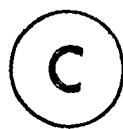


VALIDATION OF THE FIRST VOIDED URINE (FVU)  
AS A SPECIMEN FOR CULTURE  
TO IDENTIFY MALE ASYMPTOMATIC URETHRAL CARRIERS OF GONORRHOEA  
IN SIERRA LEONE - A DESIGN THESIS



by

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A Thesis

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**IDENTIFICATION OF MALE ASYMPTOMATIC URETHRAL GONORRHOEA**

)

MASTER OF SCIENCE ( MEDICAL SCIENCES)

TITLE: Validation of the First Voided Urine (FVU)  
as a Specimen for Culture to Identify Male  
Asymptomatic Urethral Carriers of Gonorrhoea  
in Sierra Leone - A Design Thesis

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## ABSTRACT

The current method presently in use for diagnosing male asymptomatic urethral gonococcal carrier is considered unsuitable for screening purposes in Sierra Leone. A search for a reliable, cheap, simple, and unobtrusive method found the first voided urine (FVU) as a specimen for culture to be the most promising. Because of methodologic weaknesses associated with previous evaluation of the FVU and the change in the population to which it would be used, blind adoption of the FVU was considered inappropriate.

A protocol for validating the FVU in Sierra Leone is presented. The major methodologic problems addressed were definition of asymptomatic male urethral gonococcal carrier, the selection of the study population, and quality control in the laboratory.

The finding of a sensitivity of 0.9 or greater for the FVU will be considered valid for the study.

## ACKNOWLEDGEMENTS

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## CHAPTER I

### BACKGROUND, ORIGIN AND SETTING OF THE STUDY

#### 1.1 Introduction

Gonorrhoea is pandemic. Current public health measures have not been very successful in combatting this infection. The problem lies in the fact that there is a pool of unidentified and untreated cases in the community continuously spreading the disease. That men are part of this pool has only been recognised in the last 20 years. However, the standard methods currently in use for identifying this pool of people are impractical for Sierra Leone. This design thesis proposes to validate a simple and unobtrusive method of identifying asymptomatic urethral gonorrhoea in men. As this is going to be the first major work on sexually transmitted diseases to be undertaken in Sierra Leone, this chapter will provide considerable detail about the setting of the study.

#### 1.2 Gonorrhoea Epidemiology

##### 1.2.1. The Extent of the Problem

Table I demonstrates the global distribution of the disease. The United Kingdom and the USA declared an epidemic of gonorrhoea in the mid-1970's.<sup>67</sup> But if the incidence data from England and Wales represents evidence of an epidemic, the situation in other countries such as Gambia, Greenland, the Bahamas and Colombia could be described as hyper-epidemic. The figures for these latter countries are astro-

nomic, Greenland having two cases of gonorrhoea per adult per year. However, the true extent of the disease in each of these countries is not known, and these incidence data cannot be compared directly because of three major methodological flaws associated with this type of data collection.

Table I - Incidence of gonorrhoea per 100,000 adults

CONTINENT	COUNTRY	YEAR	INCIDENCE
Africa	Gambia <sup>42</sup>	1976	17,780
Asia	India <sup>42</sup>	1977	150
Australia	Australia <sup>29</sup>	1977	85
Caribbean	Bahamas <sup>42</sup>	1976	6,670
Europe	England & Wales	1977	132
North America	USA <sup>9</sup>	1977	447
Polar Regions	Greenland <sup>42</sup>	1977	216,950
South America	Colombia <sup>42</sup>	1976	2,170

(1) Notification Data

The first of these three is the common problem associated with the collection of notification data, that of under-reporting of the disease, as it relies on reports by clinicians and 'non-clinicians', of cases seen and treated. The problem is evident in the data for Australia<sup>29</sup> in 1977, where the national incidence was reported as 85/100,000 whilst that of South Australia was almost twice the national figure at 150/100,000. The only explanation offered was that physicians in South Australia were more diligent in their notification than those in the rest of Australia.

In some countries such as Britain, USA and Australia, notification of gonorrhoea is a statutory mandate, whilst it is not in most developing countries.

(2) "Cases" not "individuals"

Even if the incidence of the disease is known, it may not reflect the actual proportion of people in a community that have the disease. Some individuals may contribute as many as 20 cases per year whilst many others do not contribute any. Thus if individuals instead of cases were used as the numerator, the incidence would be less. The degree to which the reporting of cases rather than affected individuals leads to a misleading estimate of the disease, varies with the social structure of the community.

(3) Method of Diagnosis

The third problem is the varying diagnostic criteria used for defining a case. The developed countries such as Britain and USA rely on

isolation of the organism on selective media before labelling a case as gonorrhoea. In the developing countries where laboratory facilities are non-existent, the diagnostic criteria are wholly clinical. As a result, other clinical entities such as non-specific urethritis and urinary tract infections sometimes get classified as cases of gonorrhoea. Thus the developing countries tend to over-estimate the incidence of gonorrhoea because of inaccurate diagnostic methods.

#### 1.2.2 Etiology, Risk factors and Transmission

Gonorrhoea is an infection caused by a gram negative bacteria, *Neisseria gonorrhoea*. Man is the only natural host.

The poor, the young, the unmarried, the urban dwellers and those sexually active with multiple sexual partners are said to be at high risk of getting the infection.<sup>69</sup>

The mode of transmission is venereal, although non-venereal transmission to children does occur.<sup>2</sup> The rate of transmission varies and the many factors affecting the outcome following exposure are shown in Fig.1.1. An uninfected female being exposed to an infected male has a higher chance of developing gonorrhoea than a non-infected male being exposed to an infected female.

#### 1.2.3 Clinical Manifestation and Pathogenesis

Uncomplicated gonorrhoea in men is characterised by dysuria, frequency of micturition and urethral discharge. The discharge is often yellow, thick, purulent and plentiful. This clinical picture of gonococcal urethritis is produced by the bacteria *Neisseria gonorrhoea* invading the transitional epithelium of the urethra. In a few cases invasion of the columnar epithelium of the prostate and epididymis does occur.

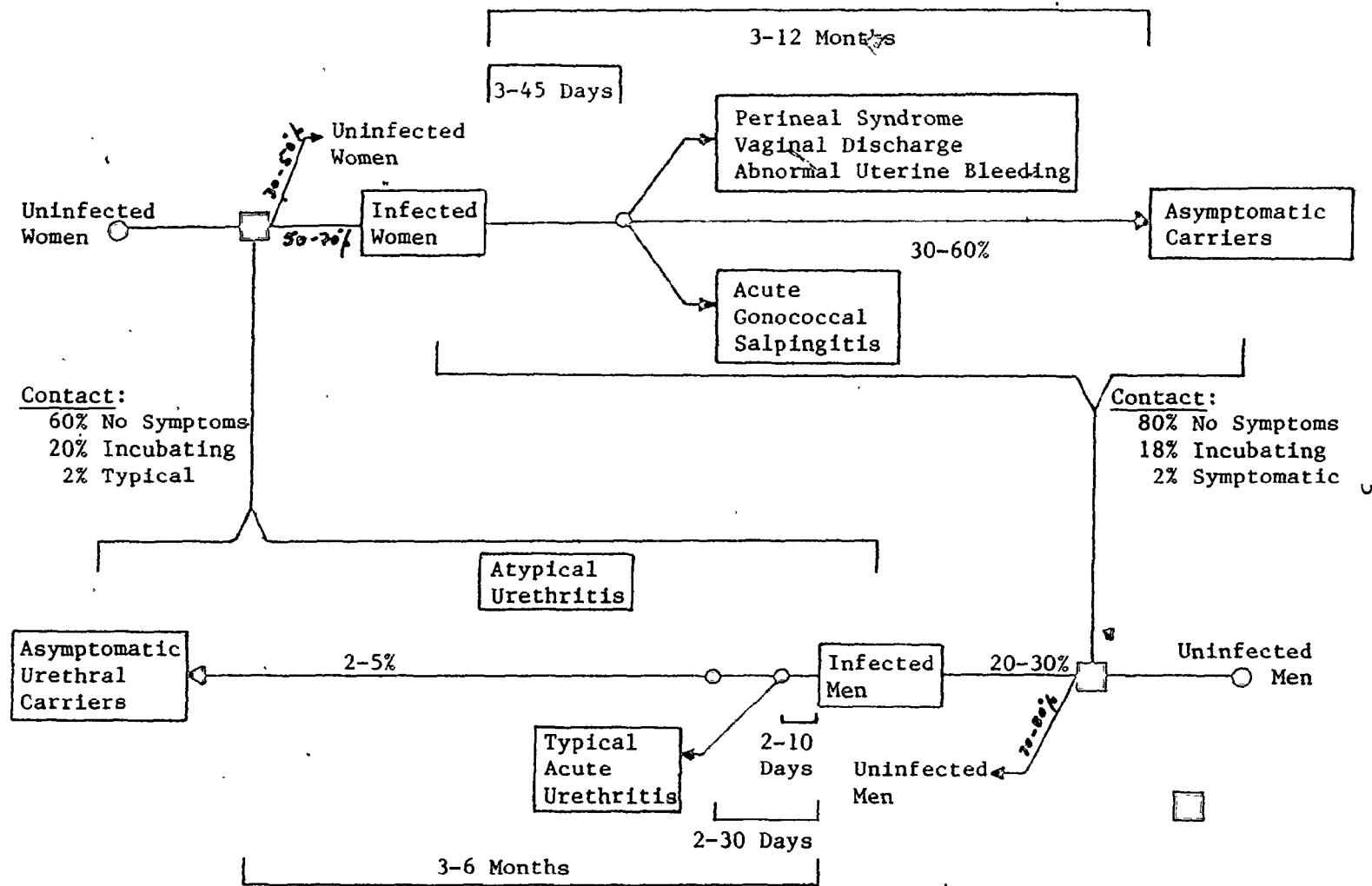


Figure 1.1 - Simplified diagram of heterosexual transmission of gonorrhoea related to disease status of infected patient.  
Extracted from Disease-a-Month69

In the female the lower genital tract infection is confined to the Bartholins glands, the periurethral glands of skene, the urethra and the endocervical canal. The upper genital tract infection results from invasion of the transitional and columnar epithelium of the endometrium, the endosalpinx and the periovarian tissues.

Gonococcal proctitis and pharyngitis are produced by infection of the glandular tissues in these areas.<sup>63</sup>

#### 1.2.4 Consequences of Gonorrhoea

The importance of gonorrhoea lies in the complications of the disease. In both sexes it produces involuntary sterility.

In the men, epididymitis and orchitis if untreated results in azoospermia. In the female, gonococcal salpingitis (or pelvic inflammatory disease) produces tubal occlusion with resultant infertility.

The sequelae of gonorrhoea is more apparent in the developing countries where inadequate and scarce health care resources do not provide adequate therapy for this disease at the early stages. It is difficult to compare infertility data directly, because of the varying definitions used in data collection. In the 1974 national census for Sierra Leone<sup>46</sup>, the infertility rate of 15% was a measure of the percentage of women aged 44-49 years that were childless at the time of the census. The national survey<sup>13</sup> of family growth in the USA reported in 1976 that 5.1% of married or previously married women classed themselves as "non-contraceptively" sterile for non-surgical reasons.

We know that the 15% for Sierra Leone and the 5.1% for the USA does represent a measure of infertility. The rate for Sierra Leone is three times that of the United States and a justifiable reason for con-

cern. The developed countries are more concerned with the economic consequences of pelvic inflammatory disease. In the United States the direct and indirect cost of PID and PID associated ectopic pregnancy was said to exceed \$1.25 billion in 1979.<sup>13</sup> It was estimated at that time that 850,000 cases of PID occur annually, require more than 212,000 hospital admissions, 115,000 surgical procedures and 2.5 million physician visits. 50% of all PID in the United States was caused by gonorrhoea.<sup>13</sup>

#### 1.2.5 Identification of Gonorrhoea

There is no fixed standard procedure in use globally. The main variation occurs around (1) availability of funds, (2) method of specimen collection, (3) the type of culture media used. Recommended methods do not reflect actual practice in clinics and offices even in the most advanced countries. In this section, the method recommended by Centre for Disease Control, Atlanta, is presented.<sup>11</sup> The literature review in the next chapter will throw some light on what is really happening in practice.

##### Women

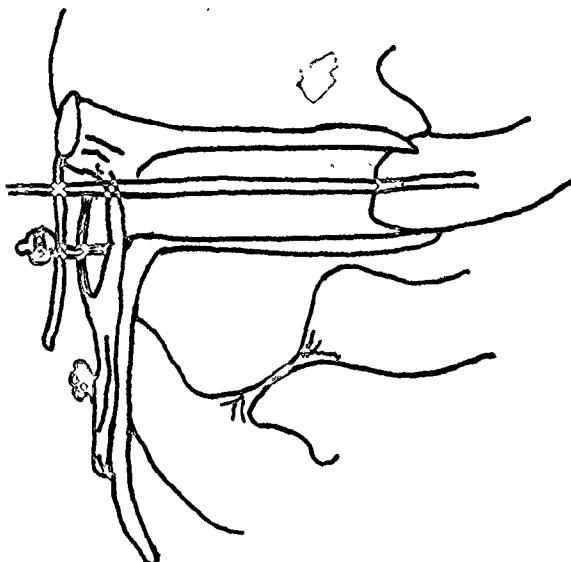
In women, the recommended method of diagnosing gonorrhoea involves collection of specimen from the endocervical canal and inoculation of the specimen on to selective media such as Modified Thayer Martin, or New York City Media in culture plates. To increase the diagnostic yield specimens could be collected from the urethra, rectum and pharynx and inoculated separately on to culture plates. The presumptive identification of *Neisseria gonorrhoea* is made if typical colonies are present on the culture plate. The colonies must be oxidase positive and show gram negative diplococci on microscopic examination.

To obtain specimen: See Figure 1.2

1. The female should be in the lithotomy position with good lighting as for any gynaecologic examination.
2. The speculum should be moistened with warm water and then inserted into the vagina.
3. Insert a sterile cotton tipped swab into the endocervical canal. The swab is moved from side to side for approximately 30 seconds to allow absorptions of organisms on to the swab.

Figure 1.2

Endocervical culture



This method of identification is recommended for symptomatic and asymptomatic women, for diagnosis, case finding and screening.

Men

1. Clinical diagnosis - a history of dysuria or urethral discharge in most developing countries is used as a method of diagnosis. In the USA

it is the reason for seeking identification of the disease.

2. Microscopic demonstration of typical gram negative intracellular diplococci on direct smear of a urethral exudate constitutes a sufficient basis for a diagnosis of gonorrhoea. The slide is prepared by rolling the swab on the side. This method is only recommended for those with visible urethral discharge.

3. In the absence of a urethral discharge or in screening of asymptomatic men for urethral infection, a culture specimen should be obtained from the anterior urethra and inoculated on to selective media. The presence of typical colonies which are oxidase positive and show gram negative diplococci on microscopic examination provides sufficient criteria for the presumptive identification of *Neisseria gonorrhoea*.

Specimen collection: See figure 1.3

The use of a calcium alginate urethral swab or a bacteriologic loop is recommended. Specimen is obtained by insertion of the swab or loop no more than 2 cm. up the anterior urethra and gently scraping the mucosa. If I may quote, "the standard cotton-tipped swab is not recommended for this procedure since it is too large to be inserted as far as required for a good specimen and results in excessive pain".<sup>11</sup>

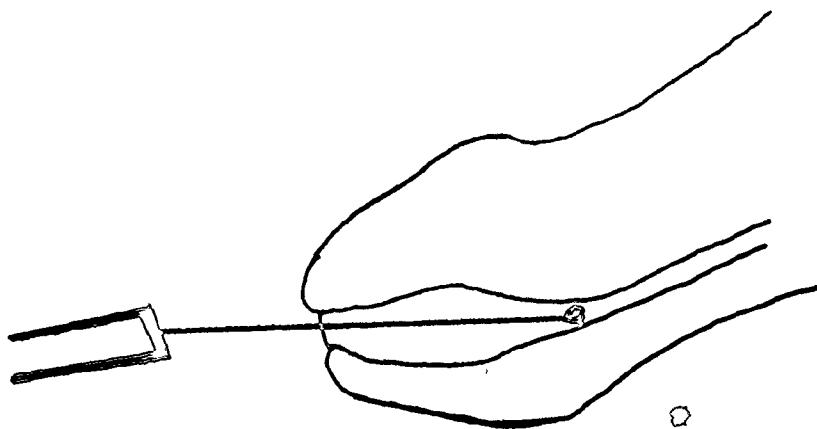
#### 1.2.6 Gonorrhoea Control

The ideal method of control is that of eradication of the organism *Neisseria gonorrhoea*. Because of the mobility of the world population, and the increasing friendliness between nations and races permitting venereal transmission, any eradication campaign has to be a global effort.

It is now recognised that the major weakness of older control pro-

Figure 1.3

Anterior urethral culture



grammes was that they completely ignored the asymptomatic cases of the disease thereby leaving a large reservoir to continue the propagation of the disease.

As asymptomatic males and females are known to exist, the ideal programme will involve seeking, identifying and treating asymptomatic as well as symptomatic disease. The United States has opted to seek asymptomatic females by screening all women having gynaecologic examination for any reason and offering epidemiologic treatment to all their contacts.<sup>9</sup> This option of screening women to reduce the pool of infection is impractical for most developing countries for the following reasons.

## (1) Cost

Gynaecologic examinations require a minimum set up. This includes a private room, sterile instruments such as vaginal speculum and forceps. These are expensive and only limited to hospital use or physician's offices.

(2) Low participation rates

Gynaecologic examinations could only be done on those who use the Health Care Facilities that have the set up and expertise to perform the examination. In Sierra Leone this will be less than 20%, as 80% of all deliveries are still done by traditional birth attendants. The traditional woman does not like gynaecologic examinations, which is often refused especially if the clinician is a man.

As a result the developing countries are left with seeking the asymptomatic males and offering epidemiologic treatment to all their contacts. The recommended standard method of identifying asymptomatic urethral disease described previously is unsuitable for Africa for the following reasons:

(1) Cost

The cost of 1,000 urogenital calcium alginate swabs is \$238.45 to hospitals in Canada. Therefore, each swab costs 23 cents in a Canadian hospital, and it will be a lot more in Sierra Leone. The latter spends less than \$3.00 (three dollars) on health per head per annum, thus it would be impossible to envisage a continuous supply of swabs being provided on such a small budget.

(2) Obtrusive

The cotton tipped swabs, though cheaper, cause excessive pain. In addition they must be sterile, thus cost will prevent a steady flow of sterile swabs.

(3) Traditional beliefs

Manipulation of the penis, the recommended milking before specimen collection and insertion of the swab into the urethra is said to decrease

their sexual powers.

Thus an unobtrusive method of identification of asymptomatic gonorrhoea was sought from the literature. The first voided urine as a specimen for culture is appealing. It involves the male voiding the first 10 ml of urine, and the urine specimen is used as an inoculum on selective media to isolate *Neisseria gonorrhoea*. This method has been validated for both symptomatic and asymptomatic urethral disease in the USA with good results. Before widespread adoption in Sierra Leone, it became apparent that the first voided urine has to be validated as a specimen for culture for identifying male asymptomatic urethral gonorrhoea in Sierra Leone.

### 1.3 Sierra Leone

#### 1.3.1 Geography

Sierra Leone is an English speaking country that lies on the west coast of Africa about 11° north of the equator. The climate is tropical, hot through the year and wet for half the year.

#### 1.3.2 The Health System

The Government tries to provide free medical care to all of its residents. Primary care at village or community level is administered by dispensers or Community Health Nurses working in dispensaries or Health Centres. These centres are supervised by the physicians working in the district hospitals. The district hospitals act as referral centres for these health centres.

The main referral centre is in the capital, Freetown. The hospitals in the capital employ more than 50% of all physicians residing in the country.

Health care is not free, as prescriptions for drugs have to be bought by the patients at primary care and hospital level.

#### 1.3.4 The Pathway to Health

Who cares for the people when they are ill? For severe illnesses, the majority of cases eventually seek care from the hospitals. For non-fatal illnesses such as gonorrhoea the pathway to health depends on the socio-economic status of the individual. The rich and well-educated seek care from private physicians which is similar in quality to that received in the developed countries. Another 15% residing in the urban areas get care from hospital outpatients and emergency rooms, or health centres. In addition, some use the private dispensaries or nurses. These paramedical workers provide cheap but ineffective care. The bulk of the population, about 80% are said to reside outside Freetown where traditional social standards still exist. 50% of people in an area surveyed in 1980/81 by the public health unit admitted to using the native doctor or herbalist during an illness. 20% used the health centre and 5.2% used the hospital. This suggests that the health care system is providing care for only 25% of the population in that area.<sup>61</sup>

#### 1.3.5 Epidemiology of Gonorrhoea in Sierra Leone

The true extent of the problem is not known as gonorrhoea is not notifiable in Sierra Leone. The only incidence data available is by Gooding, who in 1976 found 19% of the male university students sought treatment for urethritis over a 10 month period.<sup>24</sup> This incidence figure was thought to be an underestimate for two reasons. Firstly, some students were known to seek treatment from a dispenser 2 miles away from campus. Secondly, their attacks of gonorrhoea whilst they were on vacation were not

known to the health centre. The major weakness of this data is that the diagnoses were clinical and may not represent gonorrhoea alone.

However, Gooding in 1978 found that 66% of all male respondents to a self administered questionnaire survey for contraceptive use, admitted to having had urethral discharge. Most of them admitted to seeking care from private dispensers.<sup>23</sup>

Table II shows incidence and prevalence rates of gonorrhoea in selected populations of Africa. With the exception of Sierra Leone, these data are reliable as all diagnoses were based on isolation of *Neisseria gonorrhoea* by culture methods. The problem is real but as it has no associated mortality it does not receive the attention it deserves.

#### 1.3.6 The Social Setting in Sierra Leone

Sierra Leone is a polygamous society, practised concurrently in the rural areas where traditional practices and beliefs are still evident. Urbanization and Christianity has brought about consecutive polygamy. The latter is now superimposed on concurrent polygamy where transitional social changes are occurring amongst the educated and urban. This has resulted in a large pool of sexual partners for the men as it is a patriarchal society. The politicians are men at all levels of leadership. Education of women is still not a right but a privilege.

#### 1.3.7 Social consequences of gonorrhoea

##### Demographic

With a high infant mortality rate, the only way a country could increase its population is by high fertility. Between 1963-1974, Sierra Leone failed to meet its predicted population growth. Infertility, which

Table II - Incidence and Prevalence Rates for Selected Populations in Africa

COUNTRY	AUTHOR	YR. OF PUBLIC.	TYPE OF STUDY	SOURCE OF SAMPLE	SIZE OF SAMPLE OR POPULATION AT RISK	INCIDENCE OR PREVALENCE
Uganda	Arya et al <sup>3</sup>	1973	P	Teso Village (R) Ankole (R) Kampala (U) Kasangati	613	7.5 1.2 15.0 2.76
Uganda	Arya et al <sup>42</sup>	1977	I	University Students (Male)	1,200	18.8%
Sierra Leone	Gooding <sup>24</sup>	1977	I	University Students (Male)	800	19.0%
Nigeria	Osoba <sup>42</sup>	1973	P	Asymptomatic Pregnant ♀ Symptomatic ♀ Asymptomatic ♀ Infertile Women ♂	208 228 130 151	3.4 17.0 5.0 1.3
Swaziland	Meheus <sup>17</sup>	1980	I	Mbabane Hospital	30,000	3%

P - Prevalence

I - Incidence

♀ - Women

R - Rural

U - Urban

was found to be as high as 22% in the southern province, was one of the explanations offered by demographers.<sup>46</sup>

### Fertility

The people of Sierra Leone have a very strong pronatal feeling. A woman's success is measured by her production of children and a man's by his number of children. Even the most educated woman with her Ph.D. has to have a child to be accepted into the society, and get the respect of others.

On the other hand, if a woman is infertile and has no children, she is labelled a "cockerel" by the men in the society. At the village level she is denied the two most coveted positions, that of the traditional birth attendant or the head of the female secret society.<sup>60</sup> The marriage often breaks down and the female is returned to her family with a request of a refund of the dowry paid. She is often abandoned by her family as the refund of the dowry causes economic hardship for most families. The female if uneducated and unskilled may not be able to earn enough for survival and often becomes a prostitute as a source of income.

They have no security for old age as adoption is not legal in Sierra Leone. The provision the society makes for the old is through the extended family, whereby the old live with their children and care for their grandchildren. In the absence of children there is no such provision.

#### 1.3.8 Gonorrhoea Control in Sierra Leone

This is non-existent. The proposed programme by Meheus et al for developing countries will be suitable for Sierra Leone.<sup>40</sup> However, it will not be control-effective as there is no provision for identification of

asymptomatic gonorrhoea, that constitutes the reservoir of infection. The identification of asymptomatic females will be impractical for Sierra Leone as outlined previously. The male asymptomatic urethral disease should be sought to make the programme control effective. It could be argued that the women constitute a greater proportion of the reservoir and seeking them may give a higher yield in terms of cases treated than asymptomatic men. Although this may be the case for the western world, the following reasons may make the reverse true for Sierra Leone. Firstly, the men tend to have more sexual partners than the females. Secondly, the men are more effective transmitters of the disease. The women in Sierra Leone will comply with requests of male partners to receive epidemiologic treatment whilst the men will not comply with the women. From the author's experience, many wives have received epidemiologic treatment, but a male has yet to be treated when the wife gets PID. Expressing this in numbers we could use the data by Arya et al<sup>4</sup>. They found 17% of women in Teso village of Uganda with gonorrhoea. If all husbands were treated on epidemiologic grounds an equal number of men will get treatment. On the other hand, if the 15% amongst the husbands of gonococcal negative women are sought, each male will bring a minimum of two sexual partners. Because the females are much more likely to comply with the wishes of the men, the yield from seeking the men and offering epidemiologic treatment to the women will be much higher. As it is a polygamous society, more than one female per man will be treated, more so in areas where the male/female ratio is low.

Thus, the objective of this design thesis is to validate the First Voided Urine (FVU) as a specimen for culture for the identification of male asymptomatic urethral gonorrhoea in Sierra Leone. This method will

be evaluated against the standard recommended technique. An attempt will be made to determine the utility of the FVU as a screening test for asymptomatic urethral gonorrhoea.

## CHAPTER 2

### REVIEW OF PERTINENT LITERATURE

#### 2.1 SUBSTANTIVE

##### 2.1.1 Introduction

The identification of the male asymptomatic gonococcal disease is the main concern of this thesis. The literature was searched for any article addressing this problem. As its importance has only been recognised recently, few articles have been written. Most of these articles are descriptive studies discussing prevalence or incidence in selected populations. Some articles focus on one aspect such as natural history, and causal issues, whilst others deal with more than one aspect and will therefore be referred to in more than one section of this review. The review will first address the definition of asymptomatic male gonorrhoea, since there are basically two working definitions. A discussion of the extent of the problem, as described in the literature, will be followed by the natural history of the disease, on which only four reports have appeared. The possible causes of the disease will be discussed to help explain why a high prevalence of asymptomatic gonorrhoea is expected to be found in Sierra Leone. Lastly, the methods of diagnosing asymptomatic disease evaluated in the literature will be described.

##### 2.1.2 Definition of Asymptomatic Male Urethral Gonorrhoea

###### Patient's Definition

Landman<sup>34</sup> in 1959 used the term 'asymptomatic' "to indicate that subjective discomfort was either non-existent or so insignificant as to

be ignored". This definition has since been used by various other investigators in their reports.<sup>6,10,59,37,34,54,18,52,66</sup>

#### Clinician's Definition

Handsfield<sup>25</sup> included in his 1974 study of asymptomatic male gonorrhoea "only those without demonstrable exudate". This definition first appeared in a review article by Pariser in 1972<sup>48</sup> where he defined asymptomatic as "the stage in which there are no clinical signs or symptoms of the disease detected by either the patient or the examining physician". This definition has been popular among recent American investigators.<sup>25, 27,65,36,50,48,32,8,47,31,21,51,49</sup>

#### Importance of the Definition

From the public health point of view, Landman's definition is probably more useful, as it is the patient's perception of whether or not he has symptoms (rather than the clinician's classification) that will be most crucial in affecting his decision to seek health care or not. It is unfortunate that most articles have addressed the problem from the clinician's point of view. Pariser's definition as it is used underestimates the problem of asymptomatic urethral gonorrhoea. Since many patients who deny that they have symptoms - and therefore should in practise be considered asymptomatic - will be classified as symptomatic by clinicians on the basis of minimal signs. A further importance of a clear definition of 'asymptomatic' is that this is not always a static fixed state but may be a stage in a continuum. Some people may be asymptomatic today - but symptomatic a few days later.

### 2.1.3 The Extent of Male Urethral Gonococcal Disease

Studies reported before that of Carpenter and Westphal<sup>10</sup> lacked internal validity and credibility, in that they relied on history and unreliable diagnostic methods such as microscopic examination of urine sediments or prostatic secretions.

#### Incidence

The true incidence is not known but various workers have tried to record the occurrence of the disease among various venereal disease clinic patients. However, the population at risk in these studies was not defined in the reports. Table III records the percentages of asymptomatic gonorrhoea that occurred in these various populations. This ranged from a low of 1.6% in Dexter's New York clinic<sup>17</sup> to a high of 34% among patients with condylomata acuminata<sup>26</sup>. The methodology in these studies was poor, but Table III indicates that wherever male asymptomatic urethral gonococcal carriers have been sought, they have been found.

#### Prevalence among males named as contacts of symptomatic women

Table IV lists studies of men named as contacts of women with symptomatic gonorrhoea, mainly gonococcal pelvic inflammatory disease (PID). The percentage of asymptomatic infection found in these males ranges from a low of 22% in Gilstrap's study<sup>21</sup> to a high of 61% in the population studied by Philips<sup>52</sup>. This group of men has yielded the highest percentage of asymptomatic infection. The wide variety of percentages reported in the studies discussed so far cannot be explained by variations in definition, but is possibly due to the differences in the population of males examined.

TABLE III - Descriptive Statistics: Studies on asymptomatic male urethral gonorrhoea at VD clinics

COUNTRY	AUTHOR	YR. OF PUBL.	POPULATION STUDIED	TYPE OF STUDY	NO. POS.	NO. STUDIED	(%) ASYMPT.	NO. POS.
England	Sarkhel <sup>59</sup>	1975	179 with GC	Incidence	175/179			14/175      9%
USA	Dexter <sup>17</sup>	1976	2370 Asymptomatic at a VD clinic	Prevalence	37/2370	(1.6%)	37/37	
Scotland	McMillan <sup>37</sup>	1978	5076 new male	Incidence	957/5076	(18.9%)	38/957	
USA	Braff <sup>8</sup>	1978	2811 "selected" at VD clinic	Incidence	80/2811	(2.8%)	25/80	31%
USA	Osborne <sup>47</sup>	1979	841 attending STD clinic	Incidence	125/841	(14.8%)	27/125	21.6%
USA	Judson	1978	773 asymptomatic at a VD clinic	Prevalence	56/773	(7.2%)	56/56	
Indonesia	Harahap <sup>26</sup>	1979	with Condyloma acuminata	Prevalence	23/67	(34%)	23/23	
Denmark	Nellson <sup>44</sup>	1976	166 asymptomatic males with scabies	case finding	3/166	(2%)	3/3	

VD - Venereal Disease      GC - Gonorrhoea  
 FPC - Family Planning Clinic PID - Pelvic Inflammatory Disease  
 ANC - Annatal Clinic      STD - Sexually Transmitted Disease

Prevalence in populations other than STD Clinics and Health Care Clinics

In order to prepare the way for a wide spread screening or case finding effort, populations other than STD clinics have been screened for prevalence of male asymptomatic disease. Table V shows that the prevalence ranges from 0 to 2%. Thatcher<sup>65</sup> found no asymptomatic carriers in his study of American male recruits, but he used the clinician's definition which has the disadvantages already mentioned. The highest prevalence of 2.2% was found by Handsfield also amongst military personnel<sup>25</sup>.

AFRICA

Very little work has been done on asymptomatic male gonorrhoea. Arya et al conducted a survey in two villages in Uganda<sup>4</sup>, and looked at the prevalence of gonorrhoea among husbands of gonococcus-negative fertile women, and husbands of gonococcus-negative infertile women. He found a prevalence of 6.7% and 24.5% respectively. These rates are quite high when the result is compared to those reported by Handsfield<sup>25</sup> and Braff<sup>8</sup>.

2.1.4 Natural History

The natural history is concerned with the period of infectivity without symptoms and if the organisms will disappear without treatment. Bittiner and Horne<sup>6</sup> in their descriptive report of seven cases noted that 5 cases were asymptomatic carriers for at least a month, one for six weeks and the seventh case for at least four months. Handsfield<sup>25</sup> did a study on the natural course of 28 males with asymptomatic urethral gonococcal infection, age range 20-45 years, mean of 25 years. They were asked to forego treatment for up to six weeks without sexual intercourse. They were examined at weekly intervals for development of symptoms and the persistence

Table IV - Asymptomatic Urethral Gonorrhoea in Selected Populations of Men Named as Contacts of Women with Gonorrhoea

Author	Yr of Study	Type of Contact	No. Pos./No. Examined (%)	No. symp./No. Pos. (%)
Landman <sup>34</sup>	1958	with GC PID	77/82 (94%)	42/77 (55%)
Portnoy <sup>54</sup>	1974	in a VD clinic	63/133 (47%)	27/63 (43%)
Eschenbach et al <sup>18</sup>	1975	with GC PID	22/42 (52%)	10/22 (45%)
Pariser et al <sup>50</sup>	1964	in a VD clinic	43/115 (37%)	26/43 (60%)
Blount <sup>7</sup>	1972	GC +ve in FPC/ANC	228/822 (28%)	91/228 (40%)
Handsfield <sup>25</sup>	1974	with symptomatic GC	21/38 (55%)	12/21 (57%)
Gilstrap <sup>21</sup>	1977	with GC PID	63/161 (39%)	14/63 (22%)
Phillips L. <sup>52</sup>	1980	with GC (PID, FPC, ANC)	274/1008 (27.4%)	167/274 (61%)
Volkin <sup>66</sup>	1979	with GC PID	34/69 (49%)	11/34 (34%)
Penderson <sup>51</sup>	1970	with GC at STD clinic	662/748 (88.5%)	19/662 (2.8%)

VD - Venereal Disease

FPC - Family Planning Clinic  
ANC - Antenatal Clinic

GC - Gonorrhoea

PID - Pelvic Inflammatory Disease  
STD - Sexually Transmitted Disease

Table V - Cross-sectional studies on male asymptomatic Gonorrhoea

Country	Author	Year of Publication	Study Population	Type of Study	Incidence or Prevalence
USA	Carpenter & Westphal <sup>10</sup>	1940	1061 prison inmates	Prevalence	1%
USA	Mahoney et al <sup>36</sup>	1946	293 volunteers	Prevalence	1%
USA	Thatcher et al <sup>65</sup>	1969	505 Naval Military (RPE)	Prevalence	0%
USA	Handsfield et al <sup>25</sup>	1976	2628 military personnel	Prevalence	2.2%
USA	Hein et al <sup>27</sup>	1977	2664 adolescents in a Detention Centre	Prevalence	1.9%

25

(RPE) - Routine Physical Examination

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of *Neisseria gonorrhoea* in their urethra. He found 18/28 (64%) remained asymptomatic until they were treated 7 to 165 days after presumed acquisition of the infection from their GC positive female contact. Two (7%) developed urethritis diagnosed by the physician but were subjectively asymptomatic and another three (11%) developed acute urethritis. The remaining five (18%) underwent spontaneous cure. To increase the credibility of their study they located and treated all known possible consorts to exclude the possibility of re-infection even though the patients said they abstained from sexual intercourse. It would appear from these two articles that spontaneous cure occurs in only a few patients and that the only way of stopping the spread of gonorrhoea by asymptomatic carriers is by their identification and treatment.

Dewar et al<sup>16</sup> also reported two cases in negro adolescent boys with asymptomatic pyuria, that yielded *Neisseria gonorrhoea* on culture, and the last sexual encounters for these boys were two months before the diagnosis was made.

Portnoy<sup>54</sup> defined duration of asymptomatic period as "the number of days between the date of the last sexual contact and the date of examination". His subjects' duration of asymptomatic urethral disease ranged from 3 to 155 days. As the normal incubation period is said to be 2 - 10 days, the nine cases that had duration of less than 10 days could be adjusted for. His incidence of asymptomatic carrier would then be less than that reported in Table IV, if those incubating the infection are excluded or adjusted for.

#### 2.1.5 Causal Issues and Association for Asymptomatic Urethral Gonorrhoea

No experimental evidence was found to incriminate the host or the pathogen for the production of the asymptomatic carrier.

##### Host

Because of its high infectivity, gonorrhoea is a communicable disease, and its carrier state is said to be the same, in pathogenesis, as the other infectious diseases. This cannot explain all the asymptomatic carriers, as the incubation period for gonorrhoea is about 2 to 10 days and Handsfield<sup>25</sup> found cases to carry the organism, without production of symptoms, for up to 4 months.

Convalescent carrier may be defined as "a person who remains infectious after clinical recovery"<sup>70</sup>. This could explain some of the cases of asymptomatic gonorrhoea, as no drug currently in use is 100% effective, so the treatment failures, if not identified and retreated become a pool of asymptomatic carriers.<sup>49</sup> This may be as high as 5.4% with Aqueous procaine penicillin G (APPG) and probenecid<sup>28</sup> and may even be higher with prolonged therapy if compliance is low or poor.

A chronic carrier "continues to be infectious sometimes intermittently for long periods after recovery".<sup>70</sup> This was suggested by a descriptive study<sup>15</sup> of 151 cases of urethritis and prostatitis in males working in industry in South Africa. These diseases were commonly seen in the days of sulphonamide therapy. As was mentioned earlier, the latter therapy is still in use in parts of Sierra Leone.

##### Agent

Four (4) types of *Neisseria gonorrhoea* have been identified. In a retrospective case-control study, Crawford et al<sup>12</sup> reported that gonococci

with nutritional requirements for arginine, hypoxanthine and uracil (A-H-U-) were recovered for 24 of 25 men with asymptomatic gonorrhoea. The result was statistically significant. As with all case control studies, it is not as good as experimental evidence as they suffer from Neyman's bias. The results, however, do suggest that A-H-U- strains are more likely to cause asymptomatic gonorrhoea than other strains.

This A-H-U- strain has been found to be more common in disseminated gonococcal infection (DGI). These A-H-U- strains are more susceptible to penicillin and tetracycline.<sup>68</sup> Weisner et al<sup>68</sup>, Knapp & Holmes<sup>33</sup> found that 89% of their isolates from DGI are A-H-U- requiring strains compared to 38% of isolates from uncomplicated infection in Seattle, and only 18% of infection in Milwaukee. This is consistent with Crawford's findings as it is well known that most cases of male DGI are asymptomatic urethral carriers of gonorrhoea and also the male contacts of females with DGI tend to have a high risk of asymptomatic disease. The finding of low minimum inhibitory concentration for strains causing asymptomatic disease is important as procaine penicillin 0.6 ml for 2 days or Triplopen 1.2 million units in one injection without probenecid is still used for uncomplicated gonorrhoea in Sierra Leone with effective clinical response.

#### Use of Antibiotics

Widespread use of antibiotics may be a causal factor in Africa where self medication is common<sup>61</sup> and inadequate doses of antibiotic capsules are used either prophylactically or to obtain symptomatic relief.

Because of the high bacterial infection rate in the community such as tuberculosis, pneumonia, dysentery, antibiotics are widely used but not in the dosages required to treat gonorrhoea effectively.

For example, streptomycin, 1 gm twice weekly, plus isoniazid, 300 milligrams daily, is the most commonly used method of tuberculosis therapy in the rural area. This is not adequate nor effective therapy for acute gonococcal disease.

The results of Carpenter and Westphal<sup>10</sup> have important implications for Sierra Leone where sulphonamide is still used by some dispensers for treating male gonorrhoea. The use of sulphonamide is not due to ignorance but the frequent unavailability of penicillin in rural health centres.

#### Failure to treat sexual partners

Treatment of a case of gonorrhoea but not the sexual partners results in re-infection of the case. It was originally thought that all treatment failures were due to reinfection, but it is now known that treatment failure as an entity does exist. Reinfection often occurs when the sexual partners are not treated.

If the suggestion by Dexter et al<sup>46</sup> and Braff<sup>37</sup> that males who are contacts of asymptomatic women tend to be symptomatic whilst men named as contacts of symptomatic women tend to have asymptomatic gonorrhoea, then Sierra Leone should expect a high asymptomatic carrier rate as Frazer<sup>55</sup> put the prevalence of infertility at 12% and mainly due to Pelvic Inflammatory Disease (symptomatic GC).

#### Adherence to Mucosal Surface

The gonococcus has been shown to possess a capsule with various antigenic structures. Antibodies to these antigenic structures have been found.<sup>69</sup> The true significance of the capsule and the Pili and the role they play in asymptomatic disease is far from complete.

#### 2.1.6 Methods of diagnosing asymptomatic male gonococcal carrier

The ideal method will be a serologic test that will be simple, cheap, reliable and acceptable.<sup>58</sup> Suffice it to say that no gonococcal serologic test available today is useful, not even for surveys, as the result will be extremely difficult to interpret.

Handsfield<sup>25</sup> in 1974 evaluated the laboratory tests available for the diagnosis of asymptomatic gonorrhoea. Murray<sup>43</sup> et al in 1980 have looked at these tests for the purpose of diagnosing symptomatic gonorrhoea, whilst Luciano et al<sup>35</sup> in 1981 have compared three of these tests for gonorrhoea screening.

When evaluating the laboratory tests there are two aspects to be considered. The first is specimen collection from the patient and the second is the processing of the specimen in the laboratory. To diagnose asymptomatic urethral gonorrhoea, the question being asked is, "Is *Neisseria gonorrhoea* present in the urethra of the man?" Two specimens have been shown to be effective to varying degree. One is scraping or a swab that strips the urethra of any exudate and some epithelium. The other is a urine specimen that washes out exudates and possibly some epithelium as the urine passes through the urethra. These two specimens once collected could be examined either directly by a staining procedure or indirectly by inoculating the specimen onto a culture media, allowing the organism to grow into colonies and then looking at the colonies for *Neisseria gonorrhoea*. This latter method of identification by culture has proved to be reliable and accurate compared to the direct examination.<sup>25,30</sup>

The method of specimen collection has always been a source of con-

troversy. Some people use loops, some cotton swabs, some calcium alginate urogenital swabs.<sup>11,25</sup> Of all the different types of swabs available, the calcium alginate swab is the least obtrusive, its major disadvantage is that of cost and the amount of inoculum collected is small. Because of the unacceptability of this method of specimen collection by many patients, some investigators<sup>43,35,19</sup> have looked at the first voided urine as a specimen for culture. Unfortunately, their population spectrum was not wide enough; nor was the sample size adequate to make the test credible for asymptomatic cases. Secondly, their gold standard was not well defined as they did include the test results in their gold standard, which is not valid<sup>71,73</sup> and thus makes it difficult to assess the predictive values.

#### Gram Staining Technique

"Gram staining" of urethral exudate or sediment of centrifuged urine.

The stained slide is examined for intracellular gram-negative bean shaped diplococci to make a presumptive diagnosis of gonorrhoea.

#### The Advantage

1. On the spot diagnosis is possible and treatment could be instituted on the same day. It takes 10 - 15 minutes to stain and read a gram stained slide.
2. Relatively cheap - as the major outlay is a lab technician's time.

The latter is a scarce commodity.

#### Disadvantage

1. Reliability - Very poor as the staining procedure is not reproducible and varies with experience and skill. In addition, there is a high inter-observer variation and intra-observer variation with micro-

scopic examination as was well documented.<sup>14</sup>

2. The properties of the test are not impressive. Handsfield<sup>25</sup> found a sensitivity of 69%, specificity of 42%, an accuracy of 65% when the prevalence is 85%. Thus if we want to use this test in an area such as Sierra Leone where the prevalence is believed to be no less than 1%, the predictive value of a positive test result becomes 1% and for a negative test 99%. This will not be useful in a screening nor a case finding situation where a very sensitive test is required.

#### Fluorescent antibody technique - Direct

This involves staining the specimen directly. The advantages and disadvantages are similar to the gram stain, only that this is more expensive in that a special microscope is required, and the fluorescent stain costs more and requires more time, and a more qualified and experienced laboratory technician for microscopy. This method yielded a high false positive rate when evaluated against the culture, the latter being the gold standard.<sup>25</sup>

#### Anterior Urethral Swab and Culture

Handsfield<sup>25</sup> evaluated four methods of diagnosing urethral gonorrhoea: (1) the gram stain, (2) direct fluorescent antibody stain, (3) microscopic examination of sediment of first voided urine and (4) anterior urethral swab and culture and found the last to be the most reliable and accurate method of the four. The last is still used as the routine method of diagnosing gonorrhoea and is often used as the gold standard. The sensitivity is said to be 99% and the specificity 100%. The 1% loss in sensitivity is attributed to the use of selective media that contain vancomycin,

and this antibiotic has been found to be bacteriocidal for some strains of *Neisseria gonorrhoea*. The amount lost by vancomycin is reported to range from 3-4%.<sup>14</sup> It is worth noting that the sensitivity could be less than 99% if the specimen used for inoculation is not adequate. This was demonstrated by the urine samples of Murray et al<sup>43</sup> that yielded more positives than the conventional urethral swab. Thus, in any evaluation, the loss in sensitivity by vancomycin should be estimated by inoculating a random sample on to chocolate agar.

#### Urine Specimen for Asymptomatic Gonorrhoea

Urine examination has been used for the diagnosis of symptomatic gonorrhoea for years. The classical two glass tests of urine where the first is cloudy and the second clear is still a method of diagnosis of urethritis where laboratory facility is non-existent.<sup>72</sup> With the rising incidence of non-gonococcal urethritis, this method is proving to be unreliable and has been virtually abandoned for more reliable laboratory tests.

Taggart<sup>64</sup> in 1955 described gonorrhoea detection by urine examination. His diagnosis was based on the presence or absence of shreds or pus in the urine. He then allowed the specimens to sit for an hour and then gram stained the sediment looking for gram negative diplococci. Then he cultured the sediment if the microscopic examination was negative. He found a 75% sensitivity in the high prevalence population, better than gram stain of exudate alone. Unfortunately, this method is now obsolete as more reliable methods are available.

His work was ignored until Moore & Pittard<sup>41</sup> evaluated the urine

sediment culture, the bacteriologic loop, the cotton swab as specimens for inoculation. The sensitivities of these three methods he reported were 89%, 93% and 91% respectively. The specificity could not be calculated nor the predictive values as the four fold table could not be constructed from the results given.

His work was followed up by Feng et al<sup>19</sup> who looked at uncentrifuged first voided urine as centrifuging involves more time, and money. They did not have a gold standard as they reported a defect in the hospital laboratory and some urethral swab cultures were lost. The four fold table could not be completed. However, they recorded a sensitivity of 94% for symptomatic disease and noted that it was more sensitive than gram stained smear in detecting asymptomatic disease in that it identified three asymptomatic cases not identified by gram stain.

Polowski<sup>53</sup>, after reading Feng's<sup>19</sup> article, in a letter to the editor, recorded a 100% sensitivity with the FVU and 99% specificity at a prevalence of 18%. Unfortunately, the sample of patients used was not described nor their referral pattern, therefore the generalisability is in doubt. In addition, the one culture plate for urine and urethral swab (biplate) was used for each patient which could give a high diagnostic suspicion bias as growth on one side often provokes intensive search of growth on the other side, and prolonged incubation.

The Boston group headed by Murray<sup>43</sup> did a controlled follow-up study of the uncentrifuged first voided urine. The main criticism with this excellent study was that the spectrum of patient selected for eval-

uation was very narrow in that they either had to have 'symptoms', 'signs' or 'suspicion' of gonorrhoea - so that their pre-test likelihood of the disease was quite high and the result could not be generalised to a population where the pre-test likelihood will be quite low. In addition, there was no mention made of blind comparison with the gold standard. However, a four fold table has been constructed with the urethral swab culture as the gold standard. (See Figure 5.3) The predictive value of the test was calculated. In the event of a negative test, the predictive value was 100%, but in the event of a positive test, the predictive value was 99% at a prevalence of 19% of the disease. I calculated the positive predictive value as 76% and negative predictive value as 100% at a prevalence of 1% of the disease.

These findings were similar to that of Luciano and Grubin<sup>35</sup> who compared gram stained smears, urethral swab cultures and culture of first voided urine. Using the urethral swab as gold standard, the properties of the first voided urine for asymptomatic urethral gonorrhoea was calculated as 85% sensitivity, 100% for specificity, at a prevalence of 2.8%. The predictive values for positive and negative results were 100% and 99.5% respectively. Their population spectrum was wider than that of Murray et al but did not include all diseases with similar symptoms that could be confused with gonococcal urethritis. There was no mention of independent blind comparison with the gold standard nor was the order of the three tests randomised to ensure that each test had equal chances of being positive. The urethral swab was always done before the FVU. In addition, the method of urine collection was not adequately described nor was the utility of the

test determined. The sample size was 248 asymptomatic cases. This sample is too small for validity, because in Chapter 3, Table III.1, the minimum sample size required to detect a sensitivity of 87% at a prevalence of the disease of 2% is 1,540, six times the size used in his study. In addition, his sample was drawn from venereal disease clinic patients and may not represent the rest of the asymptomatic population.

Because of the methodologic weaknesses of these two studies<sup>43,35</sup>, and considering sensitivity and specificity may change in a different population, there is a need for critically evaluating this test for use in male asymptomatic urethral disease in Sierra Leone.

It must be borne in mind that the predictive value of 75% by Murray et al is because of the false positive rate attributed to the urine specimen with the urethral swab as the gold standard. With blinding and elimination of diagnostic suspicion bias, this would indicate that the urine as a specimen is a more sensitive detector of the disease than the present gold standard as false positives on culture do not exist.

In response to a criticism that if the patient had just voided urine they would be missed by the urine or urethral swab method; this was not found by Judson et al<sup>30</sup>, who found that recent micturition does not affect the detection of urethral gonorrhoea by the urethral swab method, but does affect the direct smear and gram strain technique.

## 2.2 Methodological Review

Sackett and Holland<sup>58</sup> in their excellent article "Controversy in the detection of disease" have clearly listed what should be looked for in a 'test' before it is put into use. Similarly, if one wants to evaluate a

test, the setting that is would be generalisable to or used should be borne in mind, especially after Shannon<sup>62</sup> reminded us that "sensitivity and specificity may change".

McNeil<sup>38</sup>, in her article on determining the value of diagnostic and screening tests, recommended the use of the Decision Matrix for binary outcomes. This thesis involves binary outcomes - growth is abnormal and no growth is normal. Ransohoff and Feinstein<sup>56,71</sup> have stressed the importance of spectrum and bias in evaluating the efficacy of diagnostic tests. They have mentioned that the test will not be valid if evaluated with the use of florid cases.

A mixture of totally asymptomatic, mildly symptomatic and symptomatic (such as dysuria cases in schistosomiasis, urinary tract infection (commonly confused disorders) should be selected. They have also mentioned the work-up bias that tends to underdiagnose the disease. This is associated with fixing the prevalence of the disease during test evaluation. The diagnostic review bias is important to be avoided in the study as decision as to whether the case is symptomatic or asymptomatic should be made before the test result is known.

The diagnostic suspicion bias could also be avoided by using two separate culture plates for each test and blinding the technician as to which two plates are from the same patient by using differing numbers rather than names on the plate.

Bell<sup>5</sup>, in his article "Efficacy, What's That?" has clearly stressed the importance of determining the utility of the test. This will be the second stage of the study. In designing the second phase of the study,

the article of Prorok et al<sup>55</sup> on "Concepts and Problems in the Evaluation of Screening Programmes" will be kept in mind.

## CHAPTER 3

### RESEARCH PROTOCOL

#### 3.1 THE RESEARCH QUESTION

"Is the first voided urine a valid specimen for culture to identify male asymptomatic urethral gonorrhoea?"

#### Supplementary Questions

1. What is the prevalence of asymptomatic urethral gonorrhoea in the study population?
2. What is the prevalence of urethral gonorrhoea (symptomatic and asymptomatic) amongst the study population?
3. What is the prevalence of penicillinase producing *Neisseria gonorrhoea* amongst the study population?
4. Is the prevalence of asymptomatic urethral gonorrhoea higher in a high risk group when compared to a low risk group drawn from the study population?
5. Is the prevalence of symptomatic urethral gonorrhoea higher amongst the high risk group when compared to the low risk group?

#### 3.2 RESEARCH DESIGN

The design chosen for evaluation of this test is a cross-sectional analytic study. The architecture is shown in Figure 3.1.

Figure 3.1Architecture of the StudyDesignGold StandardUrethral Swab (US) Culture

+ve                    -ve

FVU + ve	a	b	a + b
Culture -ve	c	d	c + d
	a + c	b + d	N



Sampling  
Direction

Justification of the DesignPre-defined sample

A pre-defined sample is used to ensure an appropriate spectrum of patients for the test evaluation. It enables a wide cross-section of the population to be studied thus permitting generalisability of the result.

One-shot study

There are two reasons for this choice. Firstly, the manoeuvre, collection of specimen by urethral swab and FVU will be done at the same time, thus one-shot. Secondly, it avoids the bias involved with setting the prevalence of the disease and thus the predictive values, especially if the sensitivity and specificity is known. In addition it will be unethical to wait for the result of one test, at least 48 hours before performing the second test, leaving a patient untreated, especially if he has

symptoms and signs of infection.

Urethral swab as a gold standard

The urethral swab is the recommended method of specimen collection for asymptomatic urethral disease<sup>11</sup>. Three studies<sup>25,43,35</sup> that have evaluated methods of specimen collection for urethral disease have found the urethral swab to be exceedingly reliable. Handsfield<sup>25</sup> recorded a sensitivity of 83.7% for urethral swab amongst asymptomatic contacts when the prevalence of the disease was 70%. Sixty-one specimens were studied. Murray<sup>43</sup> looked at 514 symptomatic and suspicious males and reported a sensitivity of 99%. The prevalence of gonorrhoea in Murray's sample was 19%.

Luciano<sup>35</sup> compared FVU and urethral swab and reported a 100% sensitivity for the urethral swab. He studied 248 asymptomatic cases and his prevalence was 2.8%. The urethral swab has stood the test of time and has been used for screening for asymptomatic urethral disease in men by Handsfield<sup>25</sup>, Hein<sup>27</sup> and Thatcher<sup>65</sup>. A better method has yet to be identified. Despite its limitations the urethral swab will be used as a gold standard.

3.3 DEFINITIONS

Asymptomatic male urethral gonorrhoea is any male from whom *Neisseria gonorrhoea* is isolated from culture of urethral swab and/or FVU specimens, who denies subjective signs or symptoms of urethral gonorrhoea, such as frequency of micturition, dysuria (pain on micturition), and urethral discharge (or exudate).

First Voided Urine

The first 10 ml of urine voided through the urethra into a container.

*Neisseria gonorrhoea*

A gram negative coccii, identified by typical colony morphology on selective media, oxidase positive and gram negative diplococci on microscopic examination.

Urethral Swab (US)

For this study, this will be a calcium alginate urogenital swab.

Sensitivity of the test

The incidence of true positive results obtained when the test is applied to subjects found to have *Neisseria gonorrhoea* by the gold standard.

Specificity of the test

The incidence of true negative results obtained when a test is applied to subjects shown not to have *Neisseria gonorrhoea* by the gold standard.

Predictive Value

The predictive value of a test is the ability of the test to correctly identify those with and without the disease of interest when applied to a mixed population of healthy and diseased individuals.

Susceptibility of the organism

This is the minimum inhibitory concentration (MIC) of an antibiotic required to prevent the growth of an organism.

Candle extinction container

This is an air tight container. A lighted candle is inserted before closing off the container. The candle will go off once all the oxygen in the air within the container has been used up, and produces carbon dioxide. The air within the container now contains between 5-10% carbon dioxide which is essential for growth of *Neisseria gonorrhoea*.

Urban

Those residences that are within the city limits of Freetown, and the township of each of the 12 districts at the time of independence in 1961.

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Peri-urban

Those residing in villages around the city limits now known as greater Freetown and the extended boundaries of all the original towns in the 12 districts.

Rural

Those areas in Sierra Leone not called urban or peri-urban will all be classified as rural.

3.4 SELECTION OF STUDY POPULATION

In choosing the study population the following were considered:

1. The appropriate spectrum of patients<sup>70</sup>.
2. The utility of the test
3. The feasibility of the study and the avoidance of volunteer bias.

(1) The spectrum of patient

Sexually active males of all ages are at risk of infection. The

risk only increases with increasing changes of sexual partners by both parties as the probability of meeting an infected partner increases with changes. As there is no way of telling an individual or groups of individuals with multiple changes of sex partners, a defined cross-section of the males in Sierra Leone will be represented. The disease spectrum will also be adequately represented by including those with/and without the disease, and those conditions that mimic the disease.

(2) The utility of the test

The test, if validated, would be used for screening selected populations found to have a high incidence of the disease or where a downward trend in an area of high incidence was not observed. Thus, population groups similar to those that would be used in the future will be selected.

(3) Feasibility

Setting up a fixed clinic such as an STD clinic to collect the appropriate number of patients will bias the outcome. Volunteers only would participate and they may not represent the population. Secondly, only those that use health care facilities would tend to volunteer. This bias will be greatly reduced by using a mobile clinic and taking the clinic to the study population and by improving participation through the leaders of the community.

SAMPLE

5,000 heterosexual males from the study population that meets the inclusion/exclusion criteria.

Inclusion criteria

1. Sexually active males aged 15-75 years.
2. Verbal consent to participate.
3. Has the ability to void urine through the urethra.

Exclusion criteria

1. Currently on antibiotics or use of antibiotics in the preceding three days.
2. Prior participation in the study.

3.5 Study Population

Because Sierra Leone is made up of groups with diverse social standards and socio-economic status, the sample has been stratified to give adequate representation of the population, and to ensure an appropriate spectrum of disease.

Urban Population

1. Men attending the surgical out-patient clinic at the Connaught Hospital.
2. Men working at a factory or construction site that has a health clinic or private physician for health care provision.
3. Men working as stewards in exclusive homes of the suburbia.

Rural Population

In some of the rural areas urinary schistosomiasis is a common cause of transient dysuria and bloody urine. The effect this will have on a man's perception of gonorrhoea is not known. Thus it is essential that schistosomal and schistosomal-free areas be used to see if schistosomiasis has any effect on the prevalence of gonorrhoea. This is important as schistosomiasis is known to produce infertility in women. For those not

familiar with tropical diseases, schistosomiasis is often diagnosed as non-specific urethritis, urinary calculi, urinary tract infection and even gonorrhoea.

The rural population will consist of:

1. Men living in villages in a schistosomal-free area served by a health centre in the Northern Province.
2. Men living in villages in a schistosomal area served by a health centre in the Southern Province.
3. Men working in an industrial complex in the Northern Province, e.g. Tobacco Company.
4. Men working in a diamond mining company in the Eastern Province.

### 3.6 Sample Size Consideration

The following were considered important variables when the sample size was being calculated.

1. That the sensitivity of the FVU as a specimen should be found to be no less than 80%. This appears to be a reasonable estimate as Luciano looking at 248 asymptomatic males found a sensitivity of 86%. The other workers that looked at symptomatic urethral gonorrhoea found even higher sensitivities. A sensitivity of less than 80% will not be control-effective in a screening situation. The reason is that 20% or more of this reservoir will not be identified by the test (the false negative rate). This proportion of individuals will not be treated; they will continue spreading the infection and a fall in the incidence data may not be observed, thus it will not be control-effective.
2. That the prevalence of the disease is no less than 1%. A preval-

ence of 1% was selected because screening for disease at a prevalence of less than 1% will not be cost-effective nor control-effective. The choice of 1% was thought to be important because in the USA, excluding the results of Thatcher, the prevalence was found to be no less than 1%. The higher the asymptomatic rate, the more cost-effective and the more control-effective it will be. Belsay and Muir<sup>2</sup> have reported that the prevalence of asymptomatic gonorrhoea in Africa is greater than 1% as only one report from Zaire recorded a prevalence of less than 1%. The specificity has been ignored as no false positives could exist. Isolation of *Neisseria gonorrhoea* is pathognomonic of the disease. False negatives may exist that could not be identified by either methods, thus could not be measured.

#### Sample Size Calculation

The maximum discrepancy between the sample sensitivity and the population sensitivity I am willing to tolerate is  $\pm$  10 percent (absolute percent). I want to be 99% certain that the sample sensitivity is within the lower level of the confidence interval. Thus 1% on 1-tail of the normal distribution,  $Z = 2.32$ .

The discrepancy between the sample proportion and the population proportion is given by  $100p - 100\bar{X} = \pm 10$ .

The critical ratio follows:

#### Equation 1

where  $p$  = sample sensitivity

$\bar{X}$  = population sensitivity

$$Z = \frac{CI}{100\sqrt{\frac{\bar{X}(1-\bar{X})}{n}}}$$

= 0.90 based on the results of the two evaluation studies<sup>35,43</sup>

Solving for n

$$\begin{aligned}
 n &= \left( \frac{Z_{\alpha} \times 100}{CI} \right)^2 \pi(1-\pi) \\
 &= \left( \frac{2.31 \times 100}{10} \right) 0.9 (0.1) \\
 &= 48.02
 \end{aligned}$$

n = the number with the disease that should be identified. Thus at a prevalence of 1% - the population that has to be screened will be 4802, at 2%, 2400. Allowing 15% for dropout, thus the sample size required is 5522.3.

The Sample Size N = 5523

TABLE VI: Sample Size N for Different Prevalences,  $Z_{\alpha}$  and Confidence Interval.

Confidence Interval Around II	$Z_{\alpha} = 1.96$		$Z_{\alpha} = 2.32$	
	1%	2%	1%	2%
±10.0%	3450	1725	4844	2422
± 7.5%	6146	3073	8100	4050
± 5.0%	13,829	6914	19,209	9604

### 3.7 Feasibility Study

During the training of the staff, the manoeuvre will be performed on patients attending the Fourah Bay College Health Centre. This would be in a case finding situation to see if asymptomatic disease does exist in our health centre, where the incidence of gonorrhoea is known.

### 3.8 Pre-test

Aim - To test the reliability of the manoeuvre. The pretest will involve screening all the male adults in the mountain villages near the Fourah Bay College campus. The population in these villages is similar to the study population. The mobile clinic, as it would be used during the study, will be set up at the Village Hall or school room for three weeks.

The following items will be measured.

1. Participation rate - An 80% participation rate would be aimed at. If this is not achieved in three weeks, a coercive method would have to be introduced such as free multivitamins, to increase the rate to 80%.
2. The time required for a patient to receive the questionnaire and specimen collection. This will facilitate maximization of time during the study.
3. The uniformity of specimen collection by the two clinicians, for both the FVU and the urethral swab.
4. The reliability and the validity of the study questionnaire in English and the other languages.
5. The usefulness of cross streaking the plates.

For details of questionnaire and reliability testing see appendix.

The demographic data and sexual history are constructs, whereby a high risk

group could be identified. These risk groups will become predictor variables for prevalence of gonorrhoea in Sierra Leone. The validity of these predictor variables will be tested against the prevalence of the disease as part of the secondary analysis. The symptoms and signs are concurrent variables and these will be used in the primary analysis to see how valid symptoms and signs are in predicting gonorrhoea in Sierra Leone.

#### SITE

This clinic will be set up close to the population to be studied. The site will not be fixed. Similarly, the incubator and refrigerator, the major components of the laboratory, will travel with the staff.

#### Staff of Mobile Unit

Research Assistant

Clinicians X 2

Laboratory Technician

Interviewers X 2

Driver

#### 3.9 Data to be collected

The following information will have to be collected:

1. Demographic data
2. Source of Health Care
3. Past Medical History (including STD History)
4. Current use of antibiotics
5. Presence of symptoms or sign of gonorrhoea at the time of interview
6. Date of last sexual exposure and number of sexual contacts in

last 30 days.

7. Urine Specimen (FVU)

8. Urethral Swab Specimen

**3.10 The Manoeuvre**

**Data Collection Procedure**

**Consent**

A verbal consent will be sought by a prominent member of that community, who would be assigned to the temporary job of "contact person" for that community. The presence of a male in the clinic site is a sign of willingness to participate. The head of the population would have informed the men of the importance of participation. Coercion method may have to be used, depending on the study site. This will be in the form of free multi-vitamins. The latter are relatively cheap.

**Structured Interview**

This is the method of choice for collecting the first six items listed under data to be collected. A standardised questionnaire that facilitates this data collection is found in Appendix I. The reliability of this questionnaire will be established during the pretest and the validity during the analysis of the result. The questionnaire will be administered by a trained interviewer in private in the language chosen by the participant. The interviewers will be multilingual, and the need for an interpreter should not arise, as interpreters tend to reduce confidentiality and reliability, the latter two being essential for this study.

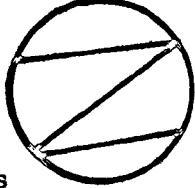
Data Collection ProcedureClinical Data Collection

Following completion of the study questionnaire, the participants would then enter one of the clinical examination rooms. A brief history and physical examination of the genitalia will be performed by the clinician.

The order of specimen collection would be alternate with all even numbers getting the FVU first and all odd numbers getting the urethral swab first. Alternate rather than random was chosen because of the ease of execution and simplicity. Although random order of specimen collection would be the ideal method to ensure equal chance between the two specimens, this will result in slowing of the process of clinical data collection as the clinicians will have to be checking a randomisation schedule before each patient. In addition, the alternate method will allow for simpler analysis if there is a systematic difference by order of specimen collection. The reasoning behind this is that in asymptomatic disease, the inoculum is quite small. Performance of any one of these tests does reduce the inoculum size. The significance of this reduced inoculum is not exactly known. However, by using alternate methods, if the error is marked it could be assessed. Details of assessment of this error is not provided as Judson<sup>30</sup> had suggested that recent micturition does not affect the culture results from urethral swab. However, he studied only 28 cases of asymptomatic urethral disease. The rest of his study patients were symptomatic. Thus, it is difficult to rely on a single report of 28 cases. Urethral swab specimen will be collected, using a sterile calcium alginate urogenital swab as detailed in Chapter 1. The swab will be rolled across

a culture plate containing modified Thayer Martin Media, to make an N - No streaking will be done by the clinician. See Appendix II. See Figure 3.2.

Figure 3.2



Chocolate Agar plates

500 specimens (5% of all the specimens collected, 10,000) will be randomly selected to be inoculated on to a second plate, the chocolate agar plate. The reasoning behind this is that the antimicrobials used in the selective media are known to reduce isolation rates of *Neisseria gonorrhoea*. The loss from selective media is reported to vary from 1 - 4%. It would thus be reasonable for this study to measure its loss in strains from use of selective media.

First Voided Urine

The participant will be asked to void the first 5 to 10 ml of urine into a 10 cc sterile bottle with a wide opening and then hand the container of urine to the clinician. A sterile cotton swab will be inserted into the urine, stirring, and the swab used to inoculate a modified Thayer Martin Culture plate. The color, consistency and appearance of the urine will be recorded by the clinician. Details of the data to be collected by the clinician are found in Appendix III.

Inoculated Plates

All plates will be labelled and put into candle extinction metal containers. This would reduce breakage during transport as glass petri

dished will be used. The metal containers will then be sent to the mobile laboratory together with laboratory request data form. See Appendix IV.

#### Laboratory Data Collection

The technician will be blinded with regards to the clinical status of the participants. The two plates inoculated from each participant will be given different numbers in order that a work-up bias will be avoided. The latter often occurs when there is pairing of plates, and growth on one plate causes intensive search for growth on the other pair. The laboratory technician, on receipt of the metal container, will check the plates so that the identification number on the plates tallies with that on the laboratory request form. A quality control plate inoculated with fresh isolates of *Neisseria gonorrhoea* will be added to each metal container, the candle re-lighted and the container incubated at 35°C. All plates will be incubated within four hours of specimen collection. The culture plates will be checked for growth at 24, 48 and 72 hours flooded before being discarded. Any growth found on the plates will have to be identified by the technician. Identification of *Neisseria gonorrhoea* will be recorded as positive and the result sent out to the research assistant/ or principal investigator. All positives would also be tested for penicillinase production.

#### IDENTIFICATION OF NEISSERIA GONORRHOEAE

1. Typical colony morphology - greyish white, opaque, small colonies on Modified Thayer Martin Media. Colony size 1-4 mm.
2. Oxidase Testing. A fresh solution of 1% tetramethyl p-phenylenediamine hydrochloride solution will be used. A few drops will be added to a few colonies suspected to be *Neisseria gonorrhoea*. A pink color, pro-

gressing to dark red to black, indicates a positive oxidase reaction - and would further support identification of *Neisseria gonorrhoea*. As the dye is toxic to *Neisseria gonorrhoea*, when only a few colonies are present the organism should be subcultured before addition of the dye to the plate.

Gram Stain

Smears of colonies will be gram stained by the Hucker method. Microscopic examination of the stained smear should reveal gram negative diplococci.

Typical colonial morphology, positive oxidase test, gram negative diplococci will be considered pathognomonic for *Neisseria gonorrhoea* in this study. No confirmatory test will be done because of cost. Carbohydrate utilisation test and fluorescent antibody staining, the two most commonly used are too expensive for this study.

Secondly, all specimens are from genital sites and the possibility of error is extremely small.

Identification of Penicillinase-producing *Neisseria gonorrhoea* strains

All *Neisseria gonorrhoea* isolated during the study will be tested for Beta-Lactamase production using Nitrocefin (Chromogenic Cephalosporin). This is being done because it would be useful to know if penicillinase producing *Neisseria gonorrhoea* does exist in Sierra Leone. This method of surveillance is chosen for the following reasons:

1. Cost - It is cheap compared to the other commercially available methods. The nitrocefin is obtained free of charge from Glaxo Research Laboratory in England, the only expenditure being handling charge for shipping.

2. Stable - Fresh solution is made up as required, whilst the commercially available paper methods (such as Beta-Lactam paper) have a shelf life, and are destroyed by warmth and moisture.

3. Simple - The method is simple and reproducible. The test could be performed on ordinary glass slides or applied directly to the colonies and thereby use minimum quantity of solution. A positive test is indicated by a yellow to red color change.

#### Susceptibility testing for the organisms

The susceptibility of the organism to the various commonly used antibiotics (penicillin and tetracycline) will be done to check the minimum inhibitory concentration (MIC) required by all isolated strains of *Neisseria gonorrhoea* during the study. This will be performed using a commercially available disc method. This is a bench method and is only used because of its simplicity. The ideal method is the agar dilution method.

It should be noted that the susceptibility testing of the organism for penicillin is standardised and the result is said to be comparable between laboratories, whilst that of tetracycline is not standardised and the results obtained should only be used as an indication of susceptibility.

#### Positive Case of *Neisseria gonorrhoea*

For ethical reasons and public health requirement, all individuals with positive cultures for *Neisseria honorrhoea* will have to be identified, contacted and treated. Treatment will be done through the health care facility available in the area. All drugs for treatment will be provided by the study. For more details see Appendix V.

### 3.11 Quality of the data

1. Questionnaire: The reliability index of the questionnaire in the various languages will be measured during pretest. The reliability index would be 80% or more before it is used in the main study.

2. Clinical Data Collection: To avoid the diagnostic suspicion bias by the clinician collecting specimens, the information on the questionnaire will not be available to the clinician. The clinicians would have been well trained and their performance assessed for accuracy and uniformity during the pretest. The variation between the two clinicians would be hard to measure because of the type of clinical condition that is being sought. However, regular check on performance to ensure uniformity will be conducted by the principal investigator.

### Quality Control in the Laboratory

The endpoint measure is a laboratory determination. Thus it is crucial that high quality control exist in the laboratory. This starts with appointment of a well trained microbiology technician with adequate training in gonococcal isolation, identification and processing. Quality control will have to involve media preparation, isolation, identification and susceptibility testing.

Media

The details of the production of uncontaminated media is found in Appendix VI. The only way to check the quality of the media is to demonstrate that *Neisseria gonorrhoea* could grow on it. This will be done by inoculating 2% (10 of 500 plates) produced by a known strain of *Neisseria gonorrhoea*. Different strength of inoculum will be used to demonstrate poor and abundant growth.

Check on VCNT

The added vancomycin, colistin, nystatin and trimethoprin are antimicrobials added to inhibit the growth of some organisms, such as *Proteus* and *Staphylococcus*, and permit the growth of *Neisseria gonorrhoea*. Another 2% of the plates, randomly selected will be inoculated with strains of *Staphylococci*, *proteus*, *Escherichia coli*, and *Candida* that are sensitive to these antimicrobials, to ensure that the latter do inhibit their growth.

Oxidase Reagent

This has to be prepared daily. The quality will be tested using *Proteus* or *Escherichia coli*, microbes that are known to be oxidase negative and also a strain of *Pseudomonas aeruginosa* as control.

Gram staining

As batches of staining solution are prepared for use, control organisms that are known to be gram positive and gram negative will be tested.

Incubation

An ordinary incubator will be used.

Candle extinction metal containers will be used to provide CO<sub>2</sub> en-

vironment. To ensure the continuous CO<sub>2</sub> atmosphere within these containers a control plate with a known strain of *Neisseria gonorrhoea* should be inserted. Failure of the growth of *Neisseria gonorrhoea* in the control plate is an indication that CO<sub>2</sub> was not present during incubation.

#### Chromogenic Cephalosporin

The solution should be tested against known positive Beta Lactamase producers such as *Staphylococcus aureus* strains and negative organisms such as non-Beta-Lactamase *Streptococcus* or *Neisseria*.

#### 3.12 The Research Plan

The execution of this protocol will follow approval of funding of the project. The appointment of a research assistant for administration, especially to maintain the time schedule is a necessity for this project. For detail of time schedule see Table III.2. The identification of study sites, estimating the size of the population identified and seeking approval from the heads of the population will follow. This will require a month as site visits have to be made, to get support from the elders and leaders of the communities, to select appropriate clinic sites and to get suggestions from the community about the best time for the study.

Table VII

Time Schedule and Steps in the Research Process

MONTH

- 
- |    |  |
|----|--|
| 0  | Funding granted                          |
| 1  | Appointment of research assistant        |
| 2  | Study sites identified                   |
| 3  | Laboratory set-up - Appointment of staff |
| 4  | Training of staff and feasibility study  |
| 5  | Pre-test                                 |
| 6  |  |
| 7  | Main study                               |
| 8  |  |
| 9  | Analysis                                 |
| 10 |  |
| 11 | Report writing                           |
| 12 | Submission of report                     |

## CHAPTER 4

### ETHICAL CONSIDERATION

#### 4.1 Introduction

Two issues are addressed in this chapter. The first is consent and the second confidentiality<sup>22</sup>. The important reasons for designing this study were considered when discussing the issues, and they are:

- (1) A valid and simple test is being sought to identify asymptomatic urethral gonorrhoea in men.
- (2) A "high risk" group may be identified that need special surveillance and control programmes.

#### 4.2 Consent

Two consents are being sought during the study.

##### 4.2.1 Group Consent

The first is group consent. This is the consent obtained from the area heads for their subjects to be participants of the study. In the case of industrial complexes, permission will have to be given by the management and their Union Officials at the site. In the case of villages it would be from the tribal heads, such as the Chiefs or Members of Parliament or the Village Health Committee. It will be a fully informed signed consent.

Group consent is a necessary but not a sufficient factor for high participation rate.

##### 4.2.2 Individual Consent

Verbal informed consent will be asked of each participant who attend the clinic site. Written consent will not be used as over 50% of men

are still illiterate in Sierra Leone. Secondly their presence in the clinic is a sign of willingness to participate. Thirdly there is very little risk involved with study participation, just a little discomfort with the urethral swab. Lastly, the benefit of such an epidemiologic study to society cannot be truly measured by an individual.

#### 4.3 Confidentiality

To achieve high participation rates, confidentiality has to be maintained and this information made known to the participants. They will be told and shown that all forms and specimens are numbered, and their names are only recorded in a book which will be kept secure and not available to national or local Government. The interview and clinical examination will be done in private rooms on a 1:1 basis. Those found positive will be informed personally by a health worker.

## CHAPTER 5

### ANALYSIS AND INTERPRETATION

#### 5.1.1 Primary Analysis

Endpoints for the Primary Analyses (See Figure 5.1)

##### Definitions

Symptom - free men(T) "are all participants that met the inclusion/exclusion criteria and replied in the negative when questioned by the interviewers about the presence of dysuria, urethral discharge and frequency of micturition."

These above symptom-free men will fall into one of four categories as shown in the fourfold table in Figure 5.1.

- (1) Cell a - 'True positives' "are those in whom *Neisseria gonorrhoea* was isolated from both the urethral swab specimen and the FVU specimen".
- (2) Cell b - 'False positives' "are those from whom *Neisseria gonorrhoea* was isolated from the FVU specimen but not from the urethral swab specimen".
- (3) Cell c - 'False negatives' "are those from whom *Neisseria gonorrhoea* was isolated from the urethral swab specimen but not from the FVU specimen".
- (4) Cell d - "True negatives" "are those from whom no *Neisseria gonorrhoea* was isolated from both the FVU and the urethral swab specimen".

These categories are essential for the evaluation of a diagnostic test but unreal for this study, because any positive result by culture is

pathognomonic for gonorrhoea. Thus, cell a, b and c are positive and the only negative cell by these two tests is cell d.

Figure 5.1

Urethral Swab

		+	-	
		a	b	
FVU	+			
	-	c	d	
				T

### 5.1.2 The Primary Analysis

#### The research question posed

"Is the first voided urine a valid specimen for culture to identify male asymptomatic urethral gonorrhoea?"

#### The Statement

For the FVU to be valid, the sensitivity of the test should be no less than 90%.

#### Calculation

Using Figure 5.1 - the sensitivity of the FVU will be calculated from Equation 1.

#### Equation 1

$$\text{Sensitivity of FVU} = \frac{a}{a + c}$$

Since this is a sample drawn from a population, it is only an estimate of the sensitivity of the test if it is applied to the general population. As sampling error has to be considered when extrapolating results from a sample to a population, the confidence interval will give the limits

within which the true population sensitivity of the test lies. To get a 95% confidence limit on the sample sensitivity for FVU equation 2 below will be used.

If the sensitivity (P) is found to be greater than 0.8, then the FVU will be considered a valid test for the identification of asymptomatic urethral gonorrhoea.

Equation 2

95% confidence interval:

$$p \pm 1.96 \sqrt{pq/n}$$

where p = sample sensitivity calculated

$$q = 1-p$$

T = symptom-free men

n = T - normal men

1.96 = Z score  
2-tailed test

The other properties of the diagnostic test will also be calculated for the FVU as follows:

$$\text{Specificity} = \frac{b}{b + d} \quad \text{Equation 3}$$

$$\text{Positive predictive value} = \frac{a}{a + b} \quad \text{Equation 4}$$

$$\text{Negative predictive value} = \frac{d}{c + d} \quad \text{Equation 5}$$

$$\text{Prevalence of the disease} = \frac{a + c}{T} \quad \text{Equation 6}$$

$$\text{'True' prevalence of the disease} = \frac{a + b + c}{T} \quad \text{Equation 7}$$

Equation 5 is important. The result of the negative predictive value determines the control effectiveness of any programme. The smaller 'c' is the higher the negative predictive value (close to 1 as possible) the better the test. A high 'c' means that the FVU is missing too many cases identified by the urethral swab. Thus, if the FVU was the only test used there will still be a reservoir in the form of 'c' continuing to spread the infection. Two prevalences are calculated but the useful one is Equation 7, as that gives us the true extent of the problem in that study population - as the presence of *Neisseria gonorrhoea* is pathognomonic.

## 5.2 Secondary Analysis

### 5.2.1 Definition of Endpoints - See Figure 5.2

#### Study population

(N) are all participants that met the inclusion/exclusion criteria.

The above will fall into the four categories of the fourfold table.

- (1) Cell  $a_1$  = 'True positives' by both tests.
- (2) Cell  $b_1$  = 'False positives' positive by FVU but negative by the urethral swab.
- (3) Cell  $c_1$  = 'False negatives' positive by the urethral swab but negative by the FVU.
- (4) Cell  $d_1$  = 'True negatives' negative by the FVU and the urethral swab.

Figure 5.2

		Urethral Swab		N
		+	-	
FVU	+	a <sub>1</sub>	b <sub>1</sub>	
	-	c <sub>1</sub>	d <sub>1</sub>	

5.2.2 The analyses in this section relate to the supplementary questions posed.

- (i) What is the prevalence of asymptomatic urethral gonorrhoea in the study population? This could be calculated as follows:

Prevalence of asymptomatic urethral gonorrhoea

$$= \frac{a + b + c}{N} \quad \text{Equation 8}$$

The numerator is all asymptomatic urethral disease found as discussed under primary analysis 5.1.2. The denominator is the study population as defined above.

- (ii) What is the prevalence of urethral gonorrhoea in the study population?

The prevalence of urethral gonorrhoea

$$= \frac{a_1 + b_1 + c_1}{N} \quad \text{Equation 9}$$

The numerators are all those found to have *Neisseria gonorrhoea* both symptomatic and asymptomatic.

The result from Equation 9 is expected to be higher than Equation 8

- as some symptomatic disease is expected to be found in the population.

The size of the symptomatic rate is dependent on the incidence rate and could be estimated if the incidence of the disease in that population is known, since prevalence is equal to incidence  $\times$  duration (number of days the study is conducted in the population.) If the symptomatic infection rate is found to exceed that estimated from the incidence in the population, an explanation should be sought for the discrepancy.

#### 5.2.3 What is the relationship between the symptomatic rate and the asymptomatic rate?

The first response to this question would be none, when the results of the studies done so far are considered. Handsfield<sup>25</sup> and Hein<sup>27</sup> did a prevalence study in healthy (asymptomatic) population, and found a rate of 2.2 and 1.9% respectively. Similarly, Dexter<sup>17</sup> in New York and Luciano<sup>35</sup> looked for asymptomatic disease in venereal disease clinics where the symptomatic gonorrhoea rate is high and found a prevalence of 1.5% and 2.8% respectively, not much different from the result of Handsfield<sup>25</sup> and Hein<sup>27</sup>.

However, the transmission of gonorrhoea as outlined in Figure 1.1 will suggest another answer - that is, the higher the symptomatic disease, and higher the asymptomatic disease. This is because it is postulated that 80% of infected men develop typical acute urethritis, 2-5% develop asymptomatic urethral gonorrhoea, and 15-18% a typical urethritis. Thus, the higher the symptomatic disease rate, the higher we should expect the asymptomatic disease.

#### 5.2.4 What happens if $b_1$ is greater than $c_1$ or $b$ is greater than $c$ ?

(from Figure 5.1 and 5.2)

The possibility of the FVU being a better method than the urethral

swab then needs to be considered. This will only be considered if  $b_1$  or  $b_1$  the 'false positives', are greater than  $c$  or  $c_1$  the 'false negatives'.

As an example, we could consider the results of Murray et al<sup>47</sup> as shown in Figure 5.3.

Figure 5.3.

Culture Results  
Urethral Swab

		+		-			
		+	99	a	b	1	100
FVU Culture Results	+	0	c	d	414	414	514
	-	99			415		

The FVU identified one case more than the urethral swab. As the latter was acting as the gold standard, the 1 in cell b became a false positive: If the FVU now becomes the gold standard, the urethral swab will have a sensitivity of 99% and FVU a specificity of 100%. Thus, if the numbers in cell b are larger than in cell c, then the FVU should be considered a better method.

#### 5.2.5 "Is the prevalence of gonorrhoea significantly higher amongst a group of men labelled 'high risk' when compared to another group labelled 'low risk'?"

##### High Risk Men

Men in the study population that are found to have all of the following characteristics, will be identified from the study questionnaire and labelled High Risk group  $N_1$ .

- (1) Young, aged 15-29 yrs.
- (2) Unmarried.

(3) Urban residence

(4) More than one sexual partner in the preceding 1 month.

In the USA, gonorrhoea is said to be higher among the young, the poor, the unmarried and those sexually active with multiple sexual partners. These characteristics, except 'poor', will be identified by constructs in the questionnaire and would thus be used as predictor variables. The purpose of this analysis is that these predictors are thought to apply world-wide and may not be the case. Poverty has been dropped out because of the difficulty in defining poverty for Sierra Leone.

Low Risk Men

Similarly men in the study population that are found to have all of the following characteristics, will be identified from the questionnaire and labelled Low Risk N<sub>2</sub>.

(1) Married

(2) Has children

(3) Rural or periurban residence

(4) Health care is normally sought from a conventional health care system, physician, hospital, health centre.

The prevalence of urethral gonorrhoea amongst these two groups of men will be compared.

Null hypothesis H<sub>0</sub>: That there is no difference in the prevalence of urethral gonorrhoea between the Low Risk and High Risk group.

Alternature Hypothesis H<sub>1</sub>: That the prevalence in the High Risk group is greater than the prevalence in the Low Risk group.

Statistical Method

This involves comparison of two independent proportions.

See Figure 5.4.

Figure 5.4

	High Risk Men	Low Risk Men	
GC +VE	a	b	
GC -VE	c	d	
	N <sub>1</sub>	N <sub>2</sub>	N <sub>1</sub> + N <sub>2</sub>

The prevalence in the High Risk group

$$= \frac{a}{N_1}$$

The prevalence in the Low Risk group

$$= \frac{b}{N_2}$$

The comparison could be done through an approximate method the 'chisquare' -see Equation 11.

$$\chi^2_{1\text{ df}} = \sum \frac{(O-E)^2}{E}$$

'p' the probability of the difference observed being due to chance is set at 0.05. Similarly, the prevalence rate of asymptomatic urethral gonorrhoea in these two groups could be compared.

5.2.6. What is the prevalence of Penicillinase producing *Neisseria gonorrhoea* (PPNG) ?

The prevalence of PPNG in the study population =

$$\frac{\text{No. of PPNG strains isolated}}{\text{study population}}$$

Equation 12

5.2.7 Is the prevalence of asymptomatic gonorrhoea higher amongst the men living in the rural schistosomal area when compared to men living in the rural non schistosomal area?

The comparison will be done by the chi-squared method as in  
5.2.5. A similar analysis will be done for symptomatic gonorrhoea.

### 5.3 Analysis of Questionnaire Results

The result of question 5 will form the basis of the primary analysis as all symptomatic cases will be excluded.

The demographic data will be used to locate positives, and together with the other social and sexual constructs will be used to identify the two mutually exclusive groups, High Risk and Low Risk.

The results of the questionnaire are tied in with the results of the laboratory tests.

## CHAPTER 6

### ADMINISTRATION AND BUDGET

#### 6.1 The Investigation Team

##### 6.1.1 The Research Assistant

The Research Assistant (R.A.) will be the key to the administration. He will be employed for the duration of the study and will have the responsibility of the day to day running of the project. The R.A. will be answerable to the Principal Investigator and work closely with the latter.

The data processing, coding of forms, completing data files for each participant in preparation for analysis will be his responsibility. In addition, the R.A. will assist in the analysis of the data.

Confidentiality is expected of the Research Assistant as he/she will have access to the name file and will have to break the codes to identify positives.

##### 6.1.2 The Principal Investigator

The overall supervision of the project is her main responsibility. Recruitment, training of staff and maintenance of quality data is also her responsibility. Reports from all the different employees could be directed to her or through the research assistant. The analysis of the data and the writing of the report she will perform at the conclusion of the study.

##### 6.1.3 Clinicians

They will be trained Male Nurses (SRN or experienced dispensers) who would receive training on the sexually transmitted diseases from the

Principal Investigator. As clinicians, they will be expected to take a brief history and perform examinations of the male genitalia and record their findings. The collection of specimens and inoculation of culture plates are their responsibility.

#### 6.1.4 Interviewers

They will be hired from the University Demography Department. They must be trained, experienced interviewers, and be able to speak two Sierra Leonean languages in addition to English and Krio. They will receive additional training from the Principal Investigator on how to collect the information on sexually transmitted diseases.

#### 6.1.5 Consultant Pathologist

This individual will act as a member of the Study Committee and will advise the Principal Investigator about the laboratory set up and quality control within the Laboratory. He will be expected to perform spot checks on the Laboratory to ensure that quality is maintained throughout the study.

#### 6.1.6 Microbiology Lab Technician

A trained Microbiology Lab Technician with at least three (3) years Laboratory experience will be used. His/her job will include media preparation in the base Lab, maintaining the culture plates at the right temperature until they are ready for use. In the mobile Laboratory, he/she will be responsible for incubation of the plates and identification of all growths found, sensitivity testing and Beta Lactamase testing.

#### 6.1.7 Driver

He should be capable of driving a heavy duty van, as a trailer for the Laboratory equipment will be attached to the vehicle used to transport the study team.

Apart from driving the vehicle, he will assist in maintaining the generator. He will be sent out to canvass for participation.

#### 6.1.8 Contact Man

This will be a local employee, who is well known and respected by the people. His job will be to inform them of the procedure, seek verbal consent and allay any fears they may have. He will be expected to give feedback as to complaints, discomforts or anxiety expressed by the participants. In a factory, this will be the shop steward or a prominent "party" man or "union" man. In the Hospital, this will be the hospital porter. In the village, the ideal person would be the herbalist or village medicine man.

#### 6.1.9 Laboratory Assistant

This would be a part-time worker, employed for 2 days a week to assist the technician with media preparation. His duties will be that of washing the petri dishes and the bottles used for urine collection prior to sterilisation.

6.2 BUDGET6.2.1 Personnel Services

Consultant Pathologist (part-time)	18/12	\$ 600	\$ 900
Research Assistant	18/12	4,000	6,000
Clerk/Typist part-time	18/12	1,500	2,250
Microbiology Lab. Tech.	12/12	6,000	6,000
Clinicians x 2	6/12	5,000	5,000
Interviewers x 2	6/12	4,000	4,000
Driver	6/12	3,000	1,500
Laboratory Assistant	6/12	1,000	500
General Purpose Man \$10.00/day x 65 days			650
			<hr/>
			\$26,800

6.2.2 TRAVEL

Overnight allowance within Sierra Leone:	Staff over 2/12 @ \$10/day x 40 x 7	2,800
	Consultant @ \$20/day x 5	100
	Principal Investigator @ \$20/day x 20	400
Out of Sierra Leone:	Principal Investigator Airfare \$1000	1,000
	Presentation of Report and final Exam @ \$100/day x 10 days	1,000
Fuel - \$4/gallon @ 20 mi. to a gallon estimated 3,000 miles		600
Vehicle Rental per day @ \$30/day x 4 months		3,600
Travel Allowance for research assistant @ \$80/month x 12		760
		<hr/>
		\$10,260

**6.2.3 Office Equipment & Furniture**

Typewriter	FREE
Calculator	FREE
Office Furniture	FREE
Office Space - FBC Health Centre	FREE

**6.2.4 Materials & Supplies**

Stationery and Office Supplies	\$ 1,000
Telephone - Local and Long Distance within Sierra Leone	120
Heavy Duty Plastic Bags	50
	<hr/>
	\$1,170

6.2.5 Equipment

	US \$
Petri Dishes - Glass - 1000	1,600
Culture Media (Dehydrated base)	5,000
Hot Air Oven	500
Urogenital Swabs (10,000)	2,500
Cotton Swabs (40,000)	200
Stains, Dyes & Sensitivity discs	2,000
Refrigerator	700
Generator (Honda - Gasoline)	1,000
Microscope - Light Microscope	3,000
Incubator	600
Candles x 200	100
Steriliser - Autoclave to sterilise media	7,400
Drugs: 1. Ampicillin (3,000 capsules)	600
2. Tetracycline (5,000)	450
3. Probenicid (1,000)	150
4. Spectinomycin (50 gm, 25 vials)	250
Water Bath	600
Flasks, pipette, cotton swabs	2,000
Stirring hot plate (Corning)	200
Beta Lactamase detection paper (1,000)	<u>550</u> 29,400
Stationery	1,170
Travel	10,260
Personnel	<u>26,800</u> \$67,630

### 6.3 Justification of Budget

#### 6.3.1 Personnel

The job description of each member of the personnel list has been outlined in Chapter 4. The consultant pathologist has to be involved from the start of the study as he would be advising on recruitment and also laboratory quality control and safety and will be involved till the very end. If the evaluation is successful, he should be able to tell us the best way of utilising the present laboratories in the country for maximisation of screening programme.

The Research Assistant also has to be involved in the feasibility study and also until the final report is concluded.

#### 6.3.2 Equipment

The Laboratory at the University Health Centre, which will be used as the reference lab is inadequately furnished as it was equipped for non-cultural work. Thus equipment for handling culture materials such as steriliser, culture plates, microscope has to be bought. A microscope, a refrigerator and an incubator that could be run on a generator is requested. Electrical supply is not a guarantee even in the capital, so provision for another source of power has to be made for the incubator, microscope and freezer.

### 6.4 Funding

#### Pre-test and Main Study

Application will be made to

1. World Health Organization Veneral Disease Branch through the regional office in Africa.

2. Planned Parenthood Association, African Region, Headquarters in Kenya.
3. Rockefeller Foundation in the U.S.A.
4. ODA - Overseas Development Agency through the British Council.

#### 6.5 Reports and Publications

The results of this investigation will be submitted to the West African Postgraduate Medical College as a report of an independent work done in Community Health, as part requirement for the final examination of the West African College of Physicians.

The findings will later be reported in one of the Venereal Diseases Publications, depending on which part of the world the funding of this proposal emerges.

## CHAPTER 7

### SUMMARY

#### 7.1 Criteria for Success

- 7.1.1 A participation rate of 80% or more in the geographically defined study population.
- 7.1.2 That the sensitivity of the FVU for asymptomatic urethral disease is greater than 90%
- 7.1.3 Finding a prevalence of 1% or more for asymptomatic disease.

#### 7.2 Suggestions for further study

- 7.2.1 If the FVU is found to be valid and the utility could be established by finding a high prevalence and a good contact tracing yield, the evaluation of this as a control programme would have to be measured in a randomized trial with pelvic inflammatory disease as the main endpoint.
- 7.2.2. The cost-effectiveness of the FVU as a routine diagnostic method.
- 7.2.3 The cost-effectiveness of the FVU as a screening tool in a control programme in conjunction with 1 above.

#### 7.3 Summary

Male urethral gonococcal carrier is now recognized as an important factor to be addressed in any successful gonorrhoea control programme. The urethral swab specimen is currently used to identify these individuals. However, it is not felt to be suitable for screening in Sierra Leone.

The literature was sought for a simple, cheap and unobtrusive method. The uncentrifuged first voided urine was appealing.

This design thesis embodies a protocol for validating the first voided urine as a specimen for culture to identify male asymptomatic urethral gonococcal carriers in Sierra Leone.

To ensure generalisability of the result, a cross-sectioned sample of the men in Sierra Leone that represent the male population and the spectrum of disease will be selected.

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**APPENDICES**

APPENDICES

- I.1 The Study Questionnaire
- I.2 Method of Determining the Reliability of the Questionnaire
- I.3 Method of Determining the Validity of the Questionnaire
- II Cross-streaking of Culture Plates
- III Clinician's Data Sheet
- IV Laboratory Request Form
- V Treatment of Positives
- VI Preparation of Uncontaminated Media

- APPENDIX I.1

THE STUDY QUESTIONNAIRE

Study No:

Date of Administration:

1. Which language would you like me to use to ask the questions?

Please tick

English

Krio

Temne

Mende

Limba

Other

2. What is your name (s)? Please list and state where commonly used.

(i)

(ii)

(iii)

(iv)

3. Addresses: Please list and state work or home and preference if we need to contact you.

(i)

(ii)

(iii)

(iv)

4. Are you presently taking or have you taken any capsules, tablets, injections or mixtures within the last 1 week?

Yes       No

If so,

Type of Medication      Duration of Use      Purpose of Medication

(i)

(ii)

(iii)

5. Have you noticed any discharge from your penis, or pain on passing urine, or frequency of micturition?

Yes       No

If so, describe

Symptom      Duration      Amount or Severity

(i)

(ii)

(iii)

Have you sought treatment for your symptoms?

Yes       No

If yes,

When      Where      Treatment given

Were you considering seeking medical care for symptoms?

Yes       No

6. Are you presently

- |               |                          |
|---------------|--------------------------|
| Married       | <input type="checkbox"/> |
| Widowed       | <input type="checkbox"/> |
| Divorced      | <input type="checkbox"/> |
| Separated     | <input type="checkbox"/> |
| Never married | <input type="checkbox"/> |

7. Do you have children?

Yes       No

If so,

How many?

8. How old are you?

Age in Years

9. Are you presently

- |            |                          |
|------------|--------------------------|
| Employed   | <input type="checkbox"/> |
| Unemployed | <input type="checkbox"/> |
| Retired    | <input type="checkbox"/> |
| Student    | <input type="checkbox"/> |

10. What type of work do you do?

Please list 1.

2.

3.

11. When was the last time you had sexual intercourse?

Date \_\_\_\_\_

12. How many women have you had sexual intercourse with in the last 30 days?

*State number*

13. How many times have you had gonorrhoea, urethral discharge or pain on passing urine during the last year?

*State number*

14. Which of the following do you use when you are ill?

- Self-Medicate
- Herbalist, witch doctor, prayer people
- Private dispensers, nurses
- Government Health Centre or Dispensaries
- Government or Mission Hospitals
- Company or Institutional Health Centres
- Company Physician
- Private Physician

## APPENDIX I.2

### METHOD OF DETERMINING THE RELIABILITY OF THE QUESTIONNAIRE

1. Each question will be considered a measuring instrument, thus the reliability of each question will have to be ascertained.
2. The reliability coefficient for each question will be measured for the various languages.
3. The Test - Retest Method will be used. This method is chosen because it is the only method available for categorical data and it is simple. During the pretest a sample of individuals will be administered the questionnaire twice, with a 2 week interval. A smaller sample will receive the questionnaire in two languages, again repeated after a 2 week interval, so that their reliability coefficient in the two languages will be compared.  
The reliability coefficient will be calculated after the second administration as follows:  
The first response will be compared to the second response as demonstrated in the four-fold table below.

		2nd Administration Response	
		Yes	No
1st Administration Response	Yes	a	b
	No	c	d
			N

N - Sample given the question.

— Continuous line - agreement boxes

----- Broken line - disagreement boxes

The numbers in 'a' and 'd' represent the agreement between the two administrations and 'b' and 'c' represent the disagreement.

*Kappa*, the agreement expected over and above that of chance agreement,

will be calculated (see Equation-A:1) and this will represent the reliability coefficient.

Equation A:1

$$K = (O-E) / (1-E)$$

Where  $K = Kappa$

$O =$  Observed % agreement

$E =$  Expected % agreement by chance alone

The  $Kappa$  will be calculated for each question, for each language, and for each interviewer. If marked differences are observed, the sources of error will be sought, the appropriate modifications made, and the instrument retested until the reliability coefficients are similar or above 0.8.

### SAMPLE SIZE CALCULATION FOR RELIABILITY COEFFICIENT

This will be calculated through the use of statistical significance of *Kappa*. The sample size will be found through the standard error of *Kappa* (Equation A:2), rather than weighted *Kappa* as the latter is appropriate only for ordinal data.

#### EQUATION A:2

$$SE(K) = \sqrt{O(1-O) / N(1-E)^2}$$

Where  $SE(K)$  = Standard error of *Kappa*

$$\Delta = z \frac{SE(K)}{\sqrt{2}}$$

$$\Delta^2 = z^2 \frac{[O(1-O)]}{\sqrt{2}} / N(1-E)^2$$

$$\therefore N = \frac{z^2}{\sqrt{2}} \frac{[O(1-O)]}{(1-E)^2}^2$$

ERRORS OR BIASES THAT COULD AFFECT THE RELIABILITY OF THE QUESTIONNAIRE

1. Two Interviewers

- (a) Interviewer bias will occur if clear and adequate instructions are not provided.
- (b) Inter-interviewer bias - variation between the two interviewers will be minimised by training before the study.

2. Questions

- (a) Attention bias - This could occur if information regarding symptoms and signs of urethritis is requested by more than one question, as the first question will direct attention to the system. To avoid this bias, Question 5 has been worded to include the 3 (three) common symptoms of gonorrhoea.

3. Different Languages

This will be reflected in the reliability coefficient. It would be difficult to get the reliability coefficient for the other languages as high as the English instrument, but all attempts will be made to make this difference as small as possible by ensuring that the interviewers are fluent in the languages.

4. Generalisability

The pretest will be done on a population that is similar to our study population. This will be a farming village just outside Freetown.

## APPENDIX I.3

### METHOD OF DETERMINING THE VALIDITY OF THE QUESTIONNAIRE

By validity I mean that the questionnaire should be able to measure precisely what it is designed to measure. The first of the validity issues concerns:

#### Face Validity

This will be evaluated by administering the questionnaire to experts in the field of asymptomatic urethral gonorrhoea to assess that all the necessary questions for this type of evaluation have been asked. They will check the questions, considering the analysis proposed in the thesis to ensure that the data that will be analysed has really been the data that ought to be collected.

#### Predictive Validity

Mass screening of any population has not been an accepted method of health care delivery. Selective screening has been found to be cost-effective. Thus the questionnaire includes questions that will provide certain characteristics that will be used to define a group of high-risk males for gonorrhoea. These two groups will be compared to see if there is a marked difference in the prevalence of asymptomatic and/or symptomatic gonorrhoea. As this is a secondary analysis, the significance of the results will require further study.

#### Concurrent Validity

This will not be useful as it is well-known that 'asymptomatic' gonorrhoea does exist. Thus for any population, the prevalence of asymptomatic urethral gonorrhoea will be an indication as to the validity of symptoms and signs of gonorrhoea. The higher the asymptomatic rate, the lower the validity.

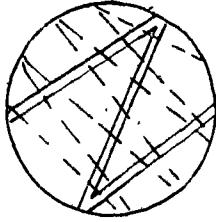
Secondly, the definition of asymptomatic used is the patient's definition, and will thus vary according to his perception of illness and disease. It would not be a useful measure to employ.

## APPENDIX II

### CROSS-STREAKING OF CULTURE PLATES

The clinicians will inoculate the media as described on page 47 and in Figure 3.2.

The inoculum contains body fluids and the latter may affect growth of *Neisseria Gonorrhoea* and the appearance of the colonies. Because of this, the technician will have to establish the need for cross-streaking of the plates during the pre-test. He will randomly select plates to be cross-streaked with a platinum loop before incubation. See below.



— — — cross-streaking  
— — — swab rolled across in the form of an 'N' by clinician

At the end of the pre-test, the technician should be able to determine if cross-streaking improves the growth and identification of *Neisseria Gonorrhoea*, in order for it to be done throughout the study.

APPENDIX III

CLINICIAN'S DATA SHEET

This will contain the manoeuvre performed by the clinician,  
his diagnosis, and treatment recommended or given.

CLINICIAN'S DATA SHEET

Date \_\_\_\_\_

Clinician's Initial \_\_\_\_\_

Participant's Study No. \_\_\_\_\_

<u>Symptoms</u>	<u>Yes</u>	<u>No</u>	<u>Describe</u>
1. Urethral Discharge	<input type="checkbox"/>	<input type="checkbox"/>	_____
2. Dysuria	<input type="checkbox"/>	<input type="checkbox"/>	_____
3. Frequency	<input type="checkbox"/>	<input type="checkbox"/>	_____
4. Genital Ulcer	<input type="checkbox"/>	<input type="checkbox"/>	_____
5. Itch	<input type="checkbox"/>	<input type="checkbox"/>	_____
6. Rash	<input type="checkbox"/>	<input type="checkbox"/>	_____
7. Allergies	<input type="checkbox"/>	<input type="checkbox"/>	_____
<u>Clinical Examination</u>	<u>Yes</u>	<u>No</u>	<u>Describe</u>
1. Urethral Discharge	<input type="checkbox"/>	<input type="checkbox"/>	_____
2. Meatus Normal	<input type="checkbox"/>	<input type="checkbox"/>	_____
3. Inguinal Lymphadenopathy	<input type="checkbox"/>	<input type="checkbox"/>	_____
4. Testes Normal	<input type="checkbox"/>	<input type="checkbox"/>	_____
5. Genital Ulcer	<input type="checkbox"/>	<input type="checkbox"/>	_____
6. Rash	<input type="checkbox"/>	<input type="checkbox"/>	_____

Clinical Diagnosis(es)

1.

2.

Treatment Recommended

1.

2.

Order of Specimen Collection

1

2

Chocolate Agar Innoculated

Yes

No

Plates No.

FVU  
MTM

US  
MTM

FVU  
Choc.

US  
Choc.

APPENDIX IV

LABORATORY REQUEST FORM

This form is designed to carry information about 10 plates. The reason for this is that each candle extinction metal container that will be used is designed to hold 10 glass petri dishes. Thus each form will go with each metal container to the laboratory.

**LABORATORY REQUEST FORM**

## **CLINICIAN**

APPENDIX V

TREATMENT OF POSITIVES

All individuals with positive cultures will be identified as described on Page 50. The treatment that will be provided by the study will be Ampicillin 3.5 grams plus 1 gram Probenicid stat (if allergic to Penicillin); then Tetracycline 500 mg, 6 hourly x 5 days (10 gm)

All sexual contacts of these individuals will be offered epidemiologic treatment. A record of all such treatment will be kept with each individual's study file. The information will be used to check the validity of Question 12 in the main study questionnaire: "How many sexual partners did you have in the last 30 days?"

A test of cure will be performed 7 days after completion of therapy on all positives treated.

If a prevalence of 10% or more is found in any village or small community, mass treatment of the adults sexually active will be considered.

## APPENDIX VI

### PREPARATION OF UNCONTAMINATED MEDIA

Two culture media has to be prepared:

- (1) Modified Thayer Martin
- (2) Chocolate Agar

They will be prepared weekly from dehydrated culture media. The manufacturer's instructions for preparation will be used.

Special precaution for this study is as follows:

- (1) The use of Tween 30 (antifoam) or Pourite to reduce the amount of frothing and to minimise loss of media.
- (2) Plate pourer. This will eliminate wastage and standardise the amount of media that goes into each plate. It will also speed the process of pouring, thus reducing the amount of time the media is exposed to the atmosphere, and thereby reducing the possibility of contamination.

#### (3) Removal of excess Moisture

In the more sophisticated lab with special air filters, the excess moisture is allowed to dry by keeping the plate half open to the atmosphere. In the tropics this results in contamination of the media. To reduce this contamination, the petri dishes will have to be closed immediately after the media is poured into them. The excess moisture will be dried out by putting the media into cooled not air oven to dry out where the risk of contamination will be minimal.

- (4) All plates will be kept refrigerated, once it has cooled and dried out, until it is ready for use.