a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis.* HSMHA Hlth. Rep. 86: No. 1, 790.

REYN A. (1965) – Laboratory Diagnosis of gonorrhoea infections. Bull. Wld. Hlth. Org. 32: 449-469.

SCHMALE J.D. et al (1969) – Observations on the culture diagnosis of gonorrhoea in women. J. Amer. Med. Ass. 210: 868.

SHROETER A.L. & REYNOLDS G. (1972) – The rectal culture as a test of cure of gonorrhoea in the females. J. Inf. Dis. 125: 5, 499.

SHROETER A.L. & LUCAS J.B. (1972) – Gonorrhoea – Diagnosis and treatment. Obstet. & Gynae. 39: 2,274.

STUART R.D. et al (1954) – The problem of transport of specimens for culture of gonococci. Canad. J. Pub. Hlth. 45: 73-83.

THAYER J.D. & MARTIN J.E. (1964) – A selective medium for the cultivation of Neisseria gonorrhoeae and Neisseria meningitidis. Publ Hlth. Rep. 79: 49-57.

THAYER J.D. & MARTIN J.E. (1966). – Improved medium selective for cultivation of *N. gonorrhoeae* and *N. meningitidis,* Pub. Hlth. Rep. 81: 6, 529.

TOW S. Y. (1964) – A study in the venereal disease statistics of the Federation of Malaya. (Unpublished data).

U.S. Dept. of Hlth., Education and Welfare (1970) – Criteria and techniques for the diagnosis of gonorrhoea. National Communicable Disease Centre, Atlanta Ca.