THE UNIVERSITY OF MANITOBA

"SEROLOGICAL STUDIES IN GONOCOCCAL INFECTION"

BY

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ABSTRACT

"SEROLOGICAL STUDIES IN GONOCOCCAL INFECTION"

The value of serological tests in the diagnosis of gonococcal infection was evaluated in 857 patients attending the Obstetric and Gynaecology Clinic at the Winnipeg General Hospital.

A comparison was made of three serological procedures for the diagnosis of gonorrhea: the Microflocculation technique (MFT), the Indirect Fluorescent Antibody technique (IFAT), and the Complement-Fixation technique (CFT). In addition, the cervix, urethra, vagina and in 204 patients the rectum were cultured for gonococci.

Of 106 patients with culturally proven gonococcal infection, sera from 80 (75.4%) reacted in MFT; 74 (69.8%) with IFAT;and 33 (31.1%) with CFT. Control sera from 31 children and adults presumed free of gonococcal infection were non-reactive in MFT and IFAT while one showed reactivity with CFT. Of sera from 55 proven meningococcal carriers, only 4 showed reactivity in MFT; 7 in IFAT and 8 in CFT. Sera from patients with gonococcal arthritis and Neisseria lactamicus were also tested.

The results of this study establish the capability of the MFT to detect gonococcal infection in the asymptomatic female. In this study, it appeared to be superior to the IFAT and CFT. Its sensitivity specificity, and simplicity make the MFT a useful screening test for large out-patient clinics. Further evaluation of its usefulness seems warranted.

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I. INTRODUCTION

Public Health authorities now admit (Guthe, 1961; Brown, 1970; Wilcox, 1961) that despite modern diagnostic methods and improved chemotherapeutics, a pandemic of gonorrhea is occurring throughout the world. Statistics show that while the incidence of syphilis and other venereal infections in many countries declined during the last decade, gonorrhea is increasing. Programmes concerned with the control of gonorrhea have depended largely on epidemiological methods and the effectiveness of penicillin. Recently, venereologists have indicated the need for more basic research as an aid in the control and eradication of this disease.

The World Health Organization Expert Committee on Gonococcal Infections (1963) reported that of the 111 countries studied, 53 experienced a significant rise in the incidence of gonorrhea. It is estimated that about 150 million cases of gonorrhea occur annually throughout the world (Toshach, 1970).

In the United States, the number of reported cases of gonorrhea increased from 270,000 in 1963 to 431,000 in 1968. (NCDC. VD Fact Sheet, 1968). Perhaps the true number of cases is as high as 1,700,000. (Brown, 1970) In Great Britain, an increase of 11% was noted from 1966 to 1967, and 7% from 1967 to 1968. (Ven. Dis. Ann. Rpt. CMO, 1969).

With the introduction of fluorescent antibody identification by Deacon et al (1959) and selective culture media by Thayer et al (1964), the diagnosis of gonococcal infection improved. However, these developments in laboratory diagnosis have failed to control gonorrhea. In remarks made to the World Health Organization, Dr.

Thorstein Guthe said that "no method as yet has been found of catching up with this heel-less Achilles." (Conger, 1964).

A number of reasons are cited as factors contributing to the dismal failure to control gonorrhea: the short incubation period of the disease, the emergence of antibiotic resistant strains, increasing sexual promiscuity, and underreporting of cases by many physicians with inadequate epidemiological investigations. However, the major deterrent to the successful control of gonorrhea is the asymptomatic untreated females (Deacon, et al, 1960; Harris et al, 1961; Brown et al, 1962), the reservoir of the disease who perpetuates infection in the population. Recently, it has also been noted that males (Moore et al, 1963; Pariser et al, 1964) with gonococcal infection can be asymptomatic. Clinically and epidemiologically, transmission by an asymptomatic male seems probable. (Thatcher, McCraney, Kellogg and Whaley, 1969).

Fig. 1, derived from the official statistical data compiled by the Epidemiology Division (Ven. Dis. Can. Ann. Rpt., 1970) Department of National Health and Welfare in Ottawa shows the incidence of gonorrhea in Canada between 1940 and 1970. The steadily increasing number of reported cases of gonorrhea shows a marked increase in 1970 to over 31,500. The rise has been particularly marked among the age group 20-39 years.

Of the ten provinces, Manitoba ranks high in the incidence of gonococcal infection. Fig. 2, depicts the number of cases during the past 25 years. A disturbing aspect of this is the presence of large

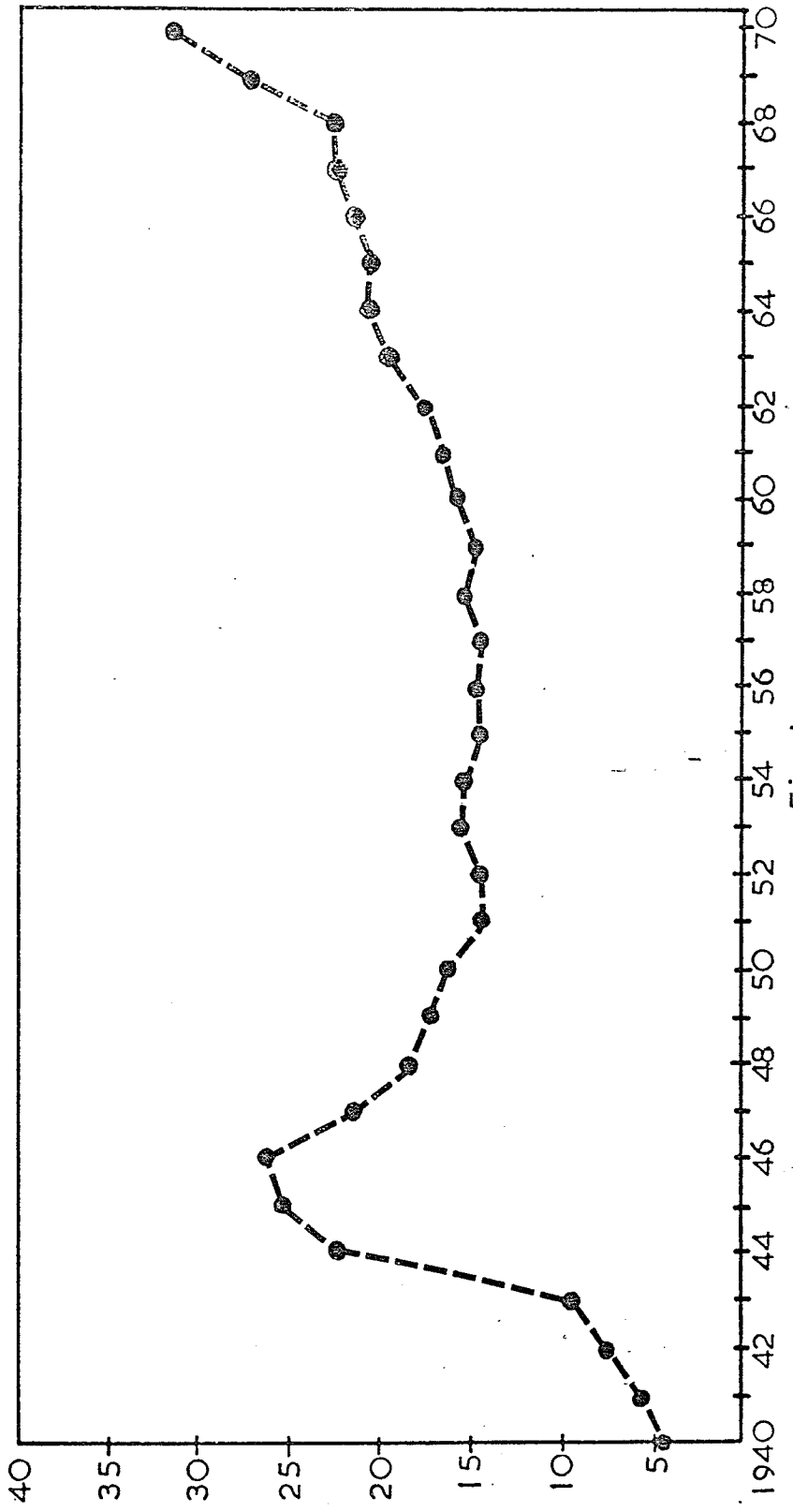


Fig. 1

CASES OF GONORRHEA IN CANADA
1940 – 1970

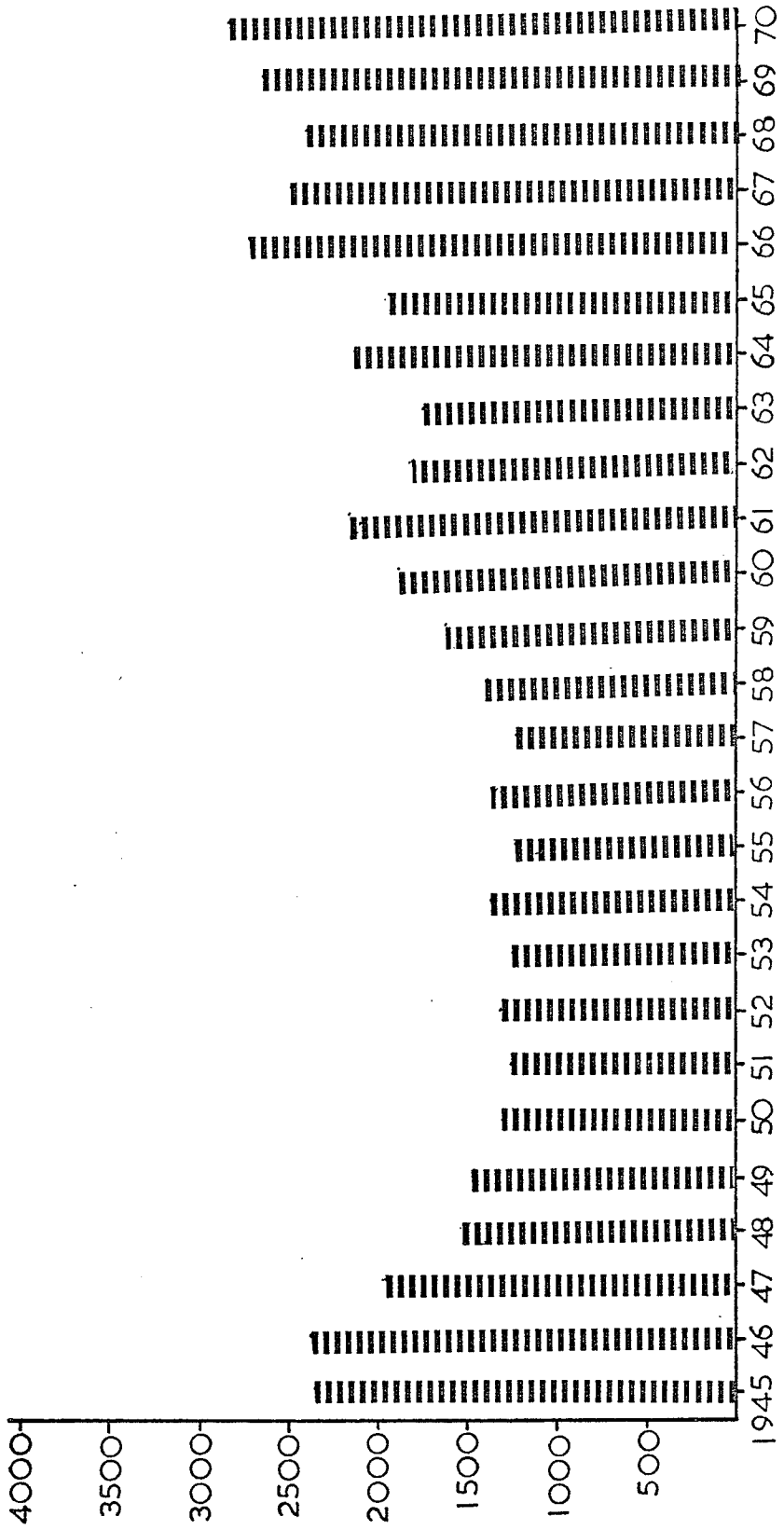


Fig. 2

REPORTED CASES OF GONORRHEA IN MANITOBA
1945 – 1970

numbers of asymptomatic females noted in a survey by Waters and Roulston (1969). Seventy-five percent of women with gonorrhea were asymptomatic.

The epidemiological virulence of gonorrhea is greater than other venereal infections. With the presently existing limitations in the cultural diagnosis of the gonococcus as a diagnostic criterion, the disease spreads faster than it is discovered. Therefore, if prevention and control of the disease is to be achieved, systematic serologic surveys of certain population groups at particular risk of infection may help locate the reservoir of the disease.

Amies and Garabedian (1967) forecast that public health and clinical laboratories will be called upon in the future to play an important part in the diagnosis and control of gonorrhea. Toshach (1970) stated that while "the role the laboratory plays is a case finding and contact tracing", she conceded that "it is an essential role since only with fast and accurate diagnosis in the laboratory can the rest of the team do its work".

Investigators at the Center for Disease Control, Atlanta, Georgia, are developing serological tests that may be able to discover asymptomatic female carriers.

A. STATEMENT OF THE PROBLEM

The problems investigated in the present study were three-fold:

First, to assess the sensitivity and specificity of three serological tests, the Microflocculation Technique (MFT), the Complement Fixation Technique (CFT), and the Indirect Fluorescent Antibody

Technique IFAT) using different antigens for the laboratory diagnosis of gonorrhea;

Second, to find a serologic screening procedure sensitive enough to detect most infected patients - a test which would be rapid, sensitive, and specific;

Third, to determine the specificity of the test in patients colonized or infected with Neisseria other than N. gonorrheae.

B. IMPORTANCE OF THE STUDY

Since asymptomatic gonorrhea has been and remains a continuing major public health problem, it is important that in Manitoba as elsewhere an efficient laboratory tool be sought for serologic detection of gonococcal infection.

Presently, Manitoba has no serological test in use for the diagnosis of gonococcal infection. The main objective of this study therefore is to find a suitable, rapid and simple serological test, readily applicable for wide scale screening in large populations and especially a test that will detect the asymptomatic female carrier in populations where the prevalence of infection is low (e.g. among prenatal patients).

The large number of sera now screened at the Manitoba Provincial Laboratory for syphilis - estimated to be over 91,000 (Maniar, Personal Communication, 1971) yearly - could also be screened for gonorrhea. In this manner, many potentially high-risk candidates might be screened without cost and time needed to culture each patient.

II. REVIEW OF LITERATURE

A. CHEMICAL ANALYSIS OF NEISSERIA GONORRHOEAE

Only a limited amount of information is available about the chemical analysis of the gonococcus. According to Stokinger et al, (1944), there are two reasons for this lack of progress in biochemical studies: (1) gonococcal infection comparable to that in man cannot be reproduced experimentally, (2) the organism cannot be readily grown in sufficient quantity to allow chemical analysis. Results of his micro-chemical analysis of six different strains of gonococcus grown in Douglas's broth reveal the dry mass of N. gonorrhoeae consist of 5-9% carbohydrate, 5-7% nucleic acids, 10-14% lipids, and 60-65% protein. Volatile and non-volatile matter constituted an additional 13-18%. Comparative analysis of cultures grown for 3, 6, and 10 days showed that phosphorus containing constituents (chiefly nucleic acid and phospholipid) are reduced to one-half their maximal value between the third and sixth day. No such change was noted in the nitrogenous components, notably proteins, certain amino acids, nitrogenous lipids, and amino sugar. Two nucleoprotein fractions have been isolated and analyzed. Of chief importance was the characterization of a relatively insoluble liponucleo-protein containing approximately 25% bound lipid and minor lipid-free soluble nucleo-protein which constituted the major part of the gonococcal cell. The lipid recovered was highly complex. It has been separated into several crystalline substances of which have been identified as lecithin, cephalin and sphingomyelin.

A discovery made by Tauber and Garson (1959), that the endotoxin of the gonococcus was not protein but consisted of a specific-lipopoly-saccharide, is believed to be of some importance since it could lead to

the development of specific antigens for diagnostic skin tests and serological testing for N. gonorrhoeae.

B. ANTIGENIC ANALYSIS OF NEISSERIA GONORRHOEAE

The antigens of N. gonorrhoeae have been subjected to a great number of serological investigations since 1906. Much has been written on antigenic properties of N. gonorrhoeae but the numerous claims in the literature are as conflicting as they are numerous and the data must be interpreted with extreme caution. It remains uncertain from the results of serological studies to date whether the different strains of N. gonorrhoeae have type-specific antigens as well as common antigens.

For most of the earlier work, agglutination test were used. Elser and Huntoon (1909) described the methods employed and the difficulties encountered. They noted that the inagglutinability of strains of N. gonorrhoeae does not "signify a total loss of agglutinogenic properties." This was confirmed by Nicolle, Jouan, and Debains (1919) who reported that inagglutinable strains become agglutinable after acidulation and heating at 100°C for 15 minutes. Tulloch (1922) noted that strains not agglutinated by antiserum may still absorb agglutinin from that antiserum.

Hermanies (1921) was able to demonstrate among 85 strains "six distinct clear-cut immunologic types having very little relationship with one another." In contrast, Segawa (1932), investigating 64 strains, found no serologically distinct types by agglutinin absorption techniques. Casper (1937) reported that the carbohydrate fraction of

the gonococcus is type specific while Torrey (1940) considered the protein fraction of the organism as the type specific antigen constituent. Wilson and Miles (1946) noted that there are probably two main serological types of gonococcus with many intermediate forms.

Price (1933) and Reyn (1949) using complement-fixation technique found that N. gonorrhoeae possess both group and type specific antigen. Chanarin (1954) demonstrated two antigens in smooth phase gonococcal strains only one of which appears to be responsible for complement-fixing in the presence of immune serum. Wilson (1954) using agglutination methods on "smooth" strains of N. gonorrhoeae, demonstrated 8 antigens: A_1, B_1, B_2 (group antigens), C (possibly another group antigen), and D, E, F, G, (type specific antigens).

C. HISTORICAL REVIEW OF THE DEVELOPMENTS OF GONOCOCCAL SEROLOGY

Serological examinations can be used in two ways, either to determine specific antigen in an organism or to test serum for antibodies. Since 1906, various serological tests for the detection of gonococcal antibodies have been reported but none have been definitive due to lack of specificity and sensitivity.

Muller and Oppenheim (1906) were first to propose complement-fixation test to detect gonococcal disease. Bruck (1906) studied the presence of immune bodies in artificially prepared gonococcal serum. He was able to demonstrate that the injection of watery extracts of gonococci into rabbits resulted in the production of specific antibodies detectable by complement-fixation technique. Since that date, numerous attempts to increase the sensitivity and

specificity of the reaction have resulted in a variety of antigens: either killed gonococcal suspensions of single or multiple strains or soluble extracts produced by lysis of the organism with sodium hydroxide. In the past, an obstacle to the use of this serological technique was the difficulty encountered in the preparation of the antigen and the fastidious cultural requirements of the organism. It seemed necessary to employ numerous strains in preparing the antigen.

Schwartz and McNeil in 1910-1911 employed gonococcal antigen made from a simple suspension of an 18-24 hour culture of 10 strains in 0.85% saline solution preserved with lysol. This antigen did not cross-react with the antisera of any of the other pathogens tested but it gave strong cross-reaction with anti-meningococcus sera. It fixed complement with practically all sera from known positive cases of gonorrhea and seemed to give very satisfactory results.

In 1912, McNeil and Olmstead succeeded in preparing aqueous extract antigen filtered through a Berkefield filter that did not bind complement with anti-meningococcus sera and which in an experimental work was much more satisfactory than the previous antigen used.

In 1916, McNeil and Wilson developed a defatted antigen prepared by treating 18-24 hour culture of gonococci with alcohol and then ether, dried and suspended in 0.9% saline solution. This proved to be very stable antigen that could be heated to 80°C without lessening its specificity and antigenic properties. This antigen did not cross-react with anti-meningococcus sera.

In 1931, Barringer, Williams and McNeil used the McNeil and Wilson antigen for a study of gonorrhea in women. The patients studied had

clinical gonorrhea. They found out that the antigen in use gave positive results in most patients during the period of antibody formation. However, if the readings were taken within 45 minutes after the antigen controls had cleared, positive readings were obtained in many sera that should have given negative readings. Even with this precaution, some sera continued to give weekly positive readings long after clinical and bacteriologic findings seemed to indicate a cure.

In 1932, Price described an antigen which was a fine colloidal suspension obtained by treating a single gonococcal strain. This was prepared by precipitating the proteins with hydrochloric acid and resuspending the precipitate with sodium hydroxide at 37.5°C adjusted to a pH 7.5. Results revealed that of the 641 sera tested, 466 agreed and 175 gave conflicting results. Unfortunately, however, with regard to the antigen, the majority of the strains treated in this way yielded antigens which were too highly anti-complementary for use and might give false positive reactions. On the other hand, individual strains selected for their relatively low anti-complementary activity might lack increase of antigenic valence and give false negative results. Price found this test of particular value in the diagnosis of the chronic form of the disease and as a test for cure. He points out that negative results may be obtained in the first 21 days of the acute disease and when the infection is limited to the anterior part of the urethra in the male or the lower genital organs of the female.

Cohn, in 1936, reported another method of preparing antigen for CFT and subsequently, Bayne-Jones suggested the use of about 12 strains

of N. gonorrhoeae originally cultivated on Levinthal's cooked blood agar (now grown on the modified filtrated cooked blood agar). This is a simple autolysate of gonococcal strains and requires about three months to ripen.

Torrey (1940) compared four gonococcal antigens - (1) Price's Antigen (1935), (2) Cohn's Antigen (1936), (3) Krueger's type undenatured Antigen (1933), and (4) McNeil's Antigen (1932) - to determine their relative sensitivity and breadth of valence. These antigens are prepared according to different methods in reference to sensitivity, breadth of valence, and anti-complementary properties specifically to determine the differences among strains of gonococcus in reference to each of these qualities. As a result, Torrey found that if the strains are employed at random, best results cannot be expected and that strains suitable for one type of antigen may be entirely unsuited for another technique. Of the four methods for producing gonococcal antigen for the complement-fixation diagnostic test which have been compared, a "dissolved" antigen obtained by the Price method is recommended because of it's superior sensitivity, stability, and immediate availability.

Labzoffsky and Kelen (1961) reported a method of preparing gonococcal antigen for use in the complement-fixation test. This antigen is prepared by extracting Neisseria gonorrhoeae cells with pyridine and then exposing the washed sediment to ultrasonic treatment. The authors reported that their method is superior to that of Price in yielding highly potent genus specific and stable antigens which are free of anti-complementary properties and possess both a broader antigenic valence and a longer range of working antigenic power.

Magnusson and Kjellander (1965) re-examined the use of GCFT. They primarily sought to determine to what extent the GCFT was positive with either complicated or uncomplicated gonococcal infection and to evaluate the specificity of the GCFT. Using an antigen made from the suspension of 20 strains of gonococci killed by heating at 60°C for 30 minutes, 50% of infected women were positive but only 21% of men. No healthy blood donors were positive. On the basis of these results it was concluded that the "reaction has unjustly fallen into far too deep obscurity and that the GCFT can still retain a place as a diagnostic tool and still be useful."

Anderson and Brendish (1965) assessed the specificity of the CFT for gonococcal infection in 500 specimens submitted for serological testing from patients not suspected to have gonococcal disease. Four reactive sera were found: one submitted for a Wasserman test from a patient with no significant history and therefore considered a false positive; one from an ante-natal patient in whom gonococcal infection was later confirmed bacteriologically; and two specimens from patients with suspicious histories but no bacteriological confirmations.

Brown (1964) employed a salt extracted antigen and found only 35% reactivity among 164 with acute gonorrheal urethritis.

Reising et al, in 1969 used the CFT as one of several research tools to detect immune response against various antigens. Two antigen preparations were investigated: the soluble antigen "A" and a "Fraction 1" antigen. This technique is particularly valuable in comparing a variety of antigens since either soluble antigens may be used. The soluble antigen was reactive with 88.2% of the sera from infected

females and Fraction 1 was reactive with 71.6% of the sera. Of sera from infected males, only 27.6% reacted with soluble antigen and only 20.4% with Fraction 1. This study suggests that these antigens might be adaptable to the detection of human gonococcal antibody especially in the female.

In 1970, Lee and Schmale in a preliminary evaluation using "B" antigen in a flocculation procedure reported that 85% of women proven by culture to have gonococcal infection were found to be sero-reactive whereas 11% of the control women thought not to be infected were sero-reactive.

Reising and Kellogg in 1965 described a "microprecipitin test" using a phenol extract antigen obtained from N. gonorrhoeae which is suitable for detecting gonococcal antibody. Of 197 females named as contacts, 81 or 60% were serologically reactive. Of control groups presumed free of gonococcal infection, none were serologically reactive to this test. The authors, however, feel this is quite inadequate as a screening test. The test provided a relatively specific, though somewhat insensitive system for detecting gonococcal antibody in human sera. It may have some value as an epidemiological tool but it does not provide information concerning the presence or absence of infection in humans.

In 1969, Chacko and Nair employed a simple microprecipitin ring test using a lipopolysaccharide antigen precipitated with alcohol from the allantoic fluid of embryonated chick eggs in which N. gonorrhoeae has been grown to maintain virulence. Results of this test reveal an overall sensitivity of 88% in 126 cases of acute untreated gonorrhea

in males. Of 146 pregnant women who had no signs or symptoms of any venereal infection and were screened during ante-natal examinations, only 4% were reactive. The specificity of this test in non-gonococcal diseases appears to be satisfactory except for its apparent cross-reactivity with patients with meningococcal infection. Carriers of meningococci may also be reactive to this test and this aspect needs more study, as does the question whether the polysaccharide antigenic constituent of N. gonorrhoeae is species-specific or group-specific.

Wallace and co-workers (1970) reported a serological test for the assay of Neisseria antibody. Such a test, using bentonite particles sensitized with phenol-extracted and acetone-prepitated antigens from N. gonorrhoeae or N. meningitidis, proved to be sensitive and specific in titrations against species-specific immune rabbit sera.

Fluorescent antibody techniques are of value for the detection of many pathogenic micro-organisms and for determination of specific antibodies. The mechanisms of the fluorescent antibody test as presented by Coons et al, in 1941 and 1942 and Kaplan in 1950 appear simple and clear in theory. However, a great number of difficulties are encountered in practice. The fluorescent antibody test has also been proposed for the diagnosis of gonorrhea but many questions remain to be answered before this test can be recommended for widespread application. (Ovcinnikov, 1962).

Cohen (1967) found that natural antibodies reactive with virulent N. gonorrhoeae were contrasted with immune antibodies (those found in the sera of infected humans). An indirect fluorescent antibody (IFA)

procedure employing fluorescent antisera specific for IgG, IgM and IgA was used to identify the immunoglobulin class of antibodies reactive with the heat labile and heat stable antigens of virulent N. gonorrhoeae. In a similar study by Cohen et al, (1969), the authors reported that humans possess "natural" antibodies to the gonococcus in all three major classes of immunoglobulin: IgG, IgM, and IgA. The "natural" IgG antibodies in the sera of the normal subjects were more reactive with heat stable than with heat labile gonococcal antigens contrasting with the IgG antibodies in the sera of infected persons which were more reactive with heat labile surface antigens. Moreover, the immune IgG antibodies were more resistant to heating than were the "natural" antibodies.

Ovcinnikov's experience (1964) with immunofluorescent method in the diagnosis of gonorrhea indicates that it cannot be recommended as yet for wide practical use as there are a number unsolved problems. It is necessary to devise an improved method for obtaining highly specific sensitive serum. It is recommended to perform immunization with separate fractions of gonococci. As their antigenic composition varies, not one but several antisera should be used for identification i.e. polysaccharidal, proteinic and lipoidic.

Thomas and Mennie (1950) used the passive hemolysis test to demonstrate antibodies against several bacterial antigen including an erythrocyte sensitizing antigen from gonococci. They found antibodies against the gonococcal antigen in sera from 10 patients with gonorrhea but not in sera from patients with non-specific urethritis.

Chanarin (1954) prepared an extract from gonococci which sensitized sheep erythrocytes. Haemolysis occurred when the sensitized erythrocytes were mixed with rabbit-immune serum and complement. Results revealed that approximately 30% of patients with uncomplicated gonorrhoea had a positive haemolysis test. However, the applicability of the test in the diagnosis was not evaluated.

Maeland (1966) reported an erythrocyte sensitizing antigen which was extracted from gonococci. This test was convenient for the estimation of the antibodies against sensitized sheep erythrocytes. This haemolysis test afforded no advantage over the complement-fixation test in the diagnosis of gonococcal infection.

III. MATERIALS AND METHODS

A. CLINICAL MATERIAL

The clinical material employed in this study consists of the following:

1. Eight-hundred and fifty-seven patients routinely cultured for gonococcus in an ongoing screening program at the Obstetric and Gynaecology Outpatient Clinic of the Winnipeg General Hospital. Materials for smear and culture were obtained from whereever possible: the cervical canal, urethra, vagina or rectal area.

2. Control material for this study consisted of: Normal sera from 21 adults who are members of the laboratory staff and who did not have a history of previous gonococcal infection. Sera from 10 children whose ages range from five months to 12 years.

3. Fifty-five nasopharyngeal carriers of meningococci from 21 adults and 24 children. (8 of Group A; 33 of Group B; 7 of Group Y; and 7 of Nongroupable). In addition, there were two adults who are known carriers of Neisseria lactamicus.

Ten (10) ml of blood were collected from patients and donors and the sera were kept frozen at -20°C until tested.

B. METHODS

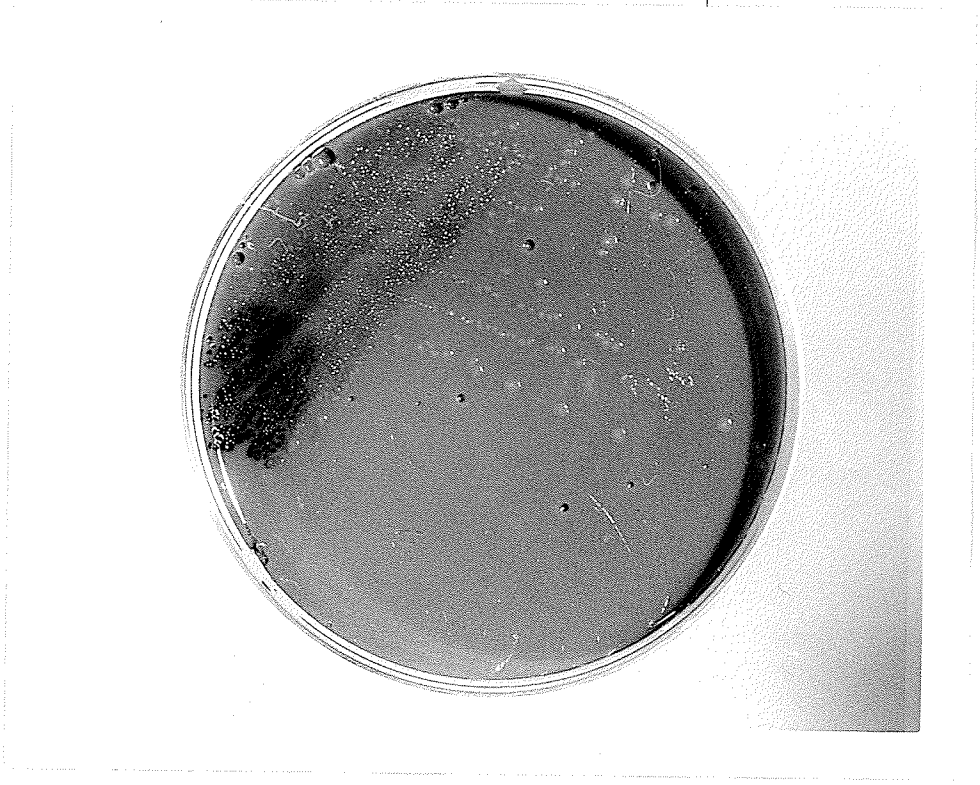
1. Selective media for N. gonorrhoeae. (a) In 1964, Thayer and Martin reported the development of a selective medium for the cultivation of gonococci and meningococci. Overgrowth by gram positive and gram negative bacteria is prevented by the addition of 10 micrograms of ristocetin and 25 units of polymyxin 8 per ml. of conventional diagnostic medium. (b). Transgrow medium which was recently introduced by the

Center for Disease Control in Atlanta, Georgia, is designed for the cultivation of the gonococci and is similar to the Thayer-Martin medium except with the addition of Isovitalex enrichment.

2. Bacterial cultures. Routinely, the specimens for culture were obtained by swab and streaked on Thayer-Martin and Trans-Grow Media. The latter medium was used in the early part of the study for comparative purposes only to determine which of the two media offers a better chance of isolating gonococcal organism. No differences were found in recovering the gonococcus, therefore, it was decided to abandon the use of the Trans-Grow medium.

3. Culture examination. After 18-24 hours incubation the plates were examined for typical oxidase positive colonies of the gonococci. (Thayer, 1968). When vancomycin-colisthin-nystatin (VCN) antibiotic were used, growth is usually apparent after 24 hours of incubation. Colonies of gonococci are usually greyish white, opaque, raised, finely granular, glistening and convex. Colony size may range from 0.5 mm to 4 mm in diameter. After 48 hours incubation, almost all strains were mucoid. The organisms were isolated and identified through fermentation tests using cysteine trypticase agar medium.

4. Oxidase test. N. gonorrhoeae produces an oxidizing enzyme which acts in the presence of air on certain aromatic amines to produce coloured compounds. Freshly prepared 1% solution of aqueous para-aminodimethylaniline monohydrochloride is poured on the plate (Fig. 3) to cover the surface of the colonies and then decanted. The colonies develop a purple colour (oxidase reaction). This method is especially useful in dealing with heavily contaiminated material containing only



(Figure 3)

Neisseria gonorrhoeae. Culture on Trans-grow media agar. Surface application of freshly prepared oxidase reagent (1% aqueous para-aminodimethylaniline monohydrochloride) to culture.

scanty gonococci. Colonies of non-pathogenic neisseria also give a positive oxidase reaction but the purple colour develops more slowly and is less intense.

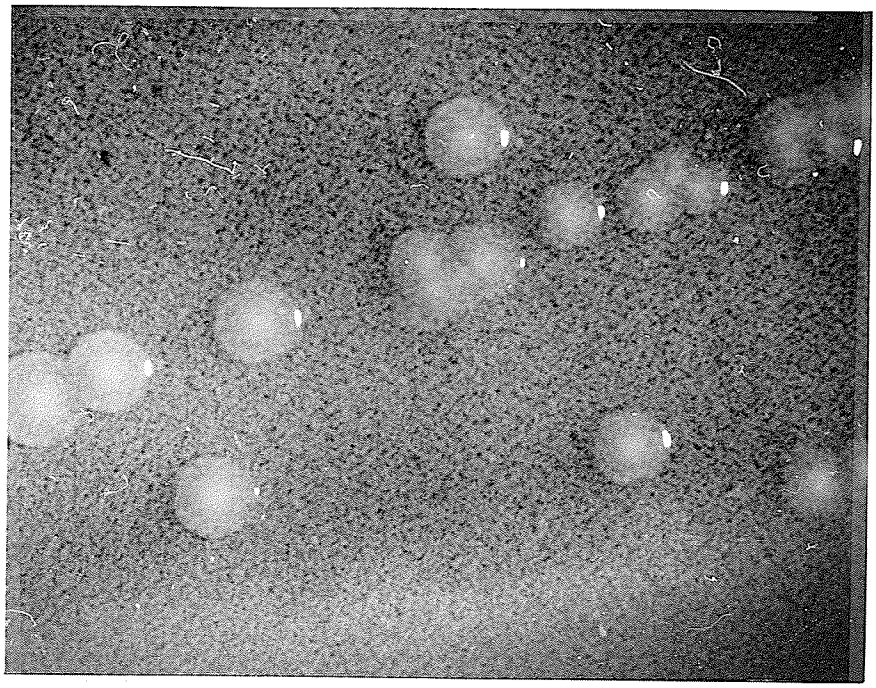
5. Antigen Preparation: Microflocculation Technique.

Virulent N. gonorrhoeae designated as F 62 Type 1 by Kellogg et al, 1963, was employed in this study (Fig. 4) because it seemed likely to reflect the antigenic stimulation responsible for antibody production in the human. The gonococcal serum was grown on G.C. medium base containing 1% modified supplement (cocarboxylase, glutamine, dextrose, and Ferric nitrate). Plates were incubated under increased carbon dioxide tension at 35°C for 20-24 hours.

Extract preparation: (Fig. 5) The cell crop was harvested into sterile, iced distilled water. The harvest was subjected to the action of Raytheon 250 watts KC Magnetostrictive Oscillator for one hour. The supernatant solution was separated from the sediment and lyphilized. (Reising, 1971)

Preparation of Antigen Suspension: 2 mg. of the dehydrated material was reconstituted in 1 ml of Sorensen's buffer pH 7.0 (Fig. 6). The reconstituted supernatant solution was transfered to a 30 ml. glass-stoppered flat bottom bottle. The bottle was tilted so that the antigen lay to one side and 0.025 ml. of cholesterol-lecithin mixture was blown to the extract. The bottle was stoppered and the mixture was shaken from the bottom to top and back approximately 30 times in 15 seconds. The mixture was allowed to stand 1 hour at room temperature. The antigen emulsion was then tested with control sera for the desired pattern.

Test procedure: 0.025 ml. of whole unheated sera were pipetted

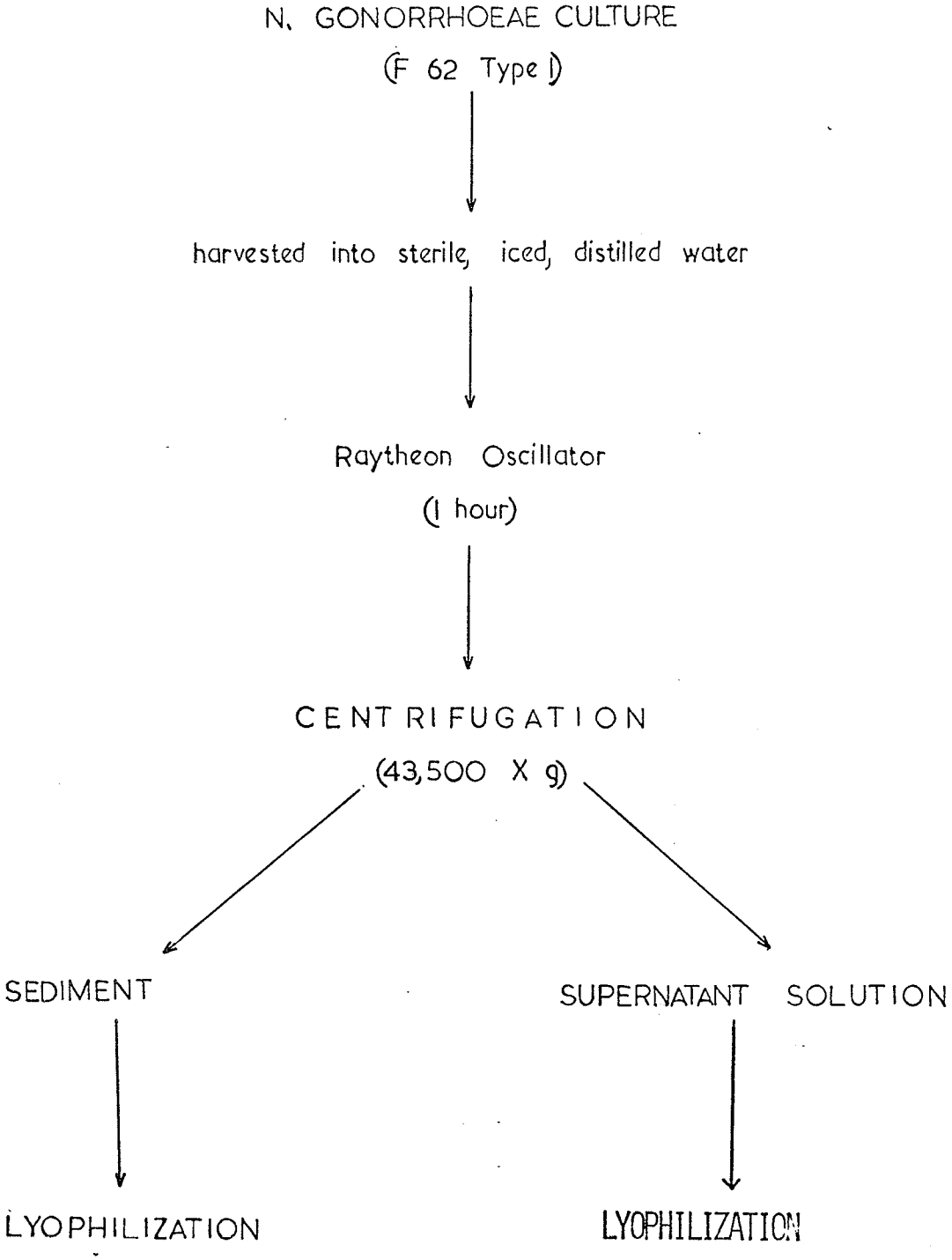


(Figure 4)

Neisseria gonorrhoeae (F 62 Type 1). Culture Thayer-Martin media agar. Colonies are round, convex, translucent, 0.5 mm. in diameter with glistening surfaces.

DIAGRAM OF BASIC PROCEDURES IN PREPARING GONOCOCCAL ANTIGEN FOR MICROFLOCCULATION TECHNIQUE

(Fig. 5)



PREPARATION OF ANTIGEN SUSPENSION
(Microflocculation Technique)

(Fig. 6)

2.mg dehydrated supernatant sol. F 62 TI

↓

reconstitute in 1ml. of Sorensen's buffer pH 7.0

↓

0.25 ml. of Cholesterol - Lecithin mixture

↓

stopper bottle and shake mixture from top to bottom and back for 30 times in 15 seconds

↓

allow mixture to stand 1 hour at room temp.

↓

TEST ANTIGEN EMULSION:

↓

Sera from diagnosed cases of gonorrhoeae – FLOCCULATION

Sera from presumed Normals – NO FLOCCULATION

into paraffin ringed glass slides (2x3 inches with paraffin rings approximately 14 mm in diameter). Then, 0.025 ml. of test antigen suspension were added to each well containing 0.025 ml. of serum. (Fig. 7). The slide was then rotated on the rotating machine at 140 rpm for 10 minutes. A 20x magnification was used to examine for evidence of agglutination.

Interpretation:

REACTIVE: - aggregation of particles into clumps of varying sizes. (Figs. 8 and 9).

NON-REACTIVE - no clumping or slight roughness. (Fig. 10).

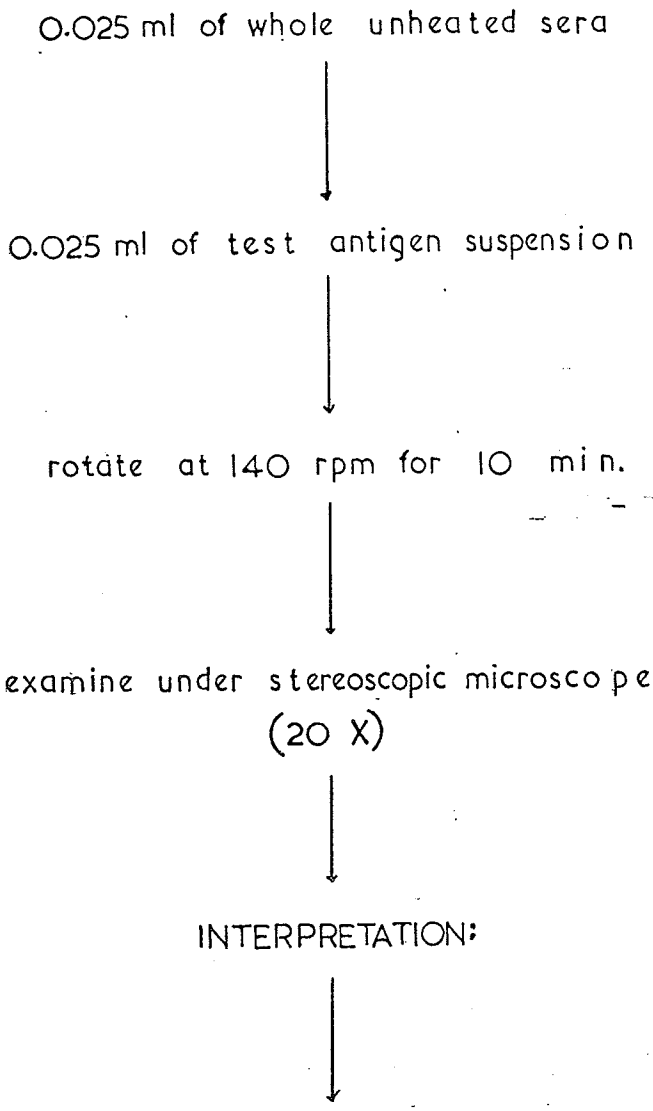
6. Antigen Preparation: Indirect Fluorescent Antibody Technique.

The organism used as an antigenic source for IFAT is an 18 hour culture of N. gonorrhoeae strain F 62 Type 1. G.C. medium enriched with defined supplement is used for isolation and cultivation of the organism incubated at 35°C under increased carbon dioxide tension. The individual colony was passaged by loop transfer, selected by morphological characteristics by means of cycloptic dissecting microscope with diffused, angled light transmitted from below up through transparent medium.

Of the four clone types of F 62, Type 1 was the most interesting since it was found only in the purulent exudate from acute gonorrhea of the male. Type 1 is characterized by glistening convexity, dark gold and small size. The other characteristic which is observed (Fig. 11) with combination of transmitted edge lighting is consistent with Type 1 colonies having a thinner surface film of reflective material than the other three types. After 18 hours incubation, the bacteria

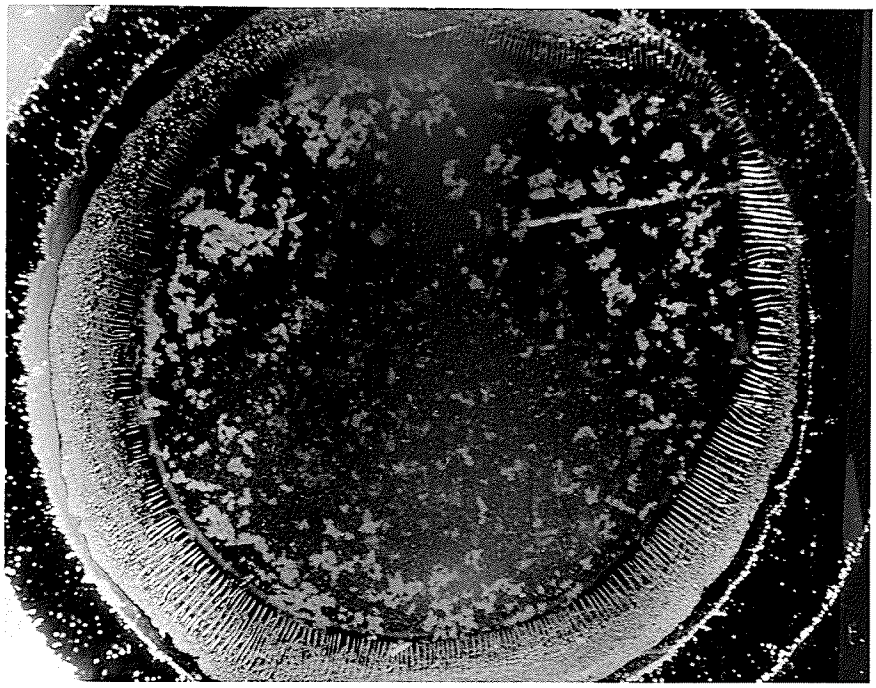
DIAGRAM OF TEST PROCEDURE FOR THE MICROFLOCCULATION TECHNIQUE

(Fig. 7)



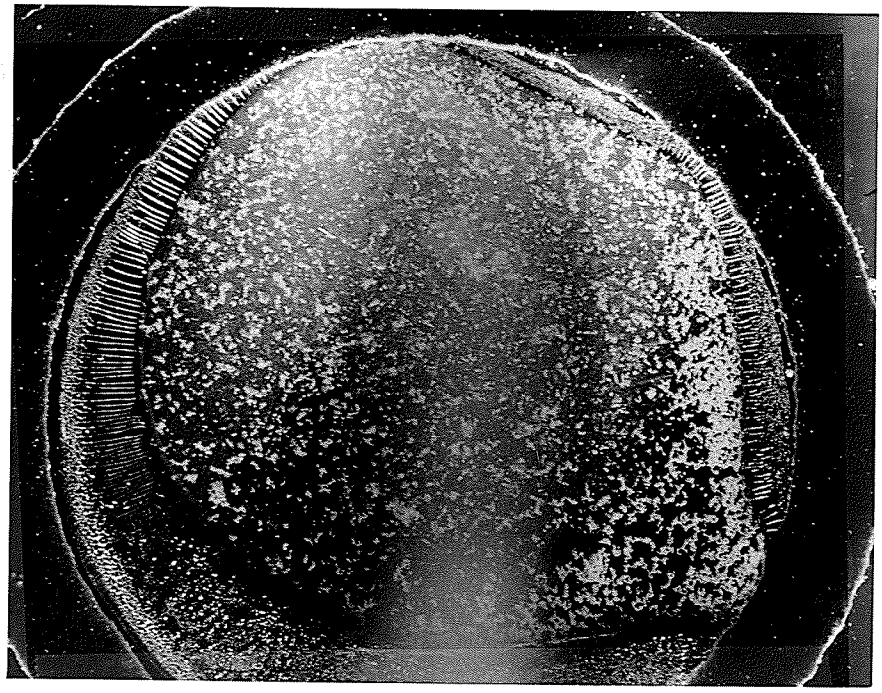
REACTIVE – aggregation of particle into clumps of varying sizes.

NON REACTIVE – no clumping or slight roughness.



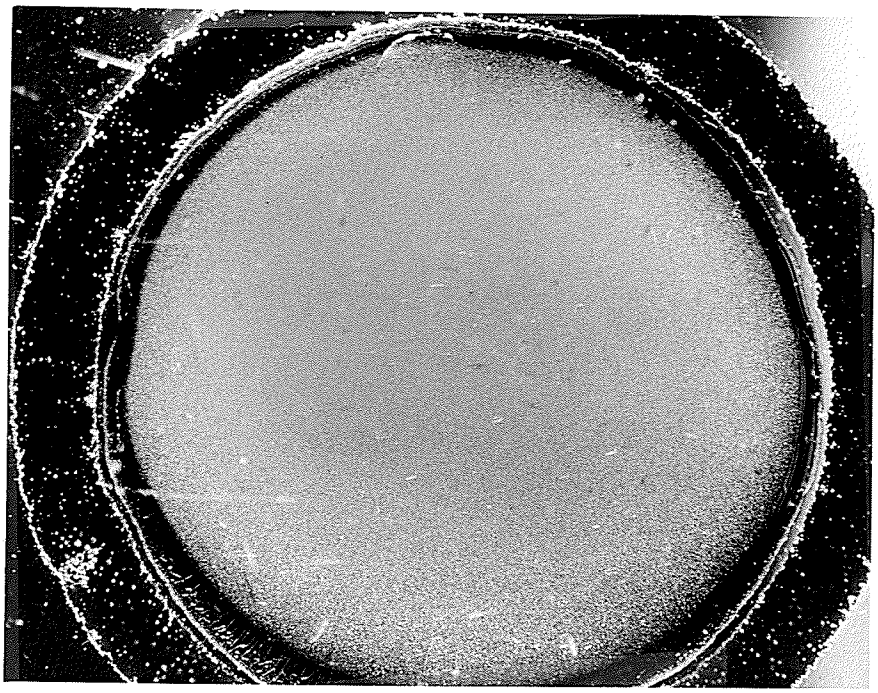
(Figure 8)

A strongly positive reaction by the Microflocculation technique.



(Figure 9)

A moderately positive reaction by the Microflocculation technique.



(Figure 10)

A Negative reaction by the Microflocculation technique.

were harvested in distilled water. The resulting bacterial suspension was adjusted to 50% light transmission by using a Beckman specthrophotometer and a 1:2 dilution of this suspension was used.

Test Procedure: Slides are prepared (Welch, in preparation) by placing a 3 mm. loopful of prepared antigen on the center of the slide. (Fig. 12). A 1:16 dilution of sera in PBS (pH 7.2) is prepared and 0.05 ml. of this serum is added to the antigen spot and incubated in a moist chamber for half an hour at 37°C. Using distilled water made alkaline pH 9.0 with 1 N NaOH, slides are rinsed and soaked for five minutes. Slides are blown dry with air pressure. A 1:200 dilution of conjugate is added to each antigen spot and incubated. Again, they are rinsed, soaked for five minutes in distilled watrer and dried with air pressure. Then slides are read using Leitz darkfield fluorescent microscope with Schott BG 12 exciter filter and OG-1 barrier filter. Control slides are read first. These include a saline, negative and positive control.

POSITIVE - stains individual separate cells brightly.

NEGATIVE - do not stain or stain very lightly.

7. Antigen Preparation: Complement-Fixation

The strain used in this antigen preparation were freshly isolated cultures submitted to the Public Health Laboratory in Toronto for bacteriological examination. The isolates were checked for purity as well as for possession of typical, morphological, cultural, and biochemical characteristics by repeated serial passages from single

DESCRIPTION OF COLONIAL MORPHOLOGY OF F 62 TYPE - 1 N. GONORRHOEAE USED AS ANTIGENIC SOURCE FOR INDIRECT FLOURESCENT ANTIBODY TECHNIQUE.

(Fig. 11)

TYPE 1 - F 62	–	OTHER TYPES (2, 3, 4)
SIZE – 1.5 mm	–	varies from 0.5 – 1 mm.
COLOR – dark gold	–	light brown to colorless
ELEVATION - convex	–	low convex
EDGES - entire	–	defined or crenated
OPACITY - translucent	–	transparent or translucent
STRUCTURE - amorphous	–	granular or amorphous

DIAGRAM OF TEST PROCEDURE FOR INDIRECT FLOURESCENT ANTIBODY TECHNIQUE

(Fig. 12)

3mm loopful of antigen
air dry

↓

0.05ml. of serum dilution (1 : 16 in PBS)
incubate 30 min at 37°C

↓

soak slides 5min in distilled water made alkaline to pH 9.0 with 1N NaOH

↓

rinse and blow dry with air pressure

↓

add 0.05ml of conjugate (1 : 200 dilution in PBS)
incubate 30 min at 37C dry with an air pressure

↓

read slides using a Leitz darkfield flourescent microscope with a Schott BG 12 exciter filter and an OG-1 barrier filter

↓

read control slides:

saline control
negative control
positive control

POSITIVE - stains brightly especially individual separate cells.
NEGATIVE - slides do not stain, or very lightly.

colonies, by microscopic examination of individual colonies and stained smears, and by the use of the oxidase reaction. The organisms (Fig. 13) were harvested into sterile distilled water containing 1% sodium azide using a bent glass rod. To remove agar lumps, the bacterial suspension was filtered through several layers of gauze, then washed three times in sterile distilled water containing 0.1% sodium azide at 200 RCF. The final suspension was adjusted to 200 mg per ml wet weight in distilled water and was passed through the RIBI Cell fractionator at 15,000 psi. The effluent from the pressure cell was not centrifuged; i.e. the final antigen preparation contained both protoplasm and cell walls. The antigen was allowed to stabilize for one month at $4^{o}C$.

Test Procedure:

a) Set up three tubes for each specimen; one for serum dilution; one for serum test; and one for the individual serum control.

b) In the serum dilution tube, make 1:4 dilution of the heat inactivated ($56^{o}C$ for 30 minutes) test serum to a final volume of 0.8 ml with saline and mix well.

c) Dispense 0.2 ml volumes of the diluted serum into both the serum test tube and the serum control tube.

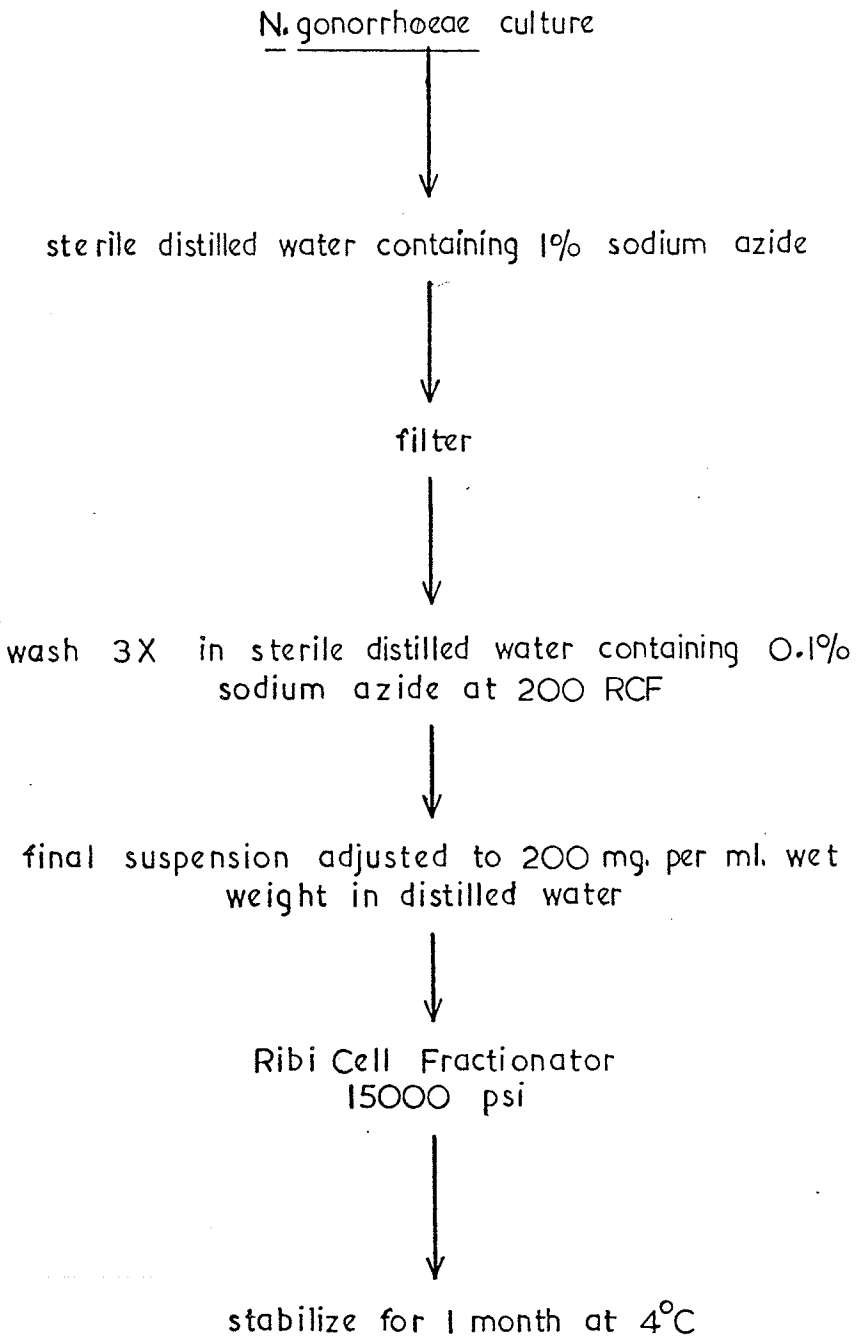
d) Add 0.2 ml of saline to the serum control tube.

e) Add 0.2 ml of the appropriate antigen to the serum test tube. (Shake antigen before dispensing).

f) Add 0.2 ml of complement (2 lytic units) to both tubes (Guinea Pig serum).

DIAGRAM OF BASIC PROCEDURES IN PREPARING GONOCOCCAL ANTIGEN FOR COMPLEMENT-FIXATION TECHNIQUE

(Fig. 13)



g) An antigen control tube is prepared containing 0.2 ml antigen, 0.2 ml saline, and 0.2 ml (2 units) complement.

h) Positive control serum should be diluted to a titre of 1:4 and 0.2 ml volumes of this serum dilution are used directly. Negative control serum is also diluted to 1:4 and used directly.

i) Shake and incubate the tubes including the positive, negative, and antigen controls at 37°C for 75 minutes.

j) Following incubation, add 0.4 ml of sensitized sheep red cells to all tubes.

k) Shake and incubate all the tubes at 37°C and read when serum controls hemolyse; not more than 30 minutes in any case.

The reaction was judged to be positive if 25% hemolysis and 75% fixation was noted. The results are presented in percent hemolysis for each serum.

IV. RESULTS

From May 1970 to June 1971, cultural examination and three serological tests were performed on sera collected from 1,121 women attending a Pre-Natal and Gynaecology Outpatient Clinic at the Winnipeg General Hospital. However, the results on 264 patients were excluded for either one or more of the following reasons: sera were not suitable for analysis as they were hemolyzed; insufficiency of serum specimens for the full range of test to be performed; anti-complementary properties of the serum; or the blood collected were not accompanied by cervical and urethral swabs for cultural verification. There remained therefore 857 sera (76%) of the total on which valid comparison could be made between the results of three serological tests and cultural examination. There were 106 cases categorized as having gonococcal disease.

A. Microflocculation Technique:

Table 1 depicts the data obtained with Microflocculation technique. It may be seen that of the 106 female patients shown to be infected with N.gonorrhoeae, 26 were non-reactive while 80 showed reactivity indicating a sensitivity of 75.4%.

Of the 751 female patients in which gonococcal infection was ruled out on first examination, 665 were non-reactive while 86 (11.4%) females who have no signs or symptoms of venereal infection showed reactivity. However, on further examination and investigation of clinical information of these patients, 40 (5%) gave a clinical history suggestive of various gynaecological disorders or histories of past gonococcal infection. Thus, of the 40 sero-reactive patients: nine patients had previous histories of gonococcal infection as revealed

SPECIMEN CATEGORY	NO. TESTED	NON REACTIVE	REACTIVE	PERCENTAGE REACTIVITY
CULTURE POSITIVE	106	26	80	75.4%
CULTURE NEGATIVE	751	665	86	11.4%

RESULTS OF MICROFLOCCULATION TECHNIQUE IN 857 FEMALE PATIENTS
(Table 1)

by records of the Venereal Disease Department of Manitoba; 3 were reactive with VDRL test for syphilis; 14 revealed gynaecological disorders, supported with historical evidence such as histories or urethritis, dysuria and vaginal discharge along with trichomonas infection; 8 patients eventually had gonococcus isolated on subsequent cultures; 2 revealed very recent gonococcal infection a week before this study was begun; 2 had previous abortions; 2 patients had gonococcus isolated while confined in the maternity ward for delivery.

The remaining 46 (6%) sero-reactive sera not supported by either cultural studies or historical evidence may be considered as biologic false positive reactors. Thus, by subtracting this from 11.4% obtained, the resulting percentage compared favourably with the study of Reising (1971) who reported a 5.4% reactivity in non-infected females and 79.0% sero-positivity in infected females.

Table 2. This table shows the results obtained from patients with gonococcal arthritis.

The MFT was carried out on sera of eight patients diagnosed with presumptive and definite acute gonococcal arthritis on the basis of clinical symptoms and cultural examination.

Of the eight sera tested, gonococcal antibodies were detected in all except one instance. Six of these gave significant strong positive reactions while one gave a weak positive reaction. Only one serum was found non-reactive where the culture was positive.

Unfortunately, in Case No. 4, cultural examination from the involved joints failed to confirm positivity. This was probably due to the fact

CASE NO.	CULTURE	REACTIVITY		
		MFT	IFAT	CFT
Case 1 P.W. 1	Positive	+	+	1:32
Case 2 W.P. 1113	Positive	+	+	–
Case 3 P.S. 261	Positive	+	+	1:16
Case 4 S.W. 242	Negative	+	–	1:32
Case 5 B.N. 1121	Positive	–	–	–
Case 6 K.F. 1115	Positive	+	–	–
Case 7 M.D. 248	Positive	+	+	1:8
Case 8 P.C. 1059	Positive	+	+	1:16

REACTIVITY OF THREE SEROLOGICAL PROCEDURES IN 8 DIAGNOSED CASES OF GONOCOCCAL ARTHRITIS

(Table 2)

that the patient was already started on therapy when cultural examinations were undertaken. This will support the claim of some investigators that 50% to 70% culture-failure rate has been the general experience in cases of gonococcal arthritis (Graber et al, 1960; Simpson et al, 1962). It is considered that when sera are found to give definite positive reactions with one or more tests further confirmation is unnecessary. In this particular case, it will be noted in this regard that if cultural methods were used alone, N. gonorrhoeae would have gone undetected.

With regards to Case No. 5 whose disease was not detected by MFT, the positive cultural report was obtained only after a series of cultural examination were done.

Although this test has been evaluated in sera with culturally and clinically diagnosed cases of uncomplicated gonorrhoea in women (Reising, 1971) and a favourable degree of reactivity has been suggested no data has previously been available on the pattern of reactivity of this test in cases of gonococcal arthritis. This data despite its small number is of value as the disease is not common.

In comparing MFT results with cultural methods, it appears that this method when properly applied has the advantage of an increase in sensitivity since joint cultures has yielded results only in about 25% of patients (Graber et al, 1960).

Table 3. This gives the data obtained when sera from fifty-five nasopharyngeal carriers of meningococci were tested by MFT. In addition, sera from two subjects carrying N. lactamicus in the pharynx were also tested.

SOURCE OF SPECIMEN	NO. TESTED	MFT		IFAT		CFT	
		NR	R	NR	R	NR	R
MENINGOCOCCAL CARRIERS Age 3 – 67 yrs.	55	51	4	48	7	47	8
GROUP A	8	8	0	8	0	6	2
GROUP B	33	32	1	31	2	31	2
GROUP Y	7	6	1	5	2	4	3
NON-GROUPABLE	7	5	2	4	3	6	1
NEISSERIA LACTAMICUS CARRIERS	2	2	0	2	0	2	0

REACTIVITY OF THE THREE SEROLOGICAL TESTS IN 57 NEISSERIA CARRIERS OTHER THAN NEISSERIA GONORRHOEAE

(Table 3)

In the sero-grouping of 55 meningococcal carriers; 8 were classified as Group A; 33 as Group B; 7 as Group Y; and 7 non-groupable. From these groups, 35 were adults and 20 were children.

Serological investigation of these sera by MFT reveals that none reacted with Group A; one reacted with Group B; one reacted with Group Y and two were reactive with the non-groupable. It is important to note that with the four reactive sera, all were adults. None of the sera from children were reactive.

With regards to the sera of carriers of N. lactamicus, no reactivity was observed in both cases.

Table 4. This table shows the results obtained from sera of individuals who were designated as "normal" on the basis of no history of previous infection with N. gonorrhoeae.

Results of this test reveal that in all apparently healthy members of the laboratory staff (12 females and 9 males) the MFT was non-reactive.

Similarly, in a group of 10 children (6 females and 4 males) aged from five months to 12 years presumed free of gonococcal infection, none were found to be reactive.

B. Indirect Fluorescent Antibody Technique:

Table 5 depicts the data obtained with IFAT.

In the group of 106 female patients with gonococcal infection, 32 were non-reactive while 74 showed reactivity indicating a sensitivity of 69.8%.

SEROLOGICAL PROCEDURE	SOURCE OF SPECIMEN	NO. TESTED	NON REACTIVE	REACTIVE
MFT	1. Laboratory Staff			
	Females	12	12	0
	Males	9	9	0
	2. Children			
	Females	6	6	0
	Males	4	4	0
IFAT	1. Laboratory Staff			
	Females	12	12	0
	Males	9	9	0
	2. Children			
	Females	6	6	0
	Males	4	4	0
CFT	1. Laboratory Staff			
	Females	12	11	1
	Males	9	9	0
	2. Children			
	Females	6	6	0
	Males	4	4	0

RESULTS OF THREE SEROLOGICAL TECHNIQUES IN APPARENTLY NORMAL SUBJECTS IN WHICH NO CULTURAL STUDIES WERE DONE

(Table 4)

SPECIMEN CATEGORY	NO. TESTED	NON REACTIVE	REACTIVE	PERCENTAGE REACTIVITY
CULTURE POSITIVE	106	32	74	69.8 %
CULTURE NEGATIVE	751	620	131	17.4 %

RESULTS OF INDIRECT FLUORESCENT ANTIBODY TECHNIQUE IN 857 FEMALE PATIENTS

(Table 5)

Of the 751 female patients that were non-infected, 620 were non-reactive while 131 (17.4%) showed reactivity. It will be noted here that the incidence of false positive reactors is higher than with MFT.

In the group of 8 patients diagnosed with gonococcal arthritis, it may be seen (Table 2) that sera of 5 tested by this technique gave a 3+ fluorescent staining at a dilution of 1:200.

In the sera of 55 nasopharyngeal carriers of meningococci, Table 3 shows that none of the eight sera from Group A reacted, however, 2 of Group B; 2 of Group Y; and 3 of the Nongroupable were reactive. With the sera from carriers of N. lactamicus, no reaction was observed.

Sera from all healthy blood donors were found to be non-reactive with this test. (Table 4).

C. Complement-Fixation Test:

Table 6 presents the results obtained with Complement-Fixation Technique (CFT).

Of the 106 sera from culturally positive patients, this test has shown a high non-reactivity of 73 (31.1%). Of the 33 sera which showed reactivity, 17 yielded a titer of 1:4; 10 a titer of 1:8; five a titer of 1:16; and one a titer of 1:32.

Of the 751 sera from culturally negative patients, 672 were non-reactive and 79 (10.5%) were reactive. Of these 79 reactive sera, 43 yielded a titer of 1:4; 22 a titer of 1:8; 12 a titer of 1:16; and two a titer of 1:32.

SPECIMEN CATEGORY	NO. TESTED	NON REACTIVE	REACTIVE	PERCENTAGE REACTIVITY
CULTURE POSITIVE	106	73	33	31.1%
CULTURE NEGATIVE	751	672	79	10.5%

RESULTS OF COMPLEMENT – FIXATION TECHNIQUE IN 857 FEMALE PATIENTS
(Table 6)

With regards to the patients with gonococcal arthritis, sera of five of the eight patients gave significant reactions; two at a titer of 1:16; and one a titer of 1:8. Three sera gave negative reactions.

Of the sera from 55 meningococcal carriers, two were reactive with Group A, (both yielding a titer of 1:4), two were reactive with Group B, (both yielding a titer of 1:4), three were reactive with Group Y, (one a titer of 1:8, and two a titer of 1:4), and one was reactive with the Nongroupable yielding a titer of 1:8.

No reactivity was noted on sera of carriers of N. lactamicus.

In testing sera with the group of presumed "normals", (Table 4) only one serum from the group of adults showed a weak reaction yielding a titer of 1:4. One serum was anti-complementary, therefore inconclusive.

Statistically, no difference in specificity could be found between the CFT (10.5%) and the MFT (11.4%) though the CFT demonstrated slightly less positive sera from the presumed non-infected group. The CFT was found to be the least sensitive (31.1%) of the three tests.

The IFAT shows a reasonable ratio of sensitivity (69.8%) but has the highest degree of non-specificity (17.4%) among the three methods used. The MFT appears to be considerably more sensitive (75.4%) than the two other tests and demonstrates an acceptable level of non-specificity (11.4%).

Table 7. Comparing Clinical Diagnosis with Serological Data.

In cases 2, 3, 4, 6 and 7, the MFT results would appear valid.

The IFAT technique appear valid only in Cases 2, 6 and 7, whereas the CFT appear valid only in two cases which are 6 and 7.

In comparing Case 1 and 5, it is important to note that the case 1 suggests a mere "immunologic status" without active infection (it obviously relates to past infection) whereas the Case 5 suggests that infection has not yet initiated a detectable immunological response or that the duration of infection is too short for the antibodies to be produced. Reports claim that two weeks or even less after gonococcal infection occurs, antibodies may develop (Chacko and Nair, 1967). But this case being a male, one probable reason for the absence of detectable antibodies is the non-absorption of the antigen due to the limitation of infection at the surface of the urethra. Therefore, it is not surprising that negative reactions could be found in cases with anterior urethral infection. Also, it is important to bear in mind the possibilities of wide discrepancy in the serological detection between the males and females. For if the case would have happened to a female, and should the antigen be asorbed because the gonococcus spread or penetrates into the deeper layers of the mucous membranes which have a greater blood and lymph supply, antibodies would have been produced.

Most important of all in cases 2, 3, and 4, the MFT strongly suggests its worthiness in tracking asymptomatic females who are the reservoir of the disease.

CASE REPORTS

(Table 7)

		Culture	MFT	IFAT	CFT
CASE 1:	A twenty yr. old female with a history of treated gonorrhea and syphilis in 1967. Admitted to the hospital in August 1970 for threatened abortion. Both MFT and IFAT were reactive but the CFT was negative. Cervical, urethral and rectal cultures were negative for gonococci.	-	+	+	-
CASE 2:	A twenty-seven yr. old female with acute urethritis, and pustular rash. Denied previous history of gonorrhea. Gonococci were isolated from pustular lesions, urine and the vagina. One blood culture grew gonococci.	+	+	+	-
CASE 3:	A sixty-nine yr. old female with no historical evidence of gonococcal infection. She appeared at the clinic for a physical examination. Urine, urethral and cervical cultures were taken but only the urethra grew G.C.	+	+	-	-
CASE 4:	Female with complaints of bleeding on voiding. Cervical culture was positive for G.C. MFT-reactive; IFA and CFT non-reactive.	+	+	-	-

		Culture	MFT	IFA	CFT
CASE 5:	Male (husband of the Case No.4) has contact with wife who was found to be asymptomatic 3 weeks before gonococcal symptoms appeared. Denies history of gonorrhea until April 17, 1971 he developed pain in left testis and swelling. Gonococci were later isolated in culture but serologic test negative.	-	-	-	-
CASE 6:	A 19 yr. old male with urethral discharge for two weeks since September 8th, 1970. Gonorrhoeae was isolated from urethral culture. Gonococcal serologic tests - MFT, IFA were reactive and CFT titer of 1:16.	+	+	+	+
CASE 7:	A 30 yr. old anemic female admitted to hospital with complaints of cervical dysplasia and bleeding. All serologic tests for G.C. were reactive. N. gonorrhoeae and Hemophilus vaginalis were isolated from the urethral and cervical cultures.	+	+	+	+

V. DISCUSSION AND CONCLUSION

A. DISCUSSION

The superiority of the conventional cultural methods over serological methods in the diagnosis of gonococcal infection especially among women has been emphasized and accepted in the literature. However, the efficiency of cultural methods depends largely on the type and sensitivity of the media employed.

It is now being suggested in the Center for Disease Control in Atlanta, Georgia, that serological tests will soon be able to discover or confirm gonococcal infection in presumptively asymptomatic women.

Since 1900, numerous investigators have studied and compared one serological test to another but none has come into routine use due to lack of sensitivity or specificity or both. So far, the complement-fixation technique appear to be the only serological procedure that was moderately specific. It seems to give the most reliable verification test and therefore has won general acceptance as a confirmatory serologic test for gonococcal disease. However, since the introduction of antibiotics such as penicillin and sulphonamide treatment, the value of this test diminished. Wallace et al, (1970) mentioned that antigen preparation in serological test for gonorrhoeae are either group specific or strain specific and therefore lack the species specificity required of a good diagnostic serological test.

Previous workers have described the following three tests for detecting gonorrhoea:

1) The microprecipitin test using phenol extracted antigen by Reising and Kellogg (1965).

2) The microprecipitin technique using a lipopolysaccharide antigen from N. gonorrhoeae by Chacko and Nair (1967) and

3) The Indirect-fluorescent antibody method using a formalin treated suspension of gonococci as antigen by Peacock et al, (1965).

None of the above methods have been proved sufficiently reliable to replace the Complement-Fixation technique.

The comparison of MFT, CFT, and IFAT results in this study reveal that the MFT was a highly effective confirmatory test in the sero-diagnosis of gonorrhea. In the majority of cases, sera which were reactive in the MFT were corroborated by either cultural examination or clinical diagnosis. Thus, the agreement of the MFT results with cultural analysis was 75.4%, whereas that of the CFT was only 31.1%. and 69.8% with IFAT. The data obtained with MFT compared well with the report of Reising (1971) which gave 79.0% as percentage sensitivity in testing 262 women infected with gonorrhea.

The data presented in Table 1, clearly indicates that of the 86 cases that were culturally negative but showed reactivity with MFT, 40 were supported either by historical evidence of gynecological disorders or past venereal infections. It is significant to note that 10 cases of asymptomatic women would have gone undetected at the time they were first examined by culture had their sera not shown reactivity to MFT.

The specificity of MFT in sera from presumed normal subjects has also been evaluated and a satisfactory level was obtained. It is as least specific as the other methods in current use. In testing sera from 51 verified meningococcal carriers, reactivity was noted only in four.

With regards to the 9 cases that were negative by culture but consistently reactive by MFT, it appears that gonococcal antibody can be present as long as 3-9 months although records from the Venereal Disease Department show that these patients had been adequately treated for their infection.

While the CFT has been widely employed as a confirmatory test for gonococcal infection, in this study, the occurrence of 31.1% sensitivity in 106 infected women has been discouraging. It appears relatively insensitive and a minority of sera give invalid results because of their anti-complementary or toxic properties.

The 73 women who were shown to be infected but were serologically non-reactive suggests undersensitivity of the test. In sera of healthy blood donors, the CFT appear to be the only one among the three tests compared that has shown reactivity in one sera. Table 3 shows that this test yielded the highest number of reactivity among the nasopharyngeal carriers of meningococci.

Since the 1930's, a vast number of investigations on gonococcal complement-fixation test has been presented and recorded. Lange et al (1965) investigated 1601 patients using the Complement-Fixation Test.

They concluded that a positive reaction without clinical signs in patients with pulmonary disease cannot be taken as an evidence of present or previous infection. However, Ovcinnikov (1963) in his review of the methods for laboratory diagnosis of gonorrhea in Russia concluded that although the CFT has fallen into disuse, it should continue to be employed provided that new and improved antigens can be developed.

IFAT for the identification of gonococcal antibodies as demonstrated in this study may be accomplished in approximately one and a half hours. Technically, it is no more difficult to perform than the MFT. This technique however, is as yet not suitable for use in small hospital laboratories because it is absolutely necessary to provide expensive equipments used in fluorescence microscopy.

The results obtained with IFAT shows that the percentage of positive findings in non-infected females was high. The reactivity with sera from the 51 meningococcal carriers totalled seven. This finding lends support to that reported by Peacock et al, (1965) who claim that the indirect FA method was ineffective in differentiating meningococcal and gonococcal antibody.

On the evidence presented in this study, it seems justifiable to claim that the MFT proved to be superior and adequate for the diagnosis of the disease especially in discovering asymptomatic women.

B. CONCLUSION

The relation of the MFT to IFAT and CFT has been discussed and the advantages of the former seem firmly established. Evaluation studies of the MFT using antigen suspension consisting of cholesterol-lechitin particles sensitized with an extract of gonococci are presented which serve to indicate its applicability of detecting human antibodies to N. gonorrhoeae especially among asymptomatic women. Thus, in this study we were able to demonstrate that the MFT is capable of detecting the gonococcus in the asymptomatic female when compared to cultural methods.

The application of MFT to the serological diagnosis of gonococcal arthritis holds promise of providing a method for specific recognition of the disease. This technique when properly applied has the advantages of increased sensitivity as the presently recommended cultural procedures. Thus, we found in this study that the results of MFT showed acceptable correlation with clinical diagnosis and cultural examination.

With regards to absolute specificity of this technique, the small number of positive results obtained which are not supported by clinical evidence may be considered to reflect cross-reactivity against related species of the Neisseria.

Our findings indicate that this technique merits field evaluation in hospital laboratories in order to define its diagnostic usefulness under routine laboratory conditions. Although further evaluation is necessary, the indications are that it will eventually prove to be an

excellent specific serological test for the detection of gonococcal antibodies.

The MFT appears to have two advantages as a serological tool: First, the convenience and simplicity of the procedure - a factor which may be important in small hospital laboratories and clinics; Second, the equipment and necessary devices are already in most laboratories and requires no special expertise.

If one considers the MFT from the practical standpoint, a saving of 2-3 days over the conventional culture is effected. Serologic identification of antibody as demonstrated by this technique can be accomplished in one hour and it is quite convenient while the patients are waiting in the clinic for examination.

VI: SUMMARY

Three serological tests -- Indirect Fluorescent Antibody Technique (IFAT); Microflocculation Technique (MFT); and the Complement-Fixation Technique (CFT) using different antigen preparations were carried out on sera of 857 female patients attending Pre-Natal and Gynaecology Clinic; 8 diagnosed cases of gonococcal arthritis; 31 sera from presumed normals; 51 bacteriologically verified nasopharyngeal carriers of meningococci and two carriers of N. lactamicus.

The results were compared and evaluated with cultural examinations and clinical diagnosis. A higher proportion of positive results were obtained by the MFT than by CFT and IFAT. In patients with G.C. arthritis, the test was reactive more often than CFT or IFAT. In the group of meningococcal carriers, it was more specific than CFT or IFAT. The specificity in normal controls was excellent.

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VIII: APPENDIX

APPENDIX 1 - MATERIALS USED

Lecithin (Beef Heart) purified from Beef Heart in not less than 3% solution in Absolute Ethyl Alcohol. (This is also completely satisfactory for the preparation of VDRL antigen in prescribed concentration of 0.2 - 0.22%.

Fluorescent Conjugated Sera - amb Anti - Human Gamma Globulin Fluorescein Labelled, is a standardized high titered reagent for use in the FTA - ABS Test. It is commercially prepared and obtained from Aerojet Medical Biological Systems.

Cholesterol (Ash - free) in Absolute Alcohol 0.9%.

REAGENTS USED

Reagents for Cholesterol - Lecithin Mixture

Stock Solution:

Bacto-Cholesterol (ash-free) (Difco #0224 - 13) 0.9%

Lecithin - Beef Heart (Difco #221376) ..... 30.3 mg/ml.

Working Solution:

Bacto-Cholesterol (0.9%) ..... 10.0 ml.

Lecithin - Beef Heart ..... 0.132 ml.

P B S Buffered Saline

Stock Solution:

Na_2HPO_4 ..... 109.6 gms.

NaH_2PO_4 ..... 31.5 gms.

Working Solution:

Stock Solution ..... 40.0 ml.

NaCl ..... 8.5 gm.

Make up the above to 1000 ml.

APPENDIX 2: SORENSEN'S BUFFER

A. Stock Solution:

Sodium Monohydrogen Phosphate ($Na_2HPO_4.2H_2O$) ---------- 11.876 gms.
in 1 liter of solution.
Potassium Dihydrogen Phosphate (KH_2PO_4) ---------- 9.076 gms.
in 1 liter of solution.

B. Working Solution:

Na_2HPO_4 ..... 60 ml.

KH_2PO_4 ..... 40 ml.

SORENSEN'S BUFFER MIXTURE

Vol. of Na_2HPO_4	Vol. of KH_2PO_4	pH of Mixtures
10.00	-	8.302
9.90	0.10	8.171
9.75	0.25	8.038
9.50	0.50	7.863
9.00	1.00	7.648
8.00	2.00	7.347
7.00	3.00	7.146
6.00	4.00	6.976
5.00	5.00	6.813
4.00	6.00	6.643
3.00	7.00	6.468
2.00	8.00	6.239
1.00	9.00	5.910
0.50	9.50	5.600
0.25	9.75	5.305
0.10	9.90	4.976
-	10.00	4.529