

SEA-STD-41
Distribution: General

Guidelines for Sexually Transmitted Infections

Prevalence Study

WHO Project : ICP RHR 001



World Health Organization
Regional Office for South-East Asia
New Delhi
August 2001

© World Health Organization 2001

This document is not a formal publication of the World Health Organization (WHO), and all rights are reserved by the Organization. The document may, however, be freely reviewed, abstracted, reproduced or translated, in part or in whole, but not for sale or for use in conjunction with commercial purposes.

The views expressed in documents by named authors are solely the responsibility of those authors.

Contents

	<i>Page</i>
Abbreviations.....	v
Acknowledgements.....	vii
1. INTRODUCTION.....	1
2. OBJECTIVES.....	2
2.1 Overall objective.....	2
2.2 Specific objectives	2
3. METHODOLOGY	3
3.1 Investigation team.....	3
3.2 STIs to be included in the study.....	4
3.3 Study population.....	4
3.4 Criteria for site selection.....	5
3.5 Sample size	6
3.6 Study design.....	6
3.7 Sampling method.....	6
3.8 Informed consent.....	8
3.9 Confidentiality.....	8
3.10 Interview and examination	9
3.11 Collection of laboratory specimens.....	10
3.12 Laboratory tests	11
3.13 Syndromic diagnosis and treatment.....	13
3.14 Time Frame.....	13
4. DATA MANAGEMENT	13

	<i>Page</i>
5. LOGISTIC MANAGEMENT	14
5.1 Transport of specimens	14
5.2 Laboratory opening hours.....	15
5.3 Staff	15
5.4 Reporting of laboratory results.....	16
5.5 Supplies	16
5.6 Forms.....	18
6. REPORT WRITING AND DISSEMINATION.....	18
7. BUDGET	19

Annexes

1. Consent Form.....	20
2. Interview and Examination	21
3. Conducting Interview, Examination and Specimen Taking.....	24
4. Laboratory Tests	31
5. Results of Laboratory Tests.....	33
6. HIV Test Results	35
7. Time Frame.....	36

Abbreviations

DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FSW	Female Sex Worker
HIV	Human Immunodeficiency Virus
HPV	Human Papilloma Virus
HSV	Herpes Simplex Virus
IDU	Injecting Drug User
LCR	Ligase Chain Reaction
NGO	Non-Governmental Organization
PCR	Polymerase Chain Reaction
PID	Pelvic Inflammatory Disease
RPR	Rapid Plasma Reagin
SEAR	South East Asia Region of WHO
STD	Sexually Transmitted Disease
STI	Sexually Transmitted Infection
TAG	Technical Advisory Group
TPHA	Treponema Pallidum Haemagglutination Assay
VDRL	Venereal Diseases Research Laboratory
WHO	World Health Organization

Acknowledgements

These Guidelines on STI Prevalence Study were originally prepared by Dr Purushottam N. Shrestha, Short-Term Professional, WHO/SEARO. It was reviewed by Drs Sudarshan Kumari and Jai P. Narain, Regional Advisers in SEARO. The guidelines were discussed at the Inter-country Workshop on Management of STI held in Yangon, Myanmar from 16 to 20 July 2001. The workshop was attended by Drs Ahammed Ali and Ehsanul Kabir of Bangladesh; Dr Pema Rinzin of Bhutan; Drs Vinay Aggarwal, Anuradha Kapur, Tarun Kapur, Sanjiv Malik, Ashish Sabharwal and Ranjit Singh Virk of India; Dr Saiful Jazan of Indonesia; Ms Nasra Ahmed of Maldives; Drs Myint Swe and Tin Aye of Myanmar; Dr Kiran Prasad Shrestha of Nepal; Drs Iyanthi Abeyewickreme and Joel Fernando of Sri Lanka; Drs Pachara Sirivongrangson and Godom Arya of Thailand; Dr Antonio Gerbase of WHO/HQ; and Drs Purushottam N. Shrestha, Lalit K. Bhutani and Ahmed Suleman Latif of WHO/SEARO.

Contributions made by the writer and all other experts are gratefully acknowledged.

1. INTRODUCTION

Sexually transmitted infections (STIs) are very common in the world both in the developing as well as the developed countries. They are among the five most important causes of adults seeking health care and of healthy productive life lost. They cause high morbidity with illness, complications and sequelae such as pelvic inflammatory disease (PID), infertility and cervical cancer. STIs, including human immunodeficiency virus (HIV), are the most important cause of illness among young adult men (15-44 years) and the second most important cause after maternal causes among young adult women. The presence of STI that causes genital inflammation or ulcer increases the risk of HIV transmission by as much as ninefold.

The exact extent of STIs in the world is not known but based on the available information, World Health Organization (WHO) estimates that about one million new cases of STIs occur daily in the world. Due to the absence of reporting system, the magnitude of STIs in the South-East Asia Region (SEAR) of WHO is not known. However, about 400,000 new cases of STIs are estimated to occur daily in South and South-East Asia.

It was common practice in the countries of the SEAR to treat people with STI based on clinical or etiological diagnosis. In some centres, the diagnosis is made on the basis of simple laboratory tests, such as microscopic examination of Gram-stained smears. Gonococcal cultures are usually not performed, and hence the pattern of antimicrobial sensitivity of *Neisseria gonorrhoeae* is not known. Tests for chlamydial infection are expensive and hence are not available in most parts of the Region. Screening of pregnant women for syphilis serology is not routinely carried out and information on screening of donated blood is not available. Recently, the syndromic approach to diagnosis and management of patients with STI, recommended by WHO, has been adopted in the countries of the SEAR in order to avoid the cost of testing and to provide treatment during the first visit of the patients. WHO's Regional Office for South East Asia has started training trainers of the Member Countries in syndromic case management.

In order to develop guidelines on STI case management using syndromic approach, it is necessary to have a knowledge of the prevalence of the various etiologic agents responsible for the STI syndrome, and of the antimicrobial sensitivity pattern of these agents. For continuing efficiency of the syndromic management approach, it is important to monitor periodically, both the pattern of prevalence of STI pathogens responsible for a syndrome and the pattern of antimicrobial susceptibility of pathogens. This information is required to make appropriate therapeutic recommendations. However, it may be stated in general that information on the pattern of STIs, the etiology of STI syndromes and the antimicrobial sensitivity pattern of pathogens in SEAR is generally lacking and needs to be obtained.

This document provides a methodology for assessing the prevalence of STIs in selected population groups. It recommends a simple and reliable design that can be widely used and implemented at the local level. The prevalence study is designed to collect basic demographic information, information on signs and symptoms related to STI and specimens for laboratory testing of STIs. To simplify the study protocol, no behavioural questions have been included. The data generated from the study could be used in planning STI control programmes, developing/refining guidelines on STI case management, and revising disease prevalence estimates for population subgroups. This document should be adapted to the local situation in the country and modified to suit the local needs and capacity.

2. OBJECTIVES

2.1 Overall objective

The overall objective is to determine the prevalence of selected STIs in the selected population subgroups.

2.2 Specific objectives

The specific objectives are:

- To determine the prevalence of selected STIs in specific population subgroups;

- To determine the antimicrobial sensitivity pattern of selected STI pathogens;
- To generate data for planning control programme and developing/refining STI case management;
- To provide baseline data for monitoring the trends of STIs and impact of the control programme.

Prevalence rate is a measure of frequency of a disease at a given time in a given population and is defined as follows:

$$\text{Prevalence} = \frac{\text{total number of cases of disease at a given time}}{\text{total population at the same time}}$$

An STI prevalence assessment is a determination of the number of persons infected with an STI among persons screened in defined populations.

3. METHODOLOGY

3.1 Investigation team

A principal investigator should be nominated soon after a decision is made to conduct the study. He/she should have past experience in epidemiological study and an epidemiological, clinical, laboratory or management background in STI or public health and should be available throughout the duration of the study. The main role of the principal investigator is to organize and conduct the prevalence study. Responsibilities will include selecting the other members of the investigation team, identifying local resources, convening a Technical Advisory Group (TAG) and adapting the study protocol to the local situation.

TAG should be convened by the principal investigator and should include members with expertise in epidemiology, laboratory methods, reproductive health, STI management and data management as well as representatives from study sites. TAG will advise the principal investigator on the specific details related to the study methods and provide guidance throughout the study.

3.2 STIs to be included in the study

The actual STIs to be included in the study should be determined according to the available laboratory facilities for testing as well as by the available budget. These include *Treponema pallidum* (syphilis), *Neisseria gonorrhoeae* (gonorrhoea), *Chlamydia trachomatis* (chlamydia), *Trichomonas vaginalis* (trichomoniasis) and HIV. The first four STIs are curable, cause considerable morbidity and mortality, are spread primarily by sexual transmission and are often asymptomatic in women. HIV testing will also be included in the survey. Other pathogens to be considered are *Haemophilus ducreyi* (chancroid), *Gardnerella vaginalis* (bacterial vaginosis), *Candida albicans* (candidiasis) and *Herpes simplex* (genital herpes).

3.3 Study population

To obtain a true prevalence of STI in any given community, it would be necessary to carry out a community-based study, but such a study will pose considerable logistic difficulties. Hence, it is suggested that the study be conducted in women attending antenatal, gynecology and family planning clinics because of their easy accessibility. A study of the prevalence of STIs in pregnant women is an approximation of the prevalence of these infections in their male counterparts. However, the limitations of this kind of study should be recognized, because pregnant women do not represent all women in the community. For example, women who do not become pregnant will not have a chance of being included in the study, and some of them may well have become infertile as a result of STI. In addition, single women who are also at risk of becoming infected with STI will not be enrolled, as they have not become pregnant. However, an advantage of enrolling pregnant women in a prevalence study is the fact that antenatal clinics are well attended by sexually active women, regardless of whether they have symptoms or not. Women attend such clinics for routine care. A study in family planning clinics will give the prevalence of STI in sexually active non-pregnant women. A study in gynecology clinics will also give the prevalence of STI in non-pregnant women, although it may also include women who are not sexually active. Such a study will determine the range of pathogens which are prevalent in a selected population subgroup and will not be a true prevalence study of STI pathogens in the whole community. Another population subgroup that may

be considered for inclusion in the study is commercial sex workers, if they are known to attend specific clinics for health care.

Men have not been included in the study because of difficulty in accessing them. However, certain subgroups, such as transport workers, military and police, may be included in the study, if it is considered to be feasible to conduct the study in such subgroups.

3.4 Criteria for site selection

The sites for the study should be selected on the basis of the following criteria:

- *Acceptability*: the study should be acceptable to the government, local authorities and the study population.
- *Commitment*: the site should have sufficient number of staff to be engaged in the study (enrolling, interviewing and examining the study population and collecting laboratory specimens) and the staff should be willing to participate and cooperate.
- *Location*: both urban and rural areas should be selected in order to provide a better representation.
- *Sample size*: the site should have an adequate number of clients available for recruitment.
- *Sector*: government sector; however, the private sector and nongovernmental organizations may also be involved if they are willing to participate and have a sufficient number of clients.
- *Local capacity*: the site should have technical and organizational skills to follow the study protocol requirements including the type of staff required, physical facilities, quality control, and maintenance of confidentiality and privacy.

For women to be recruited in the study, the sites would be antenatal, gynecology and family planning clinics with 50 new patients every month. If commercial sex workers are also included in the study, it will be necessary to find out the sites with adequate number of sample size. Military recruits may be studied in a military clinic. Suitable sites may need to be identified for transport workers, with the cooperation of their unions.

3.5 Sample size

The minimum acceptable sample size for assessing prevalence depends upon: the estimated prevalence of the disease in the population subgroup; the degree of precision/certainty wanted in that prevalence estimate; and whether or not the data generated by the study will be used to monitor the trends of the disease. Generally, the more precise the estimate desired from the study results, the larger the sample size required. A larger sample size is also required if the trend over time is to be monitored, as the sample has to be large enough to have sufficient statistical power to detect changes of a moderate size. The sample sizes should be large enough to measure changes in indicators of 15 percentage points at the 95% significance level and with 80% power. In general, a sample size of 500 would be adequate in most situations. However, a statistician should be consulted for calculation of the sample size.

3.6 Study design

The most appropriate study design for the STI prevalence study is a cross-sectional study. Cross-sectional studies are observational studies in which a sample of subjects in a population (e.g. pregnant women attending an antenatal clinic) are investigated for specific characteristics, in this instance laboratory-confirmed STI. Prevalence studies do not establish causality.

3.7 Sampling method

While deciding on the sampling method, it is necessary to consider a sampling frame that is representative of key epidemiological and socioeconomic factors; study sites that have sufficient client numbers and medical and laboratory expertise and capacity; clients that are likely to consent to participation; and government support for the prevalence study.

Usually, a population is too large for the study to examine every individual. Therefore, a sample is taken instead. If the sample is representative of the population, the findings of the sample can be generalized for the whole population it represents. The main advantages of taking a sample are in saving time, cost and personnel required for the study. The main disadvantage of a sample is the loss of precision compared to the study of the whole population.

Thus, the sample estimate will have some margin of error. There are two main types of error: *sampling errors*, which occur because only part of the population is included in the study (generally the larger the sample size, the smaller the sampling error); and *selection bias* (non-sampling errors) which occurs if the sampling procedure is flawed and the sample is not representative of the whole population. Selection bias is independent of sample size. An example of selection bias is when pregnant women of higher economic status are not included in a study of pregnant women if the sampling for the study is limited to government antenatal clinics.

A convenience sample with consecutive sampling should be used in the STI prevalence study. Enrolment should continue until the required number of subjects has been reached. A convenience sample means that the study population is already accessible to the study for a reason not related to the study itself, for example, pregnant women attending antenatal clinic for routine care. Convenience sampling is used because it is a convenient, practical and cheaper way of recruiting the study subjects. It uses existing infrastructure such as clinic facilities and staff. The disadvantage is that the sample population may not be representative of the total study population in an economic, cultural or geographic sense.

Within a convenience sample framework, sampling can either be:

- (a) random, where everyone has the equal probability of being selected, making the sample more representative of the population of interest. For random sampling, a full count of all clinic attenders for the study period is needed. The disadvantage is that it complicates recruitment, specimen collection and potentially, clinical management of asymptomatic infections; or
- (b) non-random (e.g. consecutive), where all eligible clinic attendees are recruited into the study until the sample size is reached.

Consecutive sampling is recommended because it is simpler, reduces the study period and is cheaper but it may increase selection bias.

The following criteria should be fulfilled for including the subjects in the study:

- The subjects should be aged 15 to 49 years so that only those who are likely to be sexually active are included in the study because those who are not sexually active will not get STI.

- For antenatal clinic sites, only those pregnant women who are visiting the clinic for the first time during their pregnancy should be included in the study. Similarly in other sites, only those attending the clinic for the first time for that episode of illness or service should be included in the study. In other words, those who come for follow-up should not be included in the study. This is necessary for avoiding double counting. The study participants should be included in the study only once.
- Those who had antibiotics during the past two weeks should not be included in the study, in order to avoid any interference with culture and sensitivity of the microorganisms.
- Participation by any subject in the study should be voluntary after informed consent is obtained. Refusal by any subject to participate in the study should not affect the services to be rendered to the subjects.
- The legal age of consent may need to be considered in some countries while recruiting the participants in the study. However, as this is a non-experimental study, any one coming independently to the clinic for care can be considered to be eligible for inclusion in the study.

3.8 Informed consent

The nature of the study and the conditions of study participation including interview, examination and laboratory tests should be explained to the eligible subjects and their consent should be obtained before enrolling them in the study. Consent may be written or verbal, depending upon the literacy level of the individual participants. A sample consent form is given in Annex 1. As HIV testing will be unlinked anonymous, it is not necessary to obtain consent for this test.

3.9 Confidentiality

All personal information of the study participants should be kept strictly confidential and should be protected from disclosure to unwarranted people. Warranted persons are those staff who need to know the information in order

to provide care and support to the participants. They should be made aware of their legal and ethical responsibilities to maintain confidentiality. The confidentiality of STI status must be ensured at all times including during testing and treatment. Any disclosure should be justified on the basis of law and professional ethics. The issue of breach of confidentiality of HIV status should not arise because the test will be unlinked and anonymous.

An unlinked anonymous testing methodology should be used for HIV and a voluntary confidential testing methodology for all other STIs. In unlinked anonymous testing, all personal identifying features of the study participants are removed from the specimen so that the result of the test cannot be traced back to the individual participant. This kind of testing is considered to be an accurate and effective method for public health surveillance of HIV infection. This method does not identify the infected persons.

In voluntary confidential testing of STI, individuals may themselves ask for STI tests or may consent to a test on recruitment into a study. Their identifying features are known only to the health professionals involved in their direct patient management. In some countries, STIs are notifiable, meaning thereby that they must be reported to the appropriate health authority. However, such notification may not need the names of the patients. In any case, confidentiality is maintained.

3.10 Interview and examination

The subjects enrolled in the study should be interviewed and examined to collect the following information:

- Study site
- Study identification number
- Age
- Marital status
- Occupation
- Symptoms
- Signs

The findings should be recorded in the form given in Annex 2. Details of how to conduct the interview and carry out a physical examination are outlined in Annex 3.

3.11 Collection of laboratory specimens

The specimens to be collected at the clinic will depend upon the tests to be performed at the laboratory. The following specimens should be collected at the clinic:

- A high vaginal swab should be obtained from the posterior fornix using a cotton-wool-tipped swab.
- A thin smear of the material obtained with the high vaginal swab should be made on a microscope slide.
- The high vaginal swab should then be broken off into the liquid Diamond agarless culture medium.
- An endocervical swab should be obtained using a cotton-wool-tipped swab. The material obtained should be plated out on modified Thayer -Martin medium and the swab should then be discarded. The inoculated medium should then be placed in a candle jar.
- Another endocervical swab should be obtained using a calcium alginate-tipped swab. The tip of this swab should be broken off in the chlamydia transport medium.
- In patients with genital ulcer, a swab of ulcer exudate should be obtained and the material should be inoculated onto Mueller -Hinton medium. The swab should then be discarded while the inoculated medium should be placed in a candle jar.
- 10 ml of venous blood should be obtained from each subject. 5 ml of the blood should be placed into one tube labelled only with the subject's age and initials of the clinic. This is for HIV test. The remaining 5 ml of blood should be placed in another tube which should be labelled with the initials of the clinic and subject's study number. The second sample is for syphilis test and, if selected, for Herpes simplex test.

Details of specimen collection are outlined in Annex 3.

An alternative method to vaginal and endocervical swabs is the use of self-administered tampon. However, this method may not be acceptable in many settings where women do not use tampons.

3.12 Laboratory tests

The aim of the prevalence study is to obtain the most accurate and valid laboratory based diagnoses of the specified STIs from the collected specimens. The degree of precision required in the study should be determined before decisions are made about the type of sample to be used and the laboratory tests to be performed. The choice of the samples and the tests will depend on the following:

- Local technical laboratory capacity;
- Sensitivity and specificity of the test in the proposed study population;
- Degree of difficulty in collecting samples;
- Sample size;
- Logistics management; and
- Cost of tests.

The following tests should be carried out in the laboratory. (See also Annex 4).

- The microscope slide containing the smear of high vaginal exudate should be Gram-stained and examined microscopically under oil immersion. The smear should be examined for *Candida* (Gram-positive yeasts and hyphae), lactobacilli (large Gram-positive rods) and clue cells (epithelial cells covered in small Gram-negative rods).
- A wet preparation should be made from the liquid Diamond agarless medium in which the high vaginal swab was placed. The wet preparations should be examined for motile trichomonads.
- An ELISA test for chlamydia antigen or a DNA probe should be carried out on the fluid in which the calcium alginate-tipped swab was placed.
- The modified Thayer-Martin medium plates should be placed in an incubator at 36°C in an atmosphere of 10% carbon dioxide for the culture of *N. gonorrhoeae*. Plates should be incubated for 48 hours and cultured colonies should be identified as *N. gonorrhoeae* by morphology, Gram stain, oxidase production and sugar fermentation

tests. Cultured colonies of gonococci should be tested for penicillinase production using the bromocresol purple or the chromogenic cephalosporin tests. Cultured colonies of *N. gonorrhoeae* should be subcultured and tested for antimicrobial resistance by the disc sensitivity method.

- The inoculated plates of Mueller-Hinton medium should be incubated for 72 hours at 36°C in an atmosphere of 10% carbon dioxide for the culture of *Haemophilus ducreyi*. Cultured colonies should be identified as *H. ducreyi* by morphology, gram stain, oxidase production and biochemical tests. Cultured colonies of *H. ducreyi* should be subcultured and tested for antimicrobial resistance by the disc sensitivity method.
- The blood sample marked with the subject's age and clinic initials should be tested for HIV using an ELISA method. On receipt of the samples, the laboratory should separate out the serum and then aliquot them. HIV testing should be carried out in batches once a month. Reactive samples should be subjected to another test based on another antigen or principle. The national recommended algorithm for the laboratory testing for HIV antibody should be followed.
- The second blood sample marked with study number and clinic initials should be tested for syphilis serology using rapid plasma reagin (RPR) test or Venereal Disease Research Laboratory (VDRL) test. If a decision is made to test for *Herpes simplex* too, microimmunofluorescent test against IgM specific antibody for types I and II should be performed.

There should be effective quality control and assurance of the laboratory. Quality control and quality assurance should be an integral part of the functioning of the laboratory. The laboratory must implement internal quality control measures that include all the activities that are undertaken to generate reliable results. Quality control means that the laboratory performing the tests has a programme to check its own results for various procedures, such as specimen collection, specimen handling, specimen logging, testing procedures, reproducibility of results, and documentation of results. As far as possible, the laboratory should participate in an External Quality Assessment Scheme and should register acceptable scores. Quality assurance means that the laboratory performance is checked by an external agency (reference

laboratory, WHO collaborating centre or international agency). It includes periodic monitoring of test quality, spot checking of tests and sometimes of laboratory techniques.

The results of the tests should be entered in triplicate as shown in Annexes 5 and 6. A copy each should be sent to the clinic, and the principal investigator and one copy should be kept at the laboratory. The copy to the clinic should be sent within one week for treatment of the patients, if needed, except for the results of HIV tests which are to be sent after each batch has been tested. All results should be kept confidential.

3.13 Syndromic diagnosis and treatment

If symptoms and/or signs of STI are found during interview and examination of the study subjects, syndromic diagnosis should be made and the subjects should be treated syndromically according to the national guidelines, free of charge. All study subjects should be asked to return to the clinic in one week for the test results. This will give an opportunity to review the treatment for those who were treated syndromically or to decide on the basis of laboratory tests whether any treatment is required for those who did not have any symptoms or signs.

3.14 Time Frame

The total duration of the study will depend on the country, number of sites, sample size and resources available. However, it should not take more than one year. An example of a typical time frame is given in Annex 7.

4. DATA MANAGEMENT

Data generated from the interview and examination and laboratory tests should be entered using an appropriate statistical package, such as Epi-Info, SAS and SPSS. The principal investigator should develop the data entry protocol and template with the assistance of a statistician. The data entry template should be designed in such a way as to minimize data entry errors, to restrict the range of values that can be entered for any data items, and to require mandatory entry for all data fields. About 10% of the data should be double entered to review the level of data entry error.

It is essential to ensure the confidentiality of the data. Access to data should be restricted to the principal investigator, the statistician and those who manage the data. The room should be locked, records should be kept in a locked cabinet and the access to the files in the computer should be protected with password. During entry, it is wise to save the files regularly in order to prevent loss due to power failure or other technical problems. Computer files should be backed up and hard copies should be printed every day.

Persons responsible for data entry should be trained in data entry protocol and process. The principal investigator and the statistician should supervise the data entry regularly to detect errors and take corrective measures in time.

The data should be analyzed to obtain the prevalence rates and antimicrobial susceptibility pattern. Prevalence rates should be stratified by population subgroup, clinic and age group.

- (a) **Seroprevalence rate of HIV infection and syphilis:** The proportion of study subjects with positive HIV and syphilis serology;
- (b) **Prevalence of STI pathogens:** The proportion of study subjects with positive test for the STI pathogens. The results should be used to review and if necessary, to modify the existing treatment guidelines.
- (c) **Antimicrobial sensitivity pattern:** Patterns of sensitivity to drugs of *Nisseria gonorrhoeae* and *Haemophilus ducreyi* will be known from the laboratory tests and the results should be used to review and revise the treatment guidelines.

5. LOGISTIC MANAGEMENT

5.1 Transport of specimens

Specimens that are collected daily at the clinics should be packed securely and transported to the laboratory on a daily basis. The following specimens will be collected from each study subject:

- 1 microscope slide
- 1 high vaginal swab in a liquid culture medium

- 1 endocervical swab in a chlamydia transport medium
- 1 inoculated modified Thayer -Martin medium
- 1 inoculated Mueller-Hinton medium (only from patients with genital ulcer)
- 2 tubes of clotted blood

5.2 Laboratory opening hours

There should be good coordination between the participating clinics and the laboratory regarding the timing of the delivery of specimens. In the laboratory, each specimen should be logged in on a register and processed and stored properly. This will require adequate time. Hence, a deadline for time should be set for delivering the specimens to the laboratory. However, this must not be to the detriment of enrolling the study subjects. If necessary, the laboratory working hours may be extended by an hour or so to allow enough time for processing and the staff should be compensated appropriately.

5.3 Staff

One of the criteria for selecting a study site is the availability of the staff at the site itself. Two clinical staff are required in each site. Both of them may be doctors or both may be nurses or they may be a team of a doctor and a nurse. At least one of them should be experienced in conducting pelvic examination. For female study subjects, at least one of the staff should be female. The main tasks of the staff are to enroll the study subjects, to interview and examine them, to collect specimens for laboratory tests and to diagnose and treat the subjects, if required. The staff should be identified before commencing the study and should be trained in their tasks adequately. The principal investigator should coordinate the training of the staff and should supervise their work.

Similarly, laboratory staff should also be identified and should include a person experienced in microbiology. They should be trained in handling specimens, log in the specimens on a register, label specimens, separate and aliquot specimens, perform tests, fill out laboratory result sheets and dispatch the results to the clinics and the principal investigator. The chief of the laboratory should be responsible for coordinating the training as well as for supervising the laboratory staff.

5.4 Reporting of laboratory results

Results of laboratory tests should be recorded in triplicate in the laboratory sheets (see Annexes 5 and 6). One copy should be kept at the laboratory, the second copy should be sent to the clinic and the third copy should be sent to the principal investigator. The results should be sent to the clinics and the principal investigator as soon as they become available, especially to the clinics in order to help them make a decision about the treatment of the study subjects.

5.5 Supplies

The actual quantity of supplies required for the study will depend on the sample size, but there should also be a provision to take care of wastage. Supplies required for the study include

- (a) forms for interview and examination;
- (b) forms for laboratory specimens;
- (c) laboratory sheets for entering test results;
- (d) software for data processing; and
- (e) laboratory supplies.

Supplies related to the laboratory

- Microscope slides
- Microscope cover slips
- Cotton-wool tipped swabs
- Calcium alginate-tipped swabs (for chlamydia antigen test)
- Blood tubes
- Modified Thayer-Martin agar in Petri dishes
- Blood or chocolate agar in Petri dishes for subculture of organisms
- Syringes (10 ml)
- Needles

- Serum tubes (1 ml)
- HIV ELISA tests (2 different methods)
- RPR tests
- TPHA tests
- Chlamydia ELISA antigen tests
- Herpes simplex type I and type II antibody tests
- Gonococcal identification reagents (Gram-stain reagents, culture, Oxidase, sugar fermentation, penicillinase, sensitivity)
- *H. ducreyi* identification reagents (Gram-stain reagents, culture, Oxidase, biochemical tests, disc sensitivity)
- Diamond agarless gel (for Trichomonas culture)
- Latex rubber gloves

N.B. The above are the supplies that are required specifically for the study. It is assumed that refrigerator, microscope, centrifuge and other standard laboratory equipment including those for disposal of biological wastes are already available in the laboratory.

Supplies for the clinics

- Specula
- Hot water boiler to sterilize specula
- Bucket to soak specula in detergent
- Liquid detergent
- Light source for speculum examination
- Large kidney dishes
- Sharps disposal
- Tourniquet
- Swabs

5.6 Forms

For every participant in the study, the following forms will be required:

- (a) Consent form
- (b) Form for Interview and Examination
- (c) Form for recording the despatch of specimens from the clinic to the laboratory
- (d) Results of Laboratory Tests

The number of forms required will depend on the sample size but there should be extra copies, about 20% more than the sample size, to take care of wastage and of excess in the enrollment. The laboratory will also send the HIV test results collectively in a separate form every month.

In addition to the above, various documents and protocols will be required. They include study protocol, study site log book, laboratory logbook, data entry form, operational guidelines.

6. REPORT WRITING AND DISSEMINATION

The principal investigator will be responsible for writing the report. While the process of writing may begin at any time during the study, it will be possible to write the main part only after the data have been analysed. Dummy tables should be prepared in advance and should be reviewed by the TAG. The report should include the study objectives, design, methodology, findings, discussions and recommendations. An executive summary highlighting the important components of the report should be placed at the beginning of the report.

The report should be presented to TAG for review and discussions. Subsequently, the main findings and recommendations should be presented at a workshop attended by all parties interested in STI prevention and care. These meetings will help in the finalization of the report and in advocacy for mobilization of resources.

The final report should be disseminated widely to all interested parties including those who are likely to use the findings in STI prevention and care

for various purposes, such as planning, designing interventions and reviewing treatment guidelines. The report should also be distributed to the staff who participated in the study as a token of appreciation of their work and to keep them interested in future studies.

7. BUDGET

The budget required for the study will largely be determined by the sample size, the number and location of study sites, STI selected for the study and the cost of laboratory supplies. The budget will also be influenced to some extent by the existing resources, such as human resources, laboratory facilities and clinic facilities. The study should aim at utilizing these existing resources as much as possible in order to minimize the budgetary requirement and allocate the approved budget for additional activities and supplies required exclusively for the study. For this, the principal investigator should try to seek cooperation and collaboration from the laboratories and the clinics.

In general, budget will be required for personnel, laboratory test kits and reagents, clinic supplies, drugs for treatment of STI, forms, transportation of specimens, data management, report writing and sundries.

Annex 1
CONSENT FORM

This study is designed to find out about your health status, particularly about sexually transmitted infection (gonorrhoea, syphilis, chlamydia or trichomonas). If you have an infection, it may give rise to serious pelvic infection, infertility, pregnancy complications and infections in the new-born babies. However, these infections can be treated and complications prevented by early treatment. The study is designed to find out how many persons attending the clinic have sexually transmitted infection.

All results will be kept strictly private and confidential. It is your choice to participate or not to participate in the study. If you participate, you will be interviewed and examined and blood and vaginal specimens will be taken. If the test is positive, you will receive treatment. If you refuse to participate, it will not affect in any way the services for which you have come to the clinic.

We would highly appreciate your participation in the study.

I have read the above information / The above information has been read out to me.

I have had the opportunity to ask questions about the study and the questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this study.

Signature_____

Name_____

Study number_____

Date_____

Annex 2

INTERVIEW AND EXAMINATION

A. INTERVIEW

Identification

1. Study number
2. Clinic name _____
3. Clinic number
4. Date Day Month Year

History

5. Age in years
6. Number of children
7. Marital status Married Single Divorced Widowed Separated
8. Occupation _____
9. Occupation of spouse _____

Symptoms

10. Do you have a sore in the genital area? Yes No
If "no", skip to 13
If "yes"
11. Is it painful? Yes No
12. How long have you had the sore in the genital area? Days
13. Do you have a vaginal discharge? Yes No
If "no" skip to 18.
If "yes",
14. What is the colour of the discharge? _____
15. Is it foul smelling? Yes No
16. Is it slight, moderate or copious? Slight Moderate Copious

17. How long have you had the discharge? Days
18. Do you have a burning sensation when you pass urine? Yes No
If "no", skip to 20.
If "yes",
19. How long have you had the burning sensation? Days
20. Do you have pain in the womb? Yes No
If "no", skip to 22.
If "yes",
21. How long have you had the pain in the womb? Days
22. Do you have pain during sexual intercourse? Yes No
If "no", skip to 24.
If "yes",
23. How long have you had the pain during sexual intercourse? Days
24. Do you have swelling in the groin? Yes No
If "no", skip to 27
If "yes",
25. Is it painful? Yes No
26. How long have you had the swelling in the groin? Days
27. Do you have pain deep down in the lower part of the abdomen? Yes No
If "no", skip to 29.
If "yes",
28. How long have you had the pain deep down in the low part of the abdomen? Days
29. In the past, have you ever had any of the following?
- | | | |
|--|------------------------------|-----------------------------|
| 29.1 Vaginal discharge | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| 29.2 Sore in the genital area | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| 29.3 Painful swelling in the groin | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| 29.4 Pain deep down in the lower part of the abdomen | Yes <input type="checkbox"/> | No <input type="checkbox"/> |

B. EXAMINATION

Is any of the following present?

30. Generalized lymphadenopathy Yes No
31. Skin rash Yes No
32. Abdominal tenderness Yes No
33. Abdominal mass Yes No
34. Pelvic tenderness Yes No
35. Inguinal bubo Yes No
36. Genital ulcer Yes No
- 36.1 If yes, painful Yes No
37. Vesicles in the genitalia Yes No
38. Vaginal discharge Yes No
- If yes,
- 38.1 Colour Clear White Curdy Yellow
- 38.2 Foul smelling Yes No
39. Discharge from the cervix Yes No
40. Ulcer in the cervix Yes No
41. Genital wart Yes No
42. Cervical excitation tenderness Yes No

43. Height of uterine fundus Weeks

Diagnosis:

Treatment of STI, if present

Follow up

Annex 3

CONDUCTING INTERVIEW, EXAMINATION AND SPECIMEN TAKING

I. Introduction

It is very important to conduct the study carefully and meticulously. This includes selection of the study participants and collection of information. All information should be collected and recorded completely and correctly. The procedure in the study site i.e. the clinic, has four sections:

- Recruiting study subjects
- Interviewing the subjects
- Examining the subjects
- Collecting laboratory specimens

The above work will be done by a team of two persons, at least one of whom should be a female. While one of them is asking questions, examining the subject or collecting the specimen, the other should be entering the information. They should cross-check each other to ensure that all required questions are asked and all entries are correct.

II. Recruiting study subjects

The potential study subjects would be all pregnant women attending the antenatal clinic, all women attending gynaecology clinic and all women attending family clinic. The study subjects should be visiting the clinic for the first time for the current pregnancy, current episode of illness or for obtaining family planning services respectively. If the subject has visited the clinic earlier for this pregnancy, illness or family service, do not enrol her in the study. This is important in order to avoid double counting. Enrol the subjects consecutively as they come in until the sample size is reached. However, it is

essential to ensure that only those persons who meet the criteria mentioned in the section 3.7 "sampling method" are selected as study subjects. There could be other sites according to the study population selected.

Explain to each selected study subject that this is a study about health and obtain consent. While written consent (Annex 1) should be obtained for literate subjects, verbal consent would do for illiterate subjects.

III. Interviewing the subjects

All selected subjects who agree to participate in the study should be interviewed and their responses recorded in the form given in Annex 2. The section on interview has three subsections and a total of 29 questions. All questions except those that are to be skipped as determined by the response to the preceding question should be asked and their responses should be recorded in the form. The numbers mentioned below correspond to the serial numbers of the questions in the form.

The subsection on **Identification** has four questions. They should be recorded as follows. *Do not write the name of the subject in the form.*

(1) Study number

The first subject enrolled in the study will be study number 001, the second subject will be 002 and so on. Enter it as follows:

The number should be entered clearly. This number should be written on each sheet of paper related to this study subject and also on all specimen containers (tubes, bottles, culture plate and slide) except the blood tube for HIV testing in which the only information written will be the age of the subject and clinic identifier. As soon as a subject is enrolled, the specimen containers should be labeled with the study number.

(2) Clinic name

Write the name of the clinic clearly.

(3) Clinic number

Write the clinic number in the boxes. The clinic number AB135 should be entered as follows:

(4) Date

The date of enrolling the subject should be entered, giving each of the day, month and year in two digits. If the date was 2 March 2001, it should be entered as follows:

Day Month Year

The subsection on **History** has five questions.

(5) Age

Enter the completed years of age of the study subject in the box provided. If the age is 21 years, enter it as follows:

(6) Number of children

If the subject has two children, enter it as follows:

(7) Marital status

Put the tick mark in the appropriate box according to the marital status. If the subject is married, enter as follows:

Married Single Divorced Widowed Separated

(8) Occupation

Ask the occupation of the subject and write the response clearly. It may be necessary to ask the nature of work in order to be clear about the occupation. If not employed, write "not employed".

(9) Occupation of spouse

Ask the occupation of the spouse only if the subject is married and write the response clearly. If not employed, write "not employed". If not married, leave it blank.

Symptoms

Questions 10 to 29 relate to symptoms. Ask the subject about the symptoms. If the subject has the symptom, put a tick mark in the "yes" box and then immediately ask following questions. Enter the duration of symptom i.e. number of days in two digits. For example, if the subject is having the symptom for the past 7 days, enter as follows:

Number of days

Remember that most of the subjects attending the antenatal or family planning clinic will not be having any symptom, as they would have come for routine antenatal care or family planning service respectively. If there is no symptom, put a tick mark in the "no" box and skip to the question as indicated.

IV. Examining the subjects

Before starting the examination, ensure that all required questions have been asked and their responses recorded. Also ensure that the study number is written on the interview form as well as on all specimen containers except the blood tube for HIV testing. The study number should be written on the top as well as the bottom of the culture plate because the top is loose.

The examination room should be well illuminated. The speculum and light source should be ready. Explain to the subject that you are going to examine her, including internally. Ask her to undress from chest down (it is not necessary to take off all clothes). Make sure that she is comfortable.

Ask the subject to sit on the couch. Feel her neck from the back for the enlarged lymph nodes in the anterior and posterior triangles of the neck. Then examine her axillae and the epitrochlear area for enlarged lymph nodes.

If there are palpably enlarged lymph glands measuring at least 1 cm in diameter and at more than one site, tick the “yes” box under generalized lymphadenopathy. If the lymph nodes are not present or are present at one site only or are less than 1 cm in size, tick the “no” box. Lymph nodes in the inguinal region may be ignored because they are usually palpable in most people.

Ask the patient to lie down. Look for skin rashes. Then palpate the abdomen, at first softly to find out any tenderness. If there is no tenderness, palpate deeply for any abdominal mass. In pregnant women, record the height of the fundus of uterus in weeks/40. Palpate for pelvic tenderness i.e. tenderness in the lower abdomen and deep down in the pelvis.

Palpate the inguinal and femoral regions for inguinal bubo. A bubo is a collection of acutely inflamed tender painful swollen glands in the groin. An enlarged lymph node which is neither painful nor tender to touch is not a bubo.

Ask the subject to bend the knees and to separate them. Look at the external genitalia for sores (ulcers) and if present, find out whether they are painful to touch. Next look for warts and small vesicles around the vulva. Next, separate the labia and look at the entrance to the vagina for sores, vesicles, warts or any obvious discharge.

Now insert the bivalve speculum into the vagina with its two valves closed and in a vertical plane. It is less uncomfortable in this way. Next, rotate the speculum so that the locking mechanism is towards the top. Open the blades gently and visualize the cervix. Look for sores, warts, erosions and discharge. Note the colour and smell of the vaginal discharge. Look at the cervix for any discharge. If the cervix is not clearly seen, wipe it with cotton wool attached to an ovum forceps.

While the speculum is still in place, take specimens from the vagina and the cervix (see below). After taking the specimens, remove the speculum and continue the examination. Carry out the examination of the pelvis with your fingers. Stand on the right side of the subject. Insert the index and middle fingers of your right hand into the vagina, with the thumb extended and ring and little fingers flexed. Place your left hand on the subject’s lower abdomen. Feel for any mass (swelling or lump) between your two hands. Uterus will be

felt as a mass and so also a distended urinary bladder. In pregnant women, note the size of uterus in weeks/40. Feel for any other mass. Next, gently move the cervix laterally to find out whether it causes pain. If yes, the subject has cervical excitation tenderness.

V. Taking specimens

As stated above, take the following specimens while the speculum is still in place.

Swab No.1: This is a cotton-wool-tipped swab. Take a swab from inside the cervix (endocervical). Insert the cotton wool end of the swab into the cervix and roll the swab a couple of times in order to collect endocervical exudate. The material obtained will be cultured for *Neisseria gonorrhoeae* in the laboratory. Plate this out onto the modified Thayer -Martin medium which is the agar plate marked "MTM". For plating, hold the end of the swab, let the cotton wool with the exudate touch one area of the agar, roll the swab so that any material on the cotton wool makes contact with the agar, and spread the material in a wedge on the agar by stroking it in a linear fashion. Do not dig into the agar. After plating, discard the swab.

Swab No.2: This swab has a special tip. The tip is impregnated with calcium alginate. Insert tip of the swab inside the cervix, roll it a few times to collect endocervical material, withdraw the swab and place the end with endocervical material into the special transport medium. Break off the swab and leave the tip into the medium. Close the tube. This material will be tested for chlamydia antigen.

Swab No. 3: This is a cotton-wool-tipped swab. Take a swab from the vault of the vagina behind the cervix (but not from inside the cervix). This is the high vaginal swab. The material obtained will be tested for trichomonas, candida and clue cells. After taking the swab from the vagina, make a smear of the obtained material on a microscope slide. Then, break off the swab head into the liquid medium that is provided (this is the Diamond agarless medium) for the detection and culture of trichomonas. The microscope slide should be Gram-stained and examined for candida and clue cells in the laboratory.

Swab No. 4: Take this swab only if there is an ulcer on the cervix or inside the vagina. It is a cotton-wool-tipped swab for collecting exudate from the ulcer. Roll the head of the swab on the base of the ulcer without

scratching the ulcer and making it bleed. Be gentle as it may be a painful procedure. Plate the material onto the culture medium plate containing Mueller-Hinton agar marked "MH". This will be cultured for *Haemophilus ducreyi*. After plating, discard the swab.

Candle jar: Place the culture plates, one on top of each other, in a glass bottle with a large mouth. Light a candle and place it on the top plate. Close the bottle over the burning flame. The flame will die out as soon as the oxygen in the bottle is used up. The bacteria grow better in the absence of oxygen. This is called candle jar. When you take another specimen, open the candle jar, remove the candle, put the new culture plate on top, put the lighted candle on the top and close the jar. Put all culture plates in the candle jar in this way.

After completing interview, examination and collection of vaginal and cervical specimens, ask the subject to dress. Collect 10 ml of blood and place 5 ml in one tube and 5 ml in another as follows:

Blood tube 1: Label with the subject's age and clinic identifier. This will be tested for HIV.

Blood tube 2: Label with the subject's study number and clinic identifier. This will be tested for syphilis and herpes.

Send all specimens every day to the laboratory as per the arrangement made with the laboratory.

VI. Diagnosis

For all subjects, write down the abnormal findings and note the diagnosis. If the subject has an STI, note the syndrome.

VII. Treatment

If the subject has any abnormal finding, prescribe the appropriate treatment. Remember that most of the women coming for routine antenatal care or family planning service will not have any abnormal finding. For STI syndrome, follow the national management guidelines. Ask the subject to return after a week for the test results. For those who were prescribed treatment for STI syndrome, review the treatment on the basis of test results. For those who were not prescribed treatment for STI syndrome, if the test results show an infection, initiate the treatment.

Annex 4

LABORATORY TESTS

Specimen handling: All specimens collected in the clinic during the day should be transported every day to the laboratory. The specimens should be logged in the register in the laboratory. Candle jars, culture plates and other supplies should be replenished as necessary. The blood tubes should be spun down in a centrifuge, serum should be separated and aliquots should be stored frozen. The following tests should be performed at the laboratory.

Blood Tube 1: On the serum obtained from this tube, perform an ELISA test for HIV. If positive, perform a second test based on different principle or method. Follow the national guidelines and algorithm for HIV testing.

Blood Tube 2: The serum should be tested as follows:

- Rapid plasma reagin (RPR) or VDRL test
- Herpes virus antibody test using RIBA or microimmunofluorescent methods

Microscope slide At the laboratory, the slide prepared from Swab No. 3 should be Gram -stained and examined in a microscope under oil immersion (x 1000) for

- Gram-positive yeasts and hyphae of *Candida albicans*
- Gram positive rods of *Lactobacillus* spp.
- Clue cells suggestive of *Gardnerella vaginalis*.

High vaginal swab in Diamond medium (Swab No. 3): At the laboratory, a fresh wet mount of the fluid should be examined in a microscope under low power (x 400) for motile *Trichomonas vaginalis*.

Gonococcal culture: The inoculated plates of modified Thayer -Martin medium (from Swab No. 1) should be incubated in carbon dioxide for 48 hours. Cultured colonies should be identified as *N. gonorrhoeae* by morphology, Gram staining, sugar fermentation tests and oxidase production. Cultured strains should be subcultured and tested for penicillinase production and for antimicrobial susceptibility using the disc method.

***Haemophilus ducreyi* culture:** The inoculated plates of Mueller -Hinton agar (from Swab No. 4) should be incubated in carbon dioxide for 48 to 72 hours. Cultured colonies should be identified as *H. ducreyi* by morphology, Gram staining and biochemical tests. Cultured strains should be subcultured and tested for antimicrobial susceptibility using the disc method.

Chlamydia antigen test: An ELISA test for the detection of chlamydia antigen should be performed on the cervical material carried in the transport medium (from Swab No. 2). Follow the standard procedure.

Reporting of results: Results of laboratory tests should be reported in the forms given in Annex 5 and Annex 6. Annex 6 is for the results of HIV tests, is used for a number of subjects in a single sheet and should be sent after a batch of specimens has been tested. Annex 5 is for all other tests, one sheet is used for each subject and the report should be sent after the results of all tests are available for the individual subject. One copy of the results should be sent to the clinic, a second copy should be sent to the principal investigator and the third copy should be kept at the laboratory.

Annex 5

RESULTS OF LABORATORY TESTS

Study number

Clinic name _____

Clinic number

Date specimen received Day Month Year

Date report sent out Day Month Year

High vaginal swab

Candida Positive Negative

Gardnerella Positive Negative

Gonococcal culture

N. gonorrhoeae Positive Negative

PPNG strains Positive Negative

Isolated strains of *N. gonorrhoeae* are SENSITIVE to the following antibiotics;

Isolated strains of *N. gonorrhoeae* are RESISTANT to the following antibiotics;

***Haemophilus ducreyi* culture** Positive Negative

Isolated strains of *H. ducreyi* are SENSITIVE to the following antibiotics;

Isolated strains of *H. ducreyi* are RESISTANT to the following antibiotics;

Chlamydia antigen test Positive Negative

RPR/VDRL test Positive Negative

Herpes virus antibody test

Herpes simplex virus Type I antibody Positive Negative

Herpes simplex virus Type II antibody Positive Negative

Annex 7
TIME FRAME

	Months												
	0	1	2	3	4	5	6	7	8	9	10	11	12
Appoint Principal Investigator	/-----/												
Convene Technical Advisory Group	/-----/												
Refine Study Protocol		/-----/											
Appoint Study Site Coordinator		/-----/											
Identify Laboratories		/-----/											
Procure supplies, equipment, medicines, test kits			/-----/										
Training				/-----/									
Field test questionnaire					/-----/								
Finalize questionnaire					/-----/								
Collect data						/-----/							
Laboratory tests						/-----/							
Data entry						/-----/							
Data analysis							/-----/						
Report writing								/-----/					
Report dissemination											/-----/		