PROJECT TITLE

The Etiology of Sexually Transmitted Syndromes in Zimbabwe

Principal Investigator:
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Lead Consultant:
Cornelis A. Rietmeijer, M.D., PhD

Co-Investigators:
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Gerald Shambira, M.D., MPH
David Lewis, M.D., PhD
Owen Mugurungi, M.D
Anna Machiha, BSc. Nursing

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Collaborating Organizations:

- University of Zimbabwe, College of Medicine, Department of Community Medicine (Zimbabwe)
- Ministry of Health and Child Welfare (Zimbabwe)
- Centers for Disease Control and Prevention (U.S.A)
- Colorado School of Public Health, University of Colorado (U.S.A.)
- National Institute of Communicable Diseases (South Africa)

Investigators Affiliations, Roles and Responsibilities

1. Professor Mufuta Tshimanga, MD, MPH
   - Affiliation: Department of Community Medicine, University of Zimbabwe, College of Medicine
   - Role: Principal Investigator
   - Responsibility: Prof. Tshimanga carries the end-responsibility of the project, including the development and implementation of the study protocol, oversight of study subject recruitment and welfare, data collection and analysis, and reporting of results.

2. Professor Cornelis Rietmeijer, MD, PhD, MSPH
   - Affiliation: Rietmeijer Consulting, LLC and Colorado School of Public Health, University of Colorado Denver.
   - Role: Lead Consultant
   - Responsibility: Prof. Rietmeijer is contracted to be the executive arm of the Principal Investigator. He will be the primary lead in developing the study protocol and overseeing all study activities, including selection of study sites, the orderly recruitment and follow-up of study participants, laboratory procedures, data processing and analysis, laboratory and assuring the timely dissemination of study results.

3. Dr. Peter Kilmarx, MD
   - Affiliation: Centers for Disease Control and Prevention (CDC)
   - Role: Co-Investigator
   - Responsibility: Dr. Kilmarx is the Country Director for the CDC in Zimbabwe, the sponsor of the project. He will provide fiscal oversight on behalf of CDC; will review all study documents, and will be involved in the analysis and dissemination of study results.

4. Dr. Gerald Shambira, MD, PhD
   - Affiliation: Department of Community Medicine, University of Zimbabwe, College of Medicine
   - Role: Co-Investigator
   - Responsibility: Dr. Shambira will directly oversee the study staff involved with study implementation, including staff recruitment and training, and quality assurance. He is also the lead responsible person in the development and implementation of the electronic study databases, data entry, storage and integrity. He will also be involved with the data analyses and dissemination of study results.

5. Dr. David Lewis, MD, PhD
   - Affiliation: National Institute of Communicable Diseases, Johannesburg, SA
   - Role: Co-Investigator
   - Responsibility: Dr. Lewis is the head of the STI Reference Laboratory at the National Institute of Communicable Disease in Johannesburg. He will oversee the multiplex-PCR testing of all specimens for this study. He will also be consulted on data analyses and development of study reports and manuscripts.

6. Dr. Owen Mugurungi, MD

Affiliation: Zimbabwe Ministry of Health and Child Welfare
Role: Co-Investigator

Responsibility: Dr. Mugurungi is the Director of the HIV and TB Programme at the Zimbabwe Ministry of Health and Child Welfare. He oversees the involvement of the Ministry in the development, implementation and evaluation of the study. He will also be consulted on data analyses and the development and dissemination of study reports and manuscripts.

7. Ms. Anna Machiha, BSc. Nursing

Affiliation: Zimbabwe Ministry of Health and Child Welfare
Role: Co-Investigator
Responsibility: Ms. Machiha is the STI and Condom Coordinator in the HIV and TB Programme and the Zimbabwe Ministry of Health and Child Welfare. Ms. Machiha will be involved with the implementation of the study and will function as the Ministry's liaison with participating clinics.

8. Ms. Amy Herman Roloff, MPH

Affiliation: Centers for Disease Control and Prevention
Role: Co_Investigator
Responsibility: Ms. Herman Roloff is the Chief Science Officer at CDC Zimbabwe. She will oversee project quality and integrity. She will also assist with data analyses and manuscript peparation.

I. Hypotheses and Specific Aims

Goal: To determine the current etiology of syndromes associated with sexually transmitted infections (STI) in Zimbabwe

Specific Aims:

1. To determine the prevalence of the following micro-organisms among men with urethral discharge syndrome in Zimbabwe: *Neisseria gonorrhoeae*, *Chlamydia trachomatis, Mycoplasma genitalium*, and *Trichomonas vaginalis*.
2. To determine the prevalence of the following micro-organisms among women with vaginal discharge syndrome in Zimbabwe: *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Trichomonas vaginalis*, and *Candia albicans*.
3. To determine the prevalence of bacterial vaginosis among women with vaginal discharge syndrome in Zimbabwe
4. To determine the prevalence of the following micro-organisms among men and women with genital ulcer disease in Zimbabwe: *Treponema pallidum*, *Haemophilis ducreyi*, herpes simplex virus, and *Chlamydia trachomatis* (LGV strains)
5. To assess the prevalence of HIV among men and women presenting with sexually transmitted syndromes in Zimbabwe.
6. To assess the prevalence of positive syphilis serology among men and women with sexually transmitted syndromes in Zimbabwe.
7. To assess the prevalence of urethral/vaginal *N. Gonorrhoeae* and *C. trachomatis* co-infection in men and women diagnosed with genital ulcer disease
8. To determine demographic and risk factors associated with the prevalence of the above infections.

II. Background and Significance:

Sexually transmitted infections (STI) continue to place a significant burden on the health of populations in both developed and developing countries. Besides HIV infection, with its known impact on morbidity and mortality, other STIs, including gonorrhea, chlamydia, trichomoniasis, syphilis, and genital herpes are still highly prevalent with important reproductive health consequences, especially pelvic inflammatory disease (PID) and its long-term complications among women, including infertility, ectopic pregnancy, and chronic abdominal pain. These "other" STIs can also play an important role in the transmission of HIV. Thus the diagnosis and treatment of these conditions remain a public health priority.

The World Health Organization estimates that close to 500 million cases of non-HIV "curable" STI occurred in 2011 globally, including more than 275 million cases of thrichomoniasis, 106 million cases of gonorrhea, 105 million cases of chlamydia and 10 million cases of syphilis.[1]

In Zimbabwe, as in many other developing countries, laboratory testing for the etiologic agents of STIs is largely unavailable. Thus, STI cases are not reported by their etiology but rather by the symptoms they cause. Thus in 2012, Zimbabwe reported close to 90,000 cases of women diagnosed with vaginal discharge syndrome, more than 50,000 cases of men with urethral discharge, over 50,000 cases or genital ulcer disease and close to 60,000 other STIs. In addition, 40,000 cases of PID were diagnosed – a critical indicator given that PID is the most important complication of STIs among women with significant direct and long-term morbidity.[2]

It is important to note that cases reported to health authorities represent but the tip of the STI iceberg. For example, most of the 1 million reported cases of chlamydia in the U.S. are diagnosed by screening asymptomatic women. STI screening is not available in countries like Zimbabwe and only STI cases that have progressed to the symptomatic stage and present for care are ultimately treated and reported.

As in other countries where etiologic testing is not available, most STIs in Zimbabwe are treated "syndromically", i.e. using a combination of antimicrobials that cover the most important etiologic agents of each STI-associated syndrome. Thus, men with urethral discharge syndrome are treated for gonorrhea, chlamydia, trichomoniasis and mycoplasma infections. Women with vaginal discharge syndrome are treated for the same infections as well as vaginal candidiasis and bacterial vaginosis. Finally, men and women with genital ulcerations are treated for syphilis, chancroid, and herpes infections.

Most countries follow the STI syndromic management guidelines published by the World Health Organization[3] or have published country-specific treatment protocols. Thus, governmental agencies in Zimbabwe have developed guidelines for the treatment of STI and its associated syndromes.[4,5]

An obvious drawback of using the "syndromic approach" is that it leads to both under-treatment as many asymptomatic cases are missed, as well as over-treatment since syndromic treatment at the individual level covers all possible etiologies and not the one(s) actually involved in each specific case.

Furthermore, because the epidemiology of individual STI shifts over time, treatment regimens may be outdated, or may fail to include medications to cover infections that have gained in importance. For example, over the several past decades, a significant shift has occurred in the etiology of genital ulcer disease in sub-Saharan Africa. Whereas chancroid and syphilis were responsible for a majority of cases in the past, currently the preponderance of cases is caused by genital herpes.[6,7] This shift has important consequences for the syndromic treatment of genital ulcer disease as it requires the inclusion of acyclovir (or similar antiviral medications) in the syndromic treatment regimen.

Also, during the past decades, important shifts have occurred in the etiology of urethral and vaginal discharge syndromes in the western world, with gonorrhea decreasing and chlamydia (relatively) increasing. Furthermore, the role of other micro-organisms, especially *Mycoplasma genitalium* is becoming increasingly recognized. This latter organism has an antibiotic susceptibility profile that is significantly different from that of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. For example, in a recent treatment study among men with urethritis in the U.S, where subjects were randomized to receive standard doses of either azithromycin or doxycycline, among those who were diagnosed with a *M. genitalium* infection only 40% and 30% had a microbiological cure respectively.[8] Thus, the relative prevalence of mycoplasmas as a cause of urethral and vaginal discharge syndromes should also determine the composition or priority order of the syndromic treatment regimens.

Significance for the Treatment of STIs in Zimbabwe

Together, the above findings form an argument for a more etiologically-oriented approach of STI diagnosis and treatment in developing countries like Zimbabwe. In the absence of routine testing for individual micro-organisms, periodic studies should be conducted among

representative samples of patients with sexually transmitted syndromes to best describe the current etiology of these syndromes in order to develop syndromic treatment guidelines in Zimbabwe on a scientific basis.

Capacity Building

While not primarily designed to develop clinical and laboratory capacity in Zimbabwe, the study will assist in capacity building in the following ways:

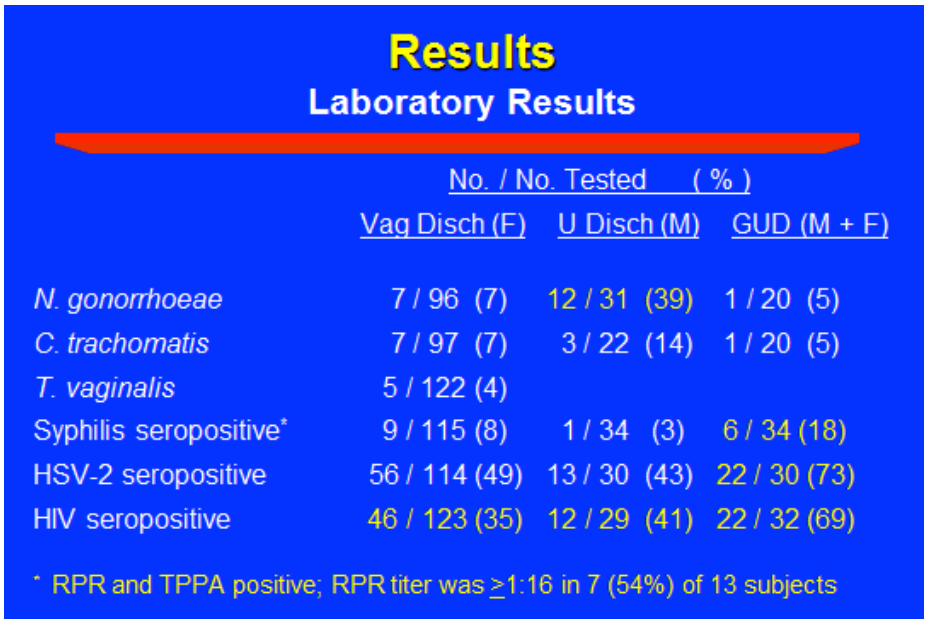
1. The clinics where the study will be implemented will benefit from the availability of research staff to train clinical personnel in current guidelines of STI management and treatment; development and implementation of research protocols, and clinic-level assessment of the prevalence of pathogens associated with STI syndromes. Details on the team approach to clinic-based research are detailed below. In addition, clinics and their staff will be compensated for research-related time and effort by being offered $5 per enrolled patient. Study subjects will likewise be compensated $5 for their time.

2. Local laboratory capacity will be used and enhanced in the following ways:
    a. Diagnostic testing for gonorrhea and chlamydia will be conducted by a local fully accredited laboratory (UCSF lab in the OB/Gyne department at the University of Zimbabwe School of Medicine), using the Becton-Dickenson ProbeTec platform.
    b. Laboratory capacity for the diagnosis of STIs will also be enhanced by the validation of the novel GeneXpert platforms for gonorrhea and chlamydia testing which is currently used for TB testing, but can also be used for chlamydia and gonorrhea testing.[9]
    c. Capacity for HIV and syphilis diagnosis will be enhanced by the validation of the Bioline DUO (combines HIV and syphilis) rapid test
    d. The study will provide a large repository of well-documented and tested specimens that can be used for further capacity building in molecular testing technologies (including multiplex PCR) at other Zimbabwe-based laboratories.

III. Preliminary Studies

This study will follow a protocol similar to previously conducted studies in the region, including Botswana[10], Zambia[11], and South Africa[7]. Of particular relevance are two studies conducted in Zimbabwe:

1. In 2007, a pilot study was conducted in Zimbabwe to determine the aetiologies of common STI syndromes. The study aimed to enroll 350 study subjects (150 women with vaginal discharge, 100 men with urethral discharge, 50 women and 50 men with genital ulcer disease). After enrolling 194 subjects, the study was ended due to societal issues that affected STI clinic staffing. The slide below shows study results.



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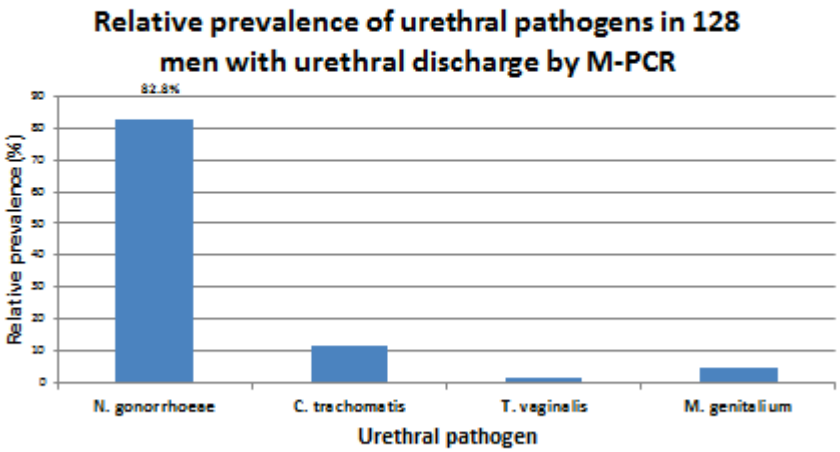
Preliminary conclusions suggested that a) chlamydia prevalence was low and marginally associated with the discharge syndromes; b) gonorrhea was more prevalence and a common cause of male discharge syndrome; 3) HSV-2 seropositivity was high and commonly associated with genital ulcer disease; 4) HIV was highly prevalent in the entire study group, especially among those with genital ulcer disease.

Conclusions of this study were weakened by the fact that the study was discontinued when relative few men with urethral discharge (N=35) and men and women with genital ulcer disease (N=35) were enrolled. Furthermore, planned laboratory tests (such as testing for *M. genitialium*) were canceled after the study was aborted.

2. Recently (2011) a study was completed to assess antimicrobial susceptibility of *N. gonorrhoeae* among men with urethral discharge recruited from 12 polyclinics in Harare. Among 128 men with urethral discharge in this study, relative prevalence rates for *N. gonorrhoeae*, *C, trachomatis*, *T. vaginalis*, and *M. genitalium* are shown on the slide below. Strikingly, close to 83% of men with discharge had gonorrhea. However, this finding may be biased by the fact that the study aimed to enroll men with gonorrhea and thus men with more typical presentations of gonococcal urethritis may have been over-enrolled.[12]



IV. Research Methods

A. Outcome Measures:

1. Urethral discharge in men (N=200)
Prevalence of *N. gonorrhoeae, C. trachomatis, U. genitalium, T. vaginalis*

2. Vaginal discharge in women (N=200)
Prevalence of *N. gonorrhoeae, C. trachomatis, U. genitalium, T. vaginalis, C. albicans*
Prevalence of bacterial vaginosis

3. Genital ulcer disease in men and women (N=200)
Prevalence of *T. pallidum, H. ducreyi, C. trachomatis* (LGV strains), HSV
Urethral/vaginal co-infection prevalence of *N. gonorrhoeae* and *C. trachomatis*

4. All syndromes (N=600)
Prevalence of HIV and syphilis (based on serology and rapid test)

B. Description of Population to be Enrolled:

1. Populations

The purpose of this study is to support and inform the Zimbabwe STI treatment recommendations. Thus, the target study population will be comprised of men and women who present for STI treatment at public health facilities.

The following, mutually exclusive, populations will be enrolled at participating clinic sites:

- Men with urethral discharge (N=200)
- Women with vaginal discharge (N=200)
- Men or women with genital ulcer disease (GUD; N=200)

Note: Men or women presenting with a combination of GUD and discharge syndromes will be enrolled in the GUD group only as GUD may be accompanied by genital discharge as well. However,

as specified above, urethral/vaginal co-infection with *N. gonorrhoeae* and *C. trachomatis* will be assessed in the GUD study group.

2. Clinic Sites

The designation of clinic sites is determined by two, partly countervailing factors. On the one hand, an unbiased assessment of STI prevalence would be served by sampling patients from a large variety of clinics representing all regions of the country. However, as explained further below, such an approach will be constrained by logistical and budgetary limitations. Thus, from a pragmatic point of view, we will use a phased approach starting in one busy clinic in Harare (Mbare Polyclinic) to pilot-test and fine-tune the study protocol and study procedures. During the next phase, the study will be implemented in other clinics inside (Budiriro) and outside Harare (Nkulumane and Khami Road clinics in Bulawayo, Dulibadzimu clinic in Beitbridge, and Gutu Road clinic in Gutu), based on regional STI surveillance data and availability of funds. More details on the selection of study sites will be provided in the next section.

C. Study Design and Research Methods

Sample Size

The main purpose of this study is to determine the relative prevalence of pathogens associated with the common STI syndromes in Zimbabwe. The stability of prevalence estimates, as determined by their 95% confidence intervals is dependent on the point estimate and the sample size. In Table 1, we have listed 95% confidence intervals as a function of a range of point estimates and sample sizes based on the formulas by Wilson and Newcombe with continuity correction.[13] Table 1 demonstrates that the largest gains in tightening the confidence intervals occur at the lower end of the sample size spectrum. For example, for a point prevalence of 15%, one would gain a 4.6% point "tightening" of the confidence interval when moving from 100 to 200 subjects, but only an additional 1.9% points when moving from 200 to 300 and 1.1% point when moving from 300 to 400. Thus the marginal gain in decreasing the confidence intervals comes at an increasingly higher marginal cost (assuming a stable per subject cost when increasing the sample size).

Based on these statistical considerations as well as the limited resources available to the study, we feel that sample sizes of 200 each for urethral discharge and vaginal discharge syndromes will be adequate. For genital ulcer disease with lower expected frequencies for both chancroid and syphilis, a larger sample size would be desirable, but might not be feasible within the time and budgetary constraints of the project given the overall lower prevalence of genital ulcer disease in comparison to the discharge syndromes. The experience with the previous study (see above) provides further support for this argument. We will thus aim to enroll 200 subjects in this category as well.

Since the study population is in essence a convenience sample, we do not intend to apply sample weights. In the presentation of the study results, we will be careful in presenting clinic comparisons as well as list caveats of combining the data.

To allow for screen failures, incomplete data, and other unforeseen events, we anticipate a maximum of 15% attrition of evaluable patients for any given analysis, and will thus over-enroll by 100 patients, for a total of 700 study subjects.

Study Sites

Study site selection is based on considerations of external and internal validity. To optimize external validity, a large number of study sites representing both urban and rural regions of the country would be desirable. However, multiple enrollment sites, especially those with low expected number of cases would compromise internal validity as it would be difficult to train a large number of study staff and provide adequate quality control of study procedures, including collection and processing of data and specimens (more on threats to internal and external validity below). Also, maintaining a large study staff to enroll patients at multiple study sites would require resources that are beyond the budget for this study. Moreover, variance of STI syndrome etiologies within a certain geographic area is likely to be small, such that the involvement of a limited number of large, geographically diverse centers will likely suffice for study purposes. In addition, if diversity is larger than anticipated, additional study sites will be identified as the study progresses.

Based on the above considerations, the study will start with a pilot site (Mbare clinic in Harare) to streamline study procedures, including the enrollment of study subjects, data and specimen collection, processing at the local staging laboratory, shipment to the reference laboratory, and receipt and processing of test data. After 50-75 subjects have been enrolled, the study will then be implemented at 4-8 subsequent urban and rural sites.

Based on STI syndromic surveillance data for 2012[2], the following (poly-) clinics in Harare, Bulawayo and other regions reported the largest number of cases:

Aetiology Study
Harare Clinic Stats - 2012

Facility	Disch F	/week	Disch M	/week	GUD F	/week	GUD M	/week
Mabelreign Satellite Clinic	579	11.1	442	8.5	306	5.9	280	5.4
Budiriro PolyClinic	891	17.1	627	12.1	191	3.7	193	3.7
Mbare PolyClinic	1507	29.0	458	8.8	219	4.2	160	3.1
Hopley Clinic	458	8.8	399	7.7	172	3.3	167	3.2
Warren Park PolyClinic	585	11.3	372	7.2	177	3.4	116	2.2
Western Triangle Clinic	426	8.2	273	5.3	135	2.6	136	2.6
Kuwadzana PolyClinic	785	15.1	384	7.4	126	2.4	115	2.2
Hatfield Clinic	469	9.0	377	7.3	127	2.4	92	1.8
Harare Central Hospital	150	2.9	17	0.3	131	2.5	84	1.6
Parirenyatwa Clinic	330	6.3	253	4.9	79	1.5	111	2.1

Aetiology Study
Bulawayo Clinic Stats - 2012

Facility	Disch F	/week	Disch M	/week	GUD F	/week	GUD M	/week
Khami Road Clinic	289	5.6	974	18.7	65	1.3	233	4.5
Princess Margaret Clinic	274	5.3	260	5.0	71	1.4	105	2.0
Mpilo Central Hospital	93	1.8	30	0.6	97	1.9	29	0.6
Luveve Clinic	359	6.9	248	4.8	76	1.5	48	0.9
Pelandaba Clinic	140	2.7	139	2.7	63	1.2	60	1.2
Mzilikazi Clinic	140	2.7	155	3.0	48	0.9	62	1.2
Emakhandeni Clinic	144	2.8	150	2.9	50	1.0	57	1.1
Cowdray Park Clinic	222	4.3	118	2.3	51	1.0	37	0.7
Nkulumane Clinic	248	4.8	184	3.5	46	0.9	41	0.8
Maqhawe Clinic	186	3.6	81	1.6	44	0.8	34	0.7

Aetiology Study
Other Clinic Stats - 2012

Facility	Disch F	/week	Disch M	/week	GUD F	/week	GUD M	/week
ms Dulibadzimu Clinic	988	19.0	1037	19.9	411	7.9	588	11.3
me Epworth Polyclinic	1056	20.3	510	9.8	318	6.1	301	5.8
me Chivhu Clinic	215	4.1	108	2.1	179	3.4	133	2.6
me Rakodzi Clinic	183	3.5	141	2.7	152	2.9	147	2.8
mc Howard Mission Hospital	678	13.0	162	3.1	140	2.7	123	2.4
mv Masvingo Provincial Hospital	361	6.9	220	4.2	147	2.8	104	2.0
mv Gutu Rural Hospital	396	7.6	237	4.6	118	2.3	125	2.4
mi Monomutapa Clinic	459	8.8	384	7.4	104	2.0	123	2.4
me Overspill Clinic	587	11.3	189	3.6	130	2.5	96	1.8
mw Kadoma District Hospital	265	5.1	286	5.5	76	1.5	138	2.7

A number of candidate clinics were visited in November, 2013. Based on these site visits, the following clinics were selected for study participation: Mbare and Budiriro clinics in Harare, Nkulumnane and Khami Road clinics in Bulawayo, Dulibadzimu clinic in Beitbridge, and Gutu Road clinic in Gutu. As mentioned above, additional clinics may be added as the study progresses.

To maximize quality control by a limited study team, the study will be conducted in a staggered fashion; deploying the research team(s) at involved clinics sequentially. Ongoing quality-assurance measures will be taken by the study team to assure the correct implementation of the protocol at each clinical site. This

will be similar to the approach taken by the ongoing PMTCT study conducted by the Department of Community Medicine at the University of Zimbabwe College of Medicine, and lessons learned in that study will be applied to the development and implementation of the STI aetiology study.

Inclusion and Exclusion Criteria

Inclusion Criteria

- Male or Female
- Sexually Active
- 18 – 55 Years of age
- English, Shona, or Ndebele speaking
- Presenting with a chief complaint of:
    - Urethral discharge (men)
    - Vaginal discharge (women)
    - Genital ulceration (men and women)

Exclusion Criteria

- Previous study enrollment
- Unable to provide consent (serious illness, under influence of drugs and/or alcohol)
- Receipt of antibiotics for STI treatment in previous 4 weeks

Note: Pregnancy is not a reason for exclusion. We will assure to enroll at least 20% of women with vaginal discharge who are currently pregnant.

Study Procedures

Enrollment

Patients who meet the enrollment criteria as identified by clinic staff, will be referred to the on-site study nurse who will introduce the study and answer initial questions. If patients express interest, they will then proceed to the consent process.

Consent Process

In a private and quiet environment, the patient will be asked to read the consent form and upon completion discuss any questions (s)he may have with the study nurse. When the patient expresses understanding of the form, the nurse will ask the patient to repeat the information in her/his own words and address any further questions and correct misunderstandings. If the patient continues to agree to participate in the study, the study nurse will then ask the patient to sign he consent form and initial the individual pages. A duplicate form or a copy from the original form will be given to the patient and the original form will be stored in the patient folder.

A single consent form will be used for all study populations (See copy in Attachment 2).

Questionnaire

Upon completion of the consent process, the study nurse will collect identifying and demographic data as well as a brief behavioral assessment and STI history. All data will be primarily entered onto hard copy

forms and subsequently submitted to an electronic database on a hand-held device, for which specific programs will be written using the Onine Data Kit (ODK) software. The research team has extensive experience with this mode of data collection in the aforementioned PMTCT study. Paper hard copies will be available should electronic data entry not be available at the clinic site.

All patients will be provided with a unique study number, however, identifying information (name, date of birth, etc.) will also be collected so that the patient can be contacted should this be necessary, for instance in case of an identified infection for which no adequate treatment has been given. However, while de-identified data may be shared with laboratories and other collaborators, identifiable data will be stored in a single, password-protected and encrypted database.

A copy of the questionnaire/data form is included in Attachment 3.
Note: While certain data will be necessary to adequately characterize the individual study subject and appropriately analyze the outcome data, for quality control and ease of replicability in a variety of clinic settings, the amount of data collected will be kept to a minimum.

Clinical examination

After completion of the questionnaire, the study nurse will ask the patient to undress and proceed to perform an external genital examination. Findings, including a characterization of discharge or ulcerations, presence of lymphadenopathy, etc. will be noted on the data collection form already mentioned above.

Note: As mentioned below, a vaginal speculum examination is not necessary for optimal specimen collection and test performance. Since a speculum examination may not be available at some of the clinics or may not be the standard of care, this examination is not a requirement for the study.

Test sampling (clinic standard + study tests)

The following samples will be taken:

- Genital ulcer disease (men and women):
    - A single swab specimen from the base of the ulcer(s) (for m-PCR)
    - A urine specimen from males (for ProbeTec and GeneXpert)
    - A vaginal swab specimen from women (for ProbeTec and GeneXpert)
- Vaginal discharge (women):
    - PH testing by test strip
    - Swab specimens from discharge at the vaginal introitus for:
        1. Gram Stain
        2. ProbeTec
        3. GeneXpert
        4. Multiplex PCR
- Urethral discharge (men):
    - Swab specimen from discharge at urethral opening for:
        1. Gram Stain

2. ProbeTec
3. GeneXpert
4. Multiplex PCR

- All patients (men and women)

  - Single blood specimen for HIV (optional) and syphilis testing

All specimens are placed in appropriately labeled (name, study number) containers and stored with the patient's study folder for transportation to the central study ("receiving") lab.

Note 1: Vaginal specimens (self-obtained or provider obtained) are the preferred specimens for nucleic acid amplification testing (NAAT) as they provide higher sensitivity levels than urine specimens or specimens obtained from the cervix. Hence, from a study perspective, speculum examination is not necessary.

Note 2: Specimens for Gram stains are smeared onto a glass slide marked with the patient's name and study number and air dried.

Note 3: If HIV and/or syphilis rapid testing is available in the clinic, these tests may be conducted on-site as per clinic protocol. Test results will be documented in the study forms. In addition, all patients consenting to HIV testing in the study consent form will have blood drawn for HIV and syphilis testing at the study laboratory whether or not they were also tested at the clinic.

Note 4. Gonococcal Resistance study.
Men presenting with urethral discharge may be co-enrolled into a study evaluating gonococcal antimicrobial resistance (Surveillance of Gonococcal Antimicrobial Susceptibility in Zimbabwe
Developed by the Zimbabwe Ministry of Health). However, this study is governed by its own protocol and consent process.

More details regarding laboratory procedures are included in Attachment 4.

Provision of stat test results (if any)

In case the clinic provides any stat laboratory tests (e.g. rapid HIV testing), patients will be given these results and managed as per clinic protocol. This information will be documented on the patient's study form.

Treatment and counseling

All patients will be treated for their condition as per clinic protocol using the current Zimbabwe guidelines for the syndromic management of sexually transmitted inefections.[5] Study staff will assure the appropriate treatment of all enrolled patients and will supply medications if necessary,

All patients will be counseled on their respective syndromes, including how to take the medications and take care of their symptoms, sexual abstinence during treatment, the need for partner notification and treatment, and prevention of repeat infections through reducing the number of sex partners and condom use. Uncircumcised men will be counseled to undergo circumcision. Women not currently on birth control

will be counseled on the use of effective birth control methods. Patients newly diagnosed with HIV infections will receive appropriate referrals as will patients with known HIV infection who are currently not receiving HIV care.

Data and Specimen Processing

Detailed information on study laboratory procedures is available in Attachment 4. Following are the most relevant details.

Upon completion of the study visit, all hard copy consent forms, written logs, data and specimens are stored together and held at the clinic under appropriate conditions until they can be shipped to the central receiving laboratory. For outlying clinics, shipment of study materials may be batched and sent once or several times per week. Storage at the clinics will be secure and only study staff will have access to patient data and specimens.

Central (receiving) laboratory

All individual data visit forms and specimens will be sent to the ZiCHIRe laboratory at Wilkins Hospital in Harare that will function as the central receiving laboratory.

The purpose of this laboratory is four-fold – each guided by standard operating procedures (SOPs).

1. Data entry: As described previously, data for each study patient are directly entered into an electronic database at the study site. These data will be reviewed at the central laboratory to assure that all specimens are appropriately linked to the database.

2. The central laboratory will also perform a limited number of tests, including HIV and syphilis testing, upon receipt of the specimens. Timely processing is necessary given the need for appropriate notification should any of these tests be positive for a person who has not been previously diagnosed with this condition and/or has not received appropriate treatment or referral. This is less urgent for the treatment of infections associated with the discharge syndromes for which testing will be performed at the reference laboratory (see below) and where test results will not be available for some time, given that syndromic management for these conditions should cover the treatment for all these pathogens.

   All patients who consent to HIV testing will be evaluated with the SD Bioline DUO (combined rapid HIV and syphilis testing) in addition to standard HIV and syphilis tested as described in Attachment 4.

   The central lab will also perform Gram staining of the dried glass slide specimens and investigate for the presence of intracellular, gram-negative diplococcic (male urethral discharge) and Nugent scoring (vaginal discharge). The results of these tests will be cross-referenced with the individual patient's treatment to assure receipt of the appropriate antibiotic regimens.

3. Next, the central lab will assure the appropriate labeling, database documentation, and storage of the specimens that will be sent to the reference laboratories identified below, under shipment specifications determined by each reference lab. All data and specimens will be de-identified before shipment.

4. Finally, the central laboratory will receive the test results from the reference laboratories and enter these into the study database. Double data entry will be performed to assure data quality.

   Test results will once again be cross-referenced with patients study files to assure that patients with positive test results have received appropriate treatment. If there are any discrepancies, efforts will be made to find the patient and bring him or her back to the clinic for re-evaluation and (additional) treatment.

   The study database will be inspected at regular intervals by lead study consultant. However, only de-identified data will be shared outside the local lab (see section on data safety below).

Laboratory for Gonorrhea and Chlamydia Testing

The laboratory in the Obstetrics/Gynecology department at the University of Zimbabwe, School of Medicine will perform diagnostic testing for *N. gonorrhoeae* and *C. trachomatis* on all vaginal and urethral specimens using the ProbeTec (Becton Dickenson) platform. This laboratory is accredited by the College of American Pathologists, requiring ongoing validation studies and quality assurance. Using this laboratory has the following advantages:

- Timely turn-around of results, benefiting study participants who may not have been adequately treated
- Supports local capacity building
- Provide reference results for validation of the GeneXpert Platform (see below)

Validation of the GeneXpert Platform

GeneXpert platforms are being installed at many laboratories in Zimbabwe for TB testing. Recently, this system has been approved for gonorrhea and chlamydia testing in the U.S. and elsewhere.[9] The advantages of this system include a relatively short (90 minute) turn-around time, the possibility of running different tests simultaneously, and starting individual tests at different times. Thus, this is a very versatile platform that can be used for many different purposes and is easy to operate. Validating existing platforms for gonorrhea and chlamydia testing presents a unique opportunity to make better use of a system that is currently under-utilized, and would greatly increase the potential to enhancing the aetiologic diagnosis of STIs in Zimbabwe. The OB/Gyne lab at the University of Zimbabwe (see above) will provide technical assistance in the validation process.

Regional Reference Laboratory

The purpose of the regional reference lab is to conduct all tests that are not available at the regional lab or at other labs in Zimbabwe. Specifically, no laboratory in Zimbabwe has the capacity to conduct multiplex polymerase chain reaction (m-PCR) tests for most of the STI pathogens evaluated in this study. Since development and maintenance of such capacity in Zimbabwe falls outside the scope of this (time-limited) etiology study, specimens will be sent to the STI reference laboratory at the National Institute of Communicable Diseases in Johannesburg, South Africa.

Specifically, the reference laboratory will conduct the following tests:

Genital Ulcer Disease: multiplex polymerase chain reaction (m-PCR) for the following pathogens: *T.pallidum, H. ducreyi,* HSV and *C. trachomatis*. Specimens positive for HSV *or C. trachomatis* may undergo further testing for HSV type specification (HSV-1 vs. HSV-2) and LGV strain identification respectively.

Vaginal and Urethral Discharge syndromes: m-PCR for*: N. gonorrheae, C. trachomatis, M. genitalium,* and *T.vaginalis.*

As indicated above, specimens will be held at -70 ^{0}F at the local laboratory in Harare and sent in batches on dry ice to the regional laboratory.

Note: While commercial nucleic acid amplification tests are available for the detection of some of these pathogens (notably *N. gonorrhoeae* and *C. trachomatis*) the multiplex PCR tests used for this study include other pathogens and are only used in the research setting. The intended laboratory at NICD in Johannesburg has validated these tests and has extensive experience in using them in a number of studies in South Africa.

Test results will be communicated by electronic communication with study staff at the local laboratory in Harare and entered into the study database as described above.

Patient Follow-up and Additional Treatment

All patients will receive information that they may be contacted should one of their test results indicate that they have an infection for which they may not have received adequate treatment and that it will thus be important that they provide study staff with accurate contact information. This is particularly true for the tests that can be performed by the study lab in Harare (see below), including HIV and syphilis (treponemal and non-treponemal) tests as well Gram-stained smears for urethral discharge that may indicate gonorrhea.

However, patients will also be explained that there is currently no capability in Zimbabwe to provide many of the other study tests quickly; that these tests will therefore be sent to South Africa and may take weeks to months before test results are known. Still, study staff will cross-reference all test results with the patient data file to assure appropriate treatment has been given at the initial visit and will attempt to follow up with clinic staff and/or patients when questions on appropriate treatment arise or if, for any reason, no treatment was given. Since all patients will be treated according syndromic treatment guidelines at enrollment, instances of non-treatment should occur rarely, if at all.

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Data Safety

All hard-copy data (specifically the signed consent forms and identifying information) will be stored at the central laboratory in locked file cabinets.

Electronic data (including the main patient database) will be stored on a secure, password protected computer at the central laboratory. Electronic back-ups of data files will be made on a daily basis and stored on a separate, password protected computer in a different location.

Transfer of files will use secure encryption programs.

Electronic records will include a unique identifier for each enrolled patient that can be used as a cross-reference to the hard copy files. However, no other patient identifiers will be included in the electronic record. Thus, the only place where identifying information is stored is in the hard copy files. Maintaining these identifiers is necessary to allow patient follow-up should this be necessary based on test results.

Risks

Given the nature of the study, no serious adverse events are anticipated. Risks include:

- Anxiety and embarrassment when answering sensitive questions, including questions on sexual behaviors, and genital examination
- Mild discomfort when obtaining specimens
- Mild pain and potential bruising as a result of needle stick for blood sample collection
- Potential breach of confidentiality regarding sensitive information

Risk Minimization

Risks are minimized by:

- Appropriate inclusion and exclusion criteria
- No unnecessary procedures
- Trained study professionals
- Patient monitoring and follow-up
- Data safety procedures (see above)

Adverse Events Monitoring

Adverse events, including patient complaints, will be documented and shared with the study coordinator, the study consultant, and the PI, who will take action on a case-by-case basis. All adverse events will be reported to the regulatory agencies overseeing this study.

Benefits

The main benefit of this study is the generation of generalizable knowledge regarding the etiology of the main STI syndromes in Zimbabwe that will inform future STI treatment and management guidelines for the country and region.

Benefits for the patient include possible detection and treatment for infections beyond current standard protocols.

Note: The patient benefit is expected to be restively small since all patients enrolled in the study should be treated for the syndrome that made them eligible for the study.

Compensation

Participants will receive $5 US in cash upon completion of the study procedures as compensation for their time.

Clinics will likewise receive $5 US for each completed study visit as compensation for time and effort of clinic staff supporting the study. This compensation will benefit the entire clinic and is in addition to salaries for research staff and other study costs.

E. Potential Scientific Problems:

As stated above, the main purpose of this study is to determine reasonably precise estimates of the relative prevalence of the most common pathogens associated with STI-associated syndromes. A number of factors could possibly affect the study's internal and external validity.

Factors affecting internal validity

These factors include appropriate enrollment of subjects and data and specimen collection, appropriate timing and conditions of transportation, processing and storage at the local laboratory, transport from local to regional laboratory, testing at the regional laboratory, and data transfer. These issues will be addressed by: 1) development of, training in and adherence to the study protocol and operating procedure and 2) monitoring of protocol breaches and adverse events.

Factors affecting external validity

These factors ultimately determine the generalizability of the results. Most importantly, there needs to be a reasonable assurance that patients enrolled in this study are representative of all persons in Zimbabwe suffering from the conditions under study. This generalizability is limited by a number of factors that will be outside the study's control. For example, persons presenting at the clinics may have more severe symptoms than those who do not present. Given that some syndromes may present with more severe and acute symptoms when caused by one compared to another, this may lead to the over-representation of the pathogen causing the more severe symptoms. For example, since gonorrhea in men is more likely to be causing urethral symptoms than chlamydia, this may lead to a relatively higher likelihood of identifying *N. gonorrhoeae* than *C. trachomatis* among men with urethral discharge syndrome. Since some pathogens are more likely to cause asymptomatic infections than others and since asymptomatic persons are not enrolled in the study, the prevalence of pathogens found in this study will only be representative of persons with the respective syndromes and not necessarily a reflection of the relative prevalence of these pathogens in the general population.

While the study cannot control which patients present themselves to the clinics, the study can control who is eligible once presenting and special care needs to be given that all persons presenting with any of the respective syndromes will have an equal chance of being enrolled in the study and not only the individuals with the most severe manifestations.

F. Data Analysis Plan:

Primary analyses include calculation of the relative prevalence of pathogens associated with genital syndromes and 95% confidence intervals using the Wilson/Newcombe method with continuity correction.[13]

Given the fact that the study population is comprised of a number of convenience samples, comparisons between clinics will be interpreted carefully and caveats to the interpretation of combined results will be identified.

Secondary analyses include the association of demographic and risk factors with the prevalence of each of the pathogens or combination of pathogens; the extent of co-infection among pathogens; and the association with HIV infection. Simple univariate (chi-square, t-test) and multivariate (logistic regression) statistical procedures will be used to test for associations between dependent and independent variables.

Study findings will be reported after completion of the study. Interim reports will be generated at regular intervals during the study to determine progress as well as to inform potentially necessary protocol changes.

It is anticipated that the study will generate at least 4 publications: the first paper will describe the study methods and overall study outcomes, and 3 subsequent manuscripts will be developed for each of the STI syndromes. Study findings will also result in abstracts to be submitted to appropriate meetings and conferences.

G. Summarization of Knowledge to be Gained:

The main outcome of the study will be the determination of the current prevalence of the most important pathogens associated with genital ulcer disease, vaginal discharge syndrome, and male urethral discharge syndrome in Zimbabwe. These findings will inform national and regional guidelines for the treatment and management of sexually transmitted infections in Zimbabwe and the southern African region.

H. Project Integrity and Quality Assurance

Data will be downloaded and analyzed by the project's lead consultant on a regular basis and reports will be provided periodically. Any discrepancies or other concerns will be shared with project staff and resolved in a timely fashion. The projects' lead consultant will also conduct regular site visits to ensure appropriate implementation of the study protocol and data/specimen collection and transport. In addition, the consultant will conduct regular visits at the study lab and perform quality assurance activities as he deems necessary.

The Centers for Disease Control and Prevention specify the following:

Sponsor Monitoring

As the study sponsor, the Centers for Disease Control (CDC) may conduct monitoring or auditing of study activities to ensure the scientific integrity of the study and to ensure the rights and protection of study participants. Monitoring and auditing activities may be conducted by:

- *CDC staff ("internal")*
- *Authorized representatives of CDC (e.g., a contracted party considered to be "external")*
- *Both internal and external parties*

Monitoring or auditing may be performed by means of on-site visits to the Investigator's facilities or through other communications such as telephone calls or written correspondence. The visits will be scheduled at mutually agreeable times, and the frequency of visits will be at the discretion of CDC. During the visit, any study-related materials may be reviewed and the Investigator along with the study staff should be available for discussion of findings.

The study may also be subject to inspection by regulatory authorities (national or foreign) as well as the IECs/IRBs to review compliance and regulatory requirements.

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I: Attachments

1. Table 1: 95% Confidence Intervals In Relation to relation Prevalence and Sample Size
2. Consent form (English version)
3. Data collection form
4. Laboratory procedures

Attachment 1

Table 1. Sample Size in Relation to Prevalence and 95% Confidence Intervals

	SAMPLE SIZE						
	50	100	200	300	400	500	1000
RATE %							
1		0.05-6.24	0.17-3.95	0.26-3.14	0.32-2.72	0.37-2.46	0.51-1.90
2	0.1-12.01	0.35-7.74	0.64-5.38	0.82-4.52	0.93-4.06	1.02-3.77	1.26-3.13
3		0.78-9.15	1.23-6.72	1.47-5.81	1.63-5.33	1.75-5.01	2.07-4.31
4	0.7-14.86	1.29-10.51	1.87-8.01	2.18-7.07	2.38-6.55	2.52-6.22	2.91-5.46
5		1.86-11.83	2.56-9.27	2.93-8.29	3.16-7.74	3.33-7.39	3.77-6.59
6	1.56-17.54	2.46-13.12	3.28-10.50	3.70-9.48	3.96-8.92	4.15-8.55	4.65-7.70
7		3.10-14.38	4.03-11.71	4.49-10.66	4.78-10.07	4.99-9.69	5.53-8.81
8	2.59-20.11	3.77-15.61	4.79-12.89	5.30-11.82	5.62-11.22	5.84-10.82	6.43-9.90
9		4.46-16.83	5.57-14.07	6.12-12.97	6.47-12.35	6.71-11.94	7.33-10.99
10	3.74-22.59	5.16-18.04	6.37-15.23	6.95-14.11	7.32-13.47	7.58-13.05	8.25-12.07
11		5.89-19.22	7.17-16.38	7.80-15.23	8.19-14.58	8.46-14.16	9.16-13.14
12	4.97-25.00	6.63-20.40	7.99-17.52	8.65-16.35	9.06-15.69	9.35-15.25	10.08-14.21
13		7.38-21.56	8.82-18.65	9.51-17.46	9.94-16.79	10.24-16.34	11.01-15.28
14	6.28-27.36	8.14-22.71	9.66-19.77	10.38-18.57	10.83-17.88	11.14-17.42	11.94-16.34
15		8.91-23.85	10.50-20.88	11.26-19.66	11.72-18.97	12.05-18.50	12.87-17.40
16	7.64-29.66	9.70-24.99	11.35-21.99	12.14-20.76	12.62-20.05	12.96-19.58	13.81-18.45
17		10.49-26.11	12.21-23.09	13.02-21.84	13.52-21.13	13.87-20.65	14.75-19.51
18	9.05-31.92	11.30-27.22	13.08-24.18	13.92-22.92	14.43-22.20	14.79-21.71	15.70-20.55
19		12.11-28.33	13.95-25.27	14.81-24.00	15.34-23.27	15.71-22.78	16.64-21.60
20	10.50-34.14	12.92-29.43	14.83-26.36	15.72-25.07	16.26-24.33	16.64-23.83	17.59-22.64
25		17.12-34.84	19.28-31.7	20.28-30.37	20.89-29.60	21.31-29.08	22.37-27.83
30	18.29-44.78	21.45-40.11	23.84-36.94	24.94-35.58	25.60-34.79	35.70-44.46	27.19-32.96
35		25.91-45.26	28.49-42.09	29.66-40.73	30.37-39.92	30.85-39.38	32.06-38.06
40	26.73-54.80	30.48-50.30	33.22-47.17	34.46-45.80	35.19-45.00	35.70-44.46	36.96-43.12
45		35.14-55.25	38.02-51.17	39.31-50.82	40.07-50.02	40.60-49.48	41.89-48.15
50	35.72-64.28	39.90-60.10	42.89-57.11	44.21-55.79	45.00-55.00	45.53-54.47	46.86-53.14

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Attachment 2 – Consent Form

ZiCHIRe Project

(Zimbabwe Community Health Intervention Research)

UNIVERSITY OF ZIMBABWE

Department of Community Medicine

ZiCHIRe Office: 28 Van Praagh, Harare, Tel: 791649/762165

Study into the Aetiology of Syndromes Associated with Sexually Transmitted infections in Zimbabwe

Co- Investigator: Prof Mufuta Tshimanga
Phone number 04 -791649/ 762165

INFORMED CONSENT FORM

What you should know about this study:

- We give you this consent form so that you may read about the purpose, risk and benefits of this research study.
- Routine care is based upon the best known treatment and is provided with the main goal of helping the individual patient. The main goal of research studies is to gain knowledge that may help future clients.
- We cannot promise that this research will benefit you. Just regular care, have side effects that can be serious or minor.
- You have the right to refuse to take part, or agree to take part now and change your mind later.
- Whatever you decide, it will not affect your regular care.
- Please review this consent form carefully. Ask many questions before you make a decision.
- Your participation is voluntary.

PURPOSE

You are being asked to participate in a research study to learn more about the causes of sexually transmitted infections in Zimbabwe. You were selected as a possible participant in this study because you are diagnosed and treated for symptoms that are likely caused by a sexually transmitted infection or STI. There are a number of infections that can cause the symptoms that you have, but testing for these infections is not widely available in Zimbabwe. Therefore patients like you are treated with a combination of antibiotics that will cure all these infections. You will receive this treatment whether you choose to be in the study or not. However, it is important that studies like this are done to determine the actual causes of STIs in Zimbabwe so that clinicians can be sure that the treatment they provide is still adequate. Up to 600 people around the country will be in the study.

PROCEDURE AND DURATION

If you decide to participate in the study, the nurse will do the following:

- Ask you questions about your personal information (name, address, date of birth, phone number) and behaviours that may be related to your condition.
- Take swabs from your vagina or penis for laboratory testing.
- Take a blood sample for HIV and syphilis testing (optional).
- Provide treatment for your condition.
- Provide counselling to prevent STI and HIV infection.

- Provide information on study follow up.

Participation in the study will take about 30 minutes.

Of the above procedures, only the samples taken for testing and some questions asked of you are part of the research study. All other procedures, including treatment for your condition are part of regular care and will be provided to you whether you chose to be in the study or not.

Samples taken from you may be tested for any of the following organisms that cause sexually transmitted infections:

Causes of genital (penile or vaginal) discharge

- *Neisseria gonorrhoeae* – the cause of gonorrhea
- *Chlamydia trachomatis* – the cause of chlamydia infections
- *Mycoplasma genitalium* – the cause of mycoplasma infections
- *Trichomonas vaginalis* – the cause of trichomoniasis

Causes of genital ulcer disease

- *Treponema pallidum* – the cause of syphilis
- *Haemophilus ducreyi* – the cause of chancroid
- Herpes simplex virus – the cause of genital herpes

Some of your samples will be tested using laboratory procedures that are not be available in Zimbabwe or have not yet been approved for use in Zimbabwe. However, all tests will be limited to the detection of organisms listed above. No other tests will be performed. All results will be reviewed by the study investigator and if the review of your study record indicates that you have not been appropriately treated, we will contact you and invite you to return for a follow-up visit to the clinic.

HIV TESTING

All patients in the study will be asked to have an HIV test. If that test is positive, showing that you are infected with HIV, we will provide counselling and advice about how to protect your health and how to prevent transmitting HIV to your sex partner or partners. If you are pregnant, we will provide advice about how to protect your baby from getting HIV. We are unable to provide treatment for HIV, but we will help refer you to a clinic where expert HIV care is available. You can choose not to have an HIV test done and still be part of the study.

I agree to be tested for HIV ❑ Yes ❑ No

RISKS AND DISCOMFORTS

Discomforts you may experience while in this study include the following:

- Taking samples from your genitalia may cause slight burning.
- Taking a blood sample will cause some burning and pain when the needle goes into your vein and may cause bruising afterwards.
- Questions about sexual behaviour and having your genitals examined by the study nurse may cause embarrassment and anxiety.
- You might be found to have HIV. This probably will be upsetting, especially if you are not expecting to have HIV.

BENEFITS
We cannot and do not guarantee or promise that you will receive any benefits from this study. This study is designed for researchers to learn more about the causes of sexually transmitted infections in Zimbabwe. While you will receive treatment for your condition, the study is not designed to treat your illness or improve your health. However there is a chance that that we detect an infection for which you have not been appropriately treated. In that case, we will contact you and we will invite you back to the clinic for follow-up evaluation and treatment.

COMPENSATION
As compensation for your time, we will waive the fee for receiving services today. Upon completion of the study procedures, we will give you $5 USD.

ALTERNATIVE PROCEDURE OR TREATMENTS
You will be treated for your illness the same way whether you are in the study or not.

CONFIDENTIALITY
The information you give us will be kept confidential. No one outside of the study including anyone in your family will know the results of your interview. All records will be locked away or kept on password-protected computers. Your name or any other data that might identify you will not be used in any reports or publications resulting from this study.

VOLUNTARY PARTICIPATION
Participation in this study is voluntary. At any time, you may refuse to be in the study, your decision will not affect your future relations with ZiCHIRe or the University of Zimbabwe, its personnel and associated partners. If you decide to participate in this study, you are free to refuse to answer any or all questions or to withdraw from the study at any time without penalty. However, we would very much like it if you participate in the study.
For questions about this study contact:

Prof Mufuta Tshimanga,Tel 791649/762165
ZiCHIRe Project, 28 Van Praagh Avenue, Milton Park, Harare.

For questions about your rights as a research participant, contact:

The National Coordinator, Medical Research Council of Zimbabwe
National Institute of Health Research, Cnr Mazoe Street/Josiah Tongogara Avenue
Ph 791792/791193, Cell 0772433166

OFFER TO ANSWER QUESTIONS
Before you sign this form please ask any questions on any aspect of this study that is unclear to you. You may take as much time as much time as necessary to think it over.

AUTHORISATION
I am making a decision whether to or not to participate in this study. My signature indicates that I have read and understood the information provided above, have had all my questions answered, and have decided to participate.
The date you sign the document to enroll in this study, which is today's date, MUST fall between the dates indicated on the approval stamp affixed to each page. These dates indicate that this form is valid when you enroll in the study but do not reflect how long you may participate in the study.

_____ _____
Name of Research Participant (please print) Date

_____ _____
Signature of Participant or legally authorised Representative AM PM Time

Names of ZiCHIRe Staff witnessing/obtaining Consent	Signature of Staff Obtaining Consent	Date

FOR AN ILLITERATE PARTICIPANT ONLY

Name of Participant Witness and surname	Signature	Date

YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM TO KEEP.

If you have any questions concerning this study or consent from beyond those answered by the investigator, including questions about the research, your rights as a research subject or research related injuries; or if you feel that you have been treated unfairly and would like to talk to someone other than a member of the research team, please feel free to contact the MEDICAL Research Council of Zimbabwe on telephone 791792 or 7911930.

Attachment 3. Specimen Storage Consent

ZiCHIRe Project

(Zimbabwe Community Health Intervention Research)
UNIVERSITY OF ZIMBABWE
Department of Community Medicine
ZiCHIRe Office: 28 Van Praagh, Harare, Tel: 791649/762165

The Etiology of Sexually Transmitted Syndromes in Zimbabwe

Principal- Investigator: Prof Mufuta Tshimanga
Phone number 04 -791649/ 762165

SPECIMEN STORAGE INFORMED CONSENT FORM

INTRODUCTION:

You have decided to take part in the investigational research study named above, sponsored by the United States Centers for Disease Control and Prevention (CDC). While you are in this study, blood and urethral fluid (if male) and vaginal fluid (if female), or fluid from genital lesions will be collected from you. You are kindly being asked to agree to the storage of these samples for use during the study and after the study have ended. We are also asking to ship some of these samples to another laboratory, the National Institute of Communicable Disease in Johannesburg, South Africa. This consent form gives you information about the collection, storage, and use of these samples. These samples may be useful for future research. The study staff will talk to you about this information. Please ask if you have any questions. You will be asked to sign or make your mark on this form to indicate whether you agree to have your samples stored and tested. You will be offered a copy of this form to keep.

YOUR PARTICIPATION IS VOLUNTARY:

Allowing your samples to be stored is completely voluntary. You may decide not to have any samples stored other than what is needed to complete this study and still be in this research study or any future study. Even if you decide now that your samples can be stored for future research, you may change your mind at any time. If this happens, you must tell the study staff that you have changed your mind. If you decide not to have your samples stored or used for future research, they will be destroyed at the end of the study.

PURPOSE:

The purpose of shipping some of the specimens to South Africa Laboratory is to conduct all tests that are not available at the regional laboratory or at other laboratories in Zimbabwe. Specifically no laboratory in Zimbabwe has the capacity to conduct multiplex polymerase chain reaction (m-PCR) tests for most of the sexually transmitted infections pathogens evaluated in this study. Since development and maintenance of such capacity in Zimbabwe falls outside the scope of this (time limited) etiology study, specimens will be sent to the STI reference laboratory at the National Institute of Communicable Disease in Johannesburg, South Africa. The following tests will be done at that laboratory. Genital ulcer disease: multiplex polymerase chain reaction (m-PCR) for the following pathogens; *Treponema pallidum*, *Haemophilus ducreyi*, herpes simplex virus and *Chlamydia trachomatis*. Specimens' positive for HSV or *Chlamydia trachomati*s may undergo

further testing for HSV type specification (HSV-1 vs. HSV-2) and lymphogranuloma venereum strain identification respectively. Vaginal and urethral discharge syndromes: multiplex polymerase chain reaction for *Neisseria gonorrhea*, *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Trichomonas vaginalis*. No other tests, including genetic testing will be done by anyone on your stored specimens without first explaining the test to you and obtaining your permission.

The study researchers do not plan to contact you or your regular doctor with any results from tests done on your stored samples. This is because research tests are often done using experimental procedures, so the results may not help for making decisions on managing your health. In case specific test result gives important information about your health, the researchers will tell the study staff and the study staff will try to contact you. If you wish to be contacted with this type of test result, you must give the study staff any change to your contact information.

Your samples will not be sold or used directly to produce commercial products. Research studies using your samples will be reviewed by a special committee at the Medical Research Council of Zimbabwe.

PROCEDURES:

When your blood is drawn from you, up to 10 ml (which is about a teaspoon) of the sample may be stored. For each sample of urethral or vaginal fluid given, part of the sample will tested immediately and the rest will be stored. Your samples will be stored safely and securely in a storage facility at the Zimbabwe Community Health Intervention Research Laboratory. Only the people who work at the facility and approved researchers will have access to your samples. The people who work at the facility will not have any information that identifies you. The approved researchers may be given information about you such as your age and sex, but they will not be given your name or any other information that identifies you. Your samples may be shipped to approved researchers, who work outside of Zimbabwe. There is no time limit on how long your samples will be stored.

RISKS and/or DISCOMFORTS:

There are few risks related to storing your samples. When tests are done on the stored samples there is a rare but possible risk to your privacy. It is possible that if others found out information about you that is learned from tests (HIV status and STIs) it could cause you problems with your family.

POTENTIAL BENEFITS:

There are no direct benefits to you from having your samples stored. You and others could benefit in the future from research done on your samples.

CONFIDENTIALITY:

To keep your information private, your samples will be labelled with a code that can only be traced back to your study clinic. Your name, where you live, and other personal information will be protected by the study clinic. When researchers are given your stored samples, they will not be given your personal information. The results of future tests will not be included in your health records. Every effort will be made to keep your personal information confidential, but we cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law.

Efforts will be made to keep your study records and test results confidential to the extent permitted by law. However, we cannot guarantee absolute confidentiality. You will be identified by a

code, and personal information from your records will not be released without your written permission. Any publication of this study will not use your name or identify you personally. However, your records may be reviewed by the sponsor of the study (U.S. Centers for Disease Control and Prevention [CDC]), the Medical Research Council of Zimbabwe (MRCZ), University of Zimbabwe, College of Medicine, Department of Community Medicine, Ministry of Health and Child Care (MOHCC), Colorado School of Public Health, University of Colorado Denver (USA), National Institute of Communicable Diseases (South Africa) and study staff.

In addition to the efforts made by the study staff to keep your personal information confidential, an Oath of Confidentiality was signed by all our staff working in this study. This Oath requires study staff not to tell people who are not connected with this study, information about you or other study participants or any other information related to the study.

PROBLEMS OR QUESTIONS:

For questions about the storage of your samples, contact:

Prof Mufuta Tshimanga, Tel 791649/762165

ZiCHIRe Project, 28 Van Praagh Avenue, Milton Park, Harare

For questions about your rights as a research subject, contact:

The National Coordinator
Medical Research Council of Zimbabwe
National Institute of Health Research
Cnr Mazoe Street/ Josiah Tongogara Avenue
Harare
Ph 791792, 791193
Cell 0772433166

CONSENT FOR SPECIMEN STORAGE AND SHIPMENT

Please carefully read the statements below (or have them read to you) and think about your choice. No matter what you decide it will not affect whether you can be in the research study, or your routine health care. (**Choose "YES" or "NO" for each statement by inserting your initials on the line**).

a) I agree to have my blood and urethral fluids (if male) and vaginal fluids(if female), and fluids from genital lesions stored in Zimbabwe after this study has ended and used for future testing related to HIV and Sexually Transmitted Infections.

YES_____ NO_____

b) I agree to have my blood and urethral fluids (if male) and vaginal fluids (if female), and fluids from genital lesions shipped to National Institute of Communicable Disease, in South Africa for tests required

YES_____ NO_____

_____ _____
Participant Name (print) Date

_____ AM_____
Signature of participant or legally authorised representative PM

_____ _____ _____
Name of ZiCHiRe Staff witnessing/obtaining consent Signature of staff obtaining consent Date

FOR AN ILLITERATE PARTICIPANT ONLY

_____ _____ _____
Name of participant witness and surname Signature Date

ETIOLOGY STUDY CLINICAL INTAKE FEMALE ASSSESMENT FORM

GENERAL

Date: Day__ __ Month __ __ Year __ __ __ __

Examiner: _____Examiner ID: _____

Study site: _____

INTRODUCTION

Thank you for agreeing to participate in this study to learn the causes of common sexually transmitted disease syndromes. In addition to the study procedures we discussed when you signed the consent form, you will be given care for the problem that brought you to the clinic.

We will start with questions about you and your sexual and medical history. Remember that all your answers will be kept completely confidential. Still, you do not have to answer any questions that you do not want to.

I. IDENTIFYING INFORMATION

1. **Surname _____**
2. **Given name _____**
3. **Date of birth: Day__ __ Month __ __ Year __ __ __ __**
4. **Address: _____Can use to contact? ___Yes ___No**
5. **Telephone/mobile number _____ Can use to contact? ___Yes ___No**
6. **Email address _____Can use to contact? ___Yes___No**

II. DEMOGRAPHICS

1. What is your ethnic background?

___ Shona
___ Ndebele
___ Kalanga
___ Suthu

___ Other _____

2. What is your current marital status?

___ Married/live with a person
___ Never married/single
___ Widowed
___ Separated
___ Divorced

3. What is your age? _____

4. What is you occupation?

___ Formal employment
___ Informal employment
___ Unemployed
___ Other, specify:_____

5. What is the highest educational level you have attained?

___ None
___ Primary
___ Secondary
___ Tertiary

6. To the best of your knowledge, are you pregnant now?

___ Yes
___ No

6a. If YES, about how far along are you? _____ Weeks

III. REASON(S) FOR TODAY'S CLINIC VISIT

1. What are the reasons you came to the clinic today?

Check all that apply

___ I have symptoms that bother me
___ My sex partner has a problem and I thought I should be checked
___ A nurse or sister told me I should be checked for STD
___ Other (specify) _____

2. What symptoms have you noticed? (Check all that apply)

	Symptom	Duration (days)
___	Abnormal vaginal discharge	_____
___	Vaginal odor	_____
___	Genital/vaginal itching	_____
___	Dysuria (pain with urination)	_____
___	Urinary frequency or urgency	_____
___	Abdominal pain or pain during sex	_____
___	Genital lesion(s) or rash	_____
___	Non-genital skin rash	_____
___	Anorectal symptoms	_____
___	Swelling or pain in the groin	_____
___	Other (specify) _____	_____

3. In the past 2 weeks, have you taken any antibiotics or other medicines?

___ Yes ___ No

3a. If yes, what medicines have you taken?_____
_____
_____

4. Who provided you with these medications?

___ Local clinic
___ Private doctor
___ Traditional healer
___ Local pharmacy
___ Self treatment
___ Other, specify: _____

5. Does your current partner or any of your other partners have STI symptoms/complaints?

___ Yes
___ No

5a. If yes, has he / have they been treated?

___ Yes
___ No

IV. SEXUAL HISTORY

Number of Partners

1. How old were you the very first time you had sex with a man? (For this and all other

questions this means vaginal or anal intercourse, that is, your received a man's penis in your vagina or rectum.)

____ Years old

2. To the best of your recollection, about how many different people have you had sex with in your *LIFETIME*? _____

3. In the last *12 MONTHS*, how many different people have you had sex with?

_____

4. In the last *3 MONTHS*, how many different people have you had sex with?

_____

4.a. Of the person or people you had sex with in the past 3 months, how many were:

Your spouse, live-in partner, or other steady partner? _____

Commercial sex partners, that is persons who paid you or gave you another benefit in order to have sex with you? _____

People with whom you had sex for the first or only time?_____

5. How many days ago did you last have sex with anybody? _____

Condom Use

6. The last time you had sex with a spouse, live-in, or steady partner did Your partner wear a male condom? ___Yes ___No

7. The last time you had sex with a new, casual, or commercial sex worker did your partner wear a male condom? ___Yes ___No

Contraception

8. Are you currently using any family planning method to delay or avoid getting pregnant?

8a. If yes, What family planning method are you currently using? (Check all that apply)

☐	Female sterilization	☐	Female condom
☐	Male sterilization (spouse/partner etc)	☐	Diaphragm
☐	Pill	☐	Foam or jelly
☐	IUD	☐	Lactational amenorrhea method
☐	Injection	☐	Rhythm or periodic abstinence
☐	Implants	☐	Withdrawal
☐	Male Condoms	☐	Emergency contraception
☐	Other: _____		

Vaginal Drying Agents

9. Have you ever used vaginal drying agents during sex? __Yes __No

9a. *IF YES:*
Have you used vaginal drying agents for sex in the past 2 months?
Yes ___No

With what type of partner have you used vaginal drying agents in the past 2 months?

___ Spouse/live-in/steady partner
___ Casual partner
___ Commercial sex partner

10. Do you ever douche? That is, do you flush your vagina with water or a cleansing fluid? __Yes __No

10a. If YES, have you douched in the last 2 months (60 days)?

___Yes ___N0

10b. If YES, how many days ago did you last douche? _____ days

V. PAST INFECTIONS

The following questions have to do with past STD infections, including infection with human immunodeficiency virus, or HIV, the cause of AIDS.

STDs

1. Have you ever been diagnosed with any of the following?

STD	Diagnosed		Date of Most Recent Diagnosis	Comments
Chlamydia	Yes	No	____ ____/____ ____	
Gonorrhea	Yes	No	____ ____/____ ____	
Bacterial vaginosis	Yes	No	____ ____/____ ____	
Trichomoniasis	Yes	No	____ ____/____ ____	
Vaginal yeast infection	Yes	No	____ ____/____ ____	
Vaginal infection, cause unknown	Yes	No	____ ____/____ ____	
Pelvic inflammatory disease	Yes	No	____ ____/____ ____	
Genital herpes	Yes	No	____ ____/____ ____	
Syphilis	Yes	No	____ ____/____ ____	
Genital warts	Yes	No	____ ____/____ ____	
Cancer of thecervix or vagina	Yes	No	____ ____/____ ____	
Hepatitis B	Yes	No	____ ____/____ ____	
Other (specify in comments)	Yes	No	____ ____/____ ____	

Comments:

HIV/AIDS

2. Have you ever had an HIV test? __Yes __No

2.a. If YES, when were you tested most recently? ____/___ (Month/Year)

2. b. If YES, what was the result? ___Positive ___Negative ___Don't know

3. Regardless of whether you actually were tested, do you believe you might have HIV or AIDS at this time? __Yes ___No ___Don't Know

VI. EXAMINATION

No Exam Done	Category	Classification				Comments/Descriptions
√		***Circle Answer***				
	1.General Appearance	No Apparent Distress		Distress		
	2. Vulva/Bus	Normal		Abnormal		
	External Lesions	No		Yes		
	3. Vagina	Normal		Abnormal		
	Discharge Amount	None	Small	Moderate	Large	
	Discharge Character	Floc	Hgn	Plq	Menses	Yellow
	6. Anal Exam	Normal		Abnormal		
	Anal lesion	No		Yes		
	7. Abdomen	Normal		Abnormal		
	8. Lymph Nodes	Normal		Abnormal		
	9. Skin Rash	No		Yes		

COMPLETE IF GENITAL AREA LESION(S) ARE OBSERVED:

1. Vesicular or pustular lesions ___Yes ___No

If YES **Number of lesions** _____

Distribution of lesions ___Single ___Scattered ___Clustered

Laterality ___Unilateral ___Bilateral __ Midline only

Tenderness of lesions (4 is most severe) __0 __1 __2 __3 __4

Location(s) (check all that apply)
___ Introitus
___ Labia minor
___ Labia major
___ Anal, perianal
___ Perineum
___ Mons pubis
___ Other (specify) _____

2. Ulcerative lesions ___ Yes ___ No

If YES **Number of lesions** _____

Size of largest lesion(s) _____ mm

Distribution of lesions ___Single ___Scattered ___Clustered

Laterality ___Unilateral ___Bilateral __ Midline only

Tenderness of lesions (4 is most severe) __0 __1 __2 __3 __4

Induration of lesions (4 is firmest) __0 __1 __2 __3 __4

Appearance of base ___ Purulent ___ "Clean" ___ Other (specify)_____

Location(s) (check all that apply)
___ Introitus
___ Labia minor
___ Labia major
___ Anal, perianal
___ Perineum
___ Mons pubis
___ Other (specify) _____

Expophytic or wart-like lesions___ Yes ___ No

If YES

Number of lesions _____

Size of largest lesion(s) _____ mm

Distribution of lesions ___Single ___Scattered ___Clustered

Laterality ___Unilateral ___Bilateral __ Midline only

Induration of lesions (4 is firmest) __0 __1 __2 __3 __4

Location(s) (check all that apply)
___ Introitus
___ Labia minor
___ Labia major
___ Anal, perianal
___ Perineum
___ Mons pubis
___ Other (specify) _____

COMPLETE IF INGUINAL LYMPHADENOPATHY IS PRESENT

Which side(s?) ___Left ___Right ___Both

Size/enlargement (4 is largest) __0 __1 __2 __3 __4

Tenderness (4 is maximum) __0 __1 __2 __3 __4

Overlying erythema __Yes __No
Fluctuance __Yes __No

VII. SPECIMEN CHECK LIST

Discharge

□ Smear for Gram Stain
□ Swab for Ct/Ng ProbeTec
□ Swab for Ct/Ng GeneXpert
□ Swab for Ct/Ng/Tv/Mg m-PCR

GUD

□ Swab for m-PCR
□ Swab for Ct/Ng ProbeTec

Blood draw for HIV/RPR

□ Yes
□ No

VIII. Treatment

Which of the following did the patient receive as treatment for his STI syndrome today? Check all that apply.

□ Kanamycin
□ Ceftriaxone
□ Spectinomycin
□ Gentamycin
□ Cefixime
□ Ofloxacin
□ Ciprofloxacin
□ Azithromycin
□ Erythromycin
□ Doxycycline
□ Tetracycline
□ Metronidazole
□ Clindamycin
□ Tinidazole
□ Benzathine Penicillin (LAB)
□ Acyclovir
□ Valacyclovir
□ Famciclovir
□ Nystatin
□ Miconazole
□ Clotrimazole

□ Other: specify:

IX.PARTNER MANAGEMENT

Please check any of the following:

□ Patient instructed to notify partner(s) and encourage them to seek medical attention
□ Partner(s) already treated
□ Partner(s) seen in clinic today

X. PATIENT INSTRUCTIONS AND FOLLOW-UP

Please check any of the following:

□ Patient given medications
□ Patient given prescription
□ Patient given instructions on how to take medications
□ Patient given information on study follow-up and who to call with questions

ETIOLOGY STUDY CLINICAL INTAKE
MALE ASSSESMENT FORM

GENERAL

Date: Day__ __ Month __ __ Year __ __ __ __

Examiner: _____Examiner ID: _____

Study site: _____

INTRODUCTION

Thank you for agreeing to participate in this study to learn the causes of common sexually transmitted disease syndromes. In addition to the study procedures we discussed when you signed the consent form, you will be given care for the problem that brought you to the clinic.

We will start with questions about you and your sexual and medical history. Remember that all your answers will be kept completely confidential. Still, you do not have to answer any questions that you do not want to.

I. IDENTIFYING INFORMATION

1. **Surname _____**
2. **Given name _____**
3. **Date of birth: Day__ __ Month __ __ Year __ __ __ __**
4. **Address: _____Can use to contact? ___Ye ___No**
5. **Telephone/mobile number _____Can use to contact? ___Yes ___No**
6. **Email address _____ Can use to contact? ___Ye ___No**

II. DEMOGRAPHICS

1. What is your ethnic background?

___ Shona
___ Ndebele
___ Kalanga
___ Suthu
___ Other _____

2. What is your current marital status?

___ Married/live with a person
___ Never married/single
___ Widowed
___ Separated
___ Divorced

3. What is your age? _____

4. What is you occupation?

___ Formal employment
___ Informal employment
___ Unemployed
___ Other, specify:_____

5. What is the highest educational level you have attained?

___ None
___ Primary
___ Secondary
___ Tertiary

III. REASON(S) FOR TODAY'S CLINIC VISIT

1. What are the reasons you came to the clinic today?

Check all that apply

___ I have symptoms that bother me
___ My sex partner has a problem and I thought I should be checked
___ A nurse or sister told me I should be checked for STD
___ Other (specify) _____

2. What symptoms have you noticed? (Check all that apply)

	Symptom	**Duration (days)**
___	Urethral discharge	_____
___	Dysuria (pain with urination)	_____
___	Other urethral symptoms	_____
___	Genital lesion(s) or rash	_____
___	Non-genital skin rash	_____
___	Anorectal symptoms	_____
___	Pain or swelling of testicle	_____

___ Swelling or pain in the groin _____
___ Other (specify) _____ _____

3. In the past 2 weeks, have you taken any antibiotics or other medicines? Yes No

3a. If yes, what medicines have you taken?_____
_____
_____

4. Who provided you with these medications?

___ Local clinic
___ Private doctor
___ Traditional healer
___ Local pharmacy
___ Self treatment
___ Other, specify: _____

5. Does your current partner or any of your other partners have STI symptoms/complaints?

___ Yes
___ No

5a. If yes, has she / have they been treated?

___ Yes
___ No

IV. SEXUAL HISTORY

Number of Partners

1. How old were you the very first time you had sex with a woman? (For this and all other questions this means vaginal or anal intercourse, that is, your penis entered your partner's vagina or rectum.)

____ Years old

2. To the best of your recollection, about how many different people have you had sex with in your *LIFETIME*? _____

3. In the last *12 MONTHS*, how many different people have you had sex with?

_____

4. In the last *3 MONTHS*, how many different people have you had sex with?

_____

4.a. Of the person or people you had sex with in the past 3 months, how many were:

Your spouse, live-in partner, or other steady partner? _____

Commercial sex partners, that is persons whom you paid or gave another benefit in order to have sex with you?

_____

People with whom you had sex for the first or only time?_____

5. How many days ago did you last have sex with anybody?

_____

Condom Use

6. The last time you had sex with a spouse, live-in, or steady partner did you wear a male condom? ___Yes ___No

7. The last time you had sex with a new, casual, or commercial sex worker did you wear a male condom? ___Yes ___No

V. PAST INFECTIONS

The following questions have to do with past STD infections, including infection with human immunodeficiency virus, or HIV, the cause of AIDS.

STDs

1. Have you ever been diagnosed with any of the following?

STD	Diagnosed		Date of Most Recent Diagnosis	Comments
Chlamydia	Yes	No	____ ____/____ ____	
Gonorrhea	Yes	No	____ ____/____ ____	
Urethral discharge, cause unknown	Yes	No	____ ____/____ ____	
Genital Herpes	Yes	No	____ ____/____ ____	
Genital Warts	Yes	No	____ ____/____ ____	
Syphilis	Yes	No	____ ____/____ ____	
Hepatitis B	Yes	No	____ ____/____ ____	
Other: specify in comments	Yes	No	____ ____/____ ____	

Comments:

HIV/AIDS

2. Have you ever had an HIV test? __Yes __No

2.a. If YES, when were you tested most recently? ____/___ (Month/Year)

2. b. If YES, what was the result? ___Positive ___Negative ___Don't know

3. Regardless of whether you actually were tested, do you believe you might have HIV or AIDS at this time? __Yes ___No ___Don't Know

VI. EXAMINATION

No Exam Done	Category	Classification				Comments/Descriptions
	1. General Appearance	No Apparent Distress		Distress		
	2. Circumcised	No		Yes		
	3. Urethral Discharge	Normal		Abnormal		
	Discharge amount	None	Small	Moderate	Large	
	Discharge character	Clear	Cloudy	Purulent		
	4. Genital lesion(s)	No		Yes		
	5. Scrotal Contents	Normal		Abnormal		
	6. Lymph Nodes	Normal		Abnormal		
	Inguinal	Normal		Abnormal		
	Other	Normal		Abnormal		
	7. Anal Exam	Normal		Abnormal		
	Anal lesion	No		Yes		
	8. Skin	Normal		Abnormal		
	9. Mouth/Throat	Normal		Abnormal		

COMPLETE IF GENITAL AREA LESION(S) ARE OBSERVED:

1. Vesicular or pustular lesions ___Yes ___No

If YES **Number of lesions** _____

Distribution of lesions___Single ___Scattered ___Clustered

Laterality ___Unilateral ___Bilateral __ Midline only

Tenderness of lesions (4 is most severe) __0 __1 __2 __3 __4

Location(s) (check all that apply) ___ Glans penis
___ Under foreskin
___ Penile shaft
___ Scrotum

___ Anal, perianal
___ Perineum
___ Mons pubis
___ Other (specify) _____

2. Ulcerative lesions ___ Yes ___ No

If YES **Number of lesions** _____

Size of largest lesion(s) _____ mm

Distribution of lesions ___Single ___Scattered ___Clustered

Laterality ___Unilateral ___Bilateral __ Midline only

Tenderness of lesions (4 is most severe) __0 __1 __2 __3 __4

Induration of lesions (4 is firmest) __0 __1 __2 __3 __4

Appearance of base ___ Purulent ___ "Clean" ___ Other (specify)_____

Location(s) (check all that apply) ___ Glans penis
___ Under foreskin
___ Penile shaft
___ Scrotum
___ Anal, perianal
___ Perineum
___ Mons pubis
___ Other (specify) _____

Expophytic or wart-like lesions___ Yes ___ No

If YES **Number of lesions** _____

Size of largest lesion(s) _____ mm

Distribution of lesions ___Single ___Scattered ___Clustered

Laterality ___Unilateral ___Bilateral __ Midline only

Induration of lesions (4 is firmest) __0 __1 __2 __3 __4

Location(s) (check all that apply) ___ Glans penis
___ Under foreskin
___ Penile shaft
___ Scrotum
___ Anal, perianal
___ Perineum
___ Mons pubis
___ Other (specify) _____

COMPLETE IF INGUINAL LYMPHADENOPATHY IS PRESENT

Which side(s?) ___Left ___Right ___Both

Size/enlargement (4 is largest)		__0 __1 __2 __3 __4
Tenderness (4 is maximum)		__0 __1 __2 __3 __4
Overlying erythema	__Yes	__No
Fluctuance	__Yes	__No

VII. SPECIMEN CHECK LIST

Discharge

□ Smear for Gram Stain
□ Swab for Ct/Ng ProbeTec
□ Swab for Ct/Ng GeneXpert
□ Swab for Ct/Ng/Tv/Mg m-PCR

GUD

□ Swab for m-PCR
□ Urine for Ct/Ng ProbeTec

Blood draw for HIV/RPR

□ Yes
□ No

VIII. Treatment

Which of the following did the patient receive as treatment for his STI syndrome today? Check all that apply.

□ Kanamycin
□ Ceftriaxone
□ Spectinomycin
□ Gentamycin
□ Cefixime
□ Ofloxacin
□ Ciprofloxacin
□ Azithromycin
□ Erythromycin
□ Doxycycline
□ Tetracycline
□ Metronidazole
□ Clindamycin
□ Tinidazole
□ Benzathine Penicillin (LAB)
□ Acyclovir
□ Valacyclovir

□ Famciclovir
□ Nystatin
□ Miconazole
□ Clotrimazole
□ Other: specify:

IX.PARTNER MANAGEMENT

Please check any of the following:

□ Patient instructed to notify partner(s) and encourage them to seek medical attention

□ Partner(s) already treated

□ Partner(s) seen in clinic today

X. PATIENT INSTRUCTIONS AND FOLLOW-UP

Please check any of the following:

□ Patient given medications

□ Patient given prescription

□ Patient given instructions on how to take medications

□ Patient given information on study follow-up and who to call with questions

Appendix 4

Laboratory Study Procedures

Standard Testing at Study Clinics
HIV and, to a certain extent, syphilis serological testing are conducted at study clinics per standard clinic protocols. While the results of these tests will be recorded for study participants after study consent has been obtained, the actual testing procedures are not part of the study and are not supervised by study staff. The following details are provided for completeness.
HIV Testing

Testing for HIV is per clinic guidelines. After obtaining patient consent, capillary blood is obtained by finger prick and processed for HIV rapid testing using the following algorithm: 1) initial test by First Response HIV1-2-O; 2) confirmatory test by SD Alere Determine HIV1/2 if the initial test is positive; and 3) INSTI HIV1/HIV2 is used as a tie breaker if the initial and confirmatory tests are discrepant. All tests are conducted following the test package inserts. Clinic staff performing the tests is trained during a 5-day workshop that covers theory and practice for rapid HIV testing conducted and accredited by the Zimbabwe Health Professions Council.

Syphilis testing
Testing for syphilis is per clinic guidelines. After obtaining patient consent, capillary blood is obtained by finger prick and processed for syphilis testing by the SD Bioline treponemal test (SD Bioline Syphilis 3.0). The test will be conducted according to the test package insert. Clinic staff performing the test is trained during a 5-day workshop that covers theory and practice for rapid HIV testing conducted and accredited by the Zimbabwe Health Professions Council.
As part of the consent process and specifically included on the consent form, participants enrolled in the STI aetiology study will be asked for permission to record HIV and syphilis test results conducted per standard clinical protocol on the study data collection form. In addition, participants will be asked to consent to having blood samples taken for HIV and syphilis testing at the study laboratory as detailed below.

In addition to standard HIV and syphilis testing, consenting patients will also be tested using the SD Bioline DUO (combined HIV and syphilis rapid test) as per the protocol that the Zimbabwe Ministry of Health has developed for the validation of this test.

Testing at Study Laboratories

The following laboratories are involved with testing for study purposes:

1. ZiCHIRe Laboratory at Wilkins Hospital, Harare (Ms. Luanne Rodgers)

The ZiCHIRe laboratory will function as the receiving, processing, and holding laboratory for all clinical specimens and hard copy data related to the STI aetiology study. This lab will also perform the following tests: Gram stain for vaginal and urethral smears; HIV and syphilis serology; GeneXpert testing for *N. gonorrhoeae* and *C. trachomatis* on urethral, vaginal, and urine specimens.

2. University of Zimbabwe/University of San Francisco laboratory in the OB/GYNE department of the University of Zimbabwe School of Medicine (Dr. Marshall Munjoma)

   This laboratory will be responsible for testing of vaginal and urine specimens for *N. gonorrhoeae* and *C. trachomatis* using the Becton Dickenson ProbeTec testing platform.

3. STI Reference Laboratory at the National Institute of Communicable Diseases, Johannesburg, South Africa (Prof. David Lewis)

Vaginal and urethral specimens will be shipped to this lab for multiplex PCR (m-PCR) testing on the following pathogens: *N. gonorrhoeae*, *C. trachomatis*, *M. genitalium*, and *T. vaginalis.* Specimens taken form genital ulcerations will be tested for: *T. pallidum*, *H. ducreyi*, and herpes simplex virus.

Specimen Collection at Study Sites

Blood Specimens

Blood specimens will be collected from all patients enrolled in the aetiology study who consent to HIV testing and from all patients who are enrolled with genital ulcer disease to test for treponemal antibodies (note: this is in addition to any testing that may have occurred per standard clinical protocol at the study site – see above).

Blood will be collected through venipuncture using standard procedures. To this effect, 7-10 ml. blood will be obtained and collected in a red-topped BD blood collection tube. The whole blood sample will be kept refrigerated (2-8 °C), until shipment in a cooler box with cooling packs and a thermometer to record max/min temperatures during transportation to the receiving laboratory. Specimens will be shipped to the ZiCHIRe receiving laboratory at least twice a week. At the receiving laboratory, the sample will then be centrifuged at 2500 RPM for 5 minutes. The serum will be aspirated and put into 2 ml cryovials, for testing and long term storage. The remaining sample will be disposed of into biohazard container and incinerated.

Genital Specimens

1. Women with Vaginal Discharge

   a. PH of vaginal discharge will be measured and recorded by study staff using ColorpHast pH strips

   b. Vaginal discharge will be obtained using a cotton-tipped swab and smeared on a glass slide and air-dried for transport to the receiving lab for Gram stain analysis.

   c. Four samples will be taken from vaginal discharge using Dacron tipped, plastic shafted swabs.
      - ProbeTec- 2 dry swabs
      - GeneXpert-1 dry swab
      - m-PCR-1 swab

The sampling kit for GeneXpert and ProbeTec will be those provided by the manufacturer and used according to the package insert.

All specimens will be stored in an on-site refrigerator until shipment to the receiving laboratory in a cooling box with cooling packs as described above.

2. Men with Urethral Discharge

   a. Urethral discharge will be obtained using a cotton-tipped swab and smeared on a glass slide and air-dried for transport to the receiving lab for Gram stain analysis.

   b. One sample for m-PCR will be taken from urethral discharge using Dacron tipped metal -shafted swabs.

c. 15 ml of first-voided urine will be obtained using collection kits for ProbeTec and GeneXpert.

The sampling kits for ProbeTec and GeneXpert will be those provided by the manufacturer and used according to the package insert.

All specimens will be stored in an on-site refrigerator until shipment to the receiving laboratory in a cooling box with cooling packs as described above.

3. Men or women with Genital Ulcer Disease

   a. A single swab specimen for m-PCR will be obtained from the genital lesion using a dry Dacron swab and stored in a plastic tube.
   b. From women, two vaginal swabs will be obtained using Dacron tipped plastic shafted swabs for ProbeTec and GeneXpert
   c. From men, 15 ml of first-voided urine will be obtained using collection kits for ProbeTec and GeneXpert.

All specimens will be stored in an on-site refrigerator until shipment to the receiving laboratory in a cooling box with cooling packs as described above.

Testing at Study Laboratories

1. ZiCHIRe Laboratory

The ZiCHIRe laboratory at Wilkins Hospital in Harare will receive all specimens and hard-copy study documentation. The ZiCHIRe lab will also conduct the following tests:

Gram Stain
Vaginal and urethral discharge slides will be Gram stained and read under high-power magnification per standard laboratory procedures.

HIV testing
Serum samples from consenting study patients received from participating clinics will be stored at 2-8 °C in the lab until tests are run. The samples will be sufficient to run both HIV and syphilis tests. The testing algorithm at the study lab will be the same as the one used at the clinics: 1) initial test by First Response HIV1-2-O; 2) confirmatory test by Alere Determine HIV1/2 if the initial test is positive; and 3) INSTI HIV1/HIV2 is used as a tie breaker if the initial and confirmatory tests are discrepant. All tests are conducted following the test package inserts and the laboratory standard operating procedures (SOP).

Syphilis testing
All serum samples are tested by the SD Bioline treponemal test, followed by the non-treponemal Rapid Plasma Reagin Test (RPR) for those specimens testing positive on the treponemal test. Patients with genital ulcer disease will have both tests performed, regardless of the result of the treponemal test. Both the SD Bioline and RPR tests will be performed using the manufacturer's instructions and the laboratory SOP's, that include standard quality assurance measures.
Serum samples remaining after HIV and syphilis testing is complete will be frozen at -85 °C for long-time storage.

In addition to the above standard HIV and syphilis tests, consenting study patients will also be tested using the Standard Diagnostics Bioline DUO (combined HIV and syphilis rapid test) per protocol developed by the Zimbabwe Ministry of Health for the validation of the DUO test. Test kits will be provided in kind to the study by the test manufacturer. DUO test results will be compared with the results of the standard HIV and syphilis tests. Conflicting results will be carefully analyzed and additional tests may be performed to resolve discrepancies.

As has been discussed above, some (but not all) patients will have been tested for HIV and/or syphilis as part of standard care at the participating clinics. Discrepant or new positive results will be carefully evaluated and, after consultation with the project's principal investigator or lead consultant, patients may be contacted and instructed to receive additional follow-up and/or care if indicated. This contingency is covered in the study consent form and patient contact information has been requested and recorded for this purpose.

2. University of Zimbabwe / University of California San Francisco Laboratory at the OB/GYNE Department of the UZ School of Medicine.

BD ProbeTec for N. gonorrhoeae and C. trachomatis

Specimens for BD ProbeTec will be stored at the ZiCHIRe receiving lab and shipped in weekly batches to the UZ/UCSF laboratory at Parirenyatwa University Hospital. This laboratory has been accredited by the College of American Pathologists to perform the BD ProbeTect combination test for the detection of *N. gonorrhoeae* and *C. trachomatis*. These tests will be run according to the manufacturer's instructions and laboratory SOP's. Results will be transmitted electronically to the ZiCHIRe laboratory and entered into the study database.

3. STI Reference Laboratory at the National Institute of Communicable Diseases, Johannesburg, South Africa.

Multiplex Polymerase Chain Reaction (m-PCR) test for N. gonorrhoeae, C. trachomatis, T. vaginalis, M. genitalium, T. pallidum, H. ducreyi, and HSV

Specimens for m-PCR (collected from patients with vaginal discharge, urethral discharge, and genital ulcer disease) will be frozen at -85 °C and stored at the ZiCHIRe receiving laboratory until ready for shipment on dry ice to the NICD lab. It is anticipated that shipment will occur in batches approximately twice per month. At the NICD laboratory, the specimens will be tested by in-house developed and validated m-PCR tests to detect the following pathogens: *N. gonorrhoeae*, *C. trachomatis*, *M. genitalium* and *T. vaginalis* (for patients with vaginal or urethral discharge syndromes) and *T. pallidum*, *H. ducreyi* and herpes simplex virus (for patients with genital ulcer disease). Testing will follow the SOP's developed for this purpose. All results will be communicated electronically to the ZiCHIRe laboratory and entered into the study database.
Of note, m-PCR testing for the above-referenced pathogens is not FDA approved and is conducted for research purposes. Given their high sensitivity, and the fact that other NAAT are not commercially available for M. genitalium, T. pallidum, H. ducreyi, and HSV, m-PCR testing is a critical research tool to establish the etiology of common STI syndromes. While we will not determine the diagnosis and treatment of individual cases based solely on the m-PCR results, we will carefully compare the results between the different testing platforms and, for positive results, also determine what if any treatment the patient received at the clinic when enrolled. In this context, it should be remembered that all patients should have received treatment for the respective syndrome they were diagnosed with as per Zimbabwe national syndromic treatment guidelines and that study staff will assure appropriate treatment as part of the study protocol. However, inadequate or insufficient treatment will be brought to the attention of the PI or lead consultant of the project and, based on their judgment, the patient may be contacted for further follow-up and treatment. This contingency is covered in the study consent form and patient contact information has been requested and recorded for this purpose.

4. Laboratory to be Determined (Flowcytometry lab, Harare)

GeneXpert

The GeneXpert platform has been FDA-approved for testing for *N. gonorrhoeae* and *C. trachomatis.* While this platform is available in Zimbabwe for purposes of tuberculosis testing, it is currently not used for *N. gonorrhhoeae* or *C. trachomatis* testing. While not conceived as a formal validation process, testing specimens by GeneXpert will allow us to gain experience with a platform that is already being used in Zimbabwe for TB testing. To this effect, Cepheid, the GeneXpert manufacturer, will provide the project with test kits at no charge. We intend to conduct GeneXpert testing on all samples that will also be tested using the Becton Dickenson ProbTec platform at the UZ/UCSF laboratory at the University of Zimbabwe School of Medicine (fully accredited by the College of American Pathologists) and the multiplex-PCR platform at the STI reference lab the National Institute of Communicable Diseases in Johannesburg, South Africa. These latter platforms will be used as gold standard comparitors, for sensitivity, specificity, and positive and negative predictive values. 95% confidence intervals for proportions will be calculated using the methods described by Newcombe and Wilson. The sample will include 200 urine samples form men with urethral discharge, 200 vaginal swab specimens form women with vaginal discharge, 100 urine specimens from men with genital ulcer disease, and 100 vaginal swabs form women with vaginal ulcer disease. Processing of specimens will follow the procedures outlined in the GeneXpert package insert. We will make no clinical or treatment decisions based on the GeneXpert results alone, however, discrepant results between GeneXpert and one or both of the other platforms will be carefully evaluated and reported to the project PI or lead consultant. Based on their judgment, the patient may be contacted for further follow-up and treatment. This contingency is covered in the study consent form and patient contact information has been requested and recorded for this purpose.

The Etiology of Sexually Transmitted Syndromes in Zimbabwe Protocol Summary

1. RESEARCH QUESTIONS TO BE ADDRESSED IN THIS PROPOSAL

The goal of this study is to determine the current etiology of syndromes associated with sexually transmitted infections (STI) in Zimbabwe. These include the causes of the following genital syndromes: urethral discharge syndrome in men, vaginal discharge syndrome in women and genital ulcer disease in both men and women.

2. RATIONALE FOR RESEARCH

As in other countries where etiologic testing is not available, most sexually transmitted infections (STI) in Zimbabwe are treated "syndromically", i.e. using a combination of antimicrobials that cover the most important etiologic agents of each syndrome. Thus, men with urethral discharge syndrome are treated for gonorrhea, chlamydia, trichomoniasis and mycoplasma infections. Women with vaginal discharge syndrome are treated for the same infections as well as vaginal candidiasis and bacterial vaginosis. Finally, men and women with genital ulcerations are treated for syphilis, chancroid, and herpes infections.

Most countries follow the STI syndromic management guidelines published by the WHO or have published country-specific treatment protocols. Thus, governmental agencies in Zimbabwe have developed guidelines for the treatment of STI and its associated syndromes.[5]

An obvious drawback of using the "syndromic approach" is that it leads to both under-treatment (as many asymptomatic cases are missed) as well as over-treatment since syndromic treatment at the individual level covers all possible etiologies and not the one(s) actually involved in each specific case.

Furthermore, as the epidemiology of individual STI shifts over time, treatment regimens may be outdated, or may fail to include medications to cover infections that have gained in importance. For example, over the several past decades, a significant shift has occurred in the etiology of genital ulcer disease in sub-Saharan Africa. Whereas chancroid and syphilis were responsible for a majority of cases in the past, currently the preponderance of cases are caused by genital herpes.[6,7]This shift has important consequences for the syndromic treatment of genital ulcer disease as it requires the inclusion of acyclovir (or similar antiviral medications) in the syndromic treatment regimen.

Also, during the past decades, important shifts have occurred in the etiology of urethral and vaginal discharge syndromes in the western world, with gonorrhea decreasing and chlamydia (relatively) increasing. Furthermore, the role of other micro-organisms, especially Mycoplasma genitalis is becoming increasingly recognized. This latter organism has an antibiotic susceptibility profile that is significantly different from that of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. [8]Thus, the relative prevalence of mycoplasma as a cause of urethral and vaginal discharge

syndromes should also determine the composition or priority order of the syndromic treatment regimens.

Together, the above findings form an argument for a more etiologically-oriented approach of STI diagnosis and treatment in developing countries like Zimbabwe. In the absence of routine testing for individual micro-organisms, periodic studies should be conducted among representative samples of patients with sexually transmitted syndromes to best describe the current etiology of these syndromes in order to develop syndromic treatment guidelines in Zimbabwe on a scientific basis.

Objectives

1. To determine the prevalence of the following micro-organisms among men with urethral discharge syndrome in Zimbabwe: *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, and *Trichomonas vaginalis*.

2. To determine the prevalence of the following micro-organisms among women with vaginal discharge syndrome in Zimbabwe*: Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Trichomonas vaginalis*, and *Candia albicans*.

3. To determine the prevalence of bacterial vaginosis among women with vaginal discharge syndrome in Zimbabwe

4. To determine the prevalence of the following micro-organisms among men and women with genital ulcer disease in Zimbabwe: *Treponema pallidum*, *Haemophilis ducreyi*, *herpes simplex virus*, and *Chlamydia trachomatis* (LGV strains)

5. To assess the prevalence of HIV among men and women presenting with sexually transmitted syndromes in Zimbabwe.

6. To assess the prevalence of positive syphilis serology among men and women with sexually transmitted syndromes in Zimbabwe.

7. To assess the prevalence of urethral/vaginal *N. Gonorrhoeae* and *C.trachomati*s co-infection in men and women diagnosed with genital ulcer disease

8. To determine demographic and risk factors associated with the prevalence of the above infections.

3. METHODS

Study Design: This study is a cross-sectional survey of patients with STI-related syndromes recruited sequentially at 4-8 clinic sites thought to be representative for the majority of patients presenting with such Syndromes in Zimbabwe. This will be a single-visit study that will take about 30 minutes to complete. Patients will be treated and followed-up per standard clinical protocol.

Population: The study will enroll 200 subjects in each of the syndromic categories, for a total of 600 subjects. These sample sizes will yield acceptable 95% confidence intervals around the expected prevalence rates for each of the pathogens. Subjects will include both men and women, aged 18-55 years who present with any of the 3 STI-associated syndromes.

Special Populations: None

Source of participating participants: The study will be first implemented at Mbare Polyclinic in Harare. Additional clinics (4-8) will be selected on the basis of reported STI cases, clinic characteristics, and diversity.

Research Procedures: Eligible patients will be asked to join the study by the study nurse and enter the consent process. After signing the consent form, the study nurse will complete the following study procedures: 1) Collect demographic and behavioral data using a standard electronic data collection form (Attached); 2) Perform a genital examination and describe characteristics of discharge or lesion(s); 3) Collect swab specimens from discharge or genital ulceration for the detection of the following micro-organisms: *N. gonorrhoeae*, *C. trachomatis*, *T. vaginalis*, and *M. genitalium* (for patients with vaginal or urethral discharge); *T. pallidum*, *H. Ducreyi*, and herpes simplex hominis virus (for patients with genital ulcer disease); 4) Collect a blood specimen for RPR and HIV testing (optional).

Laboratory testing: Testing for *N. gonorrhoeae* and *C. trachomatis* will be conducted at the OB/GYNE laboratory at the University of the Zimbabwe School of Medicine using the ProbeTec nucleic acid amplification assay. Nucleic acid amplification testing (NAAT) for other etiologic agents, including *T. vaginalis*, *M. genitalium*, *T. pallidum*, *H. ducreyi*, and herpes simplex virus can only be conducted at specialized STI research laboratories and is not currently available in Zimbabwe. Specimens will therefore also be sent to the STI reference laboratory at the National Institute of Communicable Diseases in Johannesburg (form attached). Finally, samples will also be used to validate a new testing modality available in Zimbabwe: the GeneXpert system which is currently available for Tuberculosis testing. This platform has recently been approved for *N. gonorrhoeae* and *C. trachomatis* testing in the U.S., but has not been validated in Zimbabwe.

Distinguish research from routine procedures: All the above-described research procedures are in addition to routine clinical procedures. This is not a treatment/intervention study. All patients will be treated for their condition using existing treatment guidelines whether they choose to be in the study or not.

Questionnaire/interview instrument: The study will employ a brief electronic demographic and behavioral assessment tool (attached).

Methods of Intervention: This is not an intervention study.

Methods for dealing with adverse events: All participant materials are reviewed by the study nurse and study coordinator on a weekly basis. Irregularities are reported to the Principal Investigator. Any unforeseen events and/or patient complaints will be investigated by the study coordinator and reported to the Principal Investigator. Serious adverse events resulting in direct or indirect patient injury will be promptly reported to MRCZ.

Methods of Dealing with Illegal, Reportable Activities: None anticipated.

4. RISKS/BENEFITS TO PARTICIPANTS

Risks of the study include anxiety and embarrassment when answering sensitive questions and genital examination; mild discomfort when obtaining specimens, mild pain and bruising as a results from needle stick for blood sample collection; and potential breach of confidentiality.

Risks are minimized by appropriate inclusion and exclusion criteria, no unnecessary procedures, trained study professionals, patient monitoring and follow-up, and data safety procedures (see below).

The main benefit of this study is generalizable knowledge regarding the aetiology of STI syndromes. Benefits for the patient include the possible detection and subsequent treatment for infections beyond current standard protocols.

5. COSTS AND COMPENSATION

There are no costs for patients to participate in the study. Participants will be paid $5 USD as compensation for their time.

6. CONFIDENTIALITY ASSURANCES

All hard copy data (specifically signed consent forms and identifying information) will be stored in locked file cabinets at the primary study site. Electronic data, including the main study database, will be stored on a secure, password protected computer at the primary study site. Electronic back-ups of data files will be made on a daily basis and stored on a separate, password protected computer in a different location. Electronic records will include a unique participant identifier that can be used as a cross-reference to the hard-copy files. However, no other patient identifiers will be included in the electronic record. Thus the only place where identifying information is stored is in the hard copy files. Maintaining these identifiers is necessary to allow patient follow-up should this be necessary based on test results.

The following persons will have access to the data: study coordinator (Mr. Vitalis Kupara, tel: 791649 principal investigator (Dr. Mufuta Tshimanga, tel: 733675) and lead consultant (Dr. Cornelis Rietmeijer., tel: +1 303 981-0008).

Upon completion of the study, when a determination is made that maintaining identifying information is no longer warranted, the dataset will be de-identified and identifiers will be destroyed. The de-identified dataset will be available for analyses indefinitely.

7. CONFLICT OF INTERESTS

There are no conflicts of interest.

8. COLLABORATIVE AGREEMENTS

The Medical Research Council of Zimbabwe is the single IRB on record for this study. No approval from other IRB's will be solicited.

9. INTENDED USE OF RESULTS

The results of this study will be reported to agencies in Zimbabwe that are concerned with the surveillance and development of treatment guidelines for sexually transmitted infections in Zimbabwe, including but not limited to the Zimbabwe Ministry of Health and Child Welfare, regional health departments, the University of Zimbabwe, and the sponsor of the study: the U.S. Centers for Disease Control and Prevention. Data will also be used for the development of one or more manuscripts or abstracts submitted to medical journals and local and international scientific conferences.

10. OTHER INFORMATION

None.

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